UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

GROWTH AND DEVELOPMENT OF THE LUSITANO FOAL ON EXTENSIVE SYSTEMS

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TESE DE DOUTORAMENTO EM CIÊNCIAS VETERINÁRIAS

ESPECIALIDADE DE PRODUÇÃO ANIMAL

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Declaração

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THESIS TITLE: Growth and development of the Lusitano foal on extensive systems

ABSTRACT:

The general aim of this study was to characterize the growth and development of the Lusitano foal bred and raised on extensive systems. In particular, the relationship between growth patterns and bone quality was evaluated. Additionally, nutritional status and body condition (BC) changes of the mares during the productive cycle were studied, in order to stablish appropriate scores to optimize the development of foals from pregnancy to weaning, as well as to increase the productive efficiency of the system. A longitudinal field study was conducted in four Lusitano stud-farms. Broodmares were monitored during three consecutive gestation/lactation cycles and foals were followed from birth to 42 months of age. The biomechanical properties of equine cortical bone were also assessed. Results showed that changes in broodmare body weight (BW) and BC were mainly influenced by pasture availability and quality and when the foaling season occurs in the year. The reproductive performance of mares and the growth of suckling foals were also clearly influenced by broodmare nutritional status. BC score changes at mating had a strong effect on fertility outcome of the first two estrous cycles after foaling, being highly impaired by BCS negative changes, whatever the BCS. Best fertility results were obtained with positive and greater BC changes. The Richards function was used to characterize foals' growth patterns and growth curves were obtained for BW, withers height (WH), girth and cannon circumference. Lusitano foals showed slower BW growth rates, comparable with moderate growth levels proposed for other sport breeds. In contrast, WH growth rates were similar to those presented by early maturing breeds. The validity of the quantitative ultrasonoghraphy as a non-invasive method for the *in vivo* assessment of bone mechanical properties and overall bone quality was also demonstrated. Changes in foals BW and WH growth rates were associated with the presence of radiographic findings compatible with osteochondrosis (OC) lesions at the onset of training. When compared to healthy foals, OC positive foals seem to be early maturing as regards to BW, and showed a tendency for worse cortical bone quality, lower IGF-I and higher insulin and PTH concentrations. The results underline the importance of an early monitoring of foals' growth during the first year of life in order to avoid sudden changes to the average growth rates and to promote a better osteoarticular quality of the Lusitano horse. The integrated approach in what concerns mares and foals management decisions, in particular the choice of the foaling season and the introduction of adequate feeding strategies,

will be determinant for the improvement of the efficiency and profitability of the Lusitano production systems.

Keywords: Lusitano horse breed, body condition, growth and development, blood metabolic indicators, bone quality.

TÍTULO DA DISSERTAÇÃO: O crescimento e desenvolvimento do poldro de raça Lusitana em sistemas extensivos

RESUMO:

O presente estudo teve como principal objectivo a caracterização do crescimento e do desenvolvimento do poldro de raça Lusitana, em sistemas extensivos. Em particular, foi avaliada a relação entre os padrões de crescimento e a qualidade do tecido ósseo. O estado nutricional e evolução da condição corporal (CC) das éguas de ventre ao longo do ciclo produtivo foram igualmente estudados, no sentido de estabelecer os índices mais adequados à optimização do desenvolvimento dos poldros desde a gestação até ao desmame, bem como de aumentar a eficiência produtiva do sistema. Para o efeito foi realizado um trabalho de campo longitudinal, no qual foram acompanhados animais pertencentes a quatro coudelarias. As éguas foram avaliadas durante três ciclos produtivos (gestação/lactação) consecutivos e os poldros foram seguidos desde o desmame aos 42 meses de idade. Paralelamente foram também estudadas as propriedades biomecânicas do osso cortical do cavalo. As variações do peso vivo (PV) e da CC das éguas ao longo do ciclo produtivo foram sobretudo influenciadas pela disponibilidade e qualidade da pastagem e pela época de parto. O desempenho reprodutivo das éguas e o crescimento dos poldros lactentes foram afectados pelo estado nutricional das éguas. A variação da CC no período correspondente à cobrição teve um forte efeito na fertilidade dos dois primeiros estros, sendo esta claramente prejudicada por variações negativas da CC, independentemente da nota observada. As melhores taxas de fertilidade foram obtidas com variações positivas e mais elevadas de CC. A função de Richards foi utilizada na caracterização dos padrões de crescimento dos poldros, tendo sido obtidas curvas de crescimento para o PV, altura ao garrote (AG), perímetro torácico e perímetro da canela. No que se refere ao PV, os poldros Lusitanos apresentaram taxas de crescimento mais lentas, podendo enquadrar-se nos valores propostos para um crescimento moderado em outras raças de desporto. No entanto, as taxas de crescimento para a AG foram semelhantes às observadas em raças mais precoces. A ultrasonografia quantitativa foi confirmada como técnica não invasiva para a avaliação das propriedades biomecânicas e da qualidade do osso cortical em geral. A presença de sinais radiográficos de osteocondrose (OC) ao desbaste foi associada a alterações nas taxas de crescimento (PV e AG) dos poldros. Para além de apresentarem um índice de maturidade mais elevado para o PV, os poldros com OC revelaram uma tendência para uma menor qualidade do osso cortical, menores concentrações de IGF-I e concentrações mais elevadas de insulina e de PTH. Os resultados obtidos apontam para a importância de uma monitorização precoce do crescimento dos poldros, em particular durante o primeiro ano de vida, no sentido de evitar alterações súbitas das taxas médias de crescimento e de promover uma melhor qualidade osteoarticular no cavalo Lusitano. A abordagem integrada das opções de maneio nas éguas e nos poldros, em particular no que se refere a uma melhor gestão da época de partos e à introdução de estratégias alimentares adequadas, será determinante para a melhoria da eficiência e da produtividade dos sistemas de produção do cavalo Lusitano.

Palavras-chave: Puro-sangue Lusitano, condição corporal, crescimento e desenvolvimento, indicadores metabólicos, qualidade do osso.

TÍTULO DA DISSERTAÇÃO: O crescimento e desenvolvimento do poldro de raça Lusitana em sistemas extensivos

RESUMO ALARGADO:

O reconhecimento do cavalo Lusitano como potencial cavalo de desporto e lazer é, hoje em dia, inquestionável. A combinação de um bom temperamento aliado a andamentos fáceis e confortáveis fez com que esta importante raça autóctone fosse considerada como um dos melhores cavalos de sela do mundo. Contudo, apesar de uma preocupação acentuada em produzir animais aptos para uma carreira desportiva, alguns aspectos importantes relacionados com o seu sistema de produção continuam insuficientemente estudados e caracterizados. Actualmente, a utilização desportiva do cavalo é cada vez mais intensa e precoce, podendo mesmo iniciar-se em idades em que os animais podem ainda não ter atingido o desenvolvimento completo do seu aparelho locomotor. O conhecimento dos modelos de crescimento mais adequados a cada raça e tipo de utilização, bem como dos factores que influenciam o desenvolvimento do esqueleto, tornam-se assim fundamentais para criadores e utilizadores.

O presente estudo teve como principal objectivo a caracterização do crescimento e do desenvolvimento do poldro de raça Lusitana, em sistemas extensivos. Em particular, foi avaliada a relação entre os padrões de crescimento e a qualidade do tecido ósseo. O estado nutricional e evolução da condição corporal (CC) das éguas de ventre ao longo do ciclo produtivo foram igualmente estudados, no sentido de estabelecer os índices mais adequados à optimização do desenvolvimento dos poldros desde a gestação até ao desmame, bem como de aumentar a eficiência produtiva do sistema. Para o efeito foi realizado um trabalho de campo longitudinal, no qual foram acompanhados animais pertencentes a quatro coudelarias. Em cada coudelaria foi monitorizado um grupo de éguas de ventre durante três ciclos produtivos (gestação/lactação) consecutivos e os seus poldros foram seguidos desde o nascimento aos 42 meses de idade. As éguas foram pesadas e a sua CC foi avaliada mensalmente. Os poldros foram igualmente pesados e medidos (altura ao garrote-AG, perímetro torácico-PT e perímetro da canela-PC) regularmente. Nos mesmos dias das avaliações anteriormente descritas, procedeu-se à colheita de amostras de sangue, tanto nas éguas como nos poldros, para determinação de alguns indicadores metabólicos (glucose, insulina, ácidos gordos não esterificados, ureia, albumina), leptina, IGF-I, marcadores ósseos (osteocalcina e fosfatase alcalina óssea), paratormona, cálcio, fósforo e magnésio. Os poldros foram ainda avaliados periodicamente por ultrasonografia quantitativa (QUS) na região da canela. No final do estudo, um sub-grupo de poldros foi sujeito a exames radiográficos. Ao longo do estudo

recolheu-se informação relativa aos regimes alimentares praticados e foram colhidas amostras de pastagem e dos alimentos distribuídos, para análise e determinação do seu valor nutritivo.

Paralelamente foi realizado um outro trabalho experimental para avaliar as propriedades biomecânicas do osso cortical do cavalo, o qual envolveu a validação da QUS como metodologia não invasiva para a apreciação da qualidade global do osso. O conjunto global dos dados recolhidos foi posteriormente convertido em cinco manuscritos que constituem a componente experimental deste trabalho, tendo a mesma sido dividida em quatro Capítulos (Capítulos III a VI).

No Capítulo III, dedicado à fase mãe, procurou-se estudar e caracterizar aspectos relacionados com o ciclo produtivo da égua, de forma a integrar a informação obtida na avaliação global do crescimento e desenvolvimento do poldro da gestação ao desmame. Para além disso, o estudo do estado nutricional e das variações da condição corporal das éguas ao longo do ano permitiu a obtenção de informação pertinente no sentido de promover uma maior eficiência produtiva do sistema. As variações do peso vivo (PV) e da CC das éguas ao longo do ciclo produtivo foram sobretudo influenciadas pela disponibilidade e qualidade da pastagem e pela época de parto. Nas éguas que pariram mais cedo e que dependeram essencialmente da pastagem como recurso alimentar, observou-se uma baixa ou nula recuperação da CC durante a primavera, mantendo estas éguas menores valores de CC ao longo de todo o ciclo produtivo. O desempenho reprodutivo das mães e o crescimento dos poldros lactentes foram afectados pelo estado nutricional das éguas. A variação da CC no período correspondente à cobrição teve um forte efeito na fertilidade dos dois primeiros ciclos éstricos, sendo esta claramente prejudicada por variações negativas da CC, independentemente da nota observada. As melhores taxas de fertilidade foram obtidas com variações positivas e mais elevadas de CC. Os poldros cujas mães apresentaram variações negativas da CC nos três primeiros meses após o parto apresentaram menores taxas de crescimento. Neste estudo foi também investigada a possível relação da hormona leptina com o desempenho reprodutivo das éguas. No periparto as concentrações de leptina foram influenciadas pela época, apresentando valores mais baixos para as éguas que pariram mais cedo. Contudo, os valores obtidos aparentam ter sido suficientes para o normal desempenho reprodutivo, dado não ter havido um efeito das concentrações de leptina sobre a fertilidade dos dois primeiros ciclos éstricos.

O Capítulo IV compreendeu o estudo e caracterização dos padrões de crescimento dos poldros do nascimento aos 42 meses de idade, altura em que são normalmente desbastados e iniciam a fase de treino. A função de Richards foi a equação que melhor se ajustou à evolução do PV, AG, PT e PC tendo sido obtidas as curvas de crescimento para estas medidas. No que se refere ao PV, os poldros Lusitanos apresentaram taxas de crescimento mais lentas, podendo enquadrar-se nos valores propostos para um crescimento moderado, em outras raças de desporto. No entanto, as taxas de crescimento para a AG foram semelhantes às observadas em raças mais precoces, o que aponta para um padrão de desenvolvimento do esqueleto comum entre raças e confirma a reconhecida precocidade do tecido ósseo. O dimorfismo sexual foi evidente, reflectindo-se em valores mais elevados à maturidade para os machos, nas quatro variáveis em estudo.

Numa terceira fase do trabalho (Capítulo V), estudaram-se as propriedades biomecânicas do terceiro metacarpo do cavalo através de duas metodologias: avaliação *in vivo* por ultrasonografia quantitativa (QUS) e testes mecânicos realizados *ex vivo* sobre o mesmo osso. A comparação dos resultados obtidos pelos dois métodos, nomeadamente a boa relação obtida entre as medidas da velocidade do som e o módulo de elasticidade determinado a partir dos testes mecânicos realizados no mesmo osso, permitiu confirmar a utilidade da QUS como técnica prática e não invasiva na avaliação das propriedades biomecânicas e da qualidade do osso cortical.

Por último (Capítulo VI), foram avaliadas durante um período alargado as alterações ocorridas a nível dos padrões de crescimento, da qualidade do osso, do metabolismo ósseo, de factores de crescimento e outros indicadores metabólicos envolvidos no desenvolvimento osteoarticular. Estas alterações foram posteriormente relacionadas com a presença de sinais radiográficos de osteocondrose (OC), observados ao desbaste. A associação entre as alterações nas taxas de crescimento dos poldros (PV e AG) e a presença de sinais radiográficos compatíveis com OC, confirmam o envolvimento das características de crescimento como possível factor de risco para a ocorrência desta condição. Para além de apresentarem um índice de maturidade mais elevado para o PV, os poldros com OC revelaram uma tendência para uma menor qualidade do osso cortical, menores concentrações de IGF-I e concentrações mais elevadas de insulina e paratormona. No âmbito deste estudo, importa salientar o conjunto abrangente de dados, recolhidos sequencialmente nos mesmos poldros durante três anos, em condições reais de campo, os quais poderão ser úteis para futuros trabalhos de investigação. Os resultados obtidos apontam para a importância de uma monitorização precoce do crescimento dos poldros, em particular durante o primeiro ano de vida, no sentido de evitar alterações súbitas das taxas médias de crescimento e de promover uma melhor qualidade osteoarticular no cavalo Lusitano.

A globalidade dos resultados obtidos ao longo deste trabalho contribui para um melhor conhecimento do ciclo produtivo do cavalo Lusitano e apontam para procedimentos práticos que poderão ser integrados nas opções de maneio das éguas e dos poldros. Em particular, as questões relativas a uma melhor gestão da época de partos e à introdução de estratégias alimentares adequadas em períodos chave parecem determinantes para a melhoria da eficiência e da produtividade do sistema de produção em causa.

PUBLICATIONS

Peer-reviewed papers:

- **Fradinho, M.J.**, Bessa, R.J.B., Martin-Rosset, W., Ferreira-Dias, G. & Caldeira, R.M. (2013). Nutritional status of *Lusitano* broodmares on extensive feeding systems: body condition, live weight and metabolic indicators. *Italian Journal of Animal Science*. 12(3):e71, 436-443.
- **Fradinho, M.J.**, Correia, M.J., Grácio, V., Bliebernicht, M., Farrim, A., Mateus, L., Martin-Rosset, W., Bessa, R.J.B., Caldeira, R.M. & Ferreira-Dias, G. (2014). Effects of body condition and leptin on the reproductive performance of Lusitano mares on extensive systems. *Theriogenology*. 81, 1214-1222.
- **Fradinho, M.J.**, Vale, A.C., Bernardes, N., Caldeira, R.M., Vaz, M.F. & Ferreira-Dias, G. (2015). Biomechanical properties of the equine third metacarpal bone: *in vivo* quantitative ultrasonography *vs ex vivo* compression and bending techniques. *Journal of Equine Veterinary Science*. 35, 198-205.
- **Fradinho, M.J.,** Bessa, R.J.B., Ferreira-Dias, G. & Caldeira, R.M. (2015). Growth and development of the Lusitano horse managed on grazing systems. *Livestock Science*. DOI: 10.1016/j.livsci.2015.06.006.
- **Fradinho. M.J.,** Mateus, L., Bernardes, N., Bessa, R.J.B., Caldeira, R.M. & Ferreira-Dias, G. (2015). Growth patterns and osteoarticular status in the Lusitano horse: a longitudinal field study. (preparation for submission)

Publications in peer-reviewed books:

- **Fradinho M.J.,** Fernandes, R., Gomes, T., Correia, M.J., Vila-Viçosa, M.J.C., Silva, M.F., Ferreira-Dias, G. & Caldeira, R.M. (2008). Growth and conformation changes in the young Lusitano horse: evaluation from birth to one year of age. In M. T. Saastamoinen and W. Martin-Rosset (Eds.) *Nutrition of the exercising horse*. (pp. 367-370). EAAP Publ N. 125, Wageningen, the Netherlands: Wageningen Academic Publishers.
- **Fradinho, M.J.,** Correia, M.J., Vila-Viçosa, M.J.C., Farrim, A., Harris, P.A., Bessa, R.J.B., Caldeira, R.M. & Ferreira-Dias, G. (2009). *Non-invasive bone assessment by quantitative ultrasonography in Lusitano suckling foals: A preliminary study*. In A. Lindner (Ed.) *Applied equine nutrition and training*. (pp. 191-196). ENUTRACO 2009, Wageningen, the Netherlands: Wageningen Academic Publishers.

- **Fradinho M.J.,** Barbosa, M., Vila-Viçosa, M.J.C., Rosa, A., Perestrello, F., Bessa, R.J.B., Caldeira, R.M. & Ferreira-Dias, G. (2010). Effect of growth rate on plasma leptin and bone markers, and metacarpal ultrasound measurements in the young Lusitano horse. In A. D. Ellis, A. C. Longland, M. Coenen and N. Miraglia (Eds.), *The impact of nutrition on the health and welfare of horses*, (pp. 308-313). EAAP Publ. N. 128, Wageningen, the Netherlands: Wageningen Academic Publishers.
- **Fradinho, M.J.**, Correia, M.J., Beja, F., Rosa, A., Perestrello, F., Bessa, R.J.B., Ferreira-Dias, G. & Caldeira, R.M. (2012). Effects of foaling season on growth and development of Lusitano sucking foals raised on extensive grazing systems, In M. Saastamoinen, M.J. Fradinho, A.S. Santos and N. Miraglia (Eds.), *Forage and grazing in horse nutrition*, (pp. 315-318). EAAP Publ. N. 132, Wageningen, the Netherlands: Wageningen Academic Publishers.

Publications in Conference Proceedings (International):

- **Fradinho M.J**., Agrícola, R., Correia, M.J., Vila-Viçosa, M.J.C., Silva, M.F., Ferreira-Dias, G. & Caldeira, R.M. (2007). Growth patterns in Lusitano foals: Evaluation from birth to weaning. In *Proceedings of Twentieth Symposium of Equine Science Society*. Hunt Valley, MD, USA, 5-8 June, pp. 150-151.
- **Fradinho M.J.,** Mateus, L., Agrícola, R., Correia, M.J., Vila-Viçosa, M.J.C., Caldeira, R.M. & Ferreira-Dias, G. (2007). Plasma concentrations of bone biochemical markers, insulin-like growth factor-I, leptin and metacarpal ultrasound speed in Lusitano suckling foals: A preliminary study. In *Proceedings of Twentieth Symposium of Equine Science Society*. Hunt Valley, MD, USA, 5-8 June, pp. 274-275.
- **Fradinho M.J.,** Mateus, L., Agrícola, R., Correia, M.J., Vila-Viçosa, M.J.C., Silva, M.F., Ferreira-Dias, G. & Caldeira, R.M. (2007). Influence of management on growth and development of the Lusitano horse [abstract]. In *Book of Abstracts of the 58th Annual Meeting of the European Association for Animal Production.* N.13. Dublin, Ireland, 26-29 August. p. 112. Wageningen, the Netherlands: Wageningen Academic Publishers.
- **Fradinho M.J.,** Mateus, L., Agrícola, R., Correia, M.J., Vila-Viçosa, M.J.C., Caldeira, R.M. & Ferreira-Dias, G. (2007). Body condition and leptin in Lusitano mares during late pregnancy and lactation [abstract]. In *Book of Abstracts of the 58th Annual Meeting of the European Association for Animal Production.* Nº13. Dublin,

Ireland, 26-29 August. p. 292. Wageningen, the Netherlands: Wageningen Academic Publishers.

- **Fradinho M.J.**, Fernandes, R., Mateus, L., Correia, M.J., Vila-Viçosa, M.J.C., Ferreira-Dias, G. & Caldeira, R.M. (2008). Characterization of feeding plans on extensive systems in Lusitano broodmares by body condition and metabolic indicators assessment [abstract]. In *Book of Abstracts of the 59th Annual Meeting of the European Association for Animal Production*. N.14. Vilnius, Lithuania, 24-27 August, p. 171. Wageningen, the Netherlands: Wageningen Academic Publishers.

- **Fradinho M.J.**, Gomes, T., Mateus, L., Correia, M.J., Vila-Viçosa, M.J.C., Caldeira, R.M. & Ferreira-Dias, G. (2008). Plasma calcium, phosphorus and magnesium in Lusitano broodmares under extensive feeding systems [abstract]. In *Book of Abstracts of the 59th Annual Meeting of the European Association for Animal Production.* N.14. Vilnius, Lithuania, 24-27 August, p. 171. Wageningen, the Netherlands: Wageningen Academic Publishers.

- **Fradinho M.J.**, Ferreira-Dias, G., Correia, M.J., Grácio, V., Beja, F., Perestrello, F., Rosa, A. and Caldeira, R.M. (2009). Characterization of body condition changes in Lusitano broodmares fed pasture based diets [abstract]. In *Book of Abstracts of the 60th Annual Meeting of the European Association for Animal Production.* N.15. Barcelona, Spain, 24-27 August, p. 429. Wageningen, the Netherlands: Wageningen Academic Publishers.

- Vale. A.C., **Fradinho, M.J.,** Bernardes, N., Pereira, M.F.C., Maurício, A. Amaral, P.M., Ferreira-Dias, G. & Vaz, M.F. (2010). Assessment of structural and mechanical properties of equine cortical bone with several techniques [abstract]. In *Book of Abstracts of the 61th Annual Meeting of the European Association for Animal Production.* N.16. Heraklion, Crete, 23-27 August, p. 300. Wageningen, the Netherlands: Wageningen Academic Publishers.

- **Fradinho. M.J.,** Correia, M.J., Beja, F., Perestrello, F., Mateus, L., Bessa, R.J.B. Caldeira, R.M. & Ferreira-Dias, G. (2010). Changes in biochemical markers of bone metabolism in the young Lusitano horse [abstract]. In *Book of Abstracts of the 61th Annual Meeting of the European Association for Animal Production.* N.16. Heraklion, Crete, 23-27 August, p. 301. Wageningen, the Netherlands: Wageningen Academic Publishers.

- **Fradinho, M.J.,** Correia, M.J., Beja, F., Rosa, A., Perestrello, F., Bessa, R.J.B., Ferreira-Dias, G. & Caldeira, R.M. (2011). Changes on body weight and body condition in the Lusitano broodmare [abstract]. In *Book of Abstracts of the 62nd Annual Meeting of the European Association for Animal Production.* N.17. Stavanger, Norway, 29 August-2 September, p. 38. Wageningen, the Netherlands: Wageningen Academic Publishers.
- **Fradinho M.J**., Vale A.C, Bernardes N., Caldeira R.M., Vaz M.F. & Ferreira-Dias G. (2014). Equine cortical bone: *in vivo* and *ex vivo* properties assessment [abstract]. In *Book of Abstracts of the 65th Annual Meeting of the European Association for Animal Production.* N.20, Copenhagen, Denmark. 25-28 August, p.184. Wageningen, the Netherlands: Wageningen Academic Publishers.
- **Fradinho M.J.,** Bessa R.J.B., Ferreira-Dias G. & Caldeira R.M. (2014). Growth curves in the *Lusitano* horse managed on grazing systems. In *Proceedings of 7th European Workshop on Equine Nutrition (EWEN),* Leipzig, Germany, 28 September-2 October, pp.132-134.

Publications in Conference Proceedings (National):

- **Fradinho M.J.**, Caldeira, R.M. & Ferreira-Dias, G. (2007). Crescimento e desenvolvimento do cavalo Puro-Sangue Lusitano: a leptina como possível modulador do metabolismo ósseo [abstract]. In *Jornadas do Ensino Pós-Graduado da FMV - Simpósio do CIISA.* Faculdade de Medicina Veterinária, Lisboa, 14-15 Junho. p. 17.
- **Fradinho M.J.,** Fernandes, R., Mateus, L., Correia, M.J., Vila-Viçosa, M.J.C., Ferreira-Dias, G. & Caldeira, R.M. (2008). Caracterização da variação da condição corporal de éguas Puro-Sangue Lusitano em regimes extensivos [abstract]. In *Livro de Resumos do IV Congresso de Ciências Veterinárias.* INRB, INIA/Fonte Boa, Santarém, 27-29 Novembro, p. 20.
- **Fradinho M.J.,** Fernandes, R., Gomes, T., Correia, M.J., Vila-Viçosa, M.J.C., Silva, M.F., Ferreira-Dias, G. & Caldeira, R.M. (2008). Padrões de crescimento e desenvolvimento no poldro Puro-Sangue Lusitano [abstract]. In *Livro de Resumos do IV Congresso de Ciências Veterinárias.* INRB, INIA/Fonte Boa, Santarém, 27-29 Novembro, p. 21.
- **Fradinho M.J.,** Fernandes, R., Correia, M.J., Mateus, L., Robalo, J., Caldeira, R.M. & Ferreira-Dias, G. (2008). Parâmetros endócrinos, de formação óssea e de puberdade

em poldros de raça Puro-Sangue Lusitano [abstract]. In *Livro de Resumos do IV Congresso de Ciências Veterinárias.* INRB, INIA/Fonte Boa, Santarém, 27-29 Novembro, p. 22.

- **Fradinho M.J.,** Barbosa, M., Vila-Viçosa, M.J.C., Rosa, A., Perestrello, F., Caldeira, R.M. & Ferreira-Dias, G. (2011). Efeito do crescimento nas concentrações plasmáticas de leptina, de marcadores ósseos e na qualidade do osso cortical no poldro PSL [abstract]. In *Livro de resumos (formato digital) das II Jornadas do GTIE*, Ponte de Lima, 25 de Junho.

- **Fradinho, M.J.,** Correia, M.J., Beja, F., Rosa, A., Perestrello, F., Bessa, R.J.B., Ferreira-Dias, G. & Caldeira, R.M. (2011). Evolução do peso vivo e da condição corporal ao longo da gestação em éguas Puro-Sangue Lusitano [abstract]. In *Livro de Resumos do V Congresso da Sociedade Portuguesa de Ciências Veterinárias.* INRB, INIA/Fonte Boa, Santarém, 13-15 Outubro, p.105.

- **Fradinho M.J.**, Correia, M.J., Grácio, V., Bliebernicht, M., Farrin, A., Mateus, L., Martin-Rosset, W., Bessa, R.J.B., Caldeira, R.M. & Ferreira-Dias, G. (2013). Efeito da condição corporal e da leptina na eficiência reprodutiva de éguas Lusitanas em sistemas extensivos [abstract]. In *Livro de resumos das III Jornadas do GTIE*, Golegã, 6 de Novembro, p. 21.

- **Fradinho, M.J.,** Vale, A.C., Bernardes, N., Caldeira, R.M., Vaz, M.F. & Ferreira-Dias, G. (2014). Propriedades do osso cortical em equinos: avaliação *in vivo* e *ex vivo* [abstract]. In *Livro de Resumos do VI Congresso da Sociedade Portuguesa de Ciências Veterinárias.* INIAV, Oeiras, 3-5 de Abril, p.103.

- **Fradinho, M.J.,** Bessa, R.J.B., Ferreira-Dias, G. & Caldeira, R.M. (2015). Crescimento e desenvolvimento do cavalo Lusitano em sistemas extensivos: aplicação de modelos não-lineares [abstract]. In *Livro de resumos das IV Jornadas do GTIE*, Golegã, 11 de Novembro, p. 48.

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List of abbreviations and symbols

AA – amino acids

ADF – acid detergent fibre

ADL – acid detergent lignin

ADG – average daily gain

Adrβ2 - β2 adrenergic receptors

AI – artificial insemination

AM – april-may

APSL – Associação Portuguesa de Criadores do Cavalo Puro-sangue Lusitano

BALP – bone alkaline phosphatase

BC – body condition

BCS – body condition scoring

BCScon – body condition score at conception

BCS3L – body condition score through the first three months of lactation

ΔBCS – body condition change

ΔBCScon – body condition changes at conception

ΔBCS3L - body condition changes throughout the first three months of lactation

BMD – bone mineral density

BMU – basic multicellular unit

BR – bone region

BS – bone section

BW – body weight

c – speed of a wave trough a solid

Ca – calcium

CART – cocaine- and amphetamine-regulated transcript

CaSR – calcium-sensing receptor

CC – cannon circumference

CF – crude fibre

CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal

CNS – cresty neck scoring

CP – crude protein

CT – calcitonin

Cu - copper

DE – digestible energy

DM – dry matter

DOD – developmental orthopaedic disease

DP – digestible protein

DPA – dual photon absorptiometry

D-Pyr – deoxy-pyridinoline

DR – digital radiography

DXA – dual X-ray absorptiometry

E – Young's modulus

ECM – extracellular matrix

EGS – extensive grazing systems

ELISA – enzyme-linked immune assay

ε_{max} – maximum strain

FAO – Food and Agriculture Organization of the United Nations

FM – february-march

G - girth

GH – growth hormone

Gla – gamma-carboxy glutamate

h^2 - heritability

HN – Haras Nationaux

IADG – instantaneous average daily gain

ICTP – cross-linked C-telopeptide of type I collagen

IE – Institut d'Élevage

IGF-I – insulin-like growth factor I

IGR – instantaneous growth rate

IIASA - International Institute for Applied Systems Analysis

IICC – instantaneous increase of cannon circumference

IIG – instantaneous increase of girth

IIWH – instantaneous increase of withers height

INRA – Institut National de la Recherche Agronomique

InsR – insulin receptors

ISRIC - World Soil Information

ISSCAS - Institute of Soil Science – Chinese Academy of Sciences

JRC - Joint Research Centre of the European Commission

k – maturing index

MADC – horse digestible crude protein

McIII – third metacarpal bone

Mg – magnesium

MRI – magnetic resonance imaging

NDF – neutral detergent fibre
NE – net energy
NEB – negative energy balance
NEFA – non-esterified fatty acids
NM – natural free mating in pasture
NP – Norma Portuguesa
NRC – National Research Council
NS – natural service
ns – non significant
NUT 2 – Nomenclature for territorial units for statistic, level two
Oc - osteocalcin
OC – osteochondrosis
OCD – osteochondritis dissecans
OPG - osteoprotegerin
P - phosphorus
P_i – inorganic phosphorus
PICP – carboxy terminal peptide of type I collagen
pQCT – peripheral quantitative computed tomography
PTH – parathyroid hormone
Pyr - pyridinoline
QCT – quantitative computed tomography
QUS – quantitative ultrasonography
ρ – density
RANKL – receptor activator of nuclear factor kB ligant
RIA - radioimmunoassay
ROI – region of interest
RSD – residual standard deviation
SD – standard deviation
SEM – standard error of the mean
SOS – speed of sound
SPA – single photon absoptiometry
spp. – species
σf – failure stress
σ_{max} – maximum stress
T3 - triiodothyronine
T4 - thyroxine

TB - Thoroughbred

TEC – total energy content

USA – Unitet States of America

WH – withers height or height at withers

Zn - zinc

"…o cavalo Lusitano, para além da sua beleza e do seu interesse genético, é hoje uma fonte inesgotável de prazer para o cavaleiro que tem a sorte de o montar. O seu equilíbrio psíquico e físico, que não tem paralelo em nenhuma outra raça, permite um nível de entendimento superior com o cavaleiro…"

Arsénio Raposo Cordeiro
(1940-2013)

CHAPTER I – *GENERAL INTRODUCTION AND OBJECTIVES*

1.1. Introduction

In the horse, growth models have not been studied as much as in other animal species. After birth, and depending on the breed, growth and development can last three to five years, which represents a large proportion of its productive life (Martin-Rosset et al., 2015b).

Nowadays, sportive utilization is becoming more and more intensive and begins at an earlier age, when the skeleton development may not yet be completed. Therefore the knowledge of the most adequate growth model for each breed and purpose, as well as the factors that influence skeleton development, is of high concern for horse breeding industry. The study of bone metabolism is particularly important in sport breeds, in order to provide the best adequate muscular-skeletal condition throughout the horse life. The Lusitano horse is no exception. This important native breed has been recognized as one of the world's best saddle horses, combining a good temperament with easy and comfortable gaits. Overall growth models had been proposed for other breeds (Staniar et al., 2004a; NRC, 2007; INRA, 2015). However, despite the common believe that Lusitano horse reaches its maturity at a later age, growth and development processes in the Lusitano foal remain to be characterized.

Lusitano stud farms are traditionally based on extensive grazing systems, where mares and foals are often bred outdoors throughout the year. In these systems, pastures represent a significant part of diets, but herbage production is commonly limited by Mediterranean climate conditions. Under such conditions and as other livestock females, mares will store and mobilize body reserves during their productive cycle (Martin-Rosset et al., 2006a). The effects of an adequate amount of body reserves on reproductive performance of the mare and also on productive parameters such as milk production and the consequent foals' growth have been recognized (Doreau et al., 1986; Henneke et al., 1984). Thus, gathering reliable information, under farm conditions, on nutritional status and body reserves management in the Lusitano mare will contribute for better decisions on the most appropriate feeding plans and foaling seasons, in order to improve the efficiency and profitability of these production systems. In addition, a better knowledge of the growing process, in relation to bone metabolism and quality, will be pivotal to support more adequate management options during growth period of the Lusitano foal.

The work presented in this thesis was based on a longitudinal field study performed in four Lusitano stud-farms located at the southern region of Portugal. In each stud-farm, a group of broodmares were monitored during three consecutive gestation/lactation cycles and foals from these mares were followed from birth to 42 months of age. Mares were monthly weighed and body condition was evaluated. Foals were also periodically weighed and measured. Blood samples were collected, both in mares and foals, for determination of hormones and metabolic indicators. Quantitative ultrasound measurements were periodically performed on the third metacarpal bone of foals. At the end of the study, a sub-sample of foals underwent radiographic examinations. During the study, information regarding feeding regimens was collected and samples of pastures and other distributed feeds were analyzed for determination of nutritional value.

In addition, another experiment was performed in order to evaluate the biomechanical properties of the equine cortical bone. This *in vivo / ex vivo* study involved the use and validation of the quantitative ultrasonography as a technique for the assessment of cortical bone quality.

This experimental work was converted in five manuscripts, which constitutes four chapters (Chapter III to VI) of this thesis. Four of them have already been published in international peer reviewed and indexed journals and the last one is being prepared for submission.

1.2. Objectives

The general aim of this study was to characterize growth and development of the Lusitano foal bred and raised on extensive systems, from birth to 42 months of age. In particular, the relationship between growth patterns and bone quality was evaluated.

Additionally, nutritional status and body condition changes of the mares during the productive cycle were studied, in order to stablish appropriate scores to optimize the development of foals from pregnancy to weaning, as well as to increase the productive efficiency of the system.

Therefore, the specific objectives of this work were:

(1) To evaluate the effects of feeding management and foaling season in extensive systems on the nutritional status of Lusitano broodmares throughout the gestation/lactation cycle by the assessment of body condition, body weight and some blood metabolic indicators. (Chapter III, Sub-chapter 3.1).

(2) To investigate the influence of body condition, body condition changes and plasma leptin concentrations on reproductive performance of Lusitano broodmares, bred at early postpartum period and, to evaluate the effect of mares' energy balance after foaling on growth performance of the suckling foals (Chapter III, Sub-chapter 3.2).

(3) To characterize the growth patterns of the Lusitano foal managed on grazing systems, from birth to 42 month of age, concerning body weight, withers height, girth and cannon circumference (Chapter IV).

(4) To evaluate the biomechanical properties of the equine cortical bone, comparing *in vivo/ex vivo* techniques. Specifically, this study aimed to (i) assess equine cortical bone status *in vivo* by quantitative ultrasonography; (ii) evaluate certain mechanical properties of the third metacarpal bone with destructive tests and characterize its regional variation along the bone; and (iii) compare the *in vivo* results with the *ex vivo* mechanical tests performed on the same bone (Chapter V).

(5) To evaluate the growth patterns and long-term changes on bone quality, bone metabolism, growth factors and metabolic related variables and, to assess whether these long-term changes are related to radiographic findings regarding osteochondrosis-like lesions at the onset of training (Chapter VI).

CHAPTER II – *STATE OF THE ART*

2.1. The Lusitano breed: a brief characterization

2.1.1. Origin

The findings of fossils of large ungulates in the caves of Sierra de Atapuerca, including some teeth of the species *Equus altidens* confirms the presence of horses in the Iberian Peninsula, from an age around the transition between the Early and the Middle Pleistocene (van der Made, 1999; 2013).

According to Andrade (1954), the horse began to be ridden in the Iberian Peninsula, even before the first millennium BC, continuing to be used in subsequent ages for warfare. The qualities of the Iberian riders were recognized by invaders, from the Carthaginians in the IV century BC to the Romans which army quickly adopted the Iberian cavalry system to fight on horseback (Bragança, 1997).

From the times of Roman occupation until the Middle Age, the horses raised in the plains of rivers Guadiana, Tagus and Guadalquivir, in the southern Iberian Peninsula kept the shape, the size and the character despite the successive invasions. For Europe's Renaissance, the "Genet d'Espagne" (name used then), was the true blooded horse and was exported to everywhere as enhancer of other breeds in order to produce light horses. This superiority was maintained until the seventeenth and eighteenth centuries (Andrade, 1954).

In Spain, the progressive abandonment of bullfight on horseback led to the introduction of a new selection process in the Andalusian horse (another term used for the horse in southern Europe), which became mostly used as a light draft horse, with exuberant, higher and less progressive movements. However, in Portugal, due to the continued use of the evolutions of “gineta” (way of horseback combat that evolves into the duel with a spear), perpetuated through the horseback bullfighting, the horses were mainly selected based on their functional characteristics. Those characteristics allow them to be considered until the present, as the most noble and legitimate representatives of this ancient breed (Andrade, 1988; Cordeiro, 1997).

The Lusitano designation was adopted in 1942 for horses born in Portugal, which presented the morphological and functional characteristics of the breed and with a known genealogy that allowed its acceptance under such denomination (Monteiro, 1983). In 1967, the Lusitano Studbook was formalized (Monteiro, 1983) and in 1989, the Portuguese Association of Lusitano Horse Breeders (APSL) was created in order to expand and to promote the breed.

Currently, the Lusitano horse is spread around the world with approximately 5,000 registered breeding mares (Vicente et al., 2012). The growing interest in the Lusitano breed outside

Portugal led to the formation of breeders' associations in 19 countries, with a significant number of animals in Brazil, France, Mexico, Spain, Italy, Belgium and United Kingdom among others (Vicente, 2015).

2.1.2. Morpho-functional characteristics

The great diversity of the existing horse breeds is mainly described based on characters related to their external morphology and proportions. Therefore, morphometry becomes an indispensable tool for an objective characterization of the breeds and the consequent definition of their standards (Monteiro, 1983; Oom, 1992). In addition to biometrical parameters, which are objective and quantifiable, the morphological characterization of the horse includes also some qualitative and subjective features regarding conformation (e.g. head profile, type of neck or orientation of the ears) (Vicente, 2015).

There are several methodologies that can be used in order to quantify biometrical variables. The direct measurements on live animals with simple instruments like an height measuring stick and flexible measuring tapes are the most common methods reported in biometric studies, both in growing (Kavazis & Ott, 2003; Cabral et al., 2004b; Valette et al., 2008) and in adult horses (Zeckner et al., 2001; Cabral et al., 2004a; Gómez et al., 2009; Komosa et al., 2013; Menezes et al., 2014). Other methodologies like photogrammetric methods (Thompson, 1995; Barrey et al., 2002) or the 3-D video morphometric measurements have been applied (Weller et al., 2006; Santos, 2008; Kristjansson et al., 2013; Solé et al., 2014).

Some previous studies involving biometric assessment with direct measurements on two Lusitano main strains have been reported (Monteiro, 1983; Oom & Ferreira, 1987), and a detailed morphometric characterization of the Lusitano breed, based on the same methodology, was performed by Oom (1992).

The breed Standard describes an ideal model for the Lusitano horse in which a large set of morphological characteristics are described (Annex I). According to this standard, the Lusitano is a medium shaped horse, with a middle weight of 500 kg, with a sub-convex profile throughout the body (rounded outlines), which silhouette can be fitted into a square. The height at withers, measured at six years old should be about 1.55 m for females and 1.60 m for males (APSL, 2010). To be registered in the Lusitano Studbook, males and females must be evaluated by a jury, and classified according to their closeness to the breed standard, morphological features and gaits (APSL, 2010). In the morphological evaluation, the judges score (from 1 to 10) the regions of the head and neck, shoulder and withers, chest and thorax, back and loin, croup, legs, overall impression and gaits. The sum of the partial scores, which receives coefficient weights of either 1.5 (back and loin, legs, overall impression and gaits) or 1.0 (head and neck, shoulder and withers, chest and thorax and croup) is then calculated in order to obtain a final score between 0 and 100 points (Vicente et al., 2014).

In the horse, morphologic and conformational traits have important implications on the limits or range of movements and function. Therefore, these characteristics will have an impact on performance (Barrey et al., 2002; Santos, 2008; Vicente, 2015).

The selection of the Lusitano horse was traditionally based on functional features that arose from its continued use in the field work, handling of wild cattle, and in bullfight on horseback. It is also widely accepted that the former way of combat on horse, called “gineta”, which required agility and quickness of turn back was fundamental for the maintaining of functional characteristics that were later used in bullfighting (Andrade, 1954; Cordeiro, 1997). Nowadays, the selection of the Lusitano horse for functionality has become increasingly important as the breed has been more used in high level sport competitions, achieving very good results, in dressage, driving and working equitation (Solé et al., 2014; Vicente, 2015). Recent studies in the Lusitano breed aiming at studying quantitative kinematic traits, identified some positive results in what concerns the swing phase duration and the range of motion of the elbow, hock and pelvis joints, characteristics that are objectively relevant to sports performance (Solé et al., 2014). In a previous study, it was also reported a relation between Lusitano morphologic traits like the length of the fore pastern and the slope of the hind pastern, and the ability for bullfighting, associated with a maximum elevation of fore and hind limbs (Santos, 2008).

All these morphological and functional features, together with its behavioural and temperamental characteristics, reinforce the recognition of the Lusitano as one of the best saddle horses in the world.

2.1.3. The Lusitano production systems

Livestock production systems have been defined on the basis of land use by livestock (Steinfeld et al., 2006). Grassland-based systems or grazing systems are production systems in which more than 90 % of dry matter fed to animals comes from rangelands, pastures, annual forages and purchase feeds, and at least 10 % of this dry matter is farm produced (Steinfeld et al., 2006). When grazing systems are associated to a low stocking rate, relatively low level of labor, resources or capital, and pastures are mainly rainfed or natural, they are commonly designed as extensive grazing systems (EGS) (Allen et al., 2011). In Mediterranean regions, where plant growth is limited, both by low temperatures in the cold season and by moisture availability during the vegetation period, the main feed resource has traditionally been the silvopastoral system. The silvopastoral system often combines rainfed tree crops (e.g. olive trees, cork-oaks) with extensive grazing (Seré & Steinfeld, 1996).

Historically, Lusitano horse production was mainly located in the former regions of Ribatejo and Alentejo and was generally associated to big livestock farms with large agricultural surfaces (Monteiro, 1983). In the eighties, the stud farms located in these regions had on average 15 to 30 broodmares, although there were also small breeders, spread all over the country, owning one or two broodmares (Monteiro, 1983).

Data from the "Registo Nacional de Equinos" showed that between 1987 and 2012, the average number of horses born and registered by breeder (including the Lusitano) was nine in the NUT 2 region of Lisboa and 14 in the NUT 2 region of Alentejo. These data confirm that nowadays, those regions still represent the most important centers of horse production in our country (Fontes & Jorge, 2013). According to information provided by APSL, there are about 350 Lusitano stud-farms in Portugal, totalizing around 700 worldwide.

The majority of Lusitano stud farms are based in EGS. In these systems, mares are often bred outdoors throughout the year, being pastures a significant part of diets. When grass production is scarce, supplementary feeds (concentrate feeds and preserved forages) are generally used, but farm practices vary widely (Paço & Fradinho, 2011). This type of systems contrast to the majority of sport and race horse breeds in temperate and cold regions, where the mares stay indoors during the winter and only turn out to pasture in the spring (Miraglia et al., 2006; Martin-Rosset et al., 2015c).

In some Lusitano stud farms, where water availability is not a limiting factor during the summer, grass production from irrigated pastures has become also an important feed resource for the global feed management of the herd. The introduction of irrigated pastures in the production system has several advantages: it avoids the lack of green grass during the summer, providing forage with high nutritive value during a long period and, if well managed, could be also harvested for hay or haylage production, to be used as good quality preserved forage during the winter time (Martin- Rosset et al., 2015b). In this type of system, rotational grazing is often implemented and stoking rates are normally higher than in EGS. Whatever the grazing system, one of the challenges in horse production is to cope with the specific nutritional requirements of some stages of the mare productive cycle and foals growth. The ovarian activity of the mares is seasonal. In the northern hemisphere, an anovulatory phase takes place in fall when the length of the day is decreasing, whereas ovarian cyclicity starts when day length is rising. Therefore, the breeding season occurs in the spring when the daylight, temperature and availability of feed increases (Guillaume et al., 2006). Generally, in our latitudes, most of the stud farms manage mare‘s reproductive cycle in order to concentrate foaling in spring, when pasture dry matter production is higher. As a result, the Lusitano foaling season is normally spread between January and May and mating usually takes place in the month following parturition. In ideal conditions, it is important that the mare conceives again during the first month after foaling in order to obtain a foal per year and to promote the efficiency of the system (Martin-Rosset et al., 2015c).

The type of breeding allowed for the Lusitano is the natural service and artificial insemination using fresh, refrigerated or frozen semen, although there is a fixed number of mated mares per stallion in the same year. In the last update of the Studbook Regulation also embryo transfer was allowed, but only a maximum of three animals from each donor mare may be registered per year (APSL, 2010).

The lactation peak in the Lusitano mare occurs between the first and the second postpartum month reaching a milk yield of 14kg/day. During the first fourth months of lactation the daily average milk yield is 12 kg (Santos & Silvestre, 2008). The weaning of foals occurs generally in the fall, between six to seven months of age, with the full separation from the dams on that day. Foals are bred outdoors throughout the year and are normally separated by gender after one year of age. Colts and fillies stay in the pasture until the end of the spring in which they complete three years old. From this moment on, they are stabled for breaking in and for the beginning of training (Paço & Fradinho, 2011).

2.1.3.1. Feeding practices in Portugal: broodmare and foals

Pastures and forages are the primary feed of the horse. Pasture grass and preserved forages account for 50 to 80 percent of the horse feed in a year (Miraglia et al., 2006) and may supply 100 percent of the daily ration for many horse categories (Virkajärvi et al., 2012). In a general way, the use of pastures in horse feed management has the advantage of presenting a fair nutritional balance, at a relative low cost. In addition, pastures provide free exercise areas, promoting equine health and wellbeing.

Whatever the breed, feeding practices are closely related with the type of production system and depends on pasture and forage availability and quality along the year.

Southern Europe countries have common influences that lead to some general climatic traits, like an accentuated drought in summer and a wide temperature range. Pasture production is negatively affected by climate characteristics, being the most limiting factors the low biomass production with an uneven distribution, which leads to the need of large grazing surfaces and some supplementation practices (Miraglia *et al*., 2006). However, climate has also a positive impact, since it allows grazing to be practiced all year around. Pastures can be natural or sown, temporary or permanent (renewed when necessary), irrigated or rainfed, and they are essentially constituted by plants of two botanical families: grasses and legumes (Paço & Fradinho, 2011). For southern regions under Mediterranean climate, rainfed pastures are based in annual species and have a peak production in spring, a pause in summer and a smaller peak in autumn, which further decreases due to low temperatures in winter (Figure 2.1).

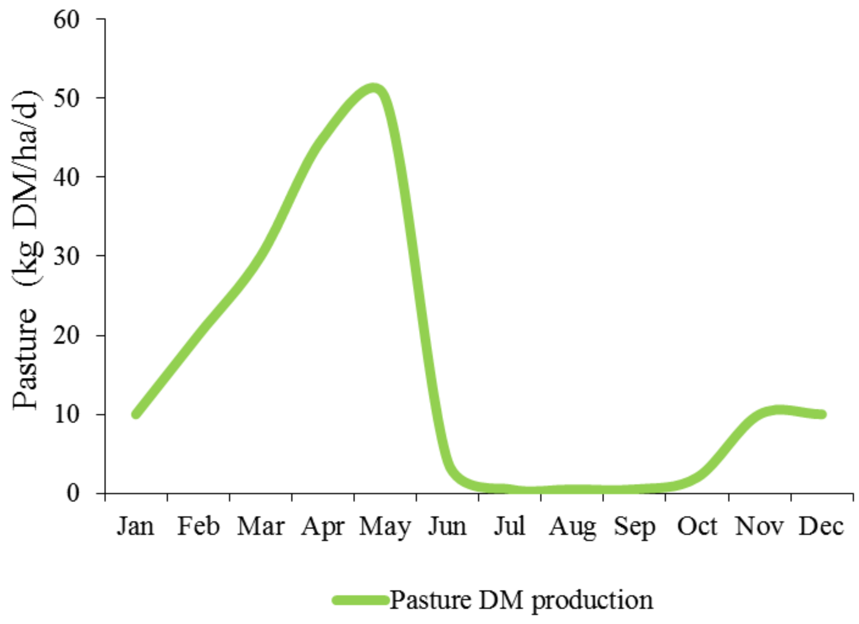

Figure 2.1 – Distribution of average dry matter production of rainfed pastures in Portugal under Mediterranean climate (adapted from Salgueiro, 1984)

Beyond the influence of summer dryness on the dry matter production and nutritive value, the quality of Mediterranean pastures is often influenced by a considerable diffusion of invasive species (Miraglia et al., 2006).

Lactating mares can adjust intake while they are fed forage-based diets to meet energy requirements (Martin-Rosset et al., 2015c). However, a study in temperate regions showed that lactating mares not supplemented at pasture, no longer meet their requirements when daily herbage allowances fall below 66 g DM/ kg BW/day (Collas et al., 2015). In the

Lusitano stud farms based on EGS, mare and foals supplementation occurs during the periods with low or even no pasture biomass production, with preserved forages (mostly grass hay or grass-legume mixed hay) and/or concentrate feeds, although the remnant dry herbage is still available as feed resource during the summer months. If foaling occurs early in the year, the period of low pasture production during winter usually matches with the last three months of gestation. On contrast, if foaling occurs after April, the early lactation stage, when mares have the highest requirements, will coincide with the decline in dry matter production during the summer. In large surface stud farms, the low stocking rates allowed a certain compromise between intake and nutrient allowances, but pasture contents in protein, minerals (*e.g.* calcium, phosphorus) and trace elements (*e.g.* copper, zinc) are often inadequate to meet requirements (Miraglia et al., 2006; Martin-Rosset et al., 2015c). Thus, in order to cope with the deficiencies of those nutrients some Lusitano breeders use commercial compound feeds as concentrates.

From birth until weaning, Lusitano foals are kept with their mothers on pasture. During the first two months, foals are mainly fed with mares' milk, but from this age on, grazing time increases and frequency of nursing decreases (Martin-Rosset et al., 2015a). In the Lusitano production systems, the last two or three months before weaning always matches with the period of low or null pasture availability. During these periods, preserved forages are commonly distributed, but the use of concentrate feeds as creep-feeding is not wide spread. A recent survey conducted in 31 sport horse stud farms in Portugal, where more than half were Lusitano stud farms, showed that the creep-feeding is only practiced in 23 % of the studs (data not published).

After weaning, foals are normally kept together during an adaptation period where they are group or individual fed with preserved forages and concentrate feeds, and turn out to pasture before the spring. Up to three years of age, foals remained outdoors and likewise broodmares, when pasture decreases in quantity and quality, supplementary feeds are timely provided. Recently, an increasing tendency to supply a daily complement to the pasture was observed in all groups of growing foals (one, two and three years old) (data not published).

2.2. Methodologies for the assessment of nutritional status

The nutrient requirements of the horse for maintenance, different physiological states (pregnancy, lactation, growth, stallion reproductive activity) and exercise were established by several feeding systems, being the most currently used the NRC (2007) and INRA (2015) systems.

The formulation of well-balanced feeding programs should be firstly based on nutrient requirements and recommended allowances tables in order to achieve the production and utilization goals. However, it is known that maintenance requirements are influenced by different factors linked to the horse and its environment, such as breed, sex, age, feeding level, climate condition and level of training (Martin-Rosset, 2008). Considering that the energy required at the maintenance level can vary up to 20% for horses with the same body weight (Martin-Rosset, 2008), some monitoring methodologies can be used in order to assess the adequacy of feeding programs to nutritional requirements (Caldeira, 2005).

Two of the most common methods for the *in vivo* assessment of nutritional status and the adequacy of diets are (1) the estimation of the amount of body reserves and respective pattern of change, and (2) the determination of some metabolites and hormones in body fluids, that can allow the early detection of nutritional unbalances (Caldeira, 2005; Caldeira et al., 2007a).

2.2.1. Body condition

2.2.1.1. Significance of body condition concept

The concept of body condition (BC) was first defined by Murray (1919) as the ratio of body fat to non-fat components in the body of a live animal. Although fat components are the main tissues that mostly change in the animal, also protein reserves in the muscle could be reflected by body condition changes (Caldeira &Vaz Portugal, 1998).

Like other mammals, body composition of horses change in accordance with age, feeding level, breed, exercise, management and other factors, reflecting different proportions of bone, muscle and fat tissues (Martin-Rosset et al., 1983; Gordon et al., 2007; Essén-Gustavsson, 2008; Fonseca et al., 2013). Some breed-related differences in fat and muscle distribution have been suggested to be linked to the fact that many horses have been bred and developed for special purposes (e.g. speed, endurance, power) (Kearns et al., 2002). Differences in fat proportion are also observed between genders. In the young Thoroughbred (162 days of age and 42% of expected mature weight), fillies were significantly fatter than colts (19.6 kg *vs.* 15.6 kg of partial empty body fat mass, respectively) (Gee et al., 2003).

Besides genetic, changes in fat mass are highly related to environmental factors, where nutrition plays a major role. Diet composition may even affect fat partition in horse, as a result of possible differences in efficiency of fat storage. In Thoroughbred geldings, diets rich in fibre and fat seem to induce significantly higher scores at neck and withers areas compared with others on diets rich in non-structural carbohydrates (starch and sugar) (Suagee et al., 2008). The level of exercise also influences fat deposition. Obese or overweight leisure or low level competing horses and ponies are commonly seen while many elite equine athletes have difficulty to meet their energy requirements and are subsequently thin (Gordon et al., 2007; Harker et al., 2011).

According to their nature, body reserves can be divided, in energy reserves (mainly triglycerides stored in the adipocytes) and protein reserves (mainly protein of muscle tissue) (Caldeira & Vaz Portugal, 1998). In the short term, between meals, or in the medium or long term, between periods of abundance and shortage of food, the physiological mechanisms of deposition and mobilisation of body reserves are an integral part of energy homeostasis (Caldeira, 1995).

As in other animal species, and particularly in pasture based systems, mares have the ability to mobilize and restore body fat reserves during their reproductive cycle. This mobilization of body reserves can be seen during the winter, at the end of pregnancy and/or early lactation periods in which nutritional needs may be greater than those provided by the diet and especially when low quality forages are used (Doreau et al., 1990; INRA, 2015). In addition, the mobilization of adipose tissue could be also in part directed towards the production of the lipid fraction of mares' milk, especially when the mares are fed limited energy (Martin-Rosset et al., 2006a). However this mobilization in early lactation is very limited when compared with cattle (Wolter, 1994). The triglycerides reserve in the horse skeletal muscle represents also important energy sources during exercise. This capacity for triglyceride storage in the muscle seems to increase with age and training (Essén-Gustavsson, 2008).

2.2.1.2. Body composition assessment in the horse

There are several methods to assess body composition in the horse. In the live animal, these methods could be divided in two major groups according to their complexity and adaptability to different situations. In research studies, methods such as dilution techniques (Fielding et al., 2004; Waller & Lindinger, 2006; Carter et al., 2010; Dugdale et al., 2010a), bioelectrical impedance analysis (Gee et al., 2003; Waller & Lindinger, 2006) and ultrasonic measurements of subcutaneous fat thickness (Westerveld et al., 1976; Gee et al., 2003; Manso Filho et al., 2009; Carter et al., 2010; Silva et al., 2012) have been used. Other techniques like hydrostatic (underwater) weighing, dual X-ray absorptiometry (DXA) or magnetic resonance imaging (MRI), frequently used in humans (Prior et al., 1997; Bosy-Westphal et al., 2008), are not applicable to the horse due to its size. The most common methods reported for the evaluation of body composition in farm conditions, has been live weighing and body condition scoring (BCS). However, body weight (BW) changes could also reflect changes in other components than body reserves. That is the case of digestive contents or foetal-placental unit weight (in particular at the end of gestation) (Martin-Rosset et al., 1986). Nevertheless, the information regarding BW could complement BCS, in particular, during stages when the variation factors are less important.

Concerning *ex vivo* techniques, carcass dissection is a hard process that provides quantitative and accurate data on body composition (Martin-Rosset et al., 1983; Gee et al., 2003; Martin-Rosset et al., 2008). However, this method cannot be used for longitudinal studies where the beginning and some middle and end time points must be measured (Kearns et al., 2002).

2.2.1.2.1. Methodologies for Body Condition Scoring

Originally developed for sheep, the concept of body condition and its application by different methods were later extended to several livestock species, including the equine and even the asinine (Caldeira & Vaz Portugal, 1998; Pearson & Ouassat, 1996). The evaluation of body condition is a simple process that identifies areas of the body where fat cover is palpable, giving a qualitative condition score. Although the differences between the scoring systems and animal species, low values always reflect emaciation and high values are associated to obesity (Kearns et al., 2002). On field conditions, BCS is a cheap and practical tool for monitoring energy balance, reflecting the abundance or shortage of nutrients on the animal recent past (Caldeira et al., 2007b; Martin-Rosset et al., 2008). Besides nutritional aspects, BCS has been also recognized as a valid indicator of global animal welfare (Christie et al., 2006; Roche et al., 2009).

One of the first scoring systems proposed for horses was developed by Henneke et al. (1983), in the United States of America (USA). This system was developed from a previous scale used on cattle and was based on visual appraisal and palpable fat cover at six body areas on

Quarter Horse mares. The scoring areas (neck, withers, lumbar region, tailhead, ribs and area behind the shoulder) were selected as being easily indicative of changes in stored body fat in the horse (Figure 2.2.1). The scale runs from 1 to 9 points, where 1 correspond to an extremely emaciated animal and 9 to an extremely fat, as described on Annex II. In this study, a positive correlation (r^2 = 0.65, P < 0.001) was found between body fat percentage (as determined by the equation developed by Westerveld et al., 1976) and body score evaluation. For practical reasons, the initial scoring scale proposed by Henneke et al. (1983) was later adapted to a more descriptive form (Annex III).

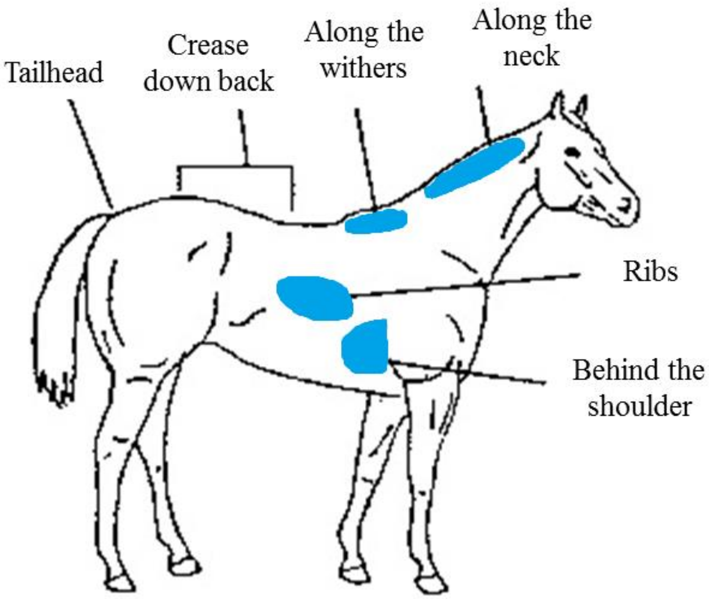

Figure 2.2.1 – Palpable areas of horse body to estimate condition score
(adapted from Henneke et al., 1983)

When some conformational aspects like prominent withers or flat backs, make difficult to apply a certain score to a specific animal, authors advise to give more emphasis on fat cover over the ribs, behind the shoulder and around the tailhead. Moreover, in the pregnant mare near term, more attention should be given to the fat deposition behind the shoulder and along the withers, since the weight of the conceptus stretched the skin and musculature of the back, ribs and tailhead, given a diminished appearance of fat cover in those areas. Although based on mares' evaluation, Henneke et al. (1983) BCS system has been widely used in USA and Anglo-Saxon countries for different categories of horses (*e.g.* young maiden mares: Gastal et al., 2004; young foals: Pagan et al., 2006; lactating mares: Huff et al., 2008; sport horses: Pagan et al., 2009; old horses: Adams et al., 2009; geldings: Carter et al., 2010).

In 1990, another BCS system was proposed by INRA for the sport and saddle horse and its fundaments and methodology were later published in 1997 (INRA-HN-IE). This method was based on studies carried out with carcass dissection. Horses were scored before slaughtered and the weight of adipose tissue (and energy content) was determined after total anatomical dissection of tissues and gross energy determination (Martin-Rosset et al., 1983). Like the Henneke system, BC is evaluated using five key anatomic regions where fat cover is perceptible and can be palpated: along the neck, along the withers, ribs (between the 10th and 14th), behind the shoulder and tailhead (Figure 2.2.2). Besides thickness, extension and consistency of the subcutaneous fat tissue on the palpable areas, this method also comprises two scores based on visual appraisal: the back line and the croup. However, factors like the winter coat emphasised the value of palpation as opposed to visual appraisal, especially when small changes in subcutaneous fat depths are present (Dugdale et al., 2010a).

The scale runs from 0 (emaciated animal) to 5 points (obese) and the use of half points are advisable to better illustrate the individual condition scores, described on the table of Annex IV. Experienced evaluators often use quarters of point, mainly on research studies, for a better accuracy of the method. In BCS systems in general, the introduction of smaller scale divisions (*e.g.* quarter point), as suggest for ewes evaluation, decreases the subjectivity inherent to each score when the perception obtained from palpation does not match with the description of a precise score (Caldeira and Vaz Portugal, 1998).

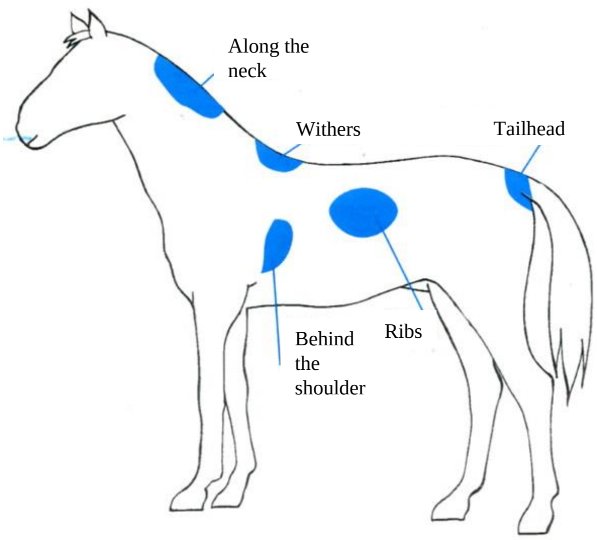

Figure 2.2.2 – Regions for manual appraisal in the French BCS system

(adapted from, INRA-HN-IE, 1997)

The INRA BCS system was validated for the sport horse with the slaughter and dissection method applied to 20 adult Selle Français and Anglo-Arab horses (Martin-Rosset et al., 2008). In this study, high correlation coefficients (ranging between 0.986 and 0.990, $P<0.001$) were obtained between the weights of total, subcutaneous, internal or intramuscular fat tissues and body condition score, showing a better accuracy than the reported in the Henneke system.

Besides Henneke et al. (1983) and INRA BCS systems, some references can be found with respect to other scales. In Australia (Ellis, 2002) and some Canadian regions (Wright et al., 1998) the method proposed by Carroll & Huntington (1988) is still used for condition scoring of horses. These authors adapted a scale (from 0 to 5 points) published in a text-book by Leighton-Hardman in 1980, which takes into account the deposition of fat in different areas by separate examination of the neck, back, ribs, pelvis and rump. Individual neck, ribs and rump assessments are then combined to give an overall condition score.

Also an adaptation of Henneke et al. (1983) system was proposed for BC evaluation in Warmblood horses by Kienzle & Schramme (2004). Although based on similar scored areas of horse body, some differences regarding methodology were introduced by these authors:

- In the neck crest, fat is measured with the head in lowered position, allowing that the neck muscles become visible as a lower borderline of crest fat; the side area (concave or convex) and the presence of a shelf where the neck meets shoulder are checked;
- In the shoulder area, the visibility of the scapula and the ribs is important and the possibility to form a small or thick skin fold is tested;
- In the back and croup area, the visibility and palpability of the bone structure of the spine and ribs are considered; it needs to be checked whether (i) the skin can be moved above the bone muscles, (ii) the subcutaneous tissue is soft and, (iii) there are fat deposits over the ribs and the croup;
- Fat deposits over the hip bones need to be scored in a scale designed for warm blood horses.

Considering the differences in regional depositions of fat between horses and ponies (*i.e.* adipose tissue deposited more heavily along the crest of the neck in some pony breeds), a specific standardised scoring system was recently proposed by Carter et al (2009). While BCS is an accepted method for the assessment of overall adiposity, cresty neck scoring (CNS) would standardise the assessment of regional fat distribution on the crest of the neck (Annex V).

2.2.1.2.2. Body Condition Scoring: practical interest

Body condition scoring can be used as a practical method for assessing the horse body reserves and their changes over the time. This is essential to promote an adequate management of feeding plans and to monitor the adjustment of diets to the different requirements of each productive phase. Regular BC evaluations allow for determining if the animal is storing, mobilizing or maintaining reserves which reflect the energy balance and the metabolic status, information that is fundamental from the nutritionist point of view (Carroll & Huntington, 1988; Caldeira, 2005). Furthermore, BCS is a complementary method for BW evaluations. For instance in the broodmare, and especially in the beginning of lactation, the weight alone may not reflect the eventual mobilization of body reserves, due to an increase of dry matter intake.

The maintenance of an adequate BC is also important for economic reasons. In normal conditions, excessive BC could represent two unnecessary expenses: first the amount of feed that was consumed to get a higher score and second, the amount of feed that is needed to maintain that score. Mature horses in fleshy body condition (7.5 – Henneke BCS system) required 2.3 kg/day more total feed than horses in moderate (5.2) body condition (Webb et al., 1990).

Besides some scores considered as ideal for several types of sport horses (Table 2.2.1), some authors advise a periodicity of BC scoring. In a stud farm, the assessment of body condition of mares and growing animals should be primarily performed at the beginning, middle and end of winter. In the case of horses at work (or other type of sport activity), the frequency of assessment should be higher (every one to two months) (INRA, 2015).

Table 2.2.1 - Advised BCS (French BCS system) for recreational and leisure horses and for sport horses (INRA, 2015)

Recreational and leisure horses	3.0 to 3.5
Endurance horses	3.0
Three day event	3.0
Show jumping	3.25 to 3.5

2.2.1.3. Body Condition and Body Weight changes in the broodmare

The live weight of broodmares can change 15-20% throughout the year, reaching the maximum and minimum just before and after foaling, respectively. The greatest variation is observed at foaling, where the loss of weight could represent 11 to 15% of the BW of the mare in good condition. If the BC is poor, additional variation could be ascribed to the mobilization of body reserves (Martin-Rosset et al., 2006b).

In Thoroughbred broodmares, Cassill et al. (2009) observed a mean weight gain throughout gestation period, of 13.9±3.2 % of initial BW, considering initial BW as the that obtained at the end of the month of the last breeding date, and the weight at the end of gestation as the last one obtained prior to foaling. The average BW gain as a percentage of BW was significantly greater for young mares (4 to 8 years) than for mature mares (9 to 18 years) (15.3 % *vs*. 12.8 %, respectively), suggesting that some of these young mares are still growing. A previous work with 12 mares, fed forage *ad libitum* during an entire gestation period, referred a total weight gain of 16 % (Lawrence et al., 1992).

Well fed mares should increase their BW on average 8% over the last three months of gestation. However, the main part of the gain takes place before the last month of gestation and a slight decrease may even occur during the last two weeks, since BW changes are also strongly related to dry matter intake. Voluntary feed intake regularly decreases on average 20% between the 5^{th} and the 1^{st} week before foaling. Thus, such level of intake allows for only a small increase (≈ 2%) in the mare BW over the last month of gestation in spite of the growing of the *foetus* during this period (Martin-Rosset et al., 2006b).

Beyond the weight gain related to gestation, changes in broodmare BW and BC along the year are also linked to seasonal and management factors. A ten years study with Thoroughbred mares in the central region of Kentucky (USA) revealed that BW, daily weight change and BCS increased in the spring (March through June) in all the mares regardless of stage of gestation/lactation (Pagan et al., 2006).

2.2.1.4. Effects of Body Condition on reproductive/productive performances

Whatever the breed, the main goal in horse production is to ensure a foal each year and to promote a short foaling-fertilisation *post-partum* interval. It is generally accepted that mares should be in good condition at foaling, for successfully rebreeding within the month following delivery. The effects of nutritional status (assessed by body condition) on reproductive performance of the mare was highlighted by Henneke et al. (1984). Mares that have foaled and maintained a moderate (5 to 6.5) to high (> 7) BCS, presented a lower interval between foaling and first *post-partum* ovulation, showed a lower number of cycles per conception and had higher pregnancy rates when compared with mares that have foaled in a BCS <5 and maintained a low BCS during the lactation period. Results from this study, pointed out to an enhancement of breeding efficiency in mares that enter the breeding season (barren or maiden mares) or foaling at a condition of 5.0 or above.

The influence of BC on the winter transition period was also studied. The anovulatory period seems to be shorter in mares which gain weight during early spring (Ginther, 1974). Considering ovarian activity, a larger maximum follicle diameter, a shorter interval from parturition to ovulation and a shorter interovulatory interval were observed in non-lactating mares that increased their BC (Godoi et al., 2002). The positive correlation between increased BC and the diameter of growing follicles was also verified in fillies, during the prepubertal phase (Gomes et al., 2002) and in the asinine species (Lemma et al., 2006).

Besides economic disadvantages, higher BCS (9, in a 1-9 scale) did not appear to adversely affect postpartum reproductive performance in the multiparous mare, when compared to a moderate degree of body fat (BCS 5.5 to 7). The intervals from foaling to first cycle ovulation, foaling to second cycle ovulation, and first to second cycle ovulations were similar between groups. All mares conceived and maintained pregnancy, and the first cycle conception rate and the number of cycles per conception did not differ (Kubiak et al., 1989). Similar results were found by Cavinder (2006). This author showed that mares maintained in a fleshy body condition (7-8) are not prone to reproductive dysfunction or lowered levels of fertility. However, altered metabolic status due to an excessive energy balance may lead to a continuous reproductive activity during the winter months. Gentry et al. (2002) demonstrated that non restricted fat mares (BCS 7.5-8.5) displayed continuous estrous cycles throughout the year.

In contrast, low BCS in lactating mares showed a negative impact on reproductive efficiency. Data obtained from a field study with 145 mares under native range conditions and with only limited supplemental feeding, indicate that lactating mares in moderate body condition (<5, 1-9 scale) tended to skip a breeding season (Gibbs & Davison, 1992). Mares under a restricted feeding level presented a winter ovarian inactivity systematically advanced, longer and finishing later than well-fed mares (Guillaume et al., 2002). Negative energy balance was also inversely correlated with growth rate of the dominant follicle and its diameter and seems to be a major factor for decreasing follicular growth during lactation (Gastal et al., 2000; Godoi et al., 2002). In the particular case of maiden mares, low body condition (<5, in a 1 to 9 points scale) was associated with reduced follicle development, during the transition between the anovulatory and ovulatory seasons and during the first interovulatory interval of the ovulatory season (Gastal et al., 2004). Even a small decrease in mares' body condition after parturition (7.6 to 6.9, in a 1 to 9 points scale) revealed a tendency for an increased interovulatory interval and for a longer second follicular phase (Nagy et al., 1998). In addition, negative energy balance seems to influence embryo loss. In a retrospective analysis over 12 breeding seasons, Newcombe & Wilson (2005) observed that 58% of the mares which experiencing embryo loss, had a net weight loss (- 8 kg) between the pre-breeding examination and the end of breeding season.

The positive effect of an adequate amount of body reserves in the mare' reproductive performance has been recognized by some authors who have tried to made suggestions about the ideal BCS at key points along the breeding cycle. Considering Henneke et al. (1983) BCS system, and mainly in the USA, it is advisable that, under ideal conditions, mares should enter the breeding season in a moderate condition (condition score of 5 - 5.5), be maintained in moderate fleshy to fleshy condition (condition score of 6 to 7 – 7.5) throughout early gestation, gain weight during late gestation, and foaled in a fleshy to fat condition (condition score 7 to 8). Under no circumstances should a mare be allowed to become obese (condition score 8.5 to 9) (Evans, 2005; Gibbs et al., 2005). In Europe, and taking into account the INRA (2015) system, it is proposed as a good standard for adult broodmares the following: a BCS of 3.5 in Autumn, after weaning, to allow some feed restriction during the winter; a BCS of 3.0 two months before foaling; and a BCS of 3.0 at foaling. The minimum admissible score at foaling and beginning of lactation should be 2.5, but as higher as possible, the earlier the mare foaled in the winter season. In this case, mares must acquire or regain a score of 3.0 to 3.5 at the end of the first month of lactation.

Mare's condition at conception appears also to have an influence on foals' gender. In a three years study, Cameron et al. (1999) reported that Kaimanawa mares that had a female foal were in poorer body condition at conception than those that had a male foal. Even mares that had foals of different sexes in different years were in significantly poorer condition when they conceived a female foal, confirming the hypothesis of Trivers & Willard (1973), where maternal condition at or around conception affects the secondary sex ratio in mammals.

Besides reproductive function, BC has been also recognized as having an influence on broodmare's productive parameters such as milk production and the consequent foals' growth. BW of suckling foals is linked with milk intake until two months of age, and average daily gain (ADG) is linearly related to milk yield (Martin-Rosset et al., 1978; Doreau et al., 1986). Milk yield and composition is affected by diet composition, feeding level and intake, and by the mare's BC. Milk yield increases in some extent with BCS. Milk from overweight mares has a higher fat content than milk from thin mares and the reverse is found for protein (Doreau et al., 1992, 1993). As a result, milk output and related growth of the foals are affected by body reserves of their dams (Martin-Rosset & Young, 2006).

2.2.2. Metabolic indicators

Together with BCS, other methodologies could be used in order to evaluate the adequacy of feeding plans and nutritional status of the animal. The assessment of some blood parameters for monitoring metabolic and nutritional status in livestock species has been widely used for a long time, either for the quality of the information that could provide or for the simplicity of collection (Doreau et al., 1981; Caldeira, 2005). However, these parameters are susceptible to circadian variations due to the kinetics of digestion and to metabolic changes (Doreau et al., 1981). In general, serum or plasma concentrations of non-esterified fatty acids, glucose and insulin provide valuable information about the energy status of the animal, while albumin and urea are good indicators of protein status (Caldeira et al., 2007a).

2.2.2.1. Energy metabolism indicators

2.2.2.1.1. Glucose

Glucose is an energy substrate for all cells and its availability in the blood is under an elegant and strong system of homeostasis. Glycemia is mainly regulated by hormones of the pancreas, anterior pituitary, adrenal cortex and medulla – such as insulin, glucagon, somatotropin, cortisol and catecholamines. The liver also functions as an important buffer system to maintain a steady blood glucose concentration. This organ and skeletal muscles are among the most important sites for post-prandial glucose uptake, where glucose is stored as glycogen. In periods when glucose availability from intestinal absorption is decreased, liver glycogen serves to maintain normal blood glucose concentrations and ensure the supply of glucose to all tissues of the body (Hyyppä, 2008). But substrates such as lactate, glycerol and certain amino acids, can be also converted into glucose in the liver, via gluconeogenesis. Up to 60% of glucose in equine blood may be synthesised in the liver from propionate that comes from the hindgut, by this pathway (Simmons & Ford, 1991).

Due to this strong homeostatic system, blood glucose levels normally stay within narrow limits (range of 3.3 to 6 mmol/l, in the horse - Ralston, 2002; Hyyppä, 2008), which makes it not so valuable as a metabolic indicator (Caldeira, 1995). Blood glucose concentrations after 12 hours of fast or longer are quite constant in healthy horses (Sticker et al., 1995b; Nadal et al., 1997; McManus & Fitzgerald, 2000). Even after moderate or intense short-term exercise, horses fasted for 12 to 16 hours maintained relatively constant blood glucose concentrations (Lawrence et al., 1995). But glucose values interpreted together with other parameters may contribute significantly to a diagnosis. Low values may indicate decreased levels of feed intake or gluconeogenesis (lack of glucose precursors) in times of greatest needs of glucose (*e.g.* late pregnancy and early lactation). Higher values generally indicate insulin resistance, common in animals with excessive BCS. Besides, animals submitted to prolonged negative energy balance could also present high glucose levels suggesting an uncontrolled homeostasis of this parameter (Caldeira, 1995). Other factors like feed type, feed processing, postprandial time, excitement or stress, illness, genetics and reproductive status, have been reported to influence blood glucose concentrations in the horse (Doreau et al., 1981; Glade et al., 1984; DePew et al., 1994; Powell et al., 2000; Ralston, 2002; Vervuert et al. 2007b). However, basal concentrations were not affected by breed, feeding frequency, energy source of feeds or pasture supplementary feeds (Nadeau et al., 2006; Douglas Wilson et al., 2007; Van Weyenberg et al., 2007; Staniar et al., 2007a).

Pregnancy and lactation result in increased metabolic demands. In fact, throughout gestation, a progressive adaptation of maternal glucose homeostasis develops and becomes maximal during the last trimester. The rate of glucose utilization by peripheral maternal tissues is lowered in late gestation indicating that the mother supplies glucose to the foetus at the expense of her own tissues. Simultaneously, an insulin-resistant state develops in the mother to help sparing glucose for the pregnant uterus (Leturque et al., 1987). These alterations in glucose homeostasis associated to gestation (homeorhesis) were confirmed in TB mares by Hoffman et al. (2003a) with larger glucose and insulin incremental areas under the response curve (AUC) and slower glucose clearance, after an oral glucose tolerance test. In early lactation (after foaling and by the end of 2nd month), basal glucose concentrations are lower than in late gestation (Hoffman et al., 2003a; Heidler et al., 2004). It is likely that the increased utilization of glucose during early lactation was influenced by mammary demands and mostly for lactose synthesis, since mares' milk is highly concentrated in this disaccharide (Santos & Silvestre, 2008).

In the growing foal, baseline plasma glucose concentrations decreased with age (from 5 to 160 days) and seem to be affected by their maternal dietary energy composition (high starch *vs.* low starch) during the last third of gestation (George et al., 2009). In fact, maternal dietary composition can influence feto-placental development by altering nutrient delivery from maternal to foetal blood. Specifically, the maternal-foetal glucose gradient, which drives glucose delivery to feto-placental tissues, can be altered by increased maternal glucose concentrations (Bell et al., 1999).

2.2.2.1.2. Non-esterified fatty acids

Non-esterified fatty acids (NEFA) result from the hydrolysis of triglycerides deposited in adipocytes. When fat stores are mobilised, triglycerides are hydrolysed into free fatty acids and glycerol, by help of a hormone-sensitive lipase. The main hormones involved in the process are catecholamines which stimulate lipolysis, whereas insulin is a potent inhibitor. NEFA are then released into the bloodstream, where they are transported by albumin (Essén-Gustavsson, 2008). In situations of negative energy balance (NEB), the amount of fatty acids released from adipocytes increases, and the circulating NEFA are quickly picked up by peripheral tissues deficient in energy (Pethick et al. 1983). In these tissues and in the liver, NEFA are completely oxidized to CO_2 via the tricarboxylic cycle, or partly oxidized giving rise to ketone bodies and acetate. Apart from those two pathways, the circulating NEFA may also be reesterified in the liver, mainly as triglycerides but also in phospholipids and cholesterol esters (Rémésy et al., 1986).

The serum concentrations of NEFA have been used in many studies as an indicator of nutritional status of animals or, more correctly, as an evaluator of the energy balance (e.g. Caldeira & Vaz Portugal, 1991, Landau et al. 1992; Cameron & Cienfuegos-Rivas, 1994; Caldeira, 1995).

The daily profile of serum concentrations of NEFA is quite known, especially in animals fed with one or two concentrate meals. Circulating NEFA drop immediately after the meal in response to the insulin increase and remains low until nutrients begin to lack. Then, NEFA blood concentration rise and keep high values until the next meal. In sedentary horses, an acrophase of the circadian rhythm of NEFA was recorded in the morning at about 07:00 hours (Orme et al., 1994), while in the athletic horse, the acrophase was observed in the afternoon, after one hour of training, showing the influence of physical activity (Piccione et al., 2009).

NEFA concentrations decreased after a meal (Doreau et al., 1981; DePew et al., 1994) but increased concentrations of NEFA were observed in several studies, after periods of short or long feed deprivation or restriction (Sticker et al., 1995a; Sticker et al., 1995b; Christensen et al., 1997; Powell et al., 2000; McManus & Fitzgerald, 2000). Factors like breed (Morgan *vs*. Thoroughbred) or meal frequency (once *vs*. three times a day) did not affect basal NEFA concentrations after an overnight fasting (Nadeau et al., 2006; Van Weyenberg et al., 2007). Also differences in diet composition seem not to affect fasting NEFA concentrations (Hallebeek & Beynen, 2001; Ropp et al., 2003). Nevertheless, in late pregnancy NEFA concentrations were higher, when compared to early lactation state (Filipović et al., 2010).

Blood NEFA concentrations increase with obesity and lack of physical activity in humans because adipose tissues reach their maximum capacity for fat storage and the inhibitory effects of insulin on hormone-responsive lipase are reduced (Boden & Laakso, 2004). Similarly, higher resting concentrations of NEFA were observed in obese horses and ponies when compared with non-obese animals (Frank et al., 2006; Dugdale et al., 2010b). In the growing horse (weanling foals), reduced circulating levels of NEFA are ascribed to a positive energy balance and to *ad libitum* access to forage (Gray & Staniar, 2011).

2.2.2.1.3. Insulin

Insulin is a hormone secreted by the β-cells of the pancreas in response mainly to blood glucose increase (Satiel & Khan, 2001). The metabolic actions of insulin maintain whole body glucose homeostasis and promote efficient glucose utilization (Geor, 2008). Insulin is essential for promoting the transport of glucose across the plasma membrane into the cell, by binding to its specific receptor. This hormone has also profound effects on the metabolism of carbohydrates. Insulin stimulates glucose uptake into skeletal muscle and into adipocytes and stimulates glycogen synthesis in muscle and liver, with simultaneous inhibition of gluconeogenesis in liver (Satiel & Khan, 2001).

Besides carbohydrate metabolism, insulin participates in a wide array of physiologic processes, including stimulation of fatty acid and triglyceride synthesis in liver and adipose tissue, inhibition of lipolysis (Essén-Gustavsson, 2008), enhancement of protein anabolism in peripheral tissues, which decreases the availability of amino acids (AA) for gluconeogenesis, cell growth and survival, regulation of vascular endothelial function, and anti-inflammatory effects (Satiel & Khan, 2001).

The maximal effect of insulin defines ‘‘insulin responsiveness’’, whereas insulin concentration that elicits a half-maximal response defines ‘‘insulin sensitivity” (Muniyappa et al., 2008). Fasting insulin concentrations are usually between values less than five and 20 μIU/mL. As with glucose, fasting concentrations of insulin are relatively constant, regardless of diet, physiologic status, or conditioning. However, in euglicemic horses, insulin secretion and clearance in response to a glucose challenge or feeding are dramatically influenced by the time since the last meal, rations fed, body condition (Hoffman et al., 2003b), physiologic status (Hoffman et al., 2003a), and circulating cortisol concentrations (Ralston, 2002).

Hyperinsulinemia, defined as plasma insulin concentrations greater than 200 μIU/mL in adult animals, is common in horses and it was frequently associated with obesity or pituitary adenomas (Ralston, 2002). Fasting hyperinsulinemia can occur when there is a compensatory response by the pancreas to peripheral tissue insulin resistance (Firshman & Valberg, 2007).

Insulin resistance is defined as the diminished ability of cells to respond to the action of insulin in transporting glucose from the bloodstream into muscle and other tissues (Valberg & Firshman, 2009), or as insulin ineffectiveness due to the disruption of glucose metabolism inside the cell (Kronfeld et al., 2005). It has been implicated as a risk factor in the pathogenesis of certain equine diseases, such as laminitis, pituitary adenoma, hyperlipidemia, and osteochondritis dissecans (Kronfeld et al., 2005).

There are marked changes in carbohydrate metabolism and in pancreatic B-cell function during pregnancy in the mare. In pregnant mares up to 270 days gestation Fowden et al. (1984) have shown, with studies of glucose tolerance, that a syndrome of insulin resistance is present. Pregnant mares exhibited higher levels of insulin release in response to exogenous and endogenous glucose, increased degradation of insulin and it appears that, after 270 days gestation, pregnant mares redirect glucose away from the maternal tissues to meet the nutritional demands of the foetus. In humans this mechanism has been described as "facilitated anabolism".

Also in the newborn foal, transient insulin resistance may develop for the first day after birth (Holdstock et al., 2004). Maturation of pancreatic β cell function occurs over time in foals with a rise in insulin concentrations during the first 24-48h *post-partum* (Fowden et al., 2005). This period of maturation of pancreatic β cell function appears to continue for approximately 3 months after birth after which similar levels of function to those of mature horses are achieved (Smyth et al., 1993).

2.2.2.2. Protein metabolism indicators

2.2.2.2.1. Urea

As in other animal species, the total protein content of the horse body ranges between 17-19% of the body weight. Depending on its location, all these proteins are continuously degraded or/and synthesized according to different turnover rates (Martin-Rosset & Tisserand, 2004). Besides the AA provided by protein content of feed stuffs, most of the AA used in synthesis have their origin in the own degradation of body protein. The AA that are not used for protein synthesis (coming either from the normal protein turnover or from an excessive or unbalanced protein intake) are catabolised in the liver (Caldeira, 2005). This process involves the removal of the amino group from the carbon skeleton which, by its turn, and together with the ammonia absorbed in the gut, entered into the urea cycle. The urea produced is then transported by the blood stream and excreted in the digestive tract and in the urine. In the digestive tract, urea is hydrolysed into ammonia which is either used by the microflora for the synthesis of AA or reabsorbed if in excess. Like in the other herbivores, this entero-hepatic urea cycle, allows the horse to spare and to supply nitrogen to the microflora of the large intestine when protein intake is too low, although with a lower efficiency when compared to ruminants (Prior et al., 1974; Glade, 1984). However, an *in vitro* fermentation study with equine caecal contents showed an increased efficiency of microbial growth, when urea was used as nitrogen source when compared to casein (Santos et al., 2013).

Diets with high levels of protein or protein with an unbalanced AA profile lead to increased values of blood urea (Miller-Graber et al., 1991; Caldeira, 2005). Uremia also increases during the prandial period (Doreau et al., 1981; DePew et al., 1994) and short term feed deprivation (48 and 72h) or a 50% restriction of energy requirements over a period of 24 days seems to induce a similar effect (Sticker et al., 1995a; 1995b; Christensen et al., 1997). On the opposite, dietary protein restrictions (50% of protein requirements) appear to decreases uremia (Sticker et al., 1995a).

Daily or circadian oscillations in urea blood concentrations were observed in two different studies, with a common acrophase after 8 a.m. (which is probably related with the morning post prandial period) (Doreau et al., 1981; Piccione et al., 2005). Doreau et al. (1981) also suggested a moderate effect of the physiological state on uremia. Non-pregnant mares presented higher values of urea when compared to gestating or lactating mares. But opposite results were reported by Harvey et al. (2005) for the nursing mare, with increased urea concentrations during lactation, when compared to nonpregnant and nonlactating mares.

These authors suggested that protein metabolism associated to the presence or absence of a foetus could influence uremia.

Other factors like the absence of normal kidney function or dehydration situations may be also in the origin of high levels of urea in the blood (Meyer, 1995).

2.2.2.2.2. Albumin

Albumin is the most abundant protein in the blood, is synthesized in the liver and its concentration in blood is used as an indicator of liver function and nutritional status. In situation of nutritional deficiency, the animal uses albumin as an important pool of labile protein. This metabolite also provides functions of binding protein and transport of other molecules in the bloodstream, including NEFA and some hormones (Kaneko, 1997).

A direct response of circulating albumin to feeding level (Tennant, 1997) and, more specifically, to the protein fraction of the diet (Hoaglund et al. 1992; Caldeira, 1995, Hoffman et al., 2001) is frequently observed. A total or a partial feed restriction often causes hypoproteinaemia (Sykes & Field, 1973, Lynch & Jackson, 1983) and hypoalbuminemia (Sykes & Field, 1973). However, this physiologic response is not immediate, as it takes several days of restriction for the decrease in serum protein or albumin levels become significant. Indeed, the levels of plasma proteins appear to be maintained during starvation until body protein reserves are markedly decreased (Ganong, 2009). In situations of malnutrition, it is required a period of about one month, to induce a significant decrease of albumin (Payne & Payne, 1987).

Generally, in animals whose liver and kidney functions are not altered, decreased levels of serum albumin indicate dietary deficiencies of protein/nitrogen in the animal' recent past. Otherwise, increased levels of serum albumin may be caused by dehydration, which leads to its concentration in blood. Feeding plans that meet the animal dietary needs in AA will necessarily allow maximum levels of albumin synthesis, leading to maximum serum concentrations, within the range of normal values (Caldeira, 2005).

In the light mare, albumin concentrations are not affected by physiological status (gestation *vs.* lactation) and there are no differences between the gestation and lactation period when compared with a reference group of nonpregnant and nonlactating mares (Harvey et al., 2005). However, in the young foal (first 60 days of live) albumin concentrations were on average 2-3 g/L lower than those of their dams (van Niekerk & van Niekerk, 1997).

2.3. Growth and development of the foal

Growth is defined as the increase in live weight and body dimensions as a function of time (Martin-Rosset et al., 2015b). At a cellular level, growth is reflected by both the increase in cell number (hyperplasia) and cell size (hypertrophy) (Owens et al., 1993; Staniar, 2013). Growth rate is usually measured by live weight expressed as mass unit per day (*e.g.* g or kg/day). Development occurs through a set of events from conception to adulthood. At its most basic level, development involves the coordinated regulation of cell proliferation, cell death (apoptosis), cell migration, and differentiation (Butler & Le Roith, 2001). During gestation, the embryo evolves through various stages to became a fetus and then, at parturition, a foal. Until adult age, morphologic, anatomic and chemical modification will follow, resulting in sexual and physical maturation. Development is measured by comparing weight, size or anatomical and chemical composition of a region or tissue at a given age to a reference element (Martin-Rosset et al., 2015b). From conception to maturity, the pattern of equine growth can generally be described as a sigmoid curve (Martin-Rosset, 2005; Staniar, 2013). However, the expression of growth is highly dependent on genetic background and environmental factors (Fitzhugh, 1976; Staniar, 2013; Martin-Rosset et al., 2015b). Among these, breeders' goals and local management, including feeding practices, has been shown to have a large influence on the life time growth pattern of the horse (Bigot et al., 1987; Pagan, 2005). After birth, and according to the genetic type and use, growth period for light breed horses lasts from 3 to 5 years of age, which represent a large percentage of their productive life (Martin-Rosset et al., 2015b). Considering the importance of this period on the future performances and productivity of the horse, the knowledge and characterization of growth and development is of utmost importance for breeders and users.

2.3.1. Pre-foaling growth and development

Most of the studies reported in literature regarding fetal growth and development in the horse were based on data from foals that were aborted or stillborn either in light breeds or ponies (Meyer & Ahlswede, 1976; Platt, 1978; 1984; Giussani et al., 2005). According to these data several equations (power, exponential and polynomial models) were proposed for predicting BW of the foetus from the day of gestation (NRC, 2007; Coenen et al., 2011; Martin-Rosset et al., 2015c). During the first half of the gestation period, there is only a small increase in weight of the fetus. The bigger increase in weight is observed in the last third of gestation whatever the model used (Figure 2.3.1).

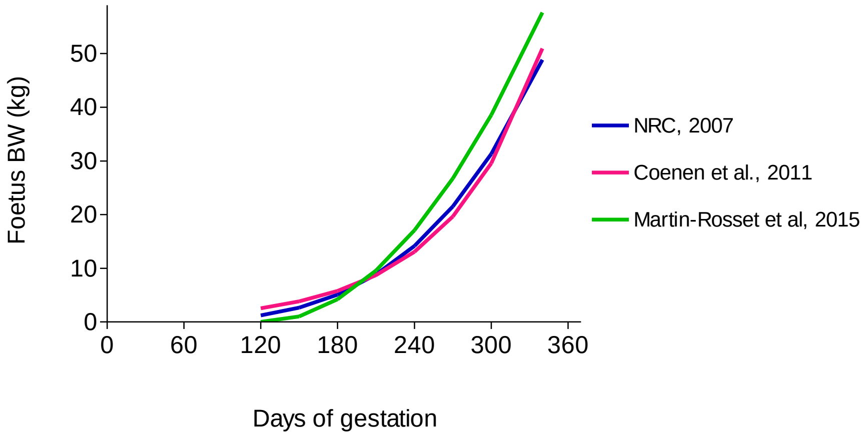

Figure 2.3.1 – Modeling of fetal body weight during gestation, considering foal birth weight as 10% of the mare body weight after foaling, and mare body weight as 500 kg. Equations:

Fetal weight (as percent of birth weight) = $1 \times 10^{-7} X^{3.5512}$ (NRC, 2007);

Fetal weight (as percent of birth weight) = $e^{0.0136\,X}$ (Coenen et al., 2011);

Fetal weight (kg) = $17.38 - 0.2885\,X + 0.001197\,X^2$ (Martin-Rosset et al., 2015c)

X = days of gestation

The development in body length observed on Thoroughbred (aborted and stillborn fetus) and measured as crown-rump length tended to precede the increase in body mass and seems to be more steady from the beginning to the end of the gestation period (Platt, 1978) (Figure 2.3.2).

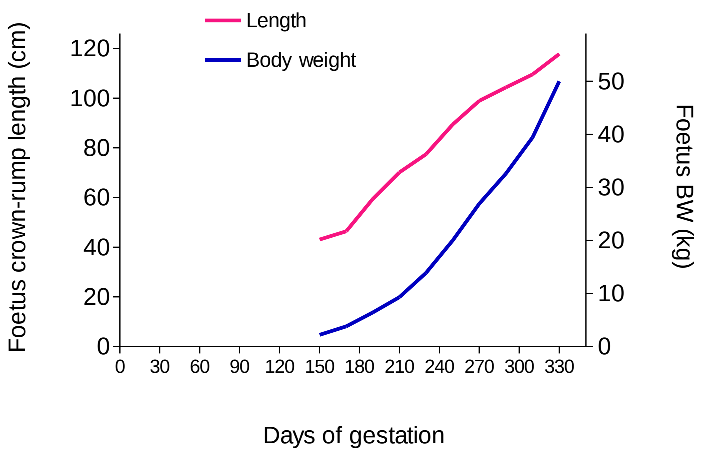

Figure 2.3.2 – Fetal growth observed in Thoroughbred (mean crown-rump length and body weight from 150 days to term of gestation.

(adapted from Platt, 1978)

The influence of maternal size on fetal development was studied based on crosses between different size horse breeds (Walton & Hammond, 1938; Tischner, 1985). These studies showed that fetal growth can be either enhanced above, or restricted below, the normal genetic potential for the breed by varying maternal size. The rate and extent of fetal growth is also influenced by the area of placental interface, and birth weight is related with the mass, gross area and volume of placenta (Allen et al., 2002).

2.3.2. Growth and development from birth to adulthood

Considering BW and body size at birth, the foal seems to be more advanced in terms of growth when compared to newborns from other livestock species, namely bovine (Martin-Rosset, 1983). According to the breed, BW at birth ranges from 8 to 12% of adult BW, representing average values of 15 to 35 kg for ponies, 45 to 55 kg for light breeds and 65 to 80 kg for draft horse breeds (Martin-Rosset et al., 2015b). In the light breeds, which includes most of the saddle horses, some birth weights were reported for Thoroughbred: 54 to 58 kg (Kavasis & Ott, 2003; Brown-Douglas et al., 2005; Pagan et al., 2006; Elliot et al., 2009), Anglo-Arab and "Selle Français": 53 kg (Bigot et al., 1988) and Purebred Arabian: 46 kg (Çilek, 2009). At birth, the skeletal of the foal is more developed than muscle or fat tissue (Martin-Rosset et al., 2015b). Therefore, for most breeds, height at withers (WH) measured at birth represents already approximately 60% of mature height (e.g. 63% for Anglo-Arab and "Selle Français", Bigot et al., 1988; 62% for Mangalarga Marchador, Cabral et al., 2004a). Average WH values observed for Thoroughbred foals at birth were 102 to 103 cm (Kavasis & Ott, 2003; Pagan et al., 2006) and 96.7 cm for Arabian foals (Çilek, 2009).

During the first months after birth growth rates are very high. Average daily gains of 1.443 kg/day and 1.233 kg/day were respectively observed in Thoroughbred and in a group of Anglo-Arab and "Selle Français" foals during the first month of life (Bigot et al., 1988; Pagan et al., 2006). From birth to six months of age ADG values of 0.900 to 1.000 kg/day were observed in the same breeds (Bigot et al., 1988; Jelan et al., 1996). At weaning (six to seven months of age), BW of foals can be almost five times its birth weight, representing about 45% of its adult BW (Martin-Rosset, 2005). After this age and depending on the breed and management conditions, ADG progressively decline until maturity. The increase in WH is also very high during the first months, declining gradually after the six months (Jelan et al., 1996; Martin-Rosset et al., 2015b). For most of the light breeds, at one year of age WH represents already 88 to 90% of mature height, reflecting the earlier development of the skeletal tissue (Cabral et al., 2004a; Martin-Rosset, 2005).

In fact, allometric coefficients obtained from the comparative slaughter method showed that bone, muscle and adipose tissue develop chronologically in this order, being respectively 0.74, 1.13 and 1.41 (Martin-Rosset et al., 2015b). Moreover, considering the different skeletal segments, it was observed that bones in the extremities (e.g. metacarpus) develop earlier in relation to the whole skeleton (Martin-Rosset et al., 1983).

The potential for growth and production performance of the horse is determined by its genetic background, but the expression of growth related traits is highly influenced by environmental factors. The individual genetic effect on body size and live weight is high, and huge differences can be found from small ponies to draft horse breeds (e.g. less than 150 kg in the Miniature horse or Shetland Ponies to 1100 kg in the Shire breed). Estimated heritability coefficients (h^2) for morphologic traits vary according to the breed and to body size parameter. In the Murgese, Noriker draught, Lipizzaner and Pura Raza Española breeds observed h^2 for WH and girth perimeter range, respectively, between 0.24 and 0.67, and 0.26 and 0.48 (Molina et al., 1999; Zechner et al., 2001; Dario et al., 2006; Druml et al., 2008). Sexual dimorphism is also reported both for growing and adult horses. At the adult age, stallions are on average 10% heavier than mares (Martin-Rosset, 2005). A study with Thoroughbred foals showed that colts were heavier than fillies at birth and the differences increased with age (Hintz et al., 1979). The same gender effect on BW and WH was found in a large study with Thoroughbred foals until weaning (Pagan et al., 2006). In the Finnhorse, gender of foal primarily influenced cannon bone circumference, and males tended to be taller, longer and wider than females (Saastamoinen, 1990).

Nutrition plays a major role among the environmental factors that influence growth and development. Body weight and body size increases with feed intake level (Cymbaluk et al., 1990; Donabédian et al., 2006) and is influenced by the nature of diet, namely by the type of forage or concentrate composition (Bigot et al., 1987; Ott & Kivipelto, 2002; Ott et al., 2005). The introduction of creep-feeding during mid-lactation can improve growth performances of the nursing foal and improve general condition after weaning (Coleman et al., 1999; Rezende et al., 2000). Growth of the nursing foal is also influenced by the month of foaling or season of the year, which is closely linked with pasture availability (Hintz et al., 1979; Pagan et al., 2006; Kocher and Staniar, 2013). In the study of Pagan et al. (2006), foals born in January and February had lower BW than foals born in March, April and May during the first three months of age, but these differences disappeared at five months of age. This observation was explained by an increase in the ADG of the lighter foals coinciding with spring pasture growth in April. In fact, like other livestock species, growing horses are able to undergo compensatory growth periods when the quantity and quality of feeds, namely pasture, becomes available after a period of energy deprivation. However, this ability to undergo compensatory growth will decrease with age (Bigot et al., 1987; Staniar, 2013; Martin-Rosset et al., 2015b).

Another environmental factor that had been reported as having an effect on growth and development of the foal is the exercise conditions (Martin-Rosset, 2005; Martin-Rosset et al., 2015b). However, as this effect is mainly observed at bone tissue level, it will be discussed in 2.4 and 2.5. Sub-chapters.

2.4. Bone tissue: general characteristics

Development of a sound and adequate skeletal support system is one of the main requirements for the locomotion and performance of any leisure or sport horse category.

The number of bones in the horse skeleton varies with age due to the fusion of some elements during growth which are separate in the fetus or foal. In the adult, a normal skeleton has on average 205 bones, although the number of coccygeal vertebrae can also vary (Goody, 1983).

The skeleton has several functions. Bones provide structural support for the body, allow movement by providing levers for the muscles and protect vital internal organs and structures. In addition, they play an important role in the maintenance of mineral homeostasis and acid-base balance, serves as a reservoir of growth factors and cytokines, and provides the suitable environment for hematopoiesis within the marrow spaces (Taichman, 2005; Clarke, 2008).

Bone is a heterogeneous composite material consisting, in decreasing order, of a mineral phase, hydroxyapatite ($Ca_{10} (PO_4)_6 (OH)_2$), an extracellular organic matrix (≈ 90% type I collagen and ≈ 5% noncollagenous proteins) cells, lipids and water (Boskey & Coleman, 2010; Boskey, 2013). The mineral content of bone, observed in different species (also referred to as "ash content"), increases during growth and development and declines with the oldest ages (Boskey & Coleman, 2010). In the horse, ash content of the third metacarpal bone reaches a maximum at 4 years of age, maintains until 7 years of age and then declines (El Shorafa et al., 1979).

Bone tissue is organized, independently of age, in a complex and hierarchical structure at many length scales. At the macroscopic level, bones can be long or short, flat or tubular, where bone tissue is arranged in two forms depending on location: cortical (compact) and cancellous or trabecular (woven and lamellar) structure. The shaft or diaphysis of long bones are primarily composed of cortical bone, whereas the metaphysis and epiphysis are composed of a honeycomb like network of trabecular plates surrounded by a relatively thin shell of dense cortical bone (Clarke, 2008).

In both bone types, the bone tissue is laid down in layers (lamellae) (Boskey & Coleman, 2010). The microstructure of cortical bone is composed of regular, cylindrically shaped lamellae, disposed in concentric layers around a canal, which contains blood vessels or nerves, giving rise to an osteon (Rho et al., 1998; Sharir et al., 2008). Primary osteons contain a less amount of lamellae and smaller vascular channels in comparison with secondary osteons, which appear due to replacement of bone by remodeling. Secondary osteons also called Haversian systems have a cylindrical shape and run parallel to the long axis of the bone

(Rho et al., 1998; Weiner & Wagner, 1998). In contrast, the microstructure produced by the compaction of cancellous bone is composed of irregular, sinuous convolutions of lamellae, giving it its characteristic spongy appearance (Rho et al., 1998). Lamellae are planar arrangements formed by parallel layers of mineralized collagen fibrils, each layer having a different orientation of fibrils (Sharir et al., 2008). The mineralized collagen fibrils are the basic building units of bone tissue at a sub-nanoscale (Weiner &Wagner, 1998). Each collagen fibril is made up of three polypeptide chains about 1,000 amino acids long. These are wound together in a triple helix. A triple-helical molecule is cylindrically shaped, with an average diameter of about 1.5 nm, and lengths of 300 nm. Collagen molecules are staggered leaving spaces between them. The calcium crystals will deposit within these gaps and around the collagen fibrils and will grow parallel to its long axes. These organic and inorganic constituents act together to give bone its unique properties (Rho et al., 1998).

2.4.1. Bone physiology and metabolism

Several types of cells can be found in bone tissue. Chondrocytes, osteoblasts and osteocytes are of mesenchymal cell origin, whereas osteoclasts are derived from precursors in the myeloid/monocyte lineage that circulate in the blood after their formation in the bone marrow (Boskey & Coleman, 2010; Boyce et al., 2009).

Chondrocytes are specialized cells which produce and maintain the extracellular matrix (ECM) of cartilage (Muir, 1995). They play a central role in the endochondral ossification process, contributing to longitudinal growth of long bones through a combination of proliferation, ECM secretion and hypertrophy (Mackie et al., 2011).

Osteoblasts are located on bone surfaces and are the cells responsible for bone formation through secretion of the organic components of the extracellular bone matrix, which consist mainly of type I collagen, but also proteoglycans, glycoproteins and γ-carboxylated proteins. In addition to bone formation, osteoblasts participate in the mineralization process, provide essential factors for differentiation of osteoclasts (bone-resorbing cells) and have also a role in the endocrine activities of the skeleton (Mackie, 2003; Lee et al., 2007; Boskey & Coleman, 2010; Lerner, 2012).

During bone formation some osteoblasts become trapped in their own bone matrix, giving rise to osteocytes. Osteocytes are the most abundant cells in mature bone (approximately 90% of total cells). These cells reside in lacunae and form a canaliculi network throughout mineralized bone tissue, communicate with each other and with the surrounding medium through dendritic processes of their plasma membrane. The lacunocanalicular system provides an ideal milieu for transfer of exogenous and endogenous signals via mechanical, electrical and chemical mechanisms. In fact, osteocytes are considered as being the primary sensors of mechanical stimuli applied to the skeleton that is assumed to modulate activity associated with remodeling and bone turnover (Mackie, 2003; Knothe Tate et al., 2004; Chen et al., 2010; Bonewald, 2011).

Osteoclasts are multinucleated giant cells that are responsible for bone resorption. Mature osteoclasts are only present on mineralized bone surfaces to which they attach by a sealing zone. A characteristic feature of osteoclasts is the development of an extensively folded cell membrane, ruffled border, in the part of the osteoclasts facing bone. In this area, proton pumps and chloride channels are expressed, which are important for the extracellular acidification and demineralization of bone. Then, proteolytic enzymes are released, which degrade the extracellular matrix proteins (Clarke, 2008; Lerner, 2012). Recently, osteoclasts precursors and osteoclasts were also implicated in the regulation of the differentiation of osteoblast precursors and the movement of hematopoietic stem cells from the bone marrow to the bloodstream, and in the immune responses by the secretion of cytokines that can affect their own functions and those of other cells in inflammatory and neoplastic processes affecting bone (Boyce et al., 2009).

Together, the integrated action of osteoblasts, osteocytes, and osteoclasts and their endo- and paracrine regulation are pivotal for the maintaining of bone mass and for the control of remodeling and modeling processes in bone (Lerner, 2012).

2.4.1.1. Bone growth and development, modeling and remodeling

Bones develop through two distinct processes, intramembranous ossification and endochondral ossification. Intramembranous ossification, which occurs in the flat bones of the skull, involves direct differentiation of embryonic mesenchymal cells into the bone-forming osteoblasts. In contrast, endochondral ossification, which occurs in the remainder of the skeleton, involves the replacement of a cartilage model by bone tissue (Mackie et al., 2011).

In this model, embryonic mesenchymal cells condense and differentiate into chondrocytes, which secrete the various components of cartilage ECM, including collagen type II and the proteoglycan aggrecan. Ossification of the cartilage model is preceded by hypertrophy of the chondrocytes in the prospective mid-shaft of the bone, and deposition of a periosteal bone collar by recently differentiated osteoblasts surrounding the mid-shaft. Blood vessels, osteoclasts, as well as bone marrow and osteoblast precursors then invade the model from the bone collar and proceed to form the primary center of ossification. The primary center, which mostly appears during fetal development, expands towards the ends of the cartilage model, as the osteoclasts remove cartilage ECM and osteoblasts deposit bone on cartilage remnants. In long bones, secondary ossification centers subsequently forms after birth at each end of the cartilage model, leaving a cartilaginous growth plate between the primary and secondary ossification centers, as well as the prospective permanent articular cartilages at each end of the bone (Figure 2.4). The growth plate chondrocyte contributes to bone elongation through a combination of proliferation, ECM secretion and hypertrophy. In most species, skeletal maturity occurs at puberty when the expanding primary center of ossification meets the secondary center of ossification, thus obliterating the growth plate. Bone grows in width (appositional bone growth) by periosteal expansion in the outer surface of cortical bone (Boskey & Coleman, 2010; Mackie et al., 2011; Kini & Nandeesh, 2012).

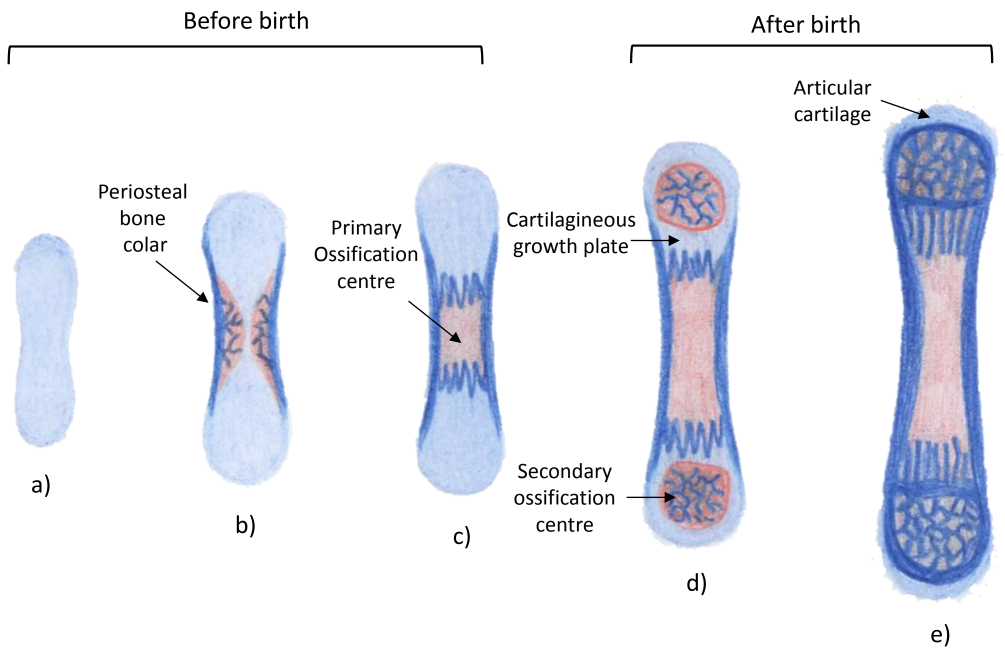

Figure 2.4.1 – Schematic development of a long bone (adapted from Mackie, 2011)

a) Cartilage model; b) Growth of cartilage model; formation of periosteal bone collar; c) Development of the primary ossification centre; it starts to expand towards the extremities of the cartilage model; d) Development of secondary ossification centres, leaving a cartilaginous growth plate between primary and secondary ossification centres; e) Skeletal maturity; complete replacement of the growth plate cartilage by bone; a layer of articular cartilage remains surrounding the epiphysis of the bone.

At birth, the development of long bones in the horse is very high. The length and density of the third metacarpal bone (McIII) observed in French saddle breed foals represents already 80% of its adult value (Bigot et al., 1990).

The radiographic closure time of the appendicular growth plates was studied in the Icelandic horse and the results were compared with previously published closure times reported for other horse breeds. According to this study, most of the examined growth plates were fully closed at the age of approximately three years. The growth plates in the digits were the first to close at 8.1 to 8.5 months of age, and those in the regions of the distal radius (27.4 to 32.0 months), tuber olecrani (31.5 to 32.2 months), and the stifle (27.0 to 40.1 months) were the last ones to close. These results were similar to the reported for other breeds (Strand et al., 2007). The observed values for the closure time of the distal radial metaphyseal growth plate in Arabian, Hucul, Thoroughbred and Anglo-Arabian horse breed ranged from 23.9 to 27 months of age (Łuszczyński et al., 2011).

After the closure of growth place, appositional bone growth continues, and this accounts for the changes in bone shape and diameter around the diaphysis, and consequent mechanical behavior (Bigot et al., 1990; Rauch, 2005). In light horse breeds the cortical area of the metacarpal bone increases sharply from birth to four years of age (4.6 cm^2 to 8.8 cm^2), remains stable until 17 years, and declines thereafter to 7.7 cm^2 (El Shorafa et al., 1979). Also a twofold increase of the cortical width was observed in the McIII of saddle French breeds between birth and 42 months of age, explaining the doubling of bone weight and volume during this period (Bigot et al., 1990).

The major systemic hormones that regulate longitudinal bone growth and development are growth hormone (GH), insulin-like factor I (IGF-I), thyroid hormones (T3 and T4), and glucocorticoids, whereas during puberty, sex steroids (androgens and estrogens) also contribute to this process (van der Eerden et al., 2003; Ohlsson et al., 2008). The behavior of growth plate chondrocytes is tightly regulated at all stages of endochondral ossification (namely proliferation, ECM secretion and hypertrophy) by a complex network of interactions between those circulating hormones, locally produced growth factors (including also IGF-I, parathyroid hormone-related protein, Indian hedgehog, WNTs, bone morphogenetic proteins and fibroblast growth factors) and the components of the ECM secreted by the chondrocytes (including collagens, proteoglycans, thrombospondins and matrilins). The death of the differentiated hypertrophic chondrocytes allows the invasion of a mixture of cells (osteoclasts, septoclasts, osteoblasts and bone marrow cells) that collectively replace the cartilage tissue with bone tissue. In this last stage, chondrocytes also secrete factors that regulate the behavior of the invading bone cells, including vascular endothelial growth factor and receptor activator of nuclear factor kB ligand (RANKL) (van der Eerden et al., 2003; Mackie et al. 2011).

In addition to longitudinal and radial growth, bone is constantly formed and resorbed during life in response to changes in mechanical loading, altered blood calcium levels and a wide range of paracrine and endocrine factors. This dynamic nature of the skeleton is achieved by a process of remodeling that occurs in both cortical and trabecular bone, although cortical bone is typically less metabolically active (Sims & Gooi, 2008; Clarke, 2008; Lerner, 2012). Besides being an integral part of calcium homeostatic system, remodeling is central to the maintenance of the mechanical integrity of the skeleton and the repair of damaged bone, without changing the size or shape of the bones (Lerner, 2012; Sims & Martin, 2014).

The remodeling process occurs always in the same sequence: bone resorption by osteoclasts, followed by bone matrix production by osteoblasts. The coordinated action of these cells is described as the "Basic Multicellular Unit" (BMU). Within the BMU, cellular activity is matched (or "coupled"), a principle that the amount of bone destroyed by osteoclasts is equal to the amount of bone formed by osteoblasts. At remodeling sites, coupling between bone formation and bone resorption is a process that involves the interaction of a wide range of cell types and control mechanisms. Osteoblast-lineage cells, including lining cells, preosteoblasts and osteocytes control their own activity as well as the initiation of osteoclast fusion, attachment, activity and apoptosis. In return, the osteoclast controls the level of osteoblast activity through release of factors within the matrix and both membrane-bound and secreted factors (Robling et al., 2006; Sims & Martin, 2014). Also signals from osteocytes and from immune cells seem to have a role in the remodeling process (Sims & Gooi, 2008).

In addition to remodeling, bone also adapts by the modeling process. Modeling changes the size and shape of bone either by bone resorption without subsequent bone formation or bone formation without previous bone resorption (e.g. development of the medullary cavity by resorption at the endosteal surface, or cortical expansion by formation at the periosteal surface). Bone modeling occurs during growth and also in response to mechanical loading (Seeman & Delmas, 2006; Clarke, 2008; Lerner, 2012; Sims & Martin, 2014; Sims & Vrahnas, 2014).

After bone matrix production, mineralization of bone occurs by a series of physicochemical and biochemical processes that together facilitate the deposition of hydroxyapatite in specific areas of the ECM (Millán, 2013).

As stated before, the mineral content of bone is mostly hydroxyapatite [Ca_{10} $(PO_4)_6$ $(OH)_2$], with small amounts of carbonate, magnesium, and acid phosphate. Bone mineral provides mechanical rigidity and load-bearing strength to bone, whereas the organic matrix provides elasticity and flexibility. Apatite crystals deposited initially in “hole” zones between the ends of collagen fibrils and develop with their long *c*-axes parallel to the fibril axis (Boskey, 2007; Bonucci, 2012).

Matrix maturation is associated with expression of alkaline phosphatase and several noncollagenous proteins, including osteocalcin, osteopontin, and bone sialoprotein. It is thought that these calcium- and phosphate-binding proteins help regulate the ordered deposition of mineral by regulating the amount and size of hydroxyapatite crystals formed (Clarke, 2008; Bonucci, 2012). As bone matures, hydroxyapatite crystals enlarge and reduce their level of impurities. Crystal enlargement occurs both by crystal growth and by aggregation. Bone matrix macromolecules, by its acidic nature, may facilitate initial crystal nucleation, sequester mineral ions to increase local concentrations of calcium and/or phosphorus, or facilitate heterogeneous nucleation. Phosphatases are implicated in the mineralization process but their functions are not completely clarified (Millán 2013). Bone alkaline phosphatase may increase local phosphorus concentrations, remove phosphate-containing inhibitors of hydroxyapatite crystal growth, or modify phosphoproteins to control their ability to act as nucleators. Vitamin D also plays an indirect role in stimulating mineralization of the newly bone matrix. The active metabolite of vitamin D (1,25-dihydroxycholecalciferol) is responsible for maintaining serum calcium and phosphorus in adequate concentrations to allow passive mineralization of unmineralized bone matrix and stimulates osteoblast expression of bone-specific alkaline phosphatase, osteocalcin, osteonectin, osteoprotegerin, and a variety of other cytokines (Clarke , 2008).

One of the main purposes of modeling and remodeling during growth is to establish the skeleton’s peak bone strength (Seeman & Delmas, 2006). In fact, bone tissue forms and is remodeled in response to the mechanical forces that it experiences, as described by Wolff’s Law. These adaptations are mediated by bone cells that sense and respond to local mechanical cues: the osteocytes and the osteoprogenitor cells (Chen et al., 2010).

Bone mineral content, cortical width and metacarpal circumference of weanling foals is positively influenced by exercise (Raub et al., 1989; Bell et al., 2001; Hiney et al., 2004). On the opposite, stall confinement and the absence of exercise in adult conditioned horses or growing yearlings result in a significant decrease of bone mineral content in the McIII (Porr et al., 1998; Hoekstra et al., 1999).

2.4.1.2. Bone metabolism and mineral homeostasis

Bones acts as an important reservoir of minerals. Ninety-nine percent of total body calcium, 85 % of phosphorus, and 60 % percent of magnesium are contained within the bones (Stewart, 2011; Toribio, 2011; Kini & Nandeesh, 2012). One of the most important metabolic functions of the skeleton is related with mineral homeostasis, namely calcium and phosphate. The regulation of calcium and phosphate metabolism results from the interactions among three important hormones – parathyroid hormone (PTH), calcitonin, and the active metabolite of vitamin D (1,25-dihydroxycholecalciferol) – at three target organs, bone, kidney, and gastrointestinal tract (Toribio, 2011).

Parathyroid hormone, a single-chain polypeptide of 84 amino acids is secreted by the chief cells of parathyroid glands. In the horse, these glands (two pairs) are in a position prior to thyroid near the carotid bifurcation trunk (Greco & Stabenfeldt, 1997). The slightest decrease in Ca^{2+} concentration in extracellular fluids cause parathyroid glands hypertrophy and stimulate PTH secretion. This effect is mediated through the parathyroid cell-plasma membrane calcium-sensing receptor for which calcium is an agonist (Favus et al., 2006; Peacock, 2010). The intact PTH molecule is rapidly metabolized by the liver and kidneys into two fragments (the N-terminal and C-terminus) and has a relatively short half-life in the blood (about 10 minutes) (Breslau, 1996; Greco & Stabenfeldt, 1997). Parathyroid hormone regulates serum calcium and phosphate concentrations through its combined actions on kidney, bone and intestine. After a decrease in serum calcium concentrations, the secreted PTH acts firstly on the kidney, increasing the reabsorption of filtered calcium in the renal distal tubule and inhibiting the reabsorption of phosphate in the proximal tubule. On bone, PTH increases the osteoclastic-mediated bone resorption through the activation of PTH receptors on stromal cells/osteoblasts. This activation stimulates the expression of macrophage colony stimulating factor and RANKL, which enhances the formation of osteoclasts from precursors and the activity of already existing mature osteoclasts. Although with a temporal delay after its secretion, PTH also enhances the synthesis of 1,25-dihydroxycholecalciferol in the renal proximal tubule (Favus et al, 2006; Brown & Jüppner, 2006; Peacock, 2010; Kini & Nandeesh, 2012). In the healthy horse, serum PTH concentrations change with age, exercise and mare physiological condition (Martin et al., 1996; Sloet van Oldruitenborgh-Ooste et al., 1999; Vervuert et al., 2005).

Vitamin D is a secosteroid that is produced in the skin through ultraviolet irradiation of 7-dehydrocholesterol. Vitamin D is biologically inert and must undergo two successive hydroxylations in the liver and kidney to become the biologically active 1,25-

dihydroxycholecalciferol. The active form of vitamin D increases the efficiency of intestinal absorption of dietary calcium, stimulates calcium reabsorption in the kidney and promotes calcium mobilization from bone. Osteoblasts have vitamin D receptors, and similarly to the PTH action, the interaction of 1,25- dihydroxycholecalciferol with the VDR receptors results in a signal transduction to induce RANKL expression. As pre-osteoclasts have the receptor (RANK) for RANKL, the direct contact of the preosteoclast's RANK with the osteoblast's RANKL results in a signal transduction to induce pre-osteoclasts to become mature osteoclasts. The mature osteoclasts release hydrochloric acid and proteolytic enzymes to dissolve bone mineral and matrix releasing calcium into the extracellular space (DeLuca, 2004; Holik & Garabedian, 2006).

Calcitonin (CT) is a 32 amino acid protein secreted by the C-cells of the thyroid gland in response to acute hypercalcemia. Calcitonin secretion leads to an inhibition of osteoclast resorption activity and to a stimulation of renal calcium excretion by the inhibition of its resorption (Deftos, 2006; Toribio, 2011). Although the principles of calcium regulation are similar for all mammals, the study of Rourke et al. (2009) showed that equine thyroid gland C-cells respond quickly (4-fold increase from baseline at 2 min) to changes in extracellular Ca^{2+} concentrations by secreting large quantities of CT into the systemic circulation.

The regulation of serum phosphate is not as precise as that of calcium. In a short term, phosphate concentration is largely determined by intestinal absorption, and therefore is more influenced by diet (Berndt & Kumar, 2009; van Doorn et al., 2011). But the main regulatory pathway is related with the efficiency of reabsorption of filtered PO4 both in the proximal and distal renal tubules, which is mediated by vitamin D and PTH (Favus et al., 2006; Berndt & Kumar, 2009; Toribio, 2011).

2.4.1.3. Bone and energy metabolism

In the last years, thanks to an integrative perspective of physiology, new relationships between organs and homeostatic functions have emerged. Beside its classical functions, bone can now be considered as a true endocrine organ implicated in the regulation of glucose and energy metabolism (Confraveux et al., 2009; Ferron & Lacombe, 2014). In fact, the finding that bone remodeling represents a high energetic cost for the body, led to the hypothesis of a coordinated relationship between bone remodeling and energy metabolism (Karsenty & Oury, 2010; Wei & Ducy, 2010; Ducy, 2011). The proposed integrative model to explain this complex interaction which, until now, was mostly demonstrated in rodents, involves the adipose tissue, the central nervous system, the bone tissue and the pancreas, and the participation of three key hormones: leptin, osteocalcin and insulin (Karsenty & Oury, 2010; Ng, 2011). However, the complete genetic and molecular mechanisms that integrate all the interactions are still under investigation.

2.4.1.3.1. Leptin, a hormone with pleiotropic characteristics

The hypothesis for the existence of a circulating, lipostatic, negative feedback signal acting centrally to alter energy expenditure and food intake was first proposed by Kennedy, in 1953. This hypothesis led to several studies with mice models where the genetically obese (*ob/ob*) mouse, a homozygous mutant that lacks a factor for regulation of body weight, was characterized (Coleman, 1973). In 1994, using positional cloning in the *ob/ob* mouse, Zhang et al. (1994) identified and sequenced the *ob* gene and its protein product, leptin (from the greek “leptos”, meaning “thin”). Leptin is secreted by adipose tissue and circulating leptin levels are correlated with the amount of body fat, reflecting energy status (Houseknecth & Portocarrero, 1998). The most significant roles of leptin include regulation of energy homeostasis, neuroendocrine, reproductive and immune functions, glucose, lipid and bone metabolism (Kelesidis et al., 2010; Dalamaga et al., 2013; Park & Ahima, 2015). Leptin mediates its effects by binding to specific leptin receptors expressed in the brain as well as in peripheral tissues (Bjorbaek & Kahn, 2004; Bjorbaek, 2009; Kalesidis et al., 2010).

In the horse, leptin receptor-long form was detected in the liver, lung, muscle, testis, ovary, choroid plexus, hypothalamus and subcutaneous adipose tissues (Buff et al., 2002). Positive correlations were found between circulating leptin concentrations and body fat mass, either by ultrasound estimation of body fat (Fitzgerald & McManus, 2000; Kearns et al., 2006) or by BCS appraisal in horses and ponies (Buff et al., 2002; Gentry et al. 2002; Gordon et al., 2007; Carter et al., 2009; Huff et al., 2009), with higher values observed in ponies (Pratt-Phillips et al., 2010).

As suggested for other species, leptin is an important link between nutrition and reproductive status, being involved in the alteration of ovarian activity (Fitzgerald & Macmanus, 2000). These authors proposed that the reproductive response to a decrease in photoperiod or a presumptive inhibitory melantonin signal is modified by energy availability which may be signalled to the hypothalamus-pituitary axis through a change in the circulating concentration of leptin. Gentry et al. (2002) demonstrated that a strong feed restriction resulting in a low body condition score (3-3.5, on a scale of 1-9) and low leptin concentrations, led to cessation of reproductive activity during the winter months. On the opposite, increased concentrations of circulating leptin in the Lusitano mare appear to be associated with the maintenance of ovarian cyclicity throughout the winter months (Ferreira-Dias et al., 2005). Leptin concentrations are influenced by the gestation and post-partum periods in the mare, by gender and season of the year (Fitzgerald & McManus, 2000; Cartmill et al., 2003, 2006; Heidler et al., 2003; Berg et al., 2007; Buff et al., 2007; Romagnoli et al., 2007).

Leptin secretion is also responsive to fasting. Short term (Piccione et al., 2004; Buff et al., 2005, 2006) and long term feed restriction (Van Weinberg et al., 2008) induce a decline in leptin concentrations, suggesting that circulating levels of this hormone are sensitive to acute disturbances in energy balance. Considering circadian patterns, leptin secretion shows clear diurnal rhythms with low levels during the light phase and high levels during the evening and the dark phase of the natural light–dark cycle (Cartmill, 2004; Piccione et al., 2004; Buff et al., 2005).

There is accumulating evidence that leptin produced by placental or fetal tissues acts through specific leptin receptors to regulate fetal growth and development. Although leptin levels are correlated with insulin and IGF-I levels, observational studies in humans indicate that its effects on fetal growth are independent of these axes and of adiposity (Christou et al., 2002). Young foals present lower leptin levels than mature animals and leptin concentrations seem to increase with age (Fitzgerald & McManus, 2000; Cebulj-Kadunc & Chestnik, 2005; 2008 Kearns et al., 2006; Berg et al., 2007). Leptin appear to have also a permissive role on the onset of puberty. Circulating leptin increases during pubertal development in rodents, human females and heifers (Hall, 2003; Zieba et al., 2005).

Leptin regulates bone mass through a central pathway comprising the hypothalamus and the central nervous system, using two neural mediators, the sympathetic tone and the neuropeptide CART (cocaine- and amphetamine-regulated transcript), both acting on the proliferation and function of one cell type, the osteoblast (Karsenty, 2006). Leptin signals to its receptors expressed on the serotonin neurons of the brainstem to inhibit synthesis and secretion of serotonin. In turn, serotonin signals in the ventromedial hypothalamus to downregulate sympathetic nervous system (Wei & Ducy, 2010; Quiros-Gonzalez & Yadav, 2014). Sympathetic output acts as a major regulator of both bone formation and bone resorption through signaling of the β2 adrenergic receptor (Adrβ2) located on osteoblast. The Adrβ2 signaling promotes two parallel regulations, osteoblast proliferation (and therefore bone formation) and the inhibition of osteoblast proliferation. The differentiation and function of osteoblast induce the expression of RANKL, which will increase osteoclast differentiation and bone resorption (Wey & Ducy, 2010).

The second neuromediator acting directly on osteoblast is CART. The expression of CART in the neurons of the arcuate hypothalamic nuclei decreases RANKL expression by osteoblasts, and therefore osteoclast differentiation and bone resorption (Karsenty, 2006; Wey & Ducy, 2010; Ng, 2011).

2.4.1.3.2. Osteocalcin, a key signal for glucose homeostasis

Osteocalcin is a small peptide exclusively secreted by osteoblasts and is one of the most abundant proteins in bone matrix (Ducy, 2011). Osteocalcin is secreted after multiple post-translational modifications which include excision of a pre-pro peptide and the vitamin K-dependent γ-carboxilation of glutamic residues into gamma-carboxy glutamate (Gla) residues. These Gla residues confer high affinity binding to hydroxyapatite, which explains the high concentration of carboxylated osteocalcin found in the bone matrix (Hauschka et al., 1989). Although both forms of osteocalcin (fully carboxylated and under-carboxylated) are present in the blood, it seems that only the uncarboxylated is the active circulating hormonal form. In the osteoblast, the osteocalcin decarboxylation is negatively regulated by the Esp gene (Lee et al., 2007). Osteocalcin is also activated by the resorption activity of osteoclasts, a process inhibited by the osteoprotegerin (OPG) produced by osteoblasts (Ducy, 2011).
Osteocalcin acts as an insulin secretagogue having a positive effect on both beta cell proliferation and insulin production. Osteocalcin can also favor adiponectin expression in adipocytes (Lee et al., 2007; Confavreux et al., 2009; Ducy, 2011). As a result, insulin sensitivity increases, lipolysis decreases and energy expenditure increases (Ng, 2011).

2.4.1.3.3. The role of insulin in osteoblast function

Insulin receptors (InsR) are abundant in osteoblasts. Insulin receptors signaling in osteoblast has a dual and positive action on osteocalcin biology. On one hand, InsR induces osteocalcin expression in osteoblasts. On other hand, it decreases OPG expression, which results in an increase of osteoclast formation and function. In order to resorb bone, proton pumps and chloride channels are expressed in the ruffled border of the osteoclast, creating an acidic extracellular milieu which is important for demineralization of bone (Lerner, 2012). This acid pH allows the decarboxylation of the osteocalcin bone matrix and promotes its activation. Undercarboxylated osteocalcin is then released into the systemic circulation acting as a circulating hormone to stimulate insulin production and secretion by pancreatic β-cells and adiponectin by adipocytes (Ducy, 2011; Ng, 2011).

The actions of insulin on osteoblasts are antagonized by several factors on which insulin resistance, the activation of InsR, leptin and some transcriptional factors within osteoblast are included (Ng, 2011).

2.4.2. Bone quality assessment

Bone quality is a broad concept that encompasses a set of characteristics that influences bone strength (Sherman & Hadley, 1993; Felsenberg & Boonen, 2005). Bone strength is mainly determined by its material composition (mainly mineral and collagen) and structural design (Seeman, 2008). The structural properties of bone include its macroscopic geometry (size and shape), as well as its microarchitecture (trabecular architecture and cortical thickness/porosity) (Felsenberg & Boonen, 2005; Donnely, 2011).

In the horse, bone quality and overall bone strength should be maximized and are particularly important in the distal limbs, in order to withstand stresses and enhance locomotory ability. The assessment of bone quality may, therefore, involve different methods that allow the evaluation of its material properties and characterization, as well as the geometric structure (Jeffcott et al., 1988).

2.4.2.1. Biomechanical properties

Bone is a complex but ordered natural composite material. As for other composite materials, the mechanical properties of bone are dependent on its composition, geometry and structure (Sharir et al., 2008; Vaz et al., 2011). Bone mechanical testing allows the direct assessment of a range of mechanical properties across multiple length scales. At the macroscopic level, the structural behavior of bones can be assessed either by whole-bone mechanical testing, or by mechanical testing of small, regularly shaped specimens (typically cylinders or cubes) excised from cortical or cancellous tissue from the whole bone. In these tests, specimens are loaded to failure in compression, bending, tension or torsion (Donnely, 2011; Vaz et al., 2011). At a more microscopic level, atomic force microscopy may be used for nanoindentation, which measures the mechanical properties of small bone units like trabeculae (in cancellous bone) or osteons (in cortical bone) (Currey, 2009; Vaz et al., 2011).

Two basic concepts are needed in order to understand the mechanical behavior of a material: the stress and the strain. The intuitive and most simple explanation of the concept of stress is load per unit area ($\sigma = F/A$). The units of stress are commonly expressed in pascal (Pa), which is equal to one newton over one square meter (N/m^2). However, since one pascal represents a very small amount of stress and physiological stresses are normally in the range of thousands of Pa, the most used unit is the megapascal (Mpa). The concept of strain is also intuitive and signifies the fractional change in length of a loaded body. Although strain is a unitless ratio (length over length), is commonly measured in units of microstrain (e.g. a strain of 0.01 (1%) would be 10.000 microstrain) (Sharir et al., 2008). During extreme activity, bones withstand high compressive strains. For example, at a speed of 16.5m/s, a mean compressive strain of 6296 µstrain was recorded in the mid-shaft of the McIII in young Thoroughbreds exercised on a treadmill (Davies & McCarthy, 1994).

When a bone sample is incrementally loaded a stress-strain curve is obtained (Figure 2.4.2).

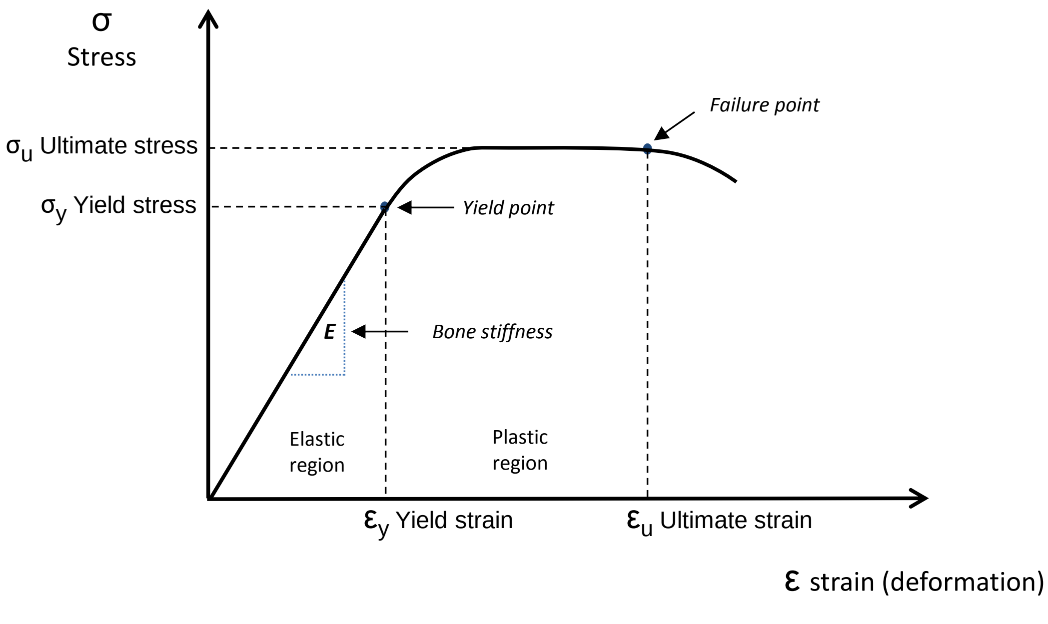

Figure 2.4.2 – Schematic stress-strain curve

(adapted from Sharir et al., 2008)

From this curve it is possible to estimate some mechanical parameters. The linear portion of the stress-strain curve is known as the elastic region. The Young´s modulus (E) is the slope of the linear portion of the curve and represents the stiffness of the bone. The yield stress is the stress attained at the end of the elastic deformation. After the yield point, the curve becomes nonlinear and corresponds to the plastic region, where the stress causes permanent damage to bone structure. Further increase in load will eventually result in breakage of the sample (failure point) and the stress at which this happens (ultimate stress) represents the strength of the bone (Viguet-Carrin et al., 2006; Sharir et al., 2008; Vaz et al., 2011).

The mechanical properties of bone are influenced by several factors. In cortical bone the mechanical properties depend mainly on the porosity, mineralization level and organization of the matrix. In turn, mechanical properties of cancellous bone vary around the periphery and along the length (Rho et al., 1998).

Studies in the horse showed that mechanical properties change with age, anatomic location, geometry, collagen fiber orientation, osteonal structure, exercise and mineral content (Lawrence et al., 1994; Mason et al., 1995; Bigot et al., 1996; Martin et al., 1996a; 1996b; Les et al., 1997; Reilly et al., 1997; Skedros et al., 2006).

The recording of bone deformation under physiological conditions has become possible with the development of *in vivo* methodologies for the assessment of bone strain. However, this technique is invasive and technically difficult. The most common method has been the direct application to the bone surface of electric resistance rosette strain gauges (Yang et al., 2011). With this technique, is was possible to show that during locomotion the equine McIII is primarily loaded in axial compression (Davies et al., 1993; Merrit et al., 2006; Rubin et al., 2013).

2.4.2.2. Non-invasive methodologies of bone assessment

The characterization of bone macrostructure and geometry can be assessed by several imaging techniques including conventional radiography, single photon absorptiometry (SPA), dual photon absorptiometry (DPA), dual X-ray absorptiometry (DXA), quantitative computed tomography (QCT), peripheral QCT (pQCT) and magnetic resonance imaging (MRI). Bone micro-architecture can only be assessed with high-resolution techniques like micro-CT, high resolution pQCT and micro magnetic resonance (Griffith & Genant, 2008; Pedrotti et al., 2010; Donelly, 2011). Most of these techniques are also densitometric and are based on the different absorption of ionizing radiations by bone and soft tissues (e.g. DXA and QCT) (Pedrotti et al., 2010). Because DXA and QCT measure a combined value of bone and bone marrow, the value obtained corresponds to the apparent bone mineral density, although it became commonly designed as bone mineral density (BMD). Quantitative computed tomography is generally considered more sensitive to detect bone density differences or changes because QCT measurements are reported as true volumetric density (mg/cm^3) in opposition to DXA measurements, which are reported as areal density (g/cm^2). However, and despite not providing information about the structural and qualitative features of bone, DXA remains one of the most used methods in human medicine to assess bone density and also as predictor of osteoporotic fracture risk (Griffith & Genant, 2008).

In the horse, high resolution techniques are usually restricted to *ex vivo* studies (Furst et al., 2008; Olstad et al., 2008; Rubio-Martinez et al., 2008; Olive et al., 2010), although one study reported the *in vivo* use of pQCT under general anaesthesia (Grace et al, 2002) . Also, due to the horse size, QCT and MRI examinations are limited to peripheral sites and measurements are always made under general anaesthesia (Dyson et al., 2003; Buhler et al., 2014). Therefore, one of the most reported non-invasive techniques for the assessment of bone mineral content in the horse was the radiographic photodensitometry (Porr et al., 1998; Hoekstra et al., 1999; Bell et al., 2001; Hiney et al., 2004). This technique was originally described by Meakim et al. (1981) and compares the optical density from a radiograph with a standardized aluminium step wedge in the radiographic field. Radiographs are scanned and evaluated with automated image analysis systems, being density results expressed in radiographic bone aluminium equivalent. The use of other techniques like SPA or DPA was punctually reported (Buckingham et al., 1992; Reichmann et al., 2004).

The application of DXA methodology to assess bone density in horses was initially limited to *ex vivo* studies (Carstanjen et al., 2003a). But the advent of portable DXA devices allowed the *in vivo* measurement of BMD with this technique. However, in order to standardize measurements on the region of interest, horses still need to be under general anaesthesia (Donabédian et al., 2005; Weisrock et al., 2011).

An alternative to radiation-based techniques for *in vivo* non-invasive bone assessment is the quantitative ultrasonography (QUS). This technique is easy to use, relatively inexpensive and in addition to bone density, provides information on bone qualitative and structural characteristics (Lepage et al., 2001; Griffith & Genant, 2008; Pedrotti et al., 2010).

2.4.2.2.1. Quantitative ultrasonography

A growing interest in bone QUS has been developing over the past years either for clinical or for research purposes (Guglielmi et al., 2009; Pedrotti et al., 2010).

This technique is based on the principle that the velocity of an ultrasound wave, also named speed of sound (SOS), relates to the properties of the medium in which it travels by the equation: SOS (m/s) = $\sqrt{E/\rho}$, where ρ is the density of bone and E is the Young's modulus (Lepage et al., 2001).

The first studies measuring ultrasound velocities in the horse were performed with two transducers applied to opposite sides of the bone (one transmiting transducer and one receiving transducer), and were based on transversal ultrasound transmission across the bone (Jeffcott & McCartney, 1985; Glade et al., 1986). However, in these systems, the accuracy of the measurements was affected by the surrounding soft tissue, as well as cross-sectional area and bone shape and corrections need to be applied (Jeffcott et al., 1988).

More recently, other QUS devices were developed allowing multisite measurements in axial transmission mode. In the horse, these devices can be used to measure superficial cortical bone properties of McIII, radius and tibia (Carstanjen et al., 2002). These devices are equipped with a hand-held probe, that contains a set of transmitters and receivers, which is percutaneously applied to the site to be measure. The velocity of an ultrasound wave propagating through a fixed distance of bone (parallel to the long axis), is calculated with a specific software. The QUS device generates pulsed acoustic waves at a centre frequency of 1.25 MHz that travels through soft tissue and thereafter along and under the bone surface, at a bone layer depth of 3 to 5 mm. The way how the sound wave is refracted (through a critical angle when entering bone), how it travels through bone tissue, and how it is scattered out of the bone and received in the probe is based on the Snell's law. With this technique, measurements are performed with the horses in a standing position and they do not require any sedation (Carstanjen et al., 2002).

Results from several studies performed on the equine McIII showed that SOS values change with anatomic region of bone (lateral vs. dorsal or medial), age, gender and exercise (Jeffcott et al., 1987; Carstanjen et al., 2002, 2003b; Tabar-Rodriguez et al., 2009), which demonstrates its usefulness in the non-invasive assessment of horse bone status.

2.4.2.2.2. Biochemical markers

The words "biomarker", "biochemical marker" and "molecular marker" have been used to describe either direct or indirect indicators of skeletal tissue metabolism. These markers are generally molecules that are the normal products and bi-products of the metabolic processes occurring within the skeleton, and their concentrations can be measured in the serum, plasma or urine (Lepage et al., 2001; McIlwraith, 2003). Because markers of bone metabolism are based on the phases of bone cycle, they may be classified either as indicators of bone formation, bone resorption, or overall bone turnover (Christenson, 1997). Bone marker measurements are non-invasive, inexpensive, and can be repeated often on the same subject (Watts, 1999).

Most of the markers first used in human medicine were assessed in urine and are, thus, of limited interest in horses due to practical difficulties of getting samples. Therefore, markers that are mainly determined in serum or plasma samples in horse studies are: (i) bone formation markers – osteocalcin (Oc), bone alkaline phosphatase (BALP) and carboxy-terminal peptide of type I procollagen (PICP); (ii) bone resorption markers – total deoxypyridinoline (TD-Pyr), cross-linked C-telopeptide of type I collagen (ICTP) and telopeptide of type I collagen (CTx). Nevertheless, pyridinium cross links of collagen (pyridinoline "Pyr" and deoxy-pyridinoline "D-Pyr") can be determined in urine samples (Lepage et al., 2001).

Several cross-sectional and longitudinal studies showed an inverse relation between both, markers of bone formation (Oc; BALP; PICP) and bone resorption (ICTP) concentrations and the age of the animals, with the most significant changes observed over the first year after birth (Lepage et al., 1990; Price et al., 1995a; 2001; Reller et al., 2003; Vervuert et al., 2007a). Bone turnover markers (Oc; ICTP) are also influenced by horse type and breed (warmblood *vs.* draught, and Thoroughbred *vs.* Quarter horse) (Lepage et al., 1997; 1998a; Reller et al., 2003), gender (Oc; ICTP) (Jackson et al., 2003b), season (Oc; BALP; PICP; ICTP) (Price et al., 2001), circadian rhythms (Oc; Pyr; D-Pyr) (Lepage et al., 1991; Black et al., 1999; Gianetto et al., 2010) and stages of the estrous cycle in the mare (Oc; ICTP) (Jackson et al., 2006).

In addition, bone marker (Oc; BALP; PICP; ICTP) determinations proved to be a sensitive and non-invasive method of monitoring skeletal adaptations during athletic training or changing of physical activity (Mäenpää et al., 1988; Price et al., 1995b; Hiney et al., 2000; Jackson et al., 2003a). More recent studies provide evidence that biomarkers of skeletal metabolism (including cartilage turnover markers) can be also related with the detection of bone or osteoarticular disease conditions (Billinghurst et al., 2004; Jackson et al. 2005; Donabédian et al., 2008; Trumble et al., 2008).

Taken into consideration the individual variability and the complex relationships between markers concentrations and age, breed, gender, season, sample time of day, single samples can be of little value for monitoring marker activity. Therefore, a series of measurements over a period of time would be a better option in order to increase the potential use of bone makers in horse studies (Price, 1998; Price et al., 2001).

2.5. Bone development and growth disorders

The musculo-skeletal structure of the horse is adapted to maximize locomotor efficiency at an early age. Foals can gallop a few hours after birth and, in most breeds, training for athletic performance starts when full growth has not yet been achieved (Firth, 2006).

Bone tissue development and quality is highly influenced by feed intake level and diet composition (Thompson et al., 1988a; 1988b; Ott & Asquith, 1989, 1995; Hoffman et al., 1999; Trillaud-Geyl et al., 2004; Martin-Rosset, 2005), but bone characteristics like mineral content, mineral density and the morphology of the mineralized tissue are also highly influenced by exercise conditions (Firth & Rogers, 2005; Firth, 2006).

Several studies showed that free or controlled exercise increases bone mineral density and content, and increase cannon bone circumference either in the weanling foals or young horses (Raub et al., 1989; McCarthy & Jeffcott, 1992; Bell et al., 2001; Hiney et al., 2004). In addition, stall confinement of young foals or even conditioned adult horses favors a decrease in bone mineral content (Porr et al., 1998; Hoekstra et al., 1999).

One of the main goals of horse breeding industry is to raise animals with a locomotor system that withstands the rigors of training and competition. Nevertheless, growth-related disorders associated to bone tissue development remain one of the biggest problems that breeders face until today (McIlwraith, 2004; Jeffcott, 2005).

2.5.1. Developmental orthopaedic diseases

The widely accepted term "Developmental orthopaedic diseases" (DOD) appeared in 1986 in order to group into a single category all the orthopaedic problems observed in the growing foal (McIlwraith, 2004). It encompasses a spectrum of conditions that are mainly associated to disturbances in the development and maturation of the musculoskeletal system, in particular in the articular and metaphyseal cartilage, during skeletal growth (Jeffcott, 2005).

The DOD designation includes all the conditions grouped under the general agreed terms of: osteochondrosis (OC); physitis; angular limb deformities; flexural deformities; cuboidal bone deformities or tarsal bone collapse; cervical vertebral malformation (Wobbler syndrome); and acquired vertebral deformities (McIlwraith, 2004; Jeffcott, 2005).

The time of onset and the predisposed sites for each of the conditions varies, and in many instances multiple sites and multiple limbs of the foals can be affected. However, a number of factors are thought to be involved in their complex aetiopathogenesis. The main features

include rapid growth, nutritional unbalances, biomechanical stress on joint and growth cartilage, hormonal changes and a genetic predisposition (Jeffcott, 2005).
Recently, the new term "Juvenile osteochondral conditions" was proposed for the particular case of the different types of epiphyseal/metaphyseal developmental disorders that can be seen in the young, growing horse. This term encompasses specific disorders according to their location at the joint level and their biomechanical origin (Denoix et al., 2013).
Among the different conditions under the common DOD designation, OC has been the main research subject in the growing horse as it affects almost all breeds and because of the economic losses it generate to the worldwide horse industry (Jeffcott, 2005).

2.5.1.1. Osteochondrosis

Osteochondrosis (OC) is a common and important joint disorder that occurs in humans and in multiple animal species, including pigs, horses, and dogs. In the young athletic horse it is a frequent cause of lameness, producing long-term negative effects on performance and longevity (Ytrehus et al., 2007; Distl et al., 2013). This condition has been described as a focal disturbance of the endochondral ossification process with a multifactorial etiology, resulting in the development of a cartilage core that can progress to subchondral bone damage (Jeffcott, 2005; van Weeren, 2005). Recently, it was proposed that the primary lesion of articular osteochondrosis should be defined as a focal ischemic necrosis of growth cartilage initiated by necrosis of cartilage canal blood vessels. Because the necrotic cartilage does not undergo mineralization or vascular penetration, a focal failure of endochondral ossification occurs when the ossification front approaches the lesion (Ytrehus et al., 2007). The biomechanical insults on these predisposal focal sites may eventually result in osteochondral fragmentation of the articular surface or at periarticular locations giving origin to the OC form known by osteochondritis dissecans (OCD) (Denoix et al., 2013; van Weeren & Jeffcott, 2013).
Other lesions in the articular surface of juvenile individuals due to biomechanical stress (*e.g.* tension on ligaments) may cause damage as well, but are not primarily due to a disturbance of the process of endochondral ossification, and thus not a form of OC (Denoix et al., 2013).
In the horse, the joints that are mainly affected by OC include the metacarpophalangeal and the metatarsophalangeal (fetlock), the tarsocrural (hock), and femoropatellar (stifle), although other joints can be also affected (Jeffcott, 1991; Ekman et al., 2009). However, the highly dynamic nature of OC in young foals became clear when young foals were monitored

longitudinally over a prolonged period. In the study of Dik et al. (1999) some initial abnormalities showed a marked tendency for regression, but the pattern of early development and regression of the OC lesions depends on the joint. These observations were confirmed by other studies and led to the conclusion that the vast majority of lesions that developed (and could be visualized radiographically at some stage) would repair spontaneously without leaving (radiographically detectable) traces or progressing to further pathology (Jacquet et al., 2013; van Weeren & Jeffcott, 2013). Physiologically, this is made possible by the high metabolic activity of juvenile cartilage that enables growth and facilitates repair of the tissue (van Weeren & Jeffcott, 2013).

Like for other DOD conditions, the complete mechanisms that lead to the OC are not fully elucidated, but its complex multifactorial etiology in which both genetic and environmental factors play a role, is widely accepted (Semeavolos & Nixon, 2007; Robert et al., 2013). Genetic studies have shown a multitude of loci on a variety of chromosomes linked to osteochondrotic phenotypes, depending on the type of manifestation of OC, the joint involved, and the breed (Distl, 2013). These characteristics of the genetic background may explain the large variation in heritability values reported for OC in the literature, which can range from 0.05 to 0.45 (see Distl, 2013). Other suggested potential factors that may be involved in OC expression include rapid growth and factors that lead to rapid growth (Pagan & Jackson, 1996; Gee et al., 2005; Donabédian et al., 2006), dietary unbalances (Savage et al., 1993a; 1993b), endocrine factors and local cartilage regulatory peptides (Jeffcott & Henson, 1998; Semevolos & Nixon, 2007), excessive biomechanical loading or lack of exercise (Barneveld & van Weeren, 1999; van Weeren & Barneveld, 1999) and failure in cartilage vascularization (Ytrehus et al., 2007).

In what concerns nutritional unbalances, the two main issues that have been appointed as contributing factors to OC development are energy intake and mineral supply (van Weeren, 2006). In the growing foal, high levels of energy intake (>130% of NRC recommendations for DE) were related with a higher incidence of lesions of dyschondroplasia (Savage et al., 1993a). Also a connection between high insulin response, especially after feeding a meal of concentrates (rich in cereal grain), and horses with OC was reported (Ralston, 1996). It was therefore hypothesized that the ingestion of feeds with large amounts of easily digestible carbohydrates may alters endocrine regulation of endochondral ossification process through insulin, IGF-I and thyroid hormones mediated-effects on chondrocytes maturation and apoptosis (Jeffcott & Henson, 1998; Vervuert & Ellis, 2013).

Among macrominerals, high levels of phosphorus (P) in the diet (388% NRC P recommendations; 100% NRC calcium (Ca) recommendations) associated with a low Ca:P ratio (0.3:1), resulted in a high incidence of OC lesions (Savage et al., 1993b). In contrast, there is no evidence that high levels of Ca in the diet (342% NRC Ca recommendations) induced OC lesions, as long as adequate P was supplied (Savage et al., 1993b). Similar conclusion regarding the potential effects of high Ca intakes (2.5-fold higher than NRC recommendation) on bone density or bone growth in weanlings was drawn by Thompson et al. (1988b).

Copper (Cu) has been recognized for a long time as having an influence in the management of joint disorders. Copper is also known to be an essential cofactor for the enzyme lysyl oxidase which cross-links collagen fibrils (Jeffcott & Davies, 1998). Several studies were performed in order to investigate the potential role of dietary Cu in cartilage and bone defects in the foal (Knight et al., 1990; Hurtig et al., 1993; Pearce et al., 1998a, 1998b, 1998c). However, some results of these studies were controversial due to an absence of a direct connection between Cu dietary intake and the pathogenesis of OC (van Weeren et al., 2003). Nevertheless, Cu supplementation of pregnant mares during last months of gestation proved to increase foal liver concentrations (Pearce et al., 1998b), and the level of Cu in the liver of new-born foals was related with the early repair of OC lesions (van Weeren et al., 2003). Therefore, it is nowadays consensual that an adequate Cu supply should be provided to broodmares during the last months of pregnancy (van Weeren & Jeffcott, 2013). In the small intestine, zinc (Zn) may compete directly for the same transport mechanism as Cu (McDowell, 2003). Therefore it was mentioned that high levels of Zn (Zn toxicosis) may reduce the absorption of Cu and play a secondary role in the occurrence of OC-like lesions (Gunson et al., 1982; van Weeren, 2006). But the experiment of Bridges & Moffitt (1990) showed that only extremely high levels of Zn in the diet (1000 and 2000 mg/kg DM) can induce hypocupremia and the associated degenerative effects on cartilage, which is unlikely to occur under practical feeding conditions (Vervuert & Ellis, 2013).

CHAPTER III – *THE MARE*

3.1. NUTRITIONAL STATUS OF LUSITANO BROODMARES ON EXTENSIVE FEEDING SYSTEMS: BODY CONDITION, LIVE WEIGHT AND METABOLIC INDICATORS.

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Contribution of Maria J. Fradinho to this article:
Maria J. Fradinho performed the field and most of the laboratory work, analyzed the data, interpreted the results and wrote the manuscript.

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3.1.1. Abstract

The present research aimed to evaluate the effects of foaling season and feeding management in extensive systems on the nutritional status of Lusitano broodmares throughout the gestation/lactation cycle, by assessment of body condition (BC), body weight (BW), and some blood metabolic indicators. Four groups of Lusitano broodmares (A, B, C and D) were monitored during four years, in a total of 119 gestation/lactation cycles. All mares were kept on pasture. A and B mares were daily supplemented. Monthly, mares were weighed and BC evaluated. Suckling foals from these mares were also monitored for BW and withers height (WH). Glucose, non-esterified fatty acids (NEFA), urea and albumin concentrations were determined in blood. BW changes were influenced by reproductive stage and foaling season ($P<0.001$), reflecting also pasture availability. Changes on BC were observed ($P<0.05$), although with small amplitudes within each group. Higher scores were reached at the end of spring, decreasing 0.25 point until late summer. Early foaling had also a marked effect, hindering the recovery of BC along the cycle. Glucose values decreased from late gestation to early lactation ($P<0.05$) and lower levels were recorded during the summer months. Uremia was mainly influenced by the reproductive stage ($P<0.05$). Under nutrition was not detected. Foals born in February-March had higher average daily gain (ADG) than those born in April-May ($P<0.05$), probably reflecting differences in milk production of the mares. BC and BW changes and, particularly, blood indicators showed an overall balanced nutritional status, reflecting an adaptation to feed availability and climate.

Keywords: Lusitano mares, body condition, body weight, metabolic indicators, nutritional status

3.1.2. Introduction

As in other livestock females, and particularly in pasture based systems, mares store and mobilize body reserves during their reproductive cycle (Martin-Rosset et al., 2006a) according to breeds and goal of production: draft and leisure breeds *vs.* race and sport breeds (Bergero et al., 2006). This mobilization can be observed during the winter, at the end of pregnancy and sometimes in early lactation periods. In these periods, nutritional requirements may exceed nutrients provided by the diet, especially when low quality forages are used. When mares are fed limited energy, the mobilization of adipose tissue is also in part directed towards the production of the lipid fraction of mares' milk (Doreau et al., 1993; Martin-Rosset et al., 2006a). But mares can restore body reserves shortly as far as feed allowances are high after foaling without any detrimental effects for its reproductive efficiency and their foals (Guillaume et al., 2006; Martin-Rosset et al., 2006a).

The most common method reported for the evaluation of body reserves, mainly regarding body fat tissue, has been Body Condition Scoring (BCS). This method has been designed for horses (Henneke et al., 1983; INRA, 1990; INRA-HN-IE, 1997). In field conditions, BCS is a cheap, practical and accurate tool for monitoring energy balance, reflecting the abundance or shortage of nutrients on the animal recent past (Caldeira et al., 2007b; Martin-Rosset et al., 2008). In addition, BC evaluation on a regular basis allow to determine the pattern of changes (deposition or mobilization of reserves), which is fundamental for diets adjustment to different requirements in each productive phase. This method could be complemented by the assessment of some metabolites in body fluids, allowing for an early detection of nutritional unbalances. In general, serum or plasma concentrations of glucose and non-esterified fatty acids (NEFA) could provide information about the energy status of the animal, while albumin and urea are good indicators of protein status in mares (Doreau et al., 1981) as in other farm animals (Caldeira et al., 2007a).

Throughout the gestation-lactation cycle, and considering different breeds, the weight of the broodmare may vary between 13 to 20%, reaching the highest and lowest values before and after foaling, respectively (Martin-Rosset et al., 1986; Lawrence et al., 1992; Cassill et al., 2009). These body weight (BW) changes could reflect variations on three different main components: weight of the foetal-placental unit (in particular at the end of gestation), digestive contents and body reserves (Martin-Rosset et al., 1986).

Changes in broodmare BW and BC along the year are also linked to seasonal and management factors. Regardless the stage of gestation/lactation, BW and BC rise during the spring, which is probably related with pasture quality and availability (Martin-Rosset et al., 2006a; Pagan et al., 2006).

Nowadays, there is an increasing worldwide interest on Lusitano breed as a sport and leisure horse, due to its functional and behaviour characteristics. Although raised in several countries, most Lusitano stud farms in Portugal are traditionally based on extensive feeding systems. On these systems, mares are often bred outdoors throughout the year, being pastures an important part of their diets. Most of these pastures (natural or sown) are under Mediterranean influence and herbage production is commonly limited by summer dryness (Miraglia et al., 2006). When grass resources are scarce, supplementary feeds are generally used, but farm practices vary widely.

To the best of our knowledge, few longitudinal studies were performed in order to access BC changes across the gestation/lactation cycle in the sport broodmare (Lawrence et al., 1992; Pagan et al., 2006), but none in Mediterranean conditions. Thus, further information on nutritional status and body reserves management in the Lusitano breed will contribute for better decisions on the most appropriate feeding plans and foaling seasons, in order to improve the efficiency and profitability of these production systems.

The present research aimed to evaluate the effects of foaling season and feeding management in extensive systems on the nutritional status of Lusitano broodmares throughout the gestation/lactation cycle by the assessment of BC, BW, and some blood metabolic indicators.

3.1.3. Materials and methods

The protocol of this study was approved by the Ethical committee of the Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal. All the animals were handled with care during the experimental procedures.

3.1.3.1. Animals and study design

This experiment was conducted in four stud-farms located at the main region of Lusitano breeding, the southern area of Portugal. The broodmares of each stud-farm, hereafter designated by groups A, B, C and D, were monitored for BCS and BW over a period of four years (A and B: 2006 to 2008; C and D: 2008 and 2009), in a total of 119 gestation/lactation cycles. Average age at the beginning of the study was 11.0 ± 3.4 (mean±SD) years for A mares (n = 17), 8.4 ± 2.9 years for B mares (n = 15), 6.5 ± 3.8 years for C mares (n = 6) and

11.0 ± 4.7 years for D mares (n = 15). The suckling foals from these mares were also monitored for BW and height at withers (WH), through early lactation months. A standardized herd health schedule with routine vaccination and deworming programs was practiced in the four stud-farms.

3.1.3.2. Feeding plans

All mares were maintained on pasture all year and had free access to water. The stud-farms are located between latitude 38º88’ to 39º29’ N and longitude 07º67’ to 08º87’W and, according to Köppen classification, are under the influence of a Mediterranean climate (Csa). The annual rainfall is 652 mm (B, C and D) and 836 mm (A). The annual mean temperature is 17ºC (B, C and D) and 16ºC (A) (Instituto de Meteorologia, I.P., Lisboa, Portugal). Temperatures range, in winter, from - 3ºC to 25.2ºC for B, C and D stud-farms and from - 4.5ºC to 21.9ºC for stud-farm A and, in summer, from 11.9ºC to 45.2ºC for B, C and D stud-farms and from 9.4ºC to 41.3ºC for stud-farm A. Pastures are settled mainly on fluvisols and regosols (B and C), cambisols (A) and podzols (D) (FAO/IIASA/ISRIC/ISSCAS/JRC, 2009). Floristic composition was typical of natural rain fed pastures of these areas, with a high biodiversity. Besides a mixture of native grasses and legumes, some forbs and weeds were also present. Among grasses (Poaceae), the main *genera* includes *Lolium* spp., *Phalaris* spp., *Bromus* spp., *Agrostis* spp. and *Poa* spp.. In the legume family (Fabaceae), plants from the *genera Trifolium* spp., *Vicia* spp., *Melilotus* spp., *Ornithopus* spp. and some annual species of *Medicago* were identified. A and B mares were daily supplemented in group with commercial compound feeds (ranging from 1.5 $kg.animal^{-1}.d^{-1}$ to 5.5 $kg.animal^{-1}.d^{-1}$), and with grass hay or cereal straw (ranging from 2 to 6 $kg.animal^{-1}.d^{-1}$), according to animals’ physiological stage and pasture availability. C and D mares were only supplemented with grass hay (ranging from 5 to 10 $kg.animal^{-1}.d^{-1}$), in periods when pasture was scarce. Dry matter production in similar pastures of the same regions ranged from 40 $kg.ha^{-1}.d^{-1}$ in February to 90 $kg.ha^{-1}.d^{-1}$ in April, and decrease to 50 $kg.ha^{-1}.d^{-1}$ in May (Paço & Fradinho, 2011). In all but B stud-farm, rotational grazing was practiced. Samples of supplementary feeds used in stud-farms A and B were regularly collected for nutritional assessment. Pasture samples (one compound sample per pasture) were also collected on the spring of 2006 (stud-farms A and B) and on the spring of 2008 (stud-farms C and D).

Chemical composition analyses were made in a reference nutrition laboratory. Samples were dried for dry matter (DM) determination in a forced-air oven, to a constant weight, at 104ºC.

Ground samples were analyzed for ash by burning overnight at 550ºC (NP 872, 1983). Crude protein (CP) was measured by Kjeldahl method (ISO 5983-1, 2005). Crude fiber (CF) analyses were conducted according to the official procedure for feed analysis (EN ISO 6865, 2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions were analyzed by sequential detergent fiber determination according to Van Soest et al. (1991). Digestible energy (DE), net energy (NE) and digestible protein (DP) were estimated using the French feed evaluation system (INRA 2012). In addition, protein value of feeds, expressed in MADC (Horse Digestible Crude Protein) was calculated according to the new prediction equations (INRA 2012).

Table 3.1.1 – Chemical composition and nutritive value of supplementary feeds (DM basis)[1] used in A and B mares

Item	Compound feed[2]		Hay		Cereal straw	
	A (*n* = 9)[3]	B (*n* = 9)[3]	A (*n* = 2)[3]	B (*n* = 1)[3]	A (*n* = 1)[3]	B (*n* = 4)[3]
DM, %	86.2±2.8	87.0±3.2	86.4±3.8	86.8	89.1	90.6±0.4
CP, %	18.4±2.3	18.4±1.9	7.1±3.5	5.8	2.7	3.2±1.1
CF, %	12.2±1.8	12.1±1.8	34.6±1.4	37.4	43.6	42.9±3.7
ADF, %	16.2±2.1	17.0±2.3	-	-	-	-
Ash, %	7.9±1.4	8.3±2.1	8.1±1.4	7.5	7.9	7.5±0.6
DE[4], MJ/kg	12.4	12.3	8.0	7.5	6.1	6.1
NE[4], MJ/kg	8.8	8.6	4.1	3.8	2.9	2.9
DP[5], g/kg	149	149	34	24	0	0
MADC[5], g/kg	132	132	29	20	0	0

[1] Values are presented as means±SD.

[2] Compound feed composition: oats (28%); soybean meal 44 (22%); alfalfa dehydrated (20%); maize (15%); carob (8%); sunflower seed meal (4%); minerals and vitamins (3%).

[3] Number of analysed samples.

[4] DE (digestible energy) and NE (net energy) were estimated according to INRA 2012 system.

[5] MADC (horse digestible crude protein) and DP (digestible protein) were estimated according to INRA 2012 system.

Table 3.1.2 – Chemical composition and nutritive value of pastures (on DM basis) sampled in the spring of 2006 (stud farms A and B) and in the spring of 2008 (stud farms C and D)

Item	Stud-farms						
	A			B		C	D
	March	April	May	March	May	April	April
DM, %	16.2	20.4	87.1	12.3	27.8	21.7	18.8
CP, %	19.8	21.1	10.3	23.1	16.8	20.3	11.7
CF, %	17.7	18.5	32.9	18.9	22.4	19.0	27.5
NDF, %	34.1	38.6	60.9	38.4	41.1	54.1	61.1
ADF, %	22.8	26.1	40.8	23.7	30.3	28.2	32.0
ADL, %	5.5	8.9	7.8	4.6	8.4	2.8	3.9
Ash, %	11.3	13.3	8.4	12.7	10.1	10.4	10.6
P, %	0.47	0.42	0.21	0.54	0.38	0.37	0.31
Ca, %	1.07	1.12	0.54	1.04	1.21	0.43	0.48
Mg, %	0.29	0.30	0.13	0.25	0.22	0.19	0.26
Zn, mg/kg	28.5	34.0	20.5	40.0	34.5	23.0	27.0
Cu, mg/kg	6.8	7.3	6.5	9.7	10.0	7.0	4.0
DE[1], MJ/kg	11.6	11.1	8.9	11.4	10.6	11.5	9.3
NE[1], MJ/kg	6.6	6.1	4.3	6.3	5.8	6.3	5.0
DP[2], g/kg	136	149	62	169	112	142	70
MADC[2], g/kg	122	134	56	152	101	128	63

[1] DE (digestible energy) and NE (net energy) were estimated according to INRA 2012 system.

[2] MADC (horse digestible crude protein) and DP (digestible protein) were estimated according to INRA 2012 system.

3.1.3.3. Body condition scoring and weight measurements

Body condition of each mare was monthly evaluated (from the 9th month of gestation to the post-weaning period) according to the BCS method of INRA-HN-IE (1997) by a single observer, blinded to previous data. This BCS system (0 to 5 points scale) is based on five palpable areas of the horse's body (along the neck, along the withers, behind the shoulder, over the rib cage, between the 10th and the 14th ribs and the area adjacent to the tail head) and on two visual appraisals (the top line of the back and the croup). A quarter of point division was used for a better accuracy of the method. On the same occasion, body weight was determined (without restriction of feed or water) using a portable electronic scale (Iconix, FX15, New Zealand), which accuracy (0.5kg) was regularly checked.

3.1.3.4. Foals measurements

In order to indirectly assess mares' milk production, growth and development of the suckling foals were monthly evaluated throughout the first months of lactation because, at least until two months of age, ADG is linearly related with milk intake (Doreau et al., 1986). This information allowed for a better understanding of mares' BC and BW changes on this period, reflecting the balance between the requirements for a higher or lower milk production and the abundance or shortage of feed. Foals' BW was assessed with the same electronic scale used for broodmares. Height at withers (WH) was measured with an aluminum standard measuring stick from the ground to the highest point of the withers.

3.1.3.5. Blood metabolites

On the days of BC and BW assessments, between 8.00h and 11.00h and before any compound feed was distributed, blood samples (≈ 18 mL) were collected from 10 mares of group A, 10 mares of group B, six mares of group C and 10 mares of group D, by jugular venipuncture into plain and heparinized tubes (Monovette Serum and Monovette Li-Heparin, SARSTEDT AG & Co., Nümbrecht, Germany) for determination of glucose, NEFA, urea and albumin concentrations. Blood was collected into plain tubes for NEFA determination and was allowed to clot at room temperature. Heparinized tubes were immediately placed on ice until centrifugation. All blood samples were transported to the laboratory on ice and centrifuged at 670 *g*, at 4ºC, for 15 minutes. Plasma and serum samples were stored at –20ºC until analysis. Plasma glucose, urea and albumin concentrations were measured by colorimetric methods in an auto-analyzer (Kone Optima Analyzer, Therma Clinical labosystems, Vantaa, Finland) with commercial kits (Albumin Monofluid, Glucose HK and Urea UV, Bradford, Kemia Cientifica S.A., Madrid, Spain). Serum NEFA concentrations were determined by an enzymatic colorimetric method with a commercial kit (NEFA-HR(2), WAKO Chemicals GmbH, Neuss, Germany).

3.1.3.6. Statistical Analyses

Each group of mares with their foals was kept in different environmental conditions which impairs the direct comparisons among them. Therefore, independent but identical statistical analysis was conducted for data obtained in each group. Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). The model considered the foaling season, the physiological stage (gestation/lactation month), and the interaction between them as fixed factors, and mares as random effect. The physiological stages (from 9th month of gestation to post-weaning) were treated as repeated measures and an autoregressive covariance matrix was used. Two foaling seasons were considered for groups A, B and C: February-March and April-May. Due to the concentration of foaling dates in group D, only one foaling season was considered: February-March. For this group the model considered the physiological stage as fixed factor and mares as random effect. When significant differences were detected, the differences among means were evaluated using the Tukey-Kramer test. Statistical significance was assumed when $P < 0.05$.

Foals data (BW and WH) were also analyzed with a mixed linear model allowing for repeated measures on time. The effects of foaling season, time (expressed in days of age), time×time and their interactions were included in the model for foals A, B and C. For D foals, only the effects of time and time×time were studied.

All results are presented as lsmeans±SEM, unless stated otherwise (*e.g.* Table 3.1.1). When variables did not follow the normal distribution, transformed data (log10 or square root) were used in the analysis. For those variables the means were back transformed and the standard error of means were replaced by back transformed confidence intervals. To evaluate relationships between variables, Spearman's correlation coefficients were calculated.

3.1.4. Results and discussion

Only mares that had a successful delivery and nursed a foal until weaning were included in the study. Globally, foaling took place between January and May. Weaning occurred in early October for A and B groups, early November for group C and middle September for group D. In each year and each group, foals were separated from their dams on the same day, regardless of foals' age.

3.1.4.1. Body weight and body condition

Variations on BW throughout the year were tightly associated with mares reproductive stage (*i.e.* gestation or lactation month) confirmed by the significant effect ($P < 0.001$) of the physiological stage on BW, in the four groups of mares. As expected, changes in BW occurred either before foaling (increasing in the two last months of gestation) or after foaling ($P < 0.01$) (Figure 3.1.1.a, b, c and d). Besides seasonal factors (*e.g.* grass abundance in the spring), major BW changes in the broodmare were normally linked with the conceptus gain (foetus + adnexa and uterus) during gestation and its reduction associated with foaling (Martin-Rosset et al., 1986; Heidler et al., 2004; Pagan et al., 2006). In the present study, and considering BW after foaling, mean weight gain observed throughout pregnancy ranged between 7.6 % and 13.9 %, while in Thoroughbred mares was 13.9 ± 3.2 % (Cassill et al., 2009). But the major increase took place in the last three months of pregnancy and could represent 7 to 9 % (Martin-Rosset et al., 1986), which coincide with the average value of 8.8 % found among the four groups of mares throughout this stage. As BCS did not change significantly during this period, it could be considered that the observed BW gain accounted for the gain of conceptus.

After foaling, it was reported for the Lipizzaner mare a weight reduction of 64.8 ± 2.4 kg, which represents about 12 % of mares BW after foaling (Heidler et al., 2004). Considering the four groups together, the weight reduction observed in the present study was 11.6 %. Also in heavy breeds, a reduction of 12% of BW after foaling was observed (Martin-Rosset et al., 1986).

BW changes were also influenced by foaling season and an interaction between foaling season and physiologic stage was found in groups B and C ($P < 0.05$). The effect of foaling season in BW was very clear in mares of group A with consistent higher values observed in Apr-May foaling mares when compared to Feb-Mar foaling mares ($P < 0.01$) (Figure 3.1.1.a). Mares of group D showed, in general, the lowest values of BW. In this group, and besides the changes observed in the two last months of gestation and after foaling, an increase in BW occurred from the 1st to the 4th month of lactation ($P < 0.01$), reflecting probably an influence of pasture spring production (Figure 3.1.1.d).

Throughout the year, changes on BCS were observed in the four groups of mares ($P < 0.05$), although with small amplitudes within each group. Generally, higher scores were reached at the end of spring, decreasing then until the end of summer (which is coincident with late lactation period), when the lowest scores were observed (Figure 3.1.1.a, b, c and d). On group A, a clear effect of foaling season on BC was observed ($P < 0.01$) with consistent higher values throughout the year in Apr-May foaling mares. BCS varied between 2.59 ± 0.08 and 2.88 ± 0.08 on Feb-Mar mares and between 2.96 ± 0.09 and 3.23 ± 0.09 on Apr-May mares. Mares of Group B showed the smallest variation in BCS, with values between 3.18 ± 0.08 and 3.29 ± 0.08 on Feb-Mar foaling mares and between 3.20 ± 0.10 and 3.44 ± 0.05 on Apr-May mares. In this group an interaction between foaling season and physiological stage was observed ($P < 0.05$), with the Apr-May foaling mares showing a BCS peak in the 11th month of gestation, which was absent in Feb-Mar foaling mares (Figure 3.1.1.b).

Although with changes throughout the year ($P < 0.01$), BCS on C mares was not influenced by foaling season. In this group, an increase in BCS was observed from late lactation (3.09 ± 0.07) to post-weaning period (3.28 ± 0.07) (Figure 3.1.1.c). Mares of group D presented the lowest BCS and the largest amplitudes, between 2.21 ± 0.9 and 2.77 ± 0.10. Higher BCS values were observed in late gestation months and there was a steady decrease during lactation period until the last months of lactation and post-weaning ($P < 0.01$) (Figure 3.1.1.d).

Considering data from all mares, a positive correlation was found between BCS and BW ($r = 0.57$; $P < 0.0001$). The higher correlation coefficient was obtained for mares of group A ($r = 0.62$; $P < 0.0001$) and the lower was obtained for mares of group D ($r = 0.34$; $P < 0.0001$).

The important role of an adequate amount of body reserves on reproductive and productive performances of the broodmare is generally recognized (Guillaume et al., 2006). The effects of nutritional status (assessed by regular BCS) on reproductive performance of the mare was highlighted in several studies (Henneke et al., 1984; Godoi et al., 2002; Guillaume et al., 2002). It was also shown that mares' intake and milk yield and composition depends on BCS (Doreau et al., 1992; Doreau et al., 1993) and, consequently, the growth of nursing foals could be influenced by the amount of body reserves of their dams (Martin-Rosset & Young, 2006). The current study provides an overview of BCS and BW changes along the year in light broodmares reared on pasture based systems of southern Europe. Like in Thoroughbred mares (Pagan et al., 2006), in our field study BC changes appear to be strongly influenced by the time of year, reflecting seasonal pasture production and quality. Regardless of gestation or lactation stage and feeding regime, higher BCS were generally found at the end of the spring (about 3.03 in earlier foaling mares and 3.33 in latter foaling mares) reflecting pasture abundance in this season. During the summer, herbage production in these regions is limited by climate conditions (high temperature and scarce rainfall). Therefore, the decrease in BCS in groups A, C and D until the end of the summer (about 0.25), when the lowest values were observed, suggests a mobilization of body reserves to match the nutritional needs throughout the last stage of lactation. According to the prediction model proposed by Martin-Rosset et al.

(2008), a decrease of 0.25 point in BCS represents a variation of 3% of body weight. Thus, considering the average body weight of A, C and D mares (first weight evaluated after foaling) as 529.4 kg, 528.9 kg and 486.3 kg, the amount of mobilized adipose tissue would have represented 15.9 kg, 15.9 kg and 14.6 kg, respectively. As the estimated net energy variation is -19.78 $MJ.kg^{-1}$ BW (Martin-Rosset et al., 2012), a deficit of 6.4% could be identified in the period between May and September regarding the maintenance requirements (INRA, 2012). Besides herbage scarcity, this deficit could also have been the consequence of some heat stress resulting from an increase of environmental temperature out of the thermo neutral zone, which is accepted to range from 5 C° to 25 C° in mild climate (Morgan et al., 1997; Morgan, 1998). For example, maintenance requirements of adult standardbred geldings in mild climate were 9% higher in summer than in winter (Martin-Rosset & Vermorel, 1991). In contrast, the absence of BCS changes in mares of group B during the summer months (Figure 3.1.1.b) was probably related to feeding practices. In fact, and besides pasture, A and B mares were daily supplemented with variable amounts of other feeds, which nutritive value is presented in Table 3.1.1. But the proportion of the requirements that was covered by compound feeds used in group B was, on average, 6% higher for energy and 10% higher for protein than in group A in the period between the 2nd and the 7th months of lactation. Unfortunately, it was not possible to collect and analyze pasture samples in the summer period. However, the results concerning the last samples taken in stud-farms A and B (May samples) indicate a better nutritive value for the pastures of stud-farm B (Table 3.1.2).

Considering global BCS throughout the study, mares of group D presented the lowest values (2.21) and the biggest amplitudes (0.56). Foaling season occurred very early in the year and, because these mares were highly dependent on grazing resources, a steady mobilization of body reserves was observed from February in order to cope with higher nutritional requirements of early lactation (Figure 3.1.1.d). In addition, it is quite clear that the nutritive value of pastures of stud farm D (April samples) is the lowest when compared with the nutritive value of pastures from stud-farms A and C in the same month (Table 3.1.2). Regardless the absence of a significant effect of foaling season on BCS of group C, mares that foaled later (Apr-May for A, B and C groups) showed, on average, higher BCS suggesting a better utilization of grazing resources. Overall, mares reached at key points of the reproductive cycle, *e.g.* foaling and weaning, an average BCS of 3.12 [2.67 to 3.36] and 2.96 [2.21 to 3.27], respectively. This level of BC at foaling is similar to the recommended (INRA, 2012) in order to optimize fertilization during the first month after foaling and to support the first months of lactation.

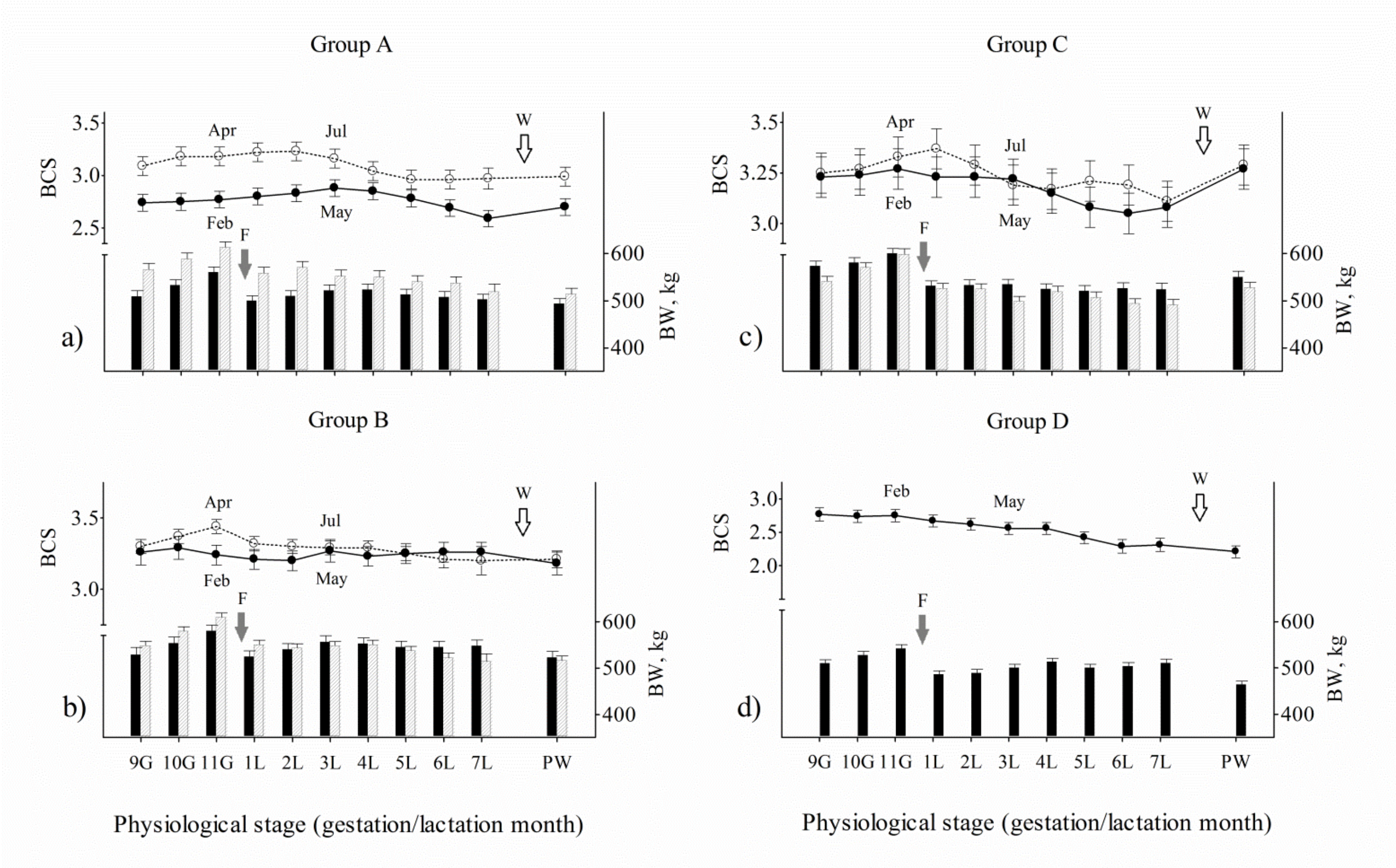

Figure 3.1.1 – BCS and BW according to physiological stage, in mares of groups A, B, C and D (figures a, b, c and d). Solid symbols and continuous lines refer to Feb-Mar foaling mares and open symbols and dotted lines refer to Apr-May foaling mares. In all the figures, foaling is represented by the solid arrow and weaning by the open arrow.

3.1.4.2. Blood metabolites

The assessment of blood parameters for monitoring metabolic and nutritional status in livestock species has been widely used for a long time because of the quality of the information that could provide and the simplicity of collection and determination (Doreau et al., 1981; Caldeira, 2005). In the present study, values of blood metabolites were, generally, within the reference ranges described in literature for horses and particularly for pregnant and nursing mares (Harvey et al., 2005).

Small changes ($P < 0.05$) in plasma glucose concentrations were observed in groups A, C and D throughout the gestation/lactation cycle. Only in mares of group B, glucose concentrations differed with foaling season ($P < 0.01$).

Blood glucose is under a powerful homeostatic control that keeps it within narrow limits. However, low values may indicate decreased feed intake or gluconeogenesis rate (due to lack of glucose precursors) in periods of greatest needs of glucose (*e.g.* early lactation). In the present study, glucose concentrations decreased from late gestation to early lactation and lower values were also recorded during the summer months ($P < 0.05$) (Figure 3.1.2). Basal lower glucose concentrations in early lactation were also observed in Thoroughbred and Lipizzaner mares when compared with values found in late gestation (Hoffman et al., 2003a; Heidler et al., 2004). It is likely that the increased use of glucose during early lactation was influenced by mammary gland demands for lactose synthesis in milk, because mares' milk is highly concentrated in this disaccharide (Doreau et al., 1993; Santos & Silvestre, 2008). The observed decrease on glucose concentrations from spring to summer months and the steady levels found during this season probably reflect the influence of pasture quality and availability, when the nutritional requirements linked to the lactation stage are still important.

Throughout the year, changes in NEFA concentrations were observed in A and B mares and an interaction between foaling season and physiological stage was found ($P < 0.01$). As observed for glucose, NEFA concentrations were only influenced by the physiological stage in groups C and D ($P < 0.001$).

Serum concentrations of NEFA have been used in several studies as a metabolic predictor of energy status. Feed deprivation or restriction leads to fat mobilization and a consequent rise in NEFA concentrations (Sticker et al., 1995a; Caldeira, 2005; Dugdale et al., 2010a). Overall, higher concentrations of NEFA were observed during periods when fat mobilization could be expected in order to cope with energy needs, due to either a specific physiologic state or a lower availability of feed. In groups A, B and D, lower NEFA concentrations were recorded in spring months, when pasture was abundant, and peaked in June-July, when pasture becomes dry.

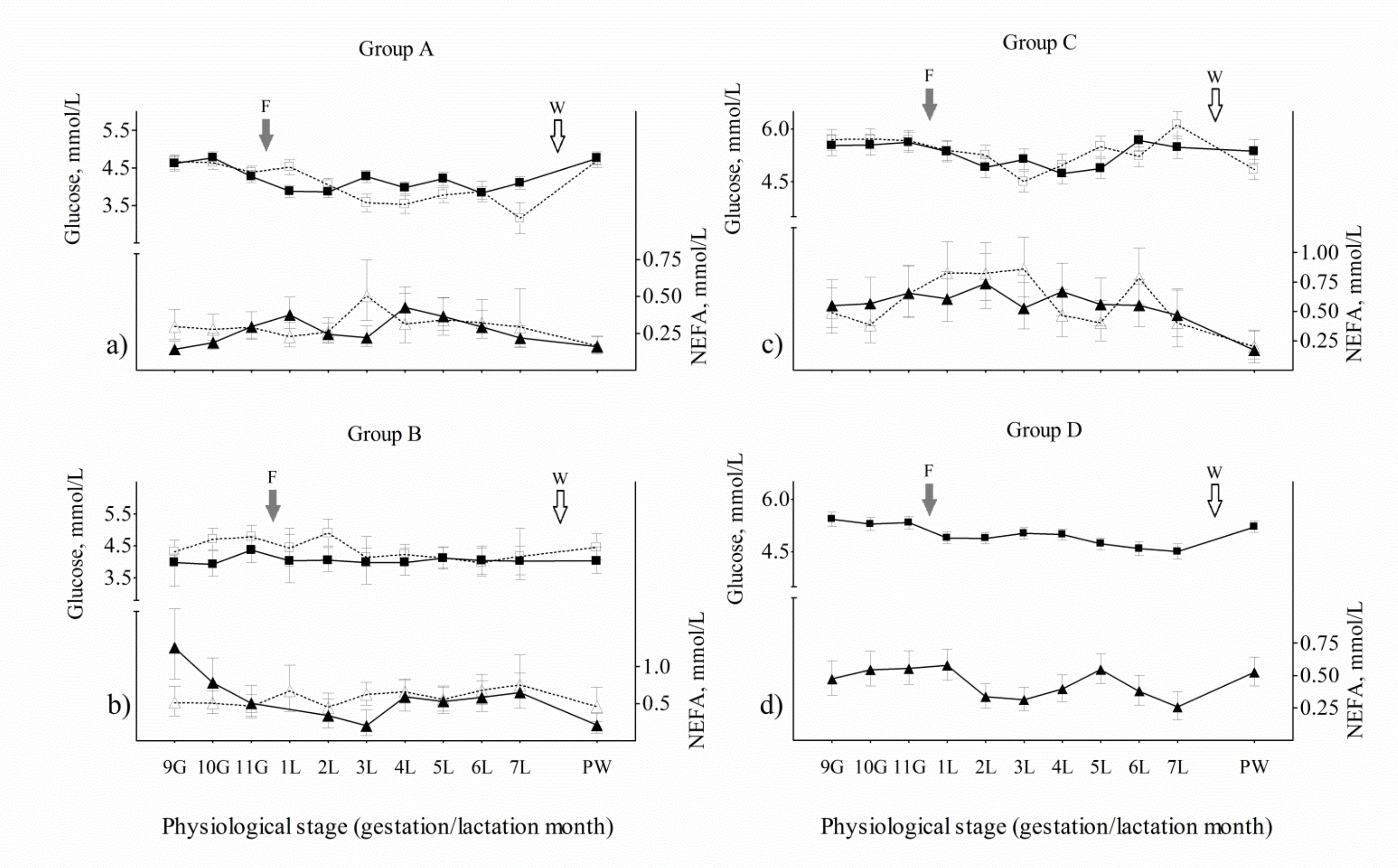

Figure 3.1.2 – Glucose and NEFA concentrations according to physiological stage, in mares of groups A, B, C and D (figures a, b, c and d). Solid symbols and continuous lines refer to Feb-Mar foaling mares and open symbols and dotted lines refer to Apr-May foaling mares. In all the figures, foaling is represented by the solid arrow and weaning is represented by the open arrow.

Concerning the metabolites associated with the protein status, some changes were found in groups A, B and C ($P < 0.05$). Urea plasma concentrations were influenced by foaling season in groups B and C ($P < 0.05$) and also by physiological stage in groups A, B and C ($P < 0.05$) (Figure 3.1.3). As for other species, previous studies in the horse have shown that uremia is sensitive to high protein dietary levels (Miller-Graber et al., 1991). In contrast, dietary protein restrictions (50% of protein requirements) appear to decrease urea concentrations in blood (Sticker et al., 1995a). The results obtained in the present study showed an influence of physiological stage on urea concentrations, which could be related with time of year. In general, uremia was higher during spring months and decreased in the summer, suggesting a relation to protein levels of pasture.

Albumin is the most abundant protein in blood and in situations of nutritional deficiency may function as an important pool of labile protein. Considering the contribution of dietary protein from compound feeds in groups A and B, it would be expected that higher values of albumin would be found in these groups. However, albumin values tended to be relatively constant along the year, and in a similar range to non-supplemented groups (C and D). In winter months, grazing is quite limited and supplementation with low quality hays could eventually justify the significant decrease in albumin values found in group C.

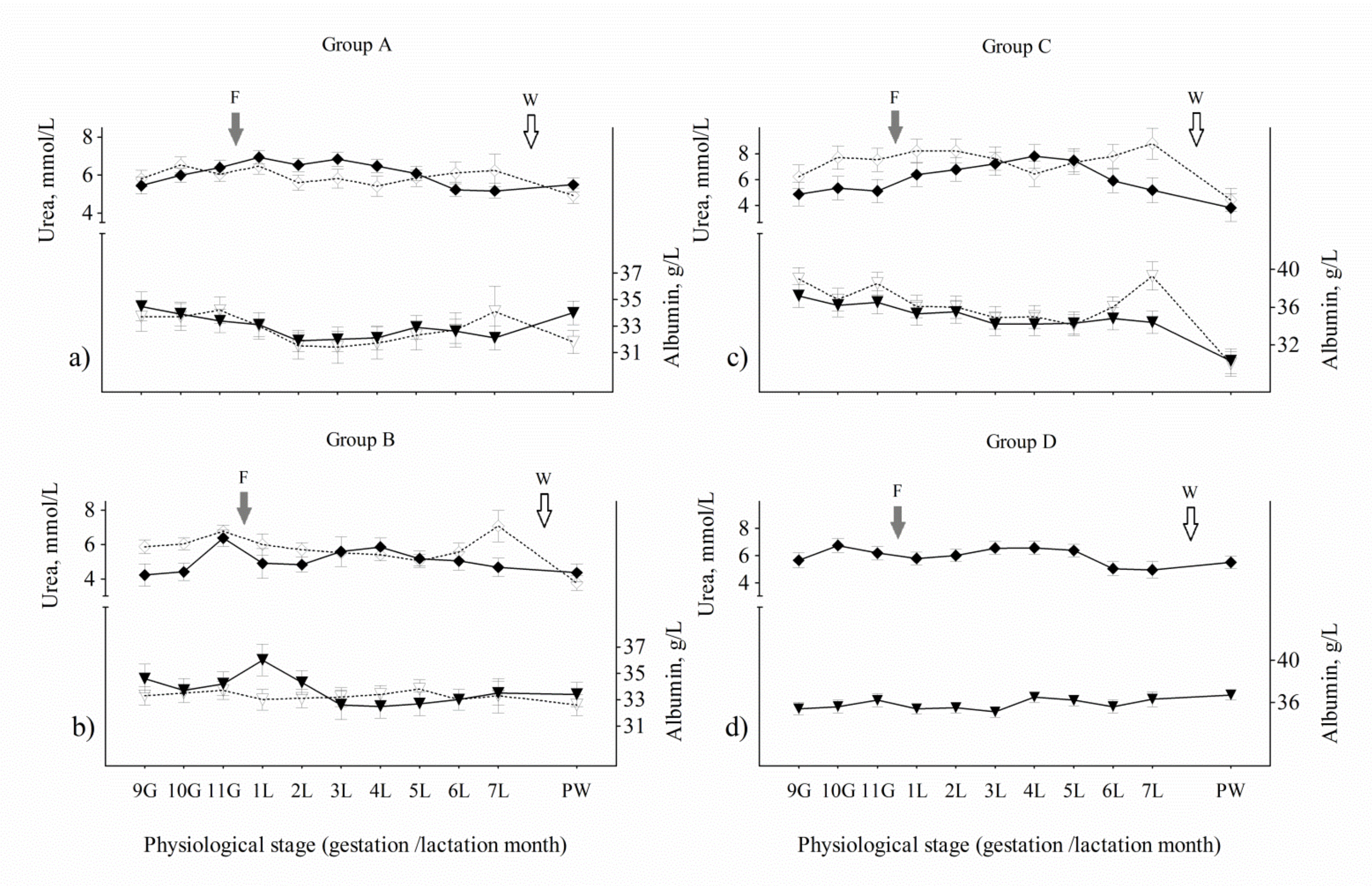

Figure 3.1.3 – Urea and albumin concentrations according to physiological stage, in mares of groups A, B, C and D (figures a, b, c and d). Solid symbols and continuous lines refer to Feb-Mar foaling mares and open symbols and dotted lines refer to Apr-May foaling mares. In all the figures, foaling is represented by the solid arrow and weaning is represented by the open arrow.

3.1.4.3. Foals' growth and development

Significant interactions between foaling season and time ($P < 0.05$) were observed on BW changes for A and C groups, indicating a different pattern of growth between foals born in Feb-Mar and foals born in Apr-May (Table 3.1.3). At 90 days of age, estimated BW varied between 140.7 kg (group B) and 160.8 kg (Feb-Mar foals of group A). Concerning WH, the influence of foaling season was only observed in group C, with an interaction between foaling season and time ($P < 0.05$) (Table 3.1.3). Estimated WH at 90 days of age varied between 120.2 cm (group B) and 123.8 cm (Feb-Mar foals of group C). Overall, higher growth performances (ADG) through the first three months of life were observed in early born foals (Feb-Mar) of groups A and C for BW and in early born foals of group C for WH.

Previous research in other geographical regions referred a clear influence of month of birth and season of year on suckling foals' growth rate, with lower values for winter born foals, when access to pasture was limited (Hintz et al., 1979; Pagan et al., 2006). Considering the growth performances of the early born A and C foals in our study, mares that foaled in Feb-Mar have, apparently, higher milk production, reflecting the influence of spring pasture quality and availability. However, the shift of nutrients for milk production at this stage caused a less effective recovery of BCS during the spring, in comparison with that observed in the mares that foaled in Apr-May, implying that Feb-Mar mares reached the summer with less body reserves.

Regardless of different feeding practices in the four groups of mares (supplemented *vs.* non-supplemented), growth performances of later born foals may suggest that other supplementation strategies (namely in what concerns some limiting nutrients) should be implemented, when pasture growth is depressed by summer dryness.

Table 3.1.3 – Prediction models for body weight and withers height in the four groups of foals, through the first four months of life.

Variable	Group	Foaling season	Model	Fixed effects (*P value*)			
				Foaling season	Time	Foaling season×Time	Time×Time
Body Weight (kg)	A	FebMar AprMay	$BW = 57.4 + 1.418\ d - 0.00299\ d^2$ $BW = 57.4 + 1.347\ d - 0.00299\ d^2$	ns	<.001	0.023	<.001
	B		$BW = 51.7 + 1.191\ d - 0.00225\ d^2$	ns	<.001	ns	<.001
	C	FebMar AprMay	$BW = 53.0 + 1.338\ d - 0.00186\ d^2$ $BW = 53.0 + 1.249\ d - 0.00186\ d^2$	ns	<.001	0.019	0.001
	D	FebMar	$BW = 50.7 + 1.358\ d - 0.00309\ d^2$	-	<.001	-	<.001
Withers Height (cm)	A		$WH = 102.0 + 0.306\ d - 0.00084\ d^2$	ns	<.001	ns	<.001
	B		$WH = 100.4 + 0.294\ d - 0.00082\ d^2$	ns	<.001	ns	<.001
	C	FebMar AprMay	$WH = 98.9 + 0.373\ d - 0.00112\ d^2$ $WH = 98.9 + 0.343\ d - 0.00112\ d^2$	ns	<.001	0.011	0.001
	D	FebMar	$WH = 99.1 + 0.345\ d - 0.00107\ d^2$	-	<.001	-	<.001

d - days of age; BW – body weight; WH – withers height.

3.1.5. Conclusions

Results show that changes in BW and BC in the Lusitano broodmare, managed on grazing systems, are mainly influenced by pasture availability and quality and the time when foaling season occurred in the year. In fact, Mediterranean pasture cycle leads to a general increase in body reserves in spring and their mobilization until autumn and winter, although this change does not represent more than half point of BC. The quality of pastures and supplementary feeds has a strong effect on the mean annual BC among stud-farms, determining almost one point of BC variance. Early foaling in the season had also a marked effect, hindering the recovery of BC and decreasing the level of BC throughout the whole cycle. BC and BW changes and, particularly, blood indicators showed an overall balanced nutritional status and an apparent metabolic welfare without any evident signs of under or over nutrition, reflecting an adaptation to feed availability and climate.

The association of these data with further studies on mares' fertility and foals' growth until weaning will contribute for better management decisions on the most suitable foaling season and the most appropriate feeding plan, in order to improve the efficiency and profitability of the Lusitano production system.

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3.2. Effects of body condition and leptin on the reproductive performance of Lusitano mares on extensive systems

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3.2.1. Abstract

The aim of this study was to investigate the effects of body condition (BC), BC changes, and plasma leptin concentrations on the reproductive performance of Lusitano broodmares on extensive systems. Data from 119 mares (ranging from 4 to 22 years of age) were collected over a period of four consecutive breeding seasons. Each case was considered as one foaled mare bred in one year. Body condition changes at conception (ΔBCScon) showed a strong effect on fertility at the first two postpartum estrous cycles and a significant interaction with body condition score at conception (BCScon) was observed ($P < 0.01$). The best fertility results were obtained with positive and greater ΔBCScon. The best predictive value of fertility (91%) was achieved when the BCScon was 3.0 and ΔBCScon was 0.375. Global foaling rate for the four-year period was 74.5%. Mean foaling interval and gestation length were, respectively, 368 ± 2.8 and 340 ± 1.0 days. Gestation length was influenced by the month of foaling ($P < 0.05$) and decreased as mares got older ($P < 0.01$). The number of estimated estrous cycles per live foal was 1.78 ± 0.12, including foal heat ovulation. Leptin was correlated with BCS (0.41; $P < 0.001$), but in the present study, plasma leptin concentrations on late gestation and early lactation did not influence fertility results. At 90 days of age, lower growth performances were obtained in foals which dams presented negative BCS changes on the first three months of lactation ($P < 0.05$). In conclusion, BC changes at early postpartum period influence the reproductive efficiency of broodmares and the growth of their suckling foals. These findings are important to help breeders on management options concerning feeding strategies in the Lusitano production systems and others under similar conditions.

Keywords: mare, body condition, fertility, leptin, growth of suckling foals, Lusitano breed

3.2.2. Introduction

The main goal in horse production is to ensure a healthy foal per mare bred every year, regardless of the breed and utilization purpose. In ideal conditions, this means that mares should conceive in the first month after foaling, to reduce the period between deliveries. However, the success of reproductive performance is generally limited since it is influenced by a wide range of factors. Among these, nutritional status and energy balance, which are reflected by body condition (BC) and its changes, were previously related with the reproductive efficiency of the mare (Henneke et al., 1984; Guillaume et al., 2006; Cavinder et al., 2009).

Over the last decade, the role of adipose tissue as an endocrine organ has become more evident. Leptin, a hormone mainly produced in the adipocytes, has been suggested to be an important link between adipose tissue and the reproductive system, signaling the adequacy of energy reserves for a normal reproductive function. In the mare, as in other species, there is a positive correlation between plasma leptin concentrations and body condition (Huff et al., 2008; Carter et al., 2009) and this hormone is also involved in ovarian activity modulation (Gentry et al., 2002; Ferreira-Dias et al., 2005).

Besides the reproductive function, the influence of BC on broodmare productive traits, such as milk production and the subsequent growth of the foal has been recognized (Martin-Rosset & Young, 2006). Up to two months of age, the average daily gain (ADG) is linearly related to milk yield (Doreau et al., 1986). In addition, milk composition also appears to be affected by mare body condition. Milk from overweight mares (body condition score (BCS) > 3.5, scale 0-5 [INRA-HN-IE, 1997]) has a higher fat content than milk from thin mares (BCS < 1.5, scale 0-5) and the reverse is found for protein (Doreau et al., 1993). Growth and development performances of the suckling foal can also be affected by foaling season. Especially on systems in which diets are based on pasture, this effect is associated with the grass production cycle and overall feed availability (Pagan et al., 2006; Fradinho et al., 2012).

Like other sport and leisure horse breeds in southern Europe, Lusitano horse breeding is mainly based on extensive production systems where pasture represents the main part of mares and foals diet. In these systems, changes in body reserves of Lusitano broodmares through the productive cycle are influenced by the time of the year in which foaling occurs (Fradinho et al., 2013). Hence, gathering reliable information, under farm conditions, about the factors that could influence management options in order to improve mares fertility is of major importance for breeders.

The hypothesis of the present study was that reproductive efficiency of Lusitano broodmares is influenced by their nutritional status and energy balance from late gestation to mid lactation. Therefore, the main objective of this work was to investigate the effects of BC, BC changes and plasma leptin concentrations on reproductive performance of Lusitano broodmares on extensive systems, bred at early postpartum period.

3.2.3. Materials and Methods

3.2.3.1. Animals and study design

A total of 119 Lusitano broodmares, ranging from 4 to 22 years of age and with a mean body weight (BW) after foaling of 524.6 ± 4.85 kg, were monitored on four stud farms (A, B, C and D) located at the southern region of Portugal (latitude between 38º88' to 39º29' N). Data were collected over a period of four consecutive breeding seasons (2006-2009) and treated on an annual basis (Table 3.2.1.). Each case was considered as one mare bred in one year. Thus, a specific mare followed in a breeding season could be also considered in a next year as another individual. At the time of breeding all mares were nursing a foal.

Different methods of breeding were used among stud farms: free natural mating (only on stud farm D), in hand natural mating, artificial insemination (AI) with fresh semen from the stud farm resident stallions, AI with cooled semen and AI with frozen semen (Table 3.2.1.). In the three stud farms where assisted reproduction was practiced, reproductive records were used to determine ovulation dates. Follicular development was monitored by sequential transrectal ultrasonography from the onset of estrus after foaling or 7d post foaling if no behavioural signs of estrus were detected. When a preovulatory follicle (≥ 35mm) was detected, mares were inseminated or mated. Pregnancy status was determined by transrectal ultrasonography on day 14 after mating on stud farms A, B and C, and every 3 weeks on stud farm D.

The day of ovulation was considered as the first day of gestation. Length of gestation was calculated as the interval from ovulation to foaling. Based on foaling dates, foaling interval was calculated for all gestations occurred on the four years. For those mares which reproductive records were not available, the number of estrous cycles between foaling and conception was estimated as the difference between foaling interval and the average gestation length found on the present study, divided by the mean inter-ovulatory interval of 21 days and considering that the foal heat ovulation occurs, on average, around the first 10 days after foaling (Ginther, 1992).

All animals were maintained on pasture and had free access to water. On stud farms A and B, mares were daily supplemented with commercial compound feeds and also with grass hay or cereal straw according to their physiologic stage and pasture availability. On stud farms C and D, mares were only supplemented during fall/winter periods with grass hay when pasture was scarce. A normal herd health schedule with routine vaccination and deworming programs was practiced on the four stud farms.

Table 3.2.1 – Source and nature of data used in the study

Stud farm	Nº (mare-year)[a]	Mares age (years) mean ± SD (range)	Breeding method[b] (n)	Mares data (reproductive season)	Foals data (year of birth)
A	43	12.1 ± 3.6 (7-19)	NS (12) AI $_{fresh}$ (31)	2006-2008	2006-2008
B	38	9.4 ± 3.0 (6-16)	AI $_{fresh}$ (35) AI $_{cooled}$ (3)	2006-2008	2006-2008
C[c]	12	8.1 ± 5.0 (4-19)	AI $_{frozen}$ (10) AI $_{cooled}$ (1)	2008 and 2009	2008 and 2009
D	26	11.3 ± 4.7 (6-22)	NM (26)	2008 and 2009	2008 and 2009

Abbreviations: AI, artificial insemination; NM, free natural mating in pasture; NS, natural service; SD, standard deviation.

[a] One mare-year was defined as one mare bred in one year.

[b] Breeding methods: NS; AI (with fresh, cooled or frozen semen), and NM.

[c] One mare of stud farm C was not included in the fertility analysis, because it was not intentionally bred in 2008

3.2.3.2. Body condition scoring

Body condition of each mare was monthly evaluated (from the ninth month of gestation to weaning) according to the BCS method of INRA-HN-IE (1997) (0-5 scale) by a single trained observer, blinded to previous data. A precision of 0.25 points was used for a better accuracy of the method.

The pattern of BC change (ΔBCS) was evaluated in two specific periods: at conception (difference between BCS evaluated before and after the probable date of fertilization: ΔBCScon) and throughout the first three months of lactation (difference between BCS evaluated at the beginning and at the end of this period: ΔBCS3L).

3.2.3.3. Blood sample and leptin assay

During the last trimester of gestation (on the ninth and 11th months) and during early lactation (on the first and third months), blood samples were collected into heparinised tubes (Monovette Li-Heparin; SARSTEDT AG & Co., Nümbrecht, Germany) between 8.00h and 11.00h, before any compound feed was given, from 59 mares (A, n=17; B, n=16; C, n=12; D, n=14), by jugular venipuncture for determination of leptin concentrations. Heparinised tubes were immediately placed on ice until centrifugation. Samples were centrifuged at 2000 x g, at 4ºC, for 15 minutes and plasma stored at –20ºC until analysis. Plasma leptin concentrations were determined by radioimmunoassay using a Multi-Species Leptin RIA kit (Multi-Species Leptin RIA; Linco, Millipore Corporation, Billerica, MA, USA.) previously validated for the horse (McManus & Fitzgerald, 2000; Cartmill et al., 2003; Ferreira-Dias et al., 2005).

3.2.3.4. Growth and development evaluation of the foals

After birth and until weaning, foals (Table 3.2.1.) were monthly weighed and measured for growth and development evaluation. Body weight (BW) was determined using a portable electronic scale (model Iconix FX, New Zealand). Withers height (WH) was obtained with a standard measuring stick from the ground to the highest point of the withers. Girth (G) and cannon circumference (CC) were measured with a flexible measuring tape. Individual growth curves were adjusted (polynomial equations, second order) and the derivatives as the instantaneous growth rate (IGR) were calculated for each animal at 90 days of age. The same methodology was used for WH, G and CC allowing for the estimate of the instantaneous increase of these biometric variables at 90 days of age.

3.2.3.5. Statistical analyses

Data analysis was conducted with SAS software (SAS 9.2 Institute Inc., Cary, NC, USA). According to foaling date, two main foaling seasons were considered in each year: February-March (FebMar) and April-May (AprMay). In order to equalize the number of mating opportunities of mares from both foaling seasons, only data concerning the first two postpartum estrous cycles were considered for fertility analysis. On a first approach, data concerning BCScon and ΔBCScon were evaluated. Because mares with a BCScon < 2.25 (n=8) were considered to be outliers, they were removed, keeping only mares with a BCScon ≥ 2.25 for further analysis. The effects of foaling season and the age of the mare on BCScon and ΔBCScon were initially tested with MIXED procedure of SAS. Fertility results concerning the first two estrous cycles were then analysed using the proc GLIMMIX of SAS, modelling the probability of a foal born alive. BCScon and ΔBCScon were treated as covariates and the fixed effects of BCScon, ΔBCScon and its interaction were tested. The breeding method was included and tested as a factor in the model. Once it was not significant, it was withdrawn from further analysis. The best fit was obtained for the model that included only the interaction between BCScon and ΔBCScon as fixed effect. Gestation length was analysed using the proc MIXED of SAS including, as fixed effects in the model, foaling season, month of birth and gender of the foal. The age of the mare was also included as covariate.

Body condition and leptin plasma concentrations during late gestation and early lactation were analysed with proc MIXED, but as repeated measures on time (ninth and 11th months of gestation, and first and third months of lactation). Foaling season, month and its interaction were included as fixed effects in the model. In the subset of mares where leptin data were available, the covariate effect of leptin on the ninth and 11th months of gestation, and first month of lactation were also tested on the number of days between foaling and conception. The fixed effects of foaling season and age of the mare were included in the model. Additionally, the covariate effect of leptin concentrations (ninth and 11th months of gestation, and first month of lactation) on fertility outcome of this subset of mares was analysed using the proc GLIMMIX.

In order to characterize foals' growth and development, polynomial regression models (second order) were adjusted with Graphpad Prism 4.0 (Graphpad Software, San Diego, CA, USA). The MIXED procedure was used for data concerning the instantaneous increase at 90 days in live weight, WH, G and CC. For these variables, foaling season, age of the mare, BCS through the first three months of lactation (BCS3L) and ΔBCS3L were tested as fixed effects, and group as random effect.

When significant differences were detected, the Tukey-Kramer test was used to evaluate the differences among means. Statistical significance was considered when P-value is less than 0.05. To evaluate the relationships among variables, Spearman's correlation coefficients were calculated. All results are presented as least square means ± standard error of the mean, unless stated otherwise.

3.2.4. Results

3.2.4.1. Reproductive parameters

In the four-year period, mares foaled between the 24th of January and the 25th of May. There was no effect of foaling season on mare BCScon and ΔBCScon, but a significant effect of age on ΔBCScon was observed, with higher BCS negative changes at conception in older mares ($P < 0.05$). Also, a trend for a negative correlation was found between the age of the mare and the mean BCS throughout lactation period (-0.190; $P = 0.062$).

Fertility results concerning the first two postpartum estrous cycles were obtained from the logit model, which estimates the probability of a foal born alive. ΔBCScon revealed to have a strong effect on fertility and a significant interaction between BCScon and ΔBCScon was observed ($P < 0.01$). Figure 3.2.1 represents the back-transform prediction values of fertility, obtained from the model, clearly showing the strong influence of ΔBCScon on the final outcome. Best fertility results were obtained with positive and greater BCScon changes. Since BCS has always a positive value, any negative change in BC at conception will lead to fertility values under 66%, whatever the BCS. Also according to this model, the greater the BCS the worse is the effect of BC negative changes at conception on fertility. Considering the range of BCScon and ΔBCScon observed in our study, the best predictive results regarding the probability to conceive during the first two estrous cycles and foaling (above 85%) were obtained for a BCScon between 3.0 and 3.75 and with a ΔBCScon of 0.25. The best predictive value of fertility (91 %) was achieved at BCScon 3.0 and ΔBCScon 0.375.

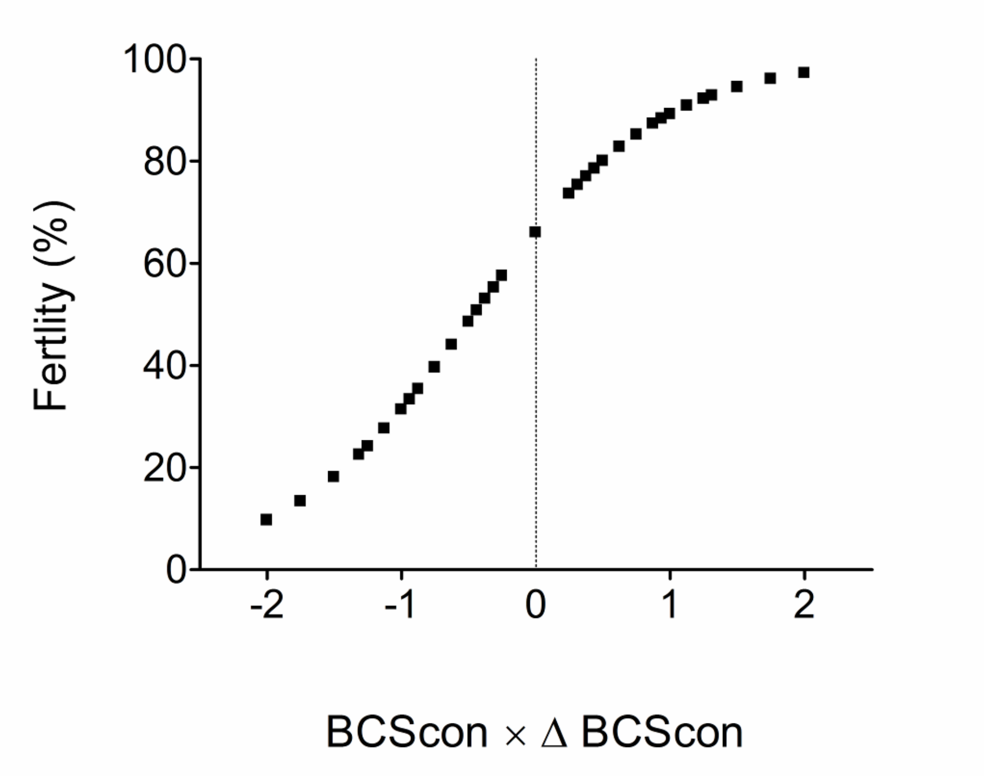

Figure 3.2.1 – Back transformed fertility results (as the probability of a foal born alive) obtained from the logit function Y = 0.6647 (± 0.2763) + 1.4488 (± 0.5331) (BCScon × Δ BCScon), (P = 0.0077). X axis represents the values resulting from the multiplication of BCS at conception (BCScon) by BCS change at conception (ΔBCScon).

For a better understanding of the meaning of ΔBCScon and ΔBCS3L and their implications on feed management, the energy content of these variations were calculated using the equation proposed by Martin-Rosset et al. (2008), regarding Total Energy Content (TEC) of empty BW (Table 3.2.2). Then the variation of TEC was expressed in percentage of maintenance requirements for a 525 kg adult mare BW evaluated according to INRA recommendations (2012). The negative variation of TEC in non-pregnant mares after the first two estrous cycles, represents a deficit of 6.4% regarding maintenance requirements (INRA, 2012) for a 525 kg reference adult BW, and a deficit of 3.9% during the first three months of lactation. On the contrary, in the mares that became pregnant on foal heat or in the second estrous cycle and produced a live foal, a positive variation was observed for both periods.

Table 3.2.2 – Body condition changes and estimates of total energy content of empty body weight during the first post-partum month and during the first three months of lactation

Periods	Mares that were pregnant after the two first estrous cycles and foaled (n=69)	Non-pregnant mares after the first two estrous cycles (n=41)
Conception:		
BCS con1	3.10	3.17
BCS con2	3.12	3.10
ΔBCScon	0.015	-0.074
Δ TEC[a]con (Mcal / MJ)	7.1 / 29.7	-18.7 / -78.2
Reqm con[b] (Mcal / MJ)	290.3 / 1214.6	290.3 / 1214.6
Δ TEC con/Reqm con (%)	2.4	- 6.4
Lactation:		
BCS 3L1	3.07	3.14
BCS 3L2	3.09	3.03
ΔBCS3L	0.023	-0.085
Δ TEC[a]3L (Mcal / MJ)	10.6 / 44.4	-22.6 / -94.6
Reqm 3L[c] (Mcal / MJ)	580.5 / 2428.8	580.5 / 2428.8
Δ TEC 3L/Reqm 3L (%)	1.8	- 3.9

Abbreviations: BC, body condition; BCS con1, body condition score evaluated before the probable date of fertilization; BCS con2, body condition score evaluated after the probable date of fertilization; BCS 3L1, body condition score evaluated at first month of lactation; BCS 3L2, body condition score evaluated at third month of lactation; BW, body weight; TEC, total energy content of empty body weight.

[a] TEC is calculated from the equation: TEC (Mcal) = 190.1 exp 0.373BCS (R^2=0.993) (Martin-Rosset et al., 2008); Then TEC was transformed in net energy for maintenance (NE_m Mcal) according to INRA system (Vermorel & Martin-Rosset, 1997; Vermorel et al., 1997; INRA, 2012);

[b] Reqm con (Requirement at maintenance for 525 kg reference BW) (INRA, 2012), for a period of 30 days (average period of two successive BC scorings) (see *3.2.3.2*).

[c] Reqm 3L (Requirement at maintenance for 525 kg reference BW) (INRA, 2012), for a period of 60 days (average period between BC scoring at first month of lactation and third month of lactation) (see *3.2.3.2.*).

Global foaling rate for the four-year period was 74.5% and the foaling interval was 368.0 ± 2.84 days. The mean gestation length was 340.3 ± 1.04 days in the 50 mares in which the day of ovulation was precisely recorded.

The age of the mare ($P < 0.01$) and the month of foaling ($P<0.05$) had a significant influence on gestation length. Gestation was longer in younger mares and decreased 0.939 days for each additional year of age (Table 3.2.3). Considering the month of foaling, the shortest gestation lengths were observed at the beginning (Feb) and in the end of the foaling period (May). The longest gestation period was recorded in mares that foaled in March. The gestation length observed in mares that gave birth to a colt was similar to mares that gave birth to a filly.

Table 3.2.3 – Effects of mare age and month of foaling on gestation length (days)

Parameters	Estimates	SEM	P value
Intercepts (month of foaling):			0.0398
February	348.1 a	3.46	
March	356.2 b	4.16	
April	349.8 ab	3.96	
May	348.2 ab	2.97	
Slope:			0.0013
Age (years)	-0.936	0.27	

Abbreviations: SEM, standard error of the mean.

Lower case letters indicate significant differences (Tukey-Kramer adjustment, $P < 0.05$).

The average number of estimated estrous cycles required per conception in the mares that had full term gestations and produced a live foal (n=82) was 1.78 ± 0.12, which includes the foal heat ovulation. The number of estrous cycles per live foal was highly correlated with the foaling interval (0.873; $P < 0.0001$). From these 82 mares, 84.1% became pregnant during the first two estrous cycles and 15.9% in the following ones.

3.2.4.2. Leptin and BCS

Plasma leptin concentrations before and after foaling are shown in Figure 3.2.2. Although with different concentrations ($P < 0.001$), the pattern of change was similar for both foaling seasons. Leptin concentrations increased from the ninth to the 11th month of gestation ($P < 0.05$), decreased to the first month of lactation ($P < 0.01$), and levelled off thereafter.

Body condition score slightly increased from the ninth month of gestation to the first month of lactation ($P < 0.05$), without any significant effect of the foaling season (Fig. 3.2.2). Overall, mean leptin concentrations were correlated with BCS (0.41; $P < 0.0001$). No significant effect was found for leptin concentrations at the ninth and 11th months of gestation and at the first month of lactation, on the number of days from parturition to conception. Also, factors like age of the mare and foaling season did not show any influence on the number of days between foaling and conception. In these mares, leptin concentrations did not influence fertility results in the early postpartum period.

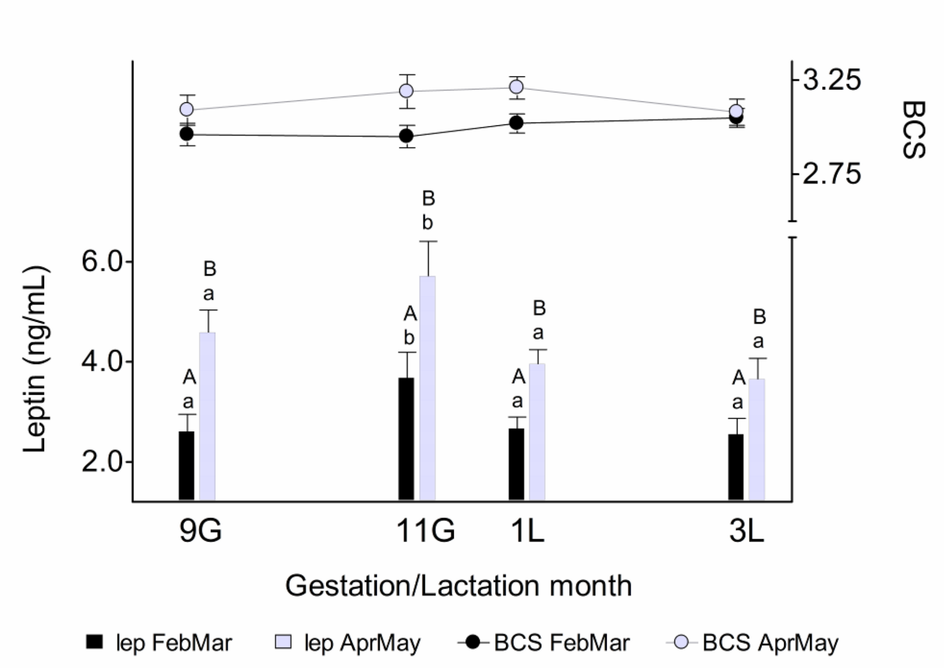

Figure 3.2.2 – Plasma leptin concentrations of mares on late gestation (9G, ninth month of gestation; 11G, 11th month of gestation) and early lactation (1L, first month of lactation; 3L, third month of lactation) and corresponding body condition score (BCS). Capital letters indicate differences between foaling seasons ($P < 0.01$). Lower case letters indicate differences throughout the gestation/lactation period ($P < 0.05$).

3.2.4.3. Growth and development of suckling foals

Instantaneous growth rate (IGR), instantaneous increase of withers height (IIWH) and instantaneous increase of girth (IIG) at 90 days of age were influenced by foaling season ($P < 0.01$), with higher values observed in foals born in FebMar (Table 3.2.4). A significant effect of ΔBCS3L on IGR was observed ($P < 0.05$), with foals that were nursed by mares with negative ΔBCS3L presenting lower growth performances. IIWH and IIG were influenced by mare age, with higher values found in foals nursed by younger mares. Quadratic models were best fitted to describe foals growth and development along the study. At 90 days of age, estimated values for body weight, withers height, girth and cannon circumference were, respectively, 149.3 ± 6.6 kg, 121.4 ± 1.3 cm, 119.5 ± 0.6 cm and 15.1 ± 0.3 cm.

Table 3.2.4 – Effects of mare body condition score changes and mare age on foal instantaneous growth rate and instantaneous increase of withers height, girth and cannon circumference, at 90 days of age.

Variable	Parameters	Estimates	SEM	P value
IGR (kg)	Intercept (foaling season):			0.0106
	FebMar	0.865	0.043	
	AprMay	0.797	0.026	
	Slope:			0.0275
	ΔBCS3L	0.165	0.074	
IIWH (cm)	Intercept (foaling season):			0.0014
	FebMar	0.180	0.006	
	AprMay	0.168	0.004	
	Slope:			0.0024
	Age of the mare (yr)	-0.002		
IIG (cm)	Intercept (foaling season):			0.0067
	FebMar	0.278	0.014	
	AprMay	0.257	0.008	
	Slope:			0.0034
	Age of the mare (yr)	-0.003	0.001	
IICC (cm)	Intercept (foaling season):			0.0740
	FebMar	0.024	0.002	
	AprMay	0.022	0.001	
	Slope:			0.0615
	Age of the mare (yr)	-0.0002	0.0001	

Abreviations: IGR, Instantaneous growth rate at 90 days of age; IIWH, Instantaneous increase of withers height at 90 days of age; IIG, Instantaneous increase of girth at 90 days of age; IICC, Instantaneous increase of cannon circumference at 90 days of age; ΔBCS3L, Mare body condition score change throughout the first three months of lactation.

3.2.5. Discussion

3.2.5.1. Reproductive parameters

These results revealed a pronounced effect of nutritional status at conception on the fertility of mares bred in early postpartum. Mares that were increasing their BC at conception and, therefore, apparently in a positive energy balance, presented higher fertility, confirming the observations made by Henneke et al. (1984). Besides extra feeding costs, high BCS did not appear to adversely affect postpartum reproductive performance in the multiparous mare of other light breeds (intervals from foaling to first cycle ovulation, inter-ovulatory interval and conception rates) when compared to a moderate degree of body fat (Kubiak et al., 1989; Cavinder et al., 2009). However, regardless the BC of the mares, any negative change in BCScon was associated with fertility values under 66%.

In the experiment by Henneke and collaborators (1984), a reduction of 2.8 in a one to nine point scale (Henneke et al., 1983) throughout the 90 days after foaling were also associated to higher pregnancy losses. Nevertheless, according to the model obtained in our study, the detrimental effect of negative changes in BCScon on fertility outcome is more pronounced the higher the BC of the mare is. According to Godoi et al. (2002), the negative energy balance during lactation is a major factor for follicular growth impairment. Also, changes in metabolic hormones associated with a negative energy balance (decrease in gonadotrophins secretion, circulating levels of insulin, IGF-I, leptin and increased circulation levels of GH) are involved in ovarian dysfunction (see Guillaume et al., 2006, for a review). We could speculate that a more intense mobilization of adipose reserves in fatter mares would influence to a large extent the release of some of these hormones, leading to a higher negative effect on fertility.

In addition, a relationship between the age of the mare and ΔBCScon was found, with older mares presenting higher negative BCS changes at conception. According to the prediction model, this observation points out to lower fertility in older mares. In fact, age is one of the factors that are extensively reported in literature as having a negative effect on the size of the pre-ovulatory follicles and on the reproductive performance of the mare (Davies Morel et al., 2010; Katila et al., 2010). Since it was suggested that the follicle size is associated with low oocyte viability, smaller pre-ovulatory follicles may partially account for the poor reproductive performance of older mares (Davies Morel et al., 2010). In the present study, there was a trend for lower BCS in older mares, remaining unclear if there was also an additional influence of BC on the follicle size of this group of mares.

Gestation length of sport and leisure light breeds has been widely studied. Mean values range from 332 days in the Arabian breed to 344 days in Thoroughbred (reviewed by Satué et al., 2011a). To the best of our knowledge, there only exists an earlier study regarding gestation length in the Lusitano breed, which refers a mean value of 344 days (Barbosa & Vidal Abreu, 1986). However, since that work was done before the current use of ultrasound scanning for monitoring ovulation, the gestation length obtained by Barbosa & Vidal Abreu (1986) was calculated from the last mating to parturition. The value found in the present study for the Lusitano (340.3 ± 1.04 days), falls within the range observed for the other breeds and could be considered more accurate, because it was calculated from ovulation to foaling.

The influence of the age of the mare on gestation length is not consensual among authors. While no effect of mare's age was observed on Thoroughbred and Quarter Horse (Davies Morel et al., 2002; Ferris & McCue, 2011), a significant influence was reported for Spanish Purebred and Arabian breeds, with longer gestation lengths in both younger (4-7 years) and older mares (> 13 years) (Valera et al., 2006; Satué et al., 2011b). The results obtained in our study were similar in what concerns younger mares, but gestation length decreased as mares got older. In fact, older mares present endometrium and placenta degenerative changes resulting in vascular alterations in fetal-maternal interface (Abd-Elnaeim et al., 2006), a condition that may result in reduced oxygenation of the fetal tissues and contribute to an early delivery, like in humans (Salafia et al., 1995; Hayes et al., 2012).

Changes in environmental and climate conditions during the reproductive season may justify the influence of the season or month of foaling on gestation length. In fact, there was no effect of foaling season on gestation length, but the gestation of mares foaled in February was shorter than the gestation of those foaled in March. Shorter gestation lengths were also observed for mares that foaled early in the year (Davies Morel et al., 2002; Ferris & McCue, 2011), as well as for mares foaling at the end of the season (May, June and July) (Davies Morel et al., 2002; Heidler et al., 2004). It has been suggested that the increase in day length and environmental temperature could have an effect on the duration of gestation. Also a relationship with the nutritional status of the mare, associated to grass availability and quality, was hypothesized, with mares attempting to bring the time of parturition to the ideal feed conditions in the early spring (Davies Morel et al., 2002). This would justify the longest gestations observed in March, but not the shorter duration found in February. Although other authors referred to a significant influence of gender of the foal on gestation length, with longer pregnancies for colts (Davies Morel et al., 2002; Valera et al., 2006; Satué et al., 2011a), no effect was observed in the current work. This could be possibly explained by the relatively low number of gestations included in the gestation length analysis (n=50).

It is recognized that different types of mating and different reproductive status of the mare influence reproductive performance (Langlois & Blouin, 2004; Katila et al., 2010). In Thoroughbred mares, it was reported a mean number of 1.86 served estrous cycles per live foal, with in-hand natural mating (Hemberg et al., 2004). But other mean values (1.64 and 1.4 cycles) were referred in studies that included mares of different reproductive status (lactating, barren and maiden) and bred by AI (Henneke et al., 1984; Camillo et al., 1997). The average number found in our study (1.78 ± 0.12 cycles) only concerns foaled and lactating mares, but includes different mating types. The use of more than three cycles per conception was only observed in mares that foaled in FebMar. This is justified by the fact that early foaling mares had more opportunities to become pregnant during the reproductive season.

Lower pregnancy rates are often observed in mares bred on foal heat when compared with mares bred on later postpartum estrous periods (Ginther, 1992; Camillo et al., 1997; Morris & Allen, 2002). In fact, in a recent study with Thoroughbred mares, where the influence of postpartum day of mating on foaling rate was investigated, a very low percentage (7%) of mares bred between 7 and 14 days postpartum was reported (Blanchard et al., 2012). However, the results found in our study contrast these observations, because the majority (52.4%) of foaled mares became pregnant during the first estrous cycle.

3.2.5.2. Leptin and BCS

An association between leptin secretion and reproductive function in the mare was first suggested by Fitzgerald & Macmanus (2000). Since then, several authors highlighted the importance of leptin as a link between nutritional status, reflected by body fat reserves and therefore energy availability, and reproductive activity (Gentry et al., 2002; Gastal et al., 2004; Ferreira-Dias et al., 2005). In the present study, changes in leptin concentrations from late gestation to early lactation followed the same pattern reported for other horse breeds. Higher leptin concentrations were observed in the last weeks of gestation, decreasing after foaling and levelled off thereafter (Heidler et al., 2003; Berg et al., 2007; Romagnoli et al., 2007). Similar changes were also reported in humans (Haugel-de Mouzon et al., 2006), rats (Garcia et al., 2000) and dairy cows (Block et al., 2001). Despite the overall positive correlation between BCS and leptin concentrations observed in our study, the increase in leptin from the 9th to the 11th month of gestation was not observed for BCS. Since leptin and its receptor were found in placental tissues from humans, rodents and farm animals, it was suggested that the placenta, rather than maternal adipose tissue, makes a substantial contribution to the rise in maternal leptin concentrations before parturition (Ashworth et al., 2000; Haugel-de Mouzon et al., 2006). Moreover, it was also hypothesized that the reduction in leptin concentrations after foaling would promote increased feed intake as a means to avoid an energy deficit throughout early lactation (Berg et al., 2007).

Foaling season revealed to have a clear influence on leptin peripartum concentrations, with consistently lower values in FebMar foaling mares. However, it seems that the levels of leptin observed in mares of both foaling seasons were sufficient to display a normal reproductive activity, since there was no effect of leptin concentrations on fertility results regarding the first two estrous cycles. Apparently, the lower values of leptin concentrations observed in FebMar foaling mares are in agreement with the range of values observed by Cavinder et al. (2009), in a study where the endocrine profile of fat versus moderately conditioned mares after foaling was investigated. According to these authors, mares maintained in a BC score of 5 or above (in a 1-9 scale) had a similar reproductive efficiency. Since no differences were found between leptin concentrations, they also concluded that mares with a BCS 5-6 have enough body fat reserves to be reproductively sound. Another study carried out in lactating mares of different breeds in order to determine the possible impact of hyperleptinemia on rebreeding success, also revealed that higher leptin concentrations throughout this stage had no effect on rebreeding results (Huff et al., 2008). Nevertheless, further studies on specific effects of leptin on fertility, should include more frequent sampling in order to strengthen the findings obtained in the present study.

3.2.5.3. Growth and development of the suckling foals

Likewise for other breeds (Saastamoinan, 1990; Pagan et al., 2006), the influence of foaling season on the growth and development of the suckling foal was previously reported for the Lusitano (Fradinho et al., 2012). The results observed in the present study confirm this effect and reinforces the evidence that better growth and development performances were obtained for foals born in FebMar when compared with AprMay foals, on our extensive production systems, where grass availability and quality considerably decrease after May. Although the BCS3L of mares does not seem to have affected foal growth and development at 90 days of age, foals that were nursed by mares with negative BCS changes through these first months of lactation presented lower growth performances. This observation contrasts with the findings of Henneke et al. (1984), where foal weights at 90 days of age were similar for foals nursed by mares in different energy balance conditions after foaling (increasing, maintaining and decreasing BCS).

Interestingly, an effect of mare age on instantaneous increase of withers height and girth at 90 days was observed, with higher values for foals nursed by younger mares. Hintz et al. (1979) also reported an effect of mare age on the growth rate of Thoroughbred foals. However, on that study, foals which dams were younger than 7 years of age were lighter, shorter and had smaller cannon bones than those from mares with ages between 7 and 11 years. Nevertheless, in the same study, foals from mares older than 15 years presented again lower values of growth measurements (weight, withers height and cannon bone) than foals born from middle aged mares (7-11 years). We can speculate if some eventual differences in milk yield and composition between younger and older mares may justify these results, even though this was not investigated. Since it was recognized that the growth of the foal is affected by body reserves of their dams (Martin-Rosset & Young, 2006), an additional explanation could be the trend for a negative correlation between mare age and BCS throughout the lactation period observed in our study (-0.190; $P = 0.062$).

At 90 days of age, the estimated values for foal body weight were in the range reported by Henneke et al. (1984), but were lower than those observed by Hintz et al. (1979) for 87 days old Thoroughbred foals (165 kg for colts and 163 kg for fillies), which could be justified by the inherent differences between breeds and production systems.

3.2.6. Conclusions

This study indicates that reproductive performance of the Lusitano broodmare in extensive systems is clearly influenced by the nutritional status in the early postpartum period. ΔBCScon had a strong effect on fertility outcome of the first two estrous cycles after foaling, being highly impaired by BCS negative changes, whatever the BCS was. On the contrary, best fertility results were obtained with positive and greater BCScon changes. Despite the effect of foaling season on leptin peripartum concentrations, the observed values seem to be sufficient to allow for a normal reproductive activity, since there was no effect of leptin concentrations on fertility regarding the first two estrous cycles. Foal growth performance appears to be influenced by mares BCS changes during the first three months of lactation with lower values obtained in foals which dams presented negative ΔBCS throughout this period. Thus, overall results suggest that foaled mares should be gaining body reserves throughout the early postpartum period in order to enhance fertility and support an adequate milk production for the growth of the suckling foal.

CHAPTER IV - *THE FOAL: GROWTH AND DEVELOPMENT*

4. Growth and development of the Lusitano horse managed on grazing systems

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Contribution of Maria J. Fradinho to this article:
Maria J. Fradinho performed the field work, analyzed the data, interpreted the results and wrote the manuscript.

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4.1. Abstract

The main objective of the present study was to characterize the growth patterns of Lusitano horse managed on grazing systems from birth to 42 month of age. A total of 4 759 records for body weight (BW), withers height (WH), girth (G) and cannon circumference (CC) were obtained from 121 Lusitano foals (62 colts and 59 fillies) born and raised at four stud-farms. Data were regularly collected between birth and 42 months of age, when foals began to be ridden. Several sigmoid growth functions were adjusted using the NLIN procedures of SAS. The Richards equation $y = A(1 - b.\exp(-kt))^M$ was chosen for further analysis because it was the best fit model for all the variables. Growth rates (ADG, kg/d or cm/d) were obtained from the first derivative of the equations. The influence of season was examined and the effect of gender was tested. The mean mature BW was 552.4±22.0 kg and the average mature sizes were 158.6±0.8 cm, 197.8±3.6 cm and 19.9±0.2 cm, respectively for WH, G and CC. Proportions (%) of mature BW at 6, 12, 18, 24, 30, 36 and 42 months of age, according to the Lusitano breed standard were, respectively, 42, 58, 69, 77, 84, 89 and 93%. The proportions (%) of mature WH for the same ages were 82, 88, 92, 94, 95, 96 and 97%. Concerning BW, Lusitano showed a slower growth rate, comparable with the moderate growth proposed for other sport breeds. In contrast, the WH growth rate was similar to those presented by early maturing breeds. A clear sexual dimorphism was observed on all variables, with higher maturity values for males (P<0.0001). The present study provides a comprehensive overview on growth patterns of the Lusitano horse managed in grazing systems under Mediterranean climate conditions. Further studies with controlled feeding levels should be done in order to verify the potential growth response of this breed.

Keywords: body measures, body weight, grazing systems, growth, Lusitano horse, nonlinear models.

4.2. Introduction

The growth pattern of each component of an animal - whether cell, tissue, organ or the whole body – is highly dependent on the genetic background and environmental factors (Fitzhugh, 1976). Among these factors, breeders' goals and local management, including feeding practices, has been shown to have a great influence on the growth patterns of the horse (Bigot et al., 1987; Thompson et al., 1988b; Ott and Kivipelto, 2002; Pagan, 2005).

In horse feeding systems, nutrient requirements and allowances of growing animals have been establish based on age, body weight (BW), expected mature BW, and growth rate (average daily gain) (NRC, 2007; INRA, 2011). Therefore, it is of major importance that accurate growth curves are known.

Growth and development of Thoroughbreds is quite well documented, both in northern and southern hemispheres (Hintz et al., 1979; Thompson, 1995; Pagan et al., 1996; Staniar et al., 2004a; Brown-Douglas et al., 2005; Kocher & Staniar, 2013; Onoda et al., 2011; 2013), but limited studies are available for long life periods in other breeds (Bigot et al., 1987; 1988; Saastamoinen, 1990; Trillaud-Geyl et al., 1992; Santos et al., 1999; Valette et al., 2008). Even within the same breed, the differences observed in the patterns of growth between horses raised in different geographical regions emphasize the importance of reference growth curves based on local management conditions (Brown-Douglas & Pagan, 2006; Morel et al., 2007).

The Lusitano horse is the most important autochthonous equine breed in Portugal, with approximately 5000 registered breeding mares around the world (Vicente et al., 2012; 2014). Given the behavioral and functional characteristics of this ancient breed, Lusitano has become more popular as a sport and leisure horse. Although raised in several countries, most Lusitano stud farms in Portugal are traditionally based on extensive grazing systems. In these systems, mares and foals are bred outdoors throughout the year, with pasture being an important part of their diets. However, in Mediterranean regions herbage production and quality is highly dependent on climate conditions (Miraglia et al., 2006; 2008). When pasture resources are scarce, supplementary feeds are often provided, but farm practices vary widely (Fradinho et al., 2013).

Growth and development of the Lusitano horse have been studied until one year of age (Fradinho et al., 2008) and the effect of the birth season was studied on the suckling foal (Fradinho et al., 2012; 2014). In these short term studies, growth patterns were described using linear regression models, including the quadratic effect of time. However, for longer periods and for larger volume of data, models which are nonlinear in their parameters adjust better to data and, usually, have an easier biological interpretation (Fitzhugh, 1976). Nonlinear functions have been extensively used to fit size-age relationships in Thoroughbreds, French Trotters, Selle Français and Pantaneiro horse (Santos et al., 1999; Staniar et al., 2004a; Valette et al., 2008; Kocher & Staniar, 2013), but to the best of our knowledge, there are no studies on longer periods aiming to determine growth curves for the Lusitano breed.

Therefore, the main objective of the present study was to characterize the growth patterns of Lusitano horses managed on grazing systems, concerning body weight (BW), withers height (WH), girth (G) and cannon circumference (CC), from birth to 42 month of age.

4.3. Materials and Methods

The protocol of this study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Lisbon, Portugal. All the animals were handled with care during the experimental procedures.

4.3.1. Animals and Management

A longitudinal study was conducted in four stud-farms located in the main region of Lusitano breeding, the southern area of Portugal (Latitude 38º88' to 39º29' N and Longitude 07º67' to 08º87'W). A total of 4759 records for BW, WH, G and CC were obtained from 121 Lusitano foals (62 colts and 59 fillies) during a period of 4 years (2006 to 2009). Data were periodically collected from birth to 42 months of age, when foals were broken in and begin to be ridden.

All animals were kept on pasture throughout the study and had *ad libitum* access to water. Floristic composition was typical of natural rainfed pastures of Mediterranean areas with a high biodiversity, but the dry matter production along the year was quite dependent on climate conditions. Detailed characterization and composition of these pastures has been previously described (Fradinho et al., 2013).

The foaling season occurred between January and May (Jan, n=8; Feb, n=43; Mar, n=28; Apr, n=30; May, n=12; mean date of birth 15th March±33d). Until weaning, mares of two stud-farms were supplemented once a day with commercial compound feeds and with grass hay or cereal straw, according to animals' lactation stage and pasture availability. On the other two stud-farms mares were only supplemented with grass hay in periods when pasture was scarce.

Foals were not creep fed, although some of them had access to their dam´s concentrate. After weaning, which occurred on average at seven months of age (208±35d, mean±SD), foals were group-fed in large paddocks, in all the four stud-farms, during an adaptation period (one to three months) with compound feeds and grass hay, and returned to pasture afterwards. After that, and according to pasture availability, foals were also supplemented with compound feeds and/or grass hay, but farm practices varied widely.

A standardized herd health schedule with routine vaccination and deworming programs was practiced in the four stud-farms.

4.3.2. Data collection

Body weight and body measurements were assessed monthly in the first year after birth, every two months in the second year, and every three months from 24 to 42 months of age. BW was determined using a portable electronic scale (Iconix, FX15, New Zealand), which accuracy was regularly checked. On the same day as weighing, WH was measured with a standard measuring stick from the ground to the highest point of the withers, and G (circumference of the girth behind the elbow) and CC (circumference taken at the midsection of the cannon bone) were measured with a flexible measuring tape. In order to estimate the mature body size of the dams and sires of foals in the present study, mares were weighed after foaling (30 to 60 days) and WH was determined. It was not possible to get the BW of sires, because they were not present at the studs. However, the individual WH of the sires was obtained from the records included in the Lusitano studbook. All measurements were taken at a similar time of day and by the same operator.

4.3.3. Data analysis

On a first approach, several sigmoid growth functions were adjusted to BW, WH, G and CC data sets using the NLIN procedures of SAS (SAS 9.3 Institute Inc., Cary, NC, USA). The growth functions corresponded to Brody (Eq.(1)), Logistic (Eq.(2)), Gompertz (Eq.(3)), von Bertalanffy (Eq.(4)) and Richards (Eq.(5)) models, where the variable y is described as a function of age t:

$$y = A(1 - b.\exp(-kt)) \qquad (1)$$

$$y = A(1 + b.\exp(-kt))^{-1} \qquad (2)$$

$$y = A.\exp(- b.\exp(-kt)) \qquad (3)$$

$$y = A(1 - b.\exp(-kt))^3 \quad (4)$$

$$y = A(1 - b.\exp(-kt))^M \quad (5)$$

The parameters of these equations can be biologically interpreted. Thus, *A* is the asymptotic value of *y* as age (*t*) approached infinity, and is commonly interpreted as the mean mature size; *b* is a scaling parameter that adjusts for situations where y_0 and/or t_0 do not equal to 0 (for example, when only postnatal observations are available and t_0 is taken as birth); *k* is a maturing index, establishing the earliness with which *y* approaches *A* (large *k* values indicate early maturing individuals and small *k* values indicate late maturing individuals); *M* determines the point of inflection where the estimate growth rate changes from an increasing to a decreasing function (for $0 < M < 1$, *M* is undefined) (Richards, 1959; Brown et al., 1976; Fitzhugh, 1976; Perotto et al., 1992; Staniar et al., 2004a).

In nonlinear models the coefficient of determination (R^2) is not readily defined. Therefore in the present study, R^2 correspond to a pseudo R^2, calculated as: 1 – (SS(Residual) / SS(Total $_{corrected}$)). To fit the nonlinear regression models, the Marquardt iterative method was used.

For each body measure, the first derivative of Richards equation was calculated with respect to time ($\delta y/\delta t$), expressing the instantaneous rate of gain (average daily gain (ADG), kg/d or cm/d) at time *t* (*t* = days of age): $y' = MAkb.\exp^{(-kt)} (1 - b.\exp^{-kt})^M (1 - b.\exp^{-kt})^{-1}$.

In order to evaluate the influence of the season of the year on growth rates, individual body measures and correspondent ADG were also represented in a different time scale. Therefore, individual data points were plotted based on the day of year on which measurements were done, considering zero as the 1st of January of the birth year. For this purpose, the second and the third years started, respectively, at days 366 and 731. This methodology was previous used by Kocher & Staniar (2013).

The effect of gender was also analyzed, using the sum of squares reduction test. The effect of stud-farm will be covered and discussed in another study. Statistical significance was assumed when $P<0.05$.

4.4. Results

Convergence criterions were met for the five growth functions. The parameter estimates of the nonlinear growth models are summarized in Table 4.1. Because Richards's equation (Eq. 5) was the best fit model for all the variables, it was chosen for further analysis. Also, the asymptotic value found for BW and WH in the Richards model seemed to be the most accurate, compared to average values for mature BW and WH given in the breed standard (APSL, 2010).

Table 4.1 – Parameter estimates of the nonlinear growth models fitted to body weight, withers height, girth and cannon circumference-age data sets in Lusitano horses.

Measure [a]	Model	Parameters [b]				R^2 [c]	RSD [d]
		A (± SE[e])	*b* (± SE[e])	*k* (± SE[e])	*M* (± SE[e])		
BW (kg)	Brody	470.3 ± 4.9	0.845 ± 0.004	0.0022 ± 0.0001	-	0.933	29.5
	Logistic	425.1 ± 2.9	3.11 ± 0.06	0.0053 ± 0.0001	-	0.916	33.1
	Gompertz	438.6 ± 3.4	1.56 ± 0.18	0.0038 ± 0.0001	-	0.925	31.3
	von Bertalanffy	445.9 ± 3.7	0.421 ± 0.004	0.0032 ± 0.0001	-	0.928	30.7
	Richards	552.4 ± 22.0	0.986 ± 0.006	0.0010 ± 0.0002	0.570 ± 0.028	0.936	28.8
WH (cm)	Brody	153.3 ± 0.3	0.320 ± 0.002	0.0043 ± 0.0001	-	0.947	3.47
	Logistic	152.5 ± 0.3	0.444 ± 0.004	0.0054 ± 0.0001	-	0.942	3.63
	Gompertz	152.8 ± 0.3	0.376 ± 0.003	0.0049 ± 0.0001	-	0.945	3.55
	von Bertalanffy	153.0 ± 0.3	0.119 ± 0.001	0.0047 ± 0.0001	-	0.945	3.53
	Richards	158.6 ± 0.8	0.942 ± 0.012	0.0018 ± 0.0002	0.164 ± 0.008	0.954	3.23
G (cm)	Brody	176.2 ± 0.5	0.461 ± 0.003	0.0035 ± 0.0001	-	0.947	5.72
	Logistic	173.8 ± 0.5	0.765 ± 0.008	0.0050 ± 0.0001	-	0.938	6.20
	Gompertz	174.8 ± 0.5	0.590 ± 0.005	0.0043 ± 0.0001	-	0.943	5.96
	von Bertalanffy	175.2 ± 0.5	0.181 ± 0.001	0.0040 ± 0.0001	-	0.944	5.88
	Richards	197.8 ± 3.6	0.985 ± 0.004	0.0008 ± 0.0001	0.204 ± 0.007	0.958	5.08
CC (cm)	Brody	19.5 ± 0.1	0.343 ± 0.004	0.0042 ± 0.0001	-	0.862	0.80
	Logistic	19.4 ± 0.1	0.490 ± 0.007	0.0053 ± 0.0001	-	0.859	0.81
	Gompertz	19.4 ± 0.1	0.408 ± 0.005	0.0048 ± 0.0001	-	0.861	0.80
	von Bertalanffy	19.4 ± 0.1	0.128 ± 0.002	0.0046 ± 0.0001	-	0.861	0.80
	Richards	19.9 ± 0.2	0.893 ± 0.036	0.0022 ± 0.0003	0.215 ± 0.026	0.866	0.79

[a] BW – body weight; WH – withers height; G –girth; CC – cannon circumference.

[b] *A* – asymptotic value for BW / WH / G / CC as age approaches infinity (interpreted as mean BW / WH / G / CC at maturity); *b* – scaling parameter that defines the degree of maturity when age = 0 d (intercept on y axis); *k* – maturing index (rate that establishes the spread of the curve along time axis); *M* – determines the point of inflexion of the curve (for $0 < M < 1$, M is undefined).

[c] R^2 correspond to a pseudo R^2, calculated as 1 – (SS(Residual) / SS(Total $_{corrected}$)).

[d] RSD – residual standard deviation.

[e] SE – approximate standard error.

Curve fit adjustments for BW, WH, G and CC using Richards equation and corresponding estimates of ADG as the first derivate of the function are presented in Figure 4.1. Predicted BW, WH, G and CC at birth (considering t = 1 day of age) were, respectively, 52 kg, 100 cm, 85 cm and 12.4 cm. At 1278 days of age (42 months) predicted BW, WH, G and CC were, respectively, 464 kg, 156 cm, 182 cm and 19.7 cm. According to this model, the asymptotic mean mature BW for the Lusitano was 552.4±22.0 kg. The average mature sizes were 158.6±0.8 cm, 197.8±3.6 cm and 19.9±0.2 cm, respectively for WH, G and CC.

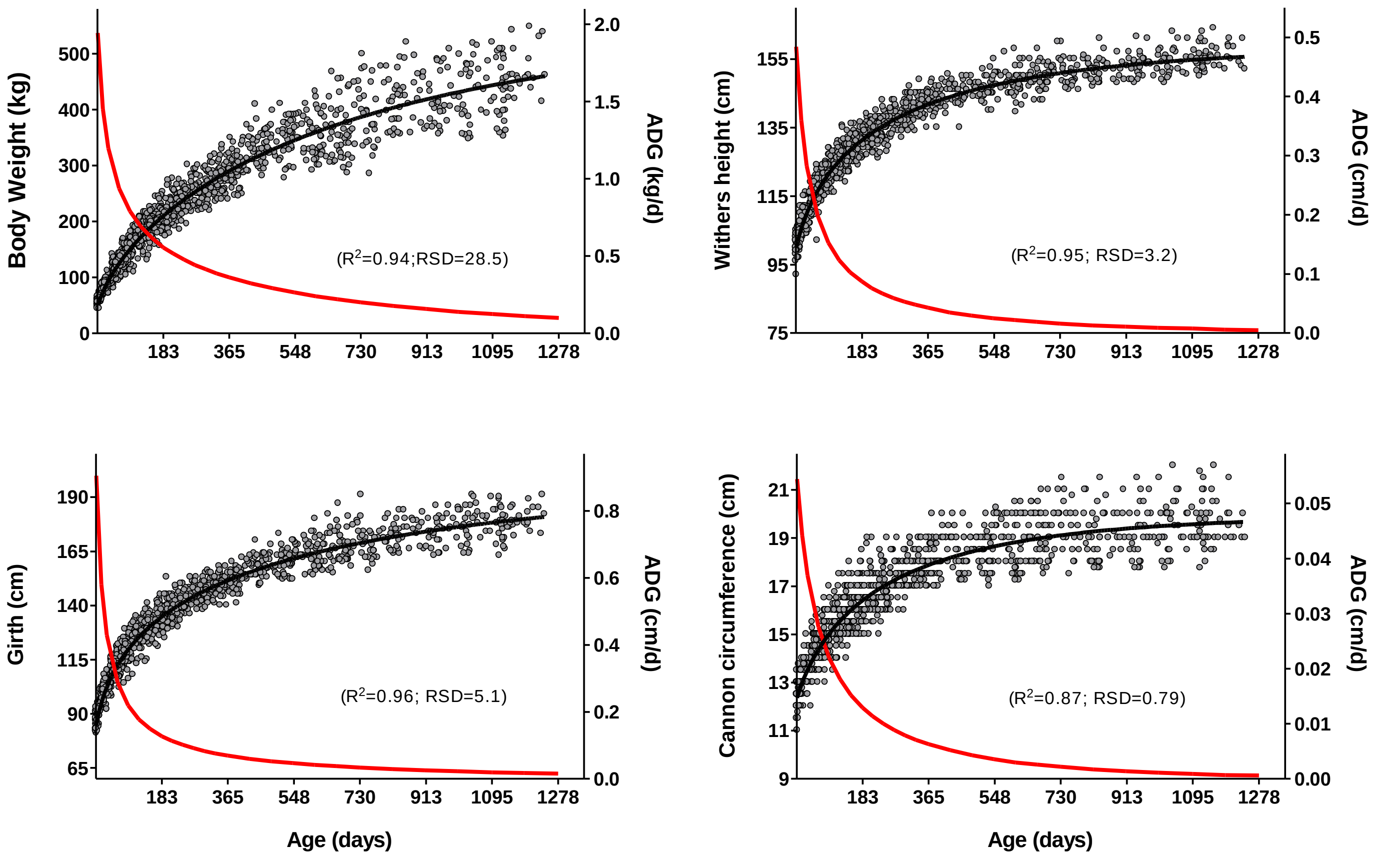

Figure 4.1 – Best fit adjustments (black lines) for individual data points regarding body weight, withers height, girth and cannon circumference using the Richards equation. Red line represents ADG (kg/d or cm/d) for each variable. In nonlinear models, R^2 correspond to a pseudo R^2 calculated as 1 – (SS(Residual) / SS(Total $_{corrected}$)).

When individual ADG were plotted against the corresponding day of year, an influence of the season on growth rates was observed (Figure 4.2). This influence was particularly noticeable in BW and G ADG from the second spring onwards. Apparently, WH growth rate was not so influenced by the season and, although with a bigger dispersion, CC followed a similar pattern (Figure 4.2).

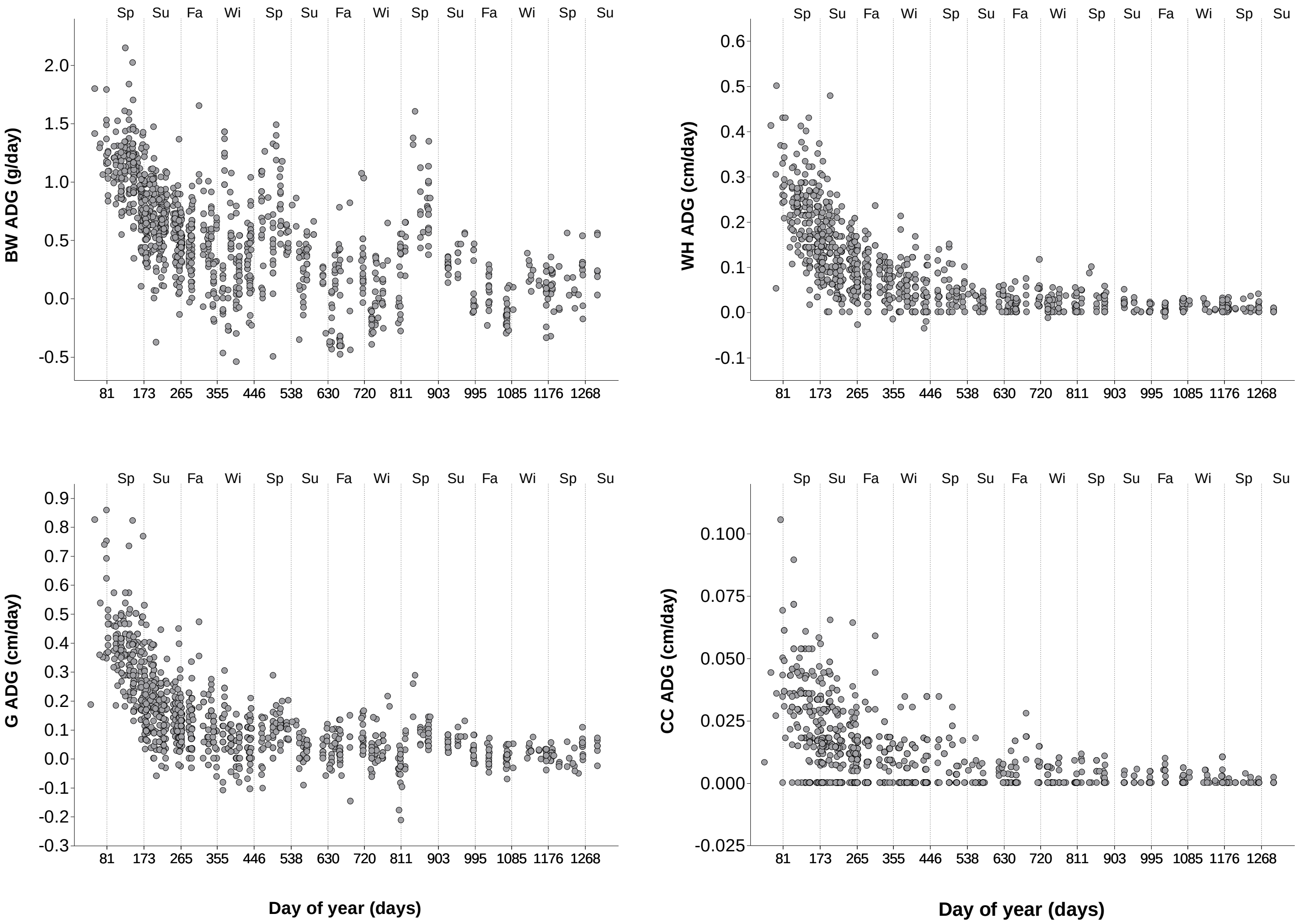

Figure 4.2 – Average daily gains for body weight (BW ADG), withers height (WH ADG), girth (G ADG) and cannon circumference (CC ADG) based on day of year, considering zero as the 1st of January of the birth year. The second and the third years start, respectively, at 366 and 731 days. Each dotted line represents the beginning of a season based on spring (Sp) (March 20th) and fall (Fa) (September 23rd) equinoxes and summer (Su) (June 21st) and winter (Wi) (December 21st) solstices in northern hemisphere.

Estimated ADG for BW at standard age-periods were compared with values published for other sport horses and for race horses in Table 4.2.

Table 4.2 – Comparison of body weight average daily gain (g/day) estimates at standard age-periods for the Lusitano horse with published values for a moderate growth in sport horses and for Thoroughbreds.

	Measure[a]	Age-periods (months)				
		3 - 6	6 - 12	18 - 24	30 - 36	36 - 42
Lusitano[b]	ADG (g/d)	669	443	233	141	111
Sport horses[c]		650-750	400-500	300-350	50-100	50-100
TB[d]		939	681	247	91	51

[a] ADG – average daily gain (g/day).

[b] ADG values for Lusitano were estimated from the resolution of the first derivative of Richards function obtained in the present study.

[c] ADG range values indicated for a moderate growth of sport horses expected to mature at 500 kg (INRA, 2011).

[d] TB – Thoroughbred; ADG values for TB were estimated from the resolution of the first derivative of Richards function obtained by Kocher & Staniar (2013).

The proportions (%) of mature BW and WH at standard ages were also compared with published data for other breeds (Table 4.3). For this purpose, 500 kg was considered as the average mature BW for the Lusitano, given the mature BW indicated in the standard of the breed, and also the big variation observed for the mares' average BW after foaling (520±52.8 kg, mean±SD). The proportions of mature WH were calculated in relation to the real average withers height (WH) of the dams and sires of our study (161±2.2 cm, mean±SD). The proportions of mature BW for the Lusitano at 6, 12, 18, 24, 30, 36 and 42 months of age were, respectively, 42, 58, 69, 77, 84, 89 and 93 %.

Table 4.3 – Proportions (%) of mature body weight and withers height at standard ages for the Lusitano and other horse breeds.

	Measure[a]	Age (months)						
		6	12	18	24	30	36	42
Lusitano [b]	BW (%)	42	58	69	77	84	89	93
NRC, 2007 [c]		43	64	78	86	91	94	97
Coenen, 2001[d]		47	64	76	85	92	98	-
INRA, 2012 [e]		48	72	86	94	97	100	100
INRA, 2012 [f]		44	60	76	88	94	97	100
Kocher & Staniar, 2013 [g]		45	67	80	88	93	96	97
Lusitano [h]	WH (%)	82	88	92	94	95	96	97
Kocher & Staniar, 2013 [i]		80	87	91	94	96	97	98

[a] BW – body weight; WH – withers height.

[b] Percentage of maturity considering the average mature BW (500 kg) indicated in the Studbook of the Lusitano horse (APSL, 2010).

[c] Percentage of maturity considering the equation proposed by NRC (2007), for horses expected to mature at 500 kg. This equation was drawn with mean data from several breeds.

[d] Percentage of maturity considering the equation proposed by Coenen (2001), for horses expected to mature at 500 kg. This equation was drawn based on a summary of data taken from the literature.

[e] Racing breeds – Percentage of maturity considering the asymptotic value (540 kg for Thoroughbred; 560 kg for French Trotter) obtained by Heugebaert et al. (2010).

[f] Sport breeds - Percentage of maturity considering the asymptotic value (590 kg for Anglo-Arab and for Selle Français) obtained by Heugebaert et al. (2010).

[g] Percentage of maturity considering the asymptotic value (556 kg) obtained for TB (Thoroughbred) by Kocher & Staniar (2013).

[h] Percentage of maturity considering the average mature WH (161 cm) of the mares and sires of the present study.

[i] Percentage of maturity considering the asymptotic value (169 cm) obtained for TB by Kocher & Staniar (2013).

A clear sexual dimorphism was observed for the four variables, with high average maturity values for males in what concerns BW and the other three body measurements (P<0.0001) (Table 4.4).

Table 4.4 – Parameters estimates and standard errors of Richards equation fitted to body weight, withers height, girth and cannon circumference as function of age (days), according to gender in Lusitano breed.

Measure [a]	Sex	Parameters [b]				R^{2} [c]	RSD [d]
		A (± SE[e])	*b* (± SE[e])	*k* (± SE[e])	*M* (± SE[e])		
BW (kg)	males	610.5 ± 39.9	0.982 ± 0.009	0.0010 ± 0.0002	0.61 ± 0.04	0.956	25.4
	females	525.3 ± 25.0	0.988 ± 0.007	0.0010 ± 0.0002	0.54 ± 0.03	0.932	28.3
WH (cm)	males	161.5 ± 1.5	0.944 ± 0.016	0.0017 ± 0.0002	0.17 ± 0.01	0.961	3.06
	females	157.6 ± 1.0	0.945 ± 0.015	0.0017 ± 0.0002	0.16 ± 0.01	0.956	3.10
G (cm)	males	202.9 ± 6.4	0.985 ± 0.006	0.0008 ± 0.0002	0.21 ± 0.01	0.969	4.52
	females	196.3 ± 4.7	0.987 ± 0.005	0.0008 ± 0.0002	0.20 ± 0.01	0.952	5.34
CC (cm)	males	21.4 ± 0.4	0.926 ± 0.035	0.0016 ± 0.0004	0.21 ± 0.03	0.895	0.77
	females	19.3 ± 0.1	0.883 ± 0.048	0.0026 ± 0.0004	0.21 ± 0.03	0.893	0.64

[a] BW – body weight; WH – withers height; G –girth; CC – cannon circumference.

[b] *A* – asymptotic value for BW / WH / G / CC as age approaches infinity (interpreted as mean BW / WH / G / CC at maturity); *b* – scaling parameter that defines the degree of maturity when age = 0 d (intercept on y axis); *k* – maturing index (rate that establishes the spread of the curve along time axis); *M* – determines the point of inflexion of the curve (for $0 < M < 1$, *M* is undefined).

[c] R^2 correspond to a pseudo R^2, calculated as 1 – (SS(Residual) / SS(Total $_{corrected}$)).

[d] RSD – residual standard deviation.

[e] SE – approximate standard error.

4.5. Discussion

The morphological type of the Lusitano horse was quite well studied and described by Oom (1992). Some results of this study were included in the Lusitano breed standard which defines this ancient breed as a “middleweight (around 500 kg), medium-lined, with a sub-convex profile throughout the body (with rounded outlines and with a silhouette that can be fitted into a square)” (APSL, 2010).

According to the genetic type and use, growth period for light breed horses lasts from 3 to 5 years of age for breeds raised in temperate zone (Martin-Rosset et al., 2012). Even though Lusitano breeders believe that this horse reaches its maturity at a later stage, there is no scientific evidence to support this opinion. The results obtained in the present study provides a comprehensive overview on growth patterns of the Lusitano horse managed in grazing systems under Mediterranean climate conditions.

In other breeds, some nonlinear models were used to describe BW and WH growth curves. For BW and WH, Brody and Weibull models were chosen as the best curve fit to describe growth of Hanoverian, Brazilian Showjumper, Thoroughbred, Crossbred and Pantaneiro horses reared in Brasil (Santos et al., 1999; McManus et al., 2010). Also the study of Valette et al. (2008) showed that monomolecular, Gompertz and logistic models were the most precise to predict WH from birth to 3 years of age of sport horses born and raised in Normandy. In contrast, all these simplified models were not considered to be well adapted to describe accurately the very high early growth of equine (Heugebaert et al., 2010). As a result, for long life periods and for large data sets, the most accurate sigmoid model that has been used to study BW and WH growth of Thoroughbreds and other sport horses was the Richards function (Staniar et al., 2004a; Heugebaert et al., 2010; Kocher & Staniar, 2013; Onoda et al., 2011; 2013).

In our study, the Richards function was also the best fit model to describe BW and WH growth of the Lusitano horse, but a detailed characterization of G and CC growth of other breeds, with the application of this nonlinear model was not found in the literature.

The maturing index (*k* parameter of the growth curve) obtained for the Lusitano (0.0010) was close to those of Anglo-Arab (0.0015) and to a lesser extent to Selle Français (0.0019) (Heugebaert et al., 2010). But the *k* value found in our study was much lower than the maturing index found for early matured Thoroughbreds (0.0028) (Kocher & Staniar, 2013). In contrast, the maturing index of WH obtained for the Lusitano was quite similar to the maturing index estimated for Thoroughbreds in the Kocher & Staniar (2013) study, revealing a comparable pattern of skeletal development between the two breeds. The more rapid decline in the estimated growth rate for WH, G and CC observed in Figure 1, also supports the early development of these body segments in contrast to a later maturing rate for BW. It was well established that allometric coefficients of the horse skeleton were much lower than 1, namely the distal bone segments of the limbs (Martin-Rosset, 2005).

There is a lack of information concerning mature BW in the Lusitano. The average value indicated in the breed standard is 500 kg, which is lower than the general asymptotic value found in the present study (552 kg), and lower than the real average BW of the mares after foaling (520 kg). Also a previous study in the Lusitano breed, reported 538.5±36.6 kg as the mature weight obtained in 67 adult males (Fradinho & Abreu, 2006), meaning that, nowadays, the value of the standard could be slightly underestimated.

In contrast, the general mature size found in our study for WH (159 cm), considering male and female together, is in accordance with what others reported in literature for Lusitano. In a recent study aiming at estimating the genetic parameters for morphology, gaits and functional traits in the Lusitano, the mean value of WH, considering 16,955 records on animals (male and female) classified in the period between 1967 and 2009 was 157.8±4.1 cm (Vicente et al., 2014). However, if we considered the asymptotic WH values by gender (161.5 cm for males and 157.6 cm for females), our results were quite in agreement with the values reported by Oom (1992) (160.2 cm for males and 157.0 cm for females), and were slightly above the values defined in the Lusitano breed standard (160 cm for males and 155 cm for females, assessed at 6 years of age).

Body weight ADG estimates at standard age-periods observed for the Lusitano were comparable with the values proposed for moderate growth of sport horses by INRA (2012). The slightly lower values obtained between 18 and 24 months in our study can probably be ascribed to an environmental effect related to the management practices during the second winter. The differences observed between the ADG estimates for the Lusitano and for Thoroughbreds until the 18 months of age, underline the early maturing index of this last breed, which can be associated to different management and feeding practices during the first year. In Thoroughbred breeding systems, weaning occurs earlier (between 4.5 and 6 months of age) than in the Lusitano studs (Thompson, 1995; Brown-Douglas et al., 2005; Morel et al., 2007). In addition, the creep-feeding practice, very common in Thoroughbred breeding has been reported to increase the growth rate of these foals (Thompson et al., 1988b; Coleman et al., 1999), while in the Lusitano systems, creep-feeding is only used by a few breeders and none of these were included in the present study.

Due to the lack of specific growth curves for most horse breeds, some equations were developed in order to predict the BW of a growing horse at a given age, from its mature BW

(Coenen, 2001; NRC, 2007). This methodology allows the prediction of growth rates for horses of different breeds and different mature BW. The comparison between the proportions of mature BW at standard ages obtained for the Lusitano, and the proportions estimated by the equations proposed by Coenen (2001) and NRC (2007) for horses expected to mature at 500 kg, as well as the proportions estimated for other breeds (Heugebaert et al., 2010), emphasize the slower growth rate and the late maturity of the Lusitano breed, managed in extensive grazing systems. A late maturity was also reported for the Anglo-Arab and the Selle Français when compared with Thoroughbreds and French trotters, considering the proportions of mature BW at 24 months of age (Heugebaert et al., 2010).

The proportions of mature WH found for the Lusitano at all considered ages were however similar to those estimated for the Thoroughbred. This observation could be explained by the recognized precocity of bone tissue development, based on the lower coefficients of allometry of the long bones (Martin-Rosset, 2005). In addition, and like other livestock species, when pasture availability is lower and some nutritional shortages are faced, bone tissue appears not be so affected as muscle and adipose tissue, which are more prone to subsequent compensatory growth (Lawrence et al., 2012).

In the present study, the Richards equation was applied to BW and to the other body measures as a continuous growth model. However, the representation of the individual ADG plotted against the day of the year on which were estimated, shows the well-known deviations on growth pattern that were related with seasonal influences. Several studies on horses raised in different geographical regions, have demonstrated that changes in ADG were closely associated with environmental influences and, particularly, with seasonal pasture availability (Bigot et al., 1987; Pagan et al., 1996; Staniar et al., 2004a; Brown-Douglas et al., 2005). In those studies, foals presented reduced growth rates during the winter season which was later compensated in the following spring, with the onset of pasture growth. The seasonal compensatory growth tended to diminish with age (Bigot et al., 1987). In our study, this seasonal influence could be observed in BW and G growth rates but was not so evident in WH and CC growth rates, probably because these measures are directly associated to bone development. It has been shown that the allometric coefficients of bone segments of the limbs increased from 0.56 for cannon bone to 1.25 for the scapula or the pelvis, from birth to three years of age (Martin-Rosset, 2005). Similar seasonal influences were previously observed on short-term growth patterns regarding BW and WH in Thoroughbreds (Kocher & Staniar, 2013).

Some of the growth studies in the horse pointed out for a sexual dimorphism in some body measures which tend to increase with time (Hintz et al., 1979; Saastamoinen, 1990). The same result was observed in the present work, with a significant effect of gender reflected in higher mature values for males, for all the studied variables. The difference between the mature BW for males and females was slightly higher than the 10% value indicated for light breeds (Martin-Rosset, 2005). Nevertheless, the adult average CC reported previously for the Lusitano breed (Oom, 1992) were very similar for females (19.4±0.8 cm), but slightly lower for males (20.1±0.7 cm), when compared with the results of our study. The only references found for G measurements in the Lusitano horse, also indicated lower values (185.7±5.9 cm, Oom, 1992; 188.6±5.3 cm, Fradinho & Abreu, 2006) than the mature G for males and females of our research. In general, we speculated that the higher mature values found for both males and females in our study could be ascribed to an improvement in the traditional feeding practices during the last years and also to the breeders' choices in terms of genetic trends.

4.6. Conclusions

The present study provides a comprehensive overview on growth patterns of the Lusitano horse managed in grazing systems, under Mediterranean climate conditions. The apparent late maturity of this breed, concerning BW, was confirmed by slower growth rates, which were comparable with the moderate growth levels proposed for other sport horses. In contrast, the results of WH growth rate were similar to Thoroughbred data, showing that skeletal development may follow a between-breed similar pattern. Seasonal influences were clearly identified on BW and G growth patterns with ADG changes generally associated with winter and spring time. The identification of periods where growth rates could be negatively affected will help breeders to develop feeding strategies in order to minimize those effects. The detailed characterization of BW, WH, G and CC growth patterns provides innovative information for the Lusitano horse. However, further studies with controlled feeding levels should be done in order to verify the potential growth response of this breed.

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CHAPTER V – *BONE QUALITY ASSESSMENT: QUANTITATIVE ULTRASONOGRAPHY AND BIOMECHANICAL PROPERTIES*

5. Biomechanical properties of the equine third metacarpal bone: *in vivo* quantitative ultrasonography *vs ex vivo* compression and bending techniques

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Contribution of Maria J. Fradinho to this article:
Maria J. Fradinho designed the study, performed the field work, analyzed the data, interpreted the results and wrote the manuscript.

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5.1. Abstract

An experiment was conducted in order to: (1) assess equine cortical bone status *in vivo* by quantitative ultrasonography (QUS); (2) evaluate certain mechanical properties of the third metacarpal bone (McIII) and to characterize its regional variation along the bone; and (3) compare the *in vivo* results with the *ex vivo* mechanical tests on the same bone. Eight adult mares were assessed with a QUS device on the mid-section of the right McIII (dorsal and lateral regions). Cortical bone samples from the dorsal and lateral regions of both McIII were collected *post-mortem*, and tested in compression and bending. Values of speed of sound (SOS) on the lateral region were higher than for the dorsal one (P<0.0001), but they were linearly correlated (r=0.803; P<0.05). In compression, the maximum stress (σ_{max}) was influenced by bone region and bone section, and an interaction was found between these two effects (P<0.01). Samples from the dorsal region were stiffer than samples from the lateral region (P<0.0001), and lower values of the Young's modulus (E) were obtained on the McIII distal sections (P<0.05). Lower bending strength was observed in the distal section of the bone, when compared with the proximal and the mid-section (P<0.01). *In vivo* SOS measurements on the dorsal region were highly related with the E obtained from *ex vivo* compression tests (r^2=0.92; P=0.0002). These results suggest that QUS could be used as a non-invasive method in the assessment of equine cortical bone mechanical properties.

Keywords: horse, third metacarpal bone, quantitative ultrasonography, bone mechanical properties, compression and bending tests.

5.2. Introduction

Most of the equestrian disciplines subject the musculoskeletal system of the horse to a high and complex loading regimen. The use of non-invasive methods that allow for a better understanding about the structure and mechanical properties of bone tissue are thus of particular importance for the sport horse industry.

Quantitative ultrasonography has been widely used for the investigation of bone status in humans. This technique is based on the principle that ultrasound propagation through the bone can be characterized by the velocity of transmission of an ultrasound signal inside the bone, either in transverse (the transmitters and receivers are placed on opposite sides of the bone) or in axial transmission (the ultrasound wave travels parallel to the shaft axis of the bone) (Gugliemi et al., 2009). It is known that the propagation speed of a wave through a solid (c) depends on the characteristics of the medium in which it travels, such as the density (ρ), being proportional to $\sqrt{E/\rho}$. Therefore, the speed of sound (SOS) measured by QUS reflects, in a general way, information about the stiffness of the material (*E* - Young's modulus) (van den Berg et al., 2000) and its density (Prevrhal et al., 2001). In the last decade, several studies have confirmed the usefulness of QUS as a non-invasive and non-ionizing radiation technique in the assessment of bone mineral status and fracture risk evaluation in human bone (Marin et al., 2006; Baroncelli, 2008; Olszynski et al., 2013). The use of QUS in horses to measure superficial cortical bone properties has been reported (Carstanjen et al., 2002; 2003a) and the effect of workload on the McIII failure in racing and training Thoroughbreds was evaluated with this technique (Tabar-Rodriguez et al., 2009).

Another approach to directly assess mechanical properties of the bone is the *ex vivo* destructive mechanical testing of bulk tissue specimen excised from whole bones. Tests of tension, compression, bending or torsion, enable measurement of the intrinsic properties of the tissue such as Young's modulus (*E*) and ultimate or maximum stress (σ_{max}) (Donnelly, 2011; Vaz et al., 2011). While Young's modulus is used to evaluate the stiffness, strength is evaluated by the maximum or yield stress (in compression) and the failure stress (in bending). In the horse, some of these mechanical tests have been used in order to characterize the mechanical properties of cortical bone excised from McIII (Bigot et al., 1996; Les et al., 1997; Shahar et al., 2007). Additionally, *in vivo* studies with strain gauges have shown evidence that the equine McIII is subjected to considerable strain during locomotion. Bending, axial compression, shear and torsion were studied at different gaits (Biewener et al., 1983; Davies et al., 1993; Merrit et al., 2006; Rubin et al., 2013) showing that the McIII is primarily loaded in axial compression (longitudinal to the shaft axis of the bone) at each gait. However, the use of strain gauges for strain measurements is invasive and technically challenging (Yang et al., 2011).

To the best of our knowledge, there are no reports about the comparison between *in vivo* QUS assessment of the McIII and *ex vivo* mechanical properties evaluated on the same bone. Therefore, the aims of this study were: (1) to assess equine cortical bone status *in vivo* by quantitative ultrasonography; (2) to evaluate certain mechanical properties of the McIII with destructive tests and characterize its regional variation along the bone; and (3) to compare the *in vivo* results with the *ex vivo* mechanical tests performed on the same bone.

5.3. Materials and Methods

The protocol of this study was approved by the Ethical committee of the Faculty of Veterinary Medicine, University of Lisbon, Portugal. All the animals were handled with care during the experimental procedures.

5.3.1. Horses

Eight Lusitano mares with an average age of 6.4±2.6 years old (3 years, n=5; 4 years, n=1; 8 years, n=1; 24 years, n=1) and with no history of bone disease were used in this study. Before the measurements, the animals were observed by a veterinarian for any evidence of lameness at the three gaits: walk, trot and gallop, and by external examination of the limbs. All mares belonged to the same stud farm and were managed under the same conditions, with regards to feeding and exercise regimen (free ranging exercise at pasture).

5.3.2. Quantitative ultrasound measurements

Ultrasound measurements were performed with a quantitative ultrasound multisite device (Sunlight EQUS, BeamMed, Ltd., Petah Tikva, Israel) developed to measure the speed of sound in axial transmission mode through bone (Carstanjen et al., 2002). Before any SOS measurements were made, the accuracy of the system was calibrated using a phantom (provided by the manufacturer), to obtain temperature corrected SOS values. Two regions of interest (ROI) were marked with a pen on the mid-section of the right McIII (dorsal and lateral regions), and silicon oil was applied to the cleaned region as a coupling medium, without shaving. Mares were squared-up, standing still on the four limbs, with no sedation. Static measurements with the probe in axial position (parallel to the axis of the bone) were performed in each mare on the two ROIs, both dorsally and laterally (Figure 5.1). In order to obtain the most accurate result, the computer software required 3 to 5 consecutive measurement cycles, searching for the best consistency. The final average value of each SOS measurement was recorded. All measurements were performed by the same operator.

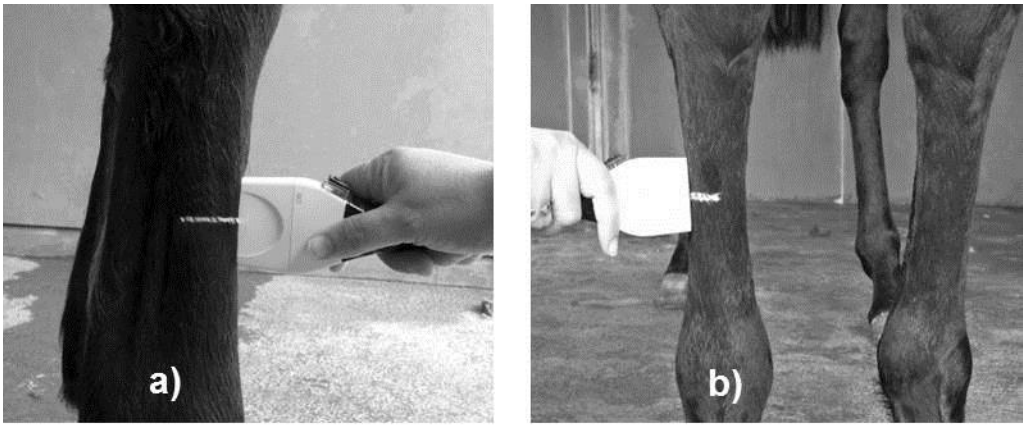

Figure 5.1 – *In vivo* dorsal (a) and lateral (b) speed of sound measurements on the mid-section of the right third metacarpal bone. The horizontal line was drawn for a precise placement of the probe at the regions of interest, indicating the midway between the proximal end of the second and fourth metacarpal bones and the apex of the proximal medial and lateral sesamoid bones.

5.3.3. Mechanical tests

The mares were slaughtered at the abattoir for reasons unrelated to this study. Both forelimbs of each mare were immediately collected distally to the middle carpal joint, wrapped in plastic and stored at -20ºC until sample preparation for mechanical testing.

5.3.3.1. Compression

After thawing at room temperature, all external soft tissue of the forelimbs was meticulously removed and 20 samples of cortical bone were cut with an electric saw from each right McIII (ten from the dorsal region and ten from the lateral region). The specimens were cubes with approximate dimensions of 10 mm side (mean height 9.94 ±0.54 mm and mean sectional area

117.13±2.73 mm^2). Samples were denoted from +5 (most proximal level) to -4 (most distal level) as represented in Figure 5.2. The orientation of the cubes was carefully marked, so that distinct axial, radial and transverse faces could be identified (Figure 5.3). Samples 2 and -2 were kept for a later study on structural evaluation. Compression tests were carried out in an Instron 8502 machine (Instron Corporation, Norwood, Massachusetts, USA), with a load cell of 250 kN and a cross-head speed of 2 mm/min (Figure 5.4.a). Slow cross-head rates are common in bone compression tests (Vale et al., 2013). Since these tests have a short duration, therefore unlikely to dehydrate, no hydration fluid was added. The specimens were placed directly between the steel plates and were compressed in an axial direction corresponding to the longitudinal axis of the bone (Figure 5.4.b). From the load F-displacement Δl curves, it was possible to calculate the stress as $\sigma = F / A$ and the strain $\varepsilon = \Delta l / l_0$, where A is the area of the cross section and l_0 the initial length of the sample.

The parameters taken from the compressive curves are the maximum or yield stress (σ_{max}), maximum strain (ε_{max}), and the slope of the initial elastic regime denoted by the Young's modulus (E). While the Young's modulus is used to evaluate the stiffness, the yield stress assesses compressive bone strength.

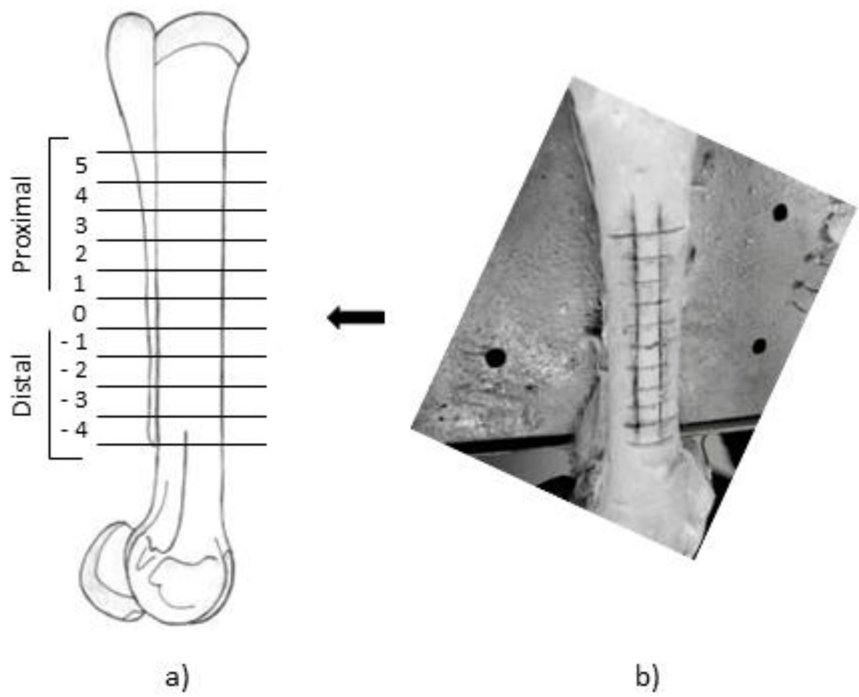

Figure 5.2 - Schematic layout localization (a) and photograph of *post-mortem* sample preparation (b) for compression tests.

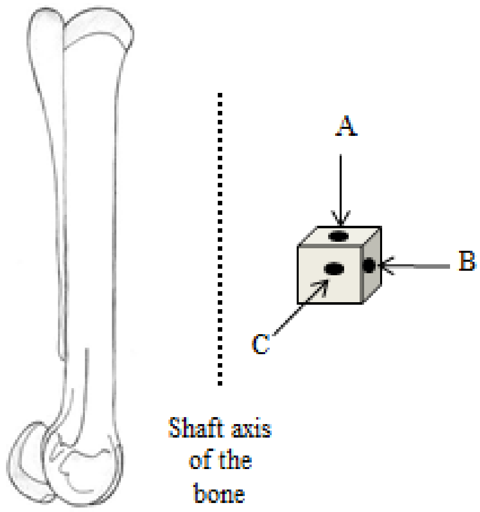

Figure 5.3 - Schematic diagram with orientation and identification of the faces on cube samples for compression: A) axial face; B) radial face; C) transverse face.

5.3.3.2. Bending

The samples for bending tests were obtained from the dorsal and lateral regions of the left McIII bone of each mare. Three sections I, II and III were defined in correspondence to the compression sample notation by: I (from +5 to +3), II (from +2 to -1) and III (from -2 to -4). The three specimens from each region (I, II, III, noted from proximal to distal level) were cut in a rectangular shape with the following size: thickness around 10 mm, width from 10 to 15 mm and length from 40 to 60 mm (average 52.16±8.86 mm).

Three point bending tests were performed in an Instron 5566 universal testing machine (Instron Corporation, Norwood, Massachusetts, USA) (Figure 5.4.c), with a cross-head speed of 0.05 mm/s and with a load cell of 10 kN. Load was applied on the external surface, perpendicularly to the shaft axis of the bone (Figure 5.4.d). The fracture/failure stress σ_f was determined by:

$$\sigma_f = \frac{3}{2} F \frac{L}{bh^2}$$

where F is the maximum load, L is the support span, b is the width and h the thickness of the sample. In the tests, the support span or distance between supports was L=30mm. The failure stress, σ_f, which may also be denoted by maximum or yield stress, evaluates the bending strength.

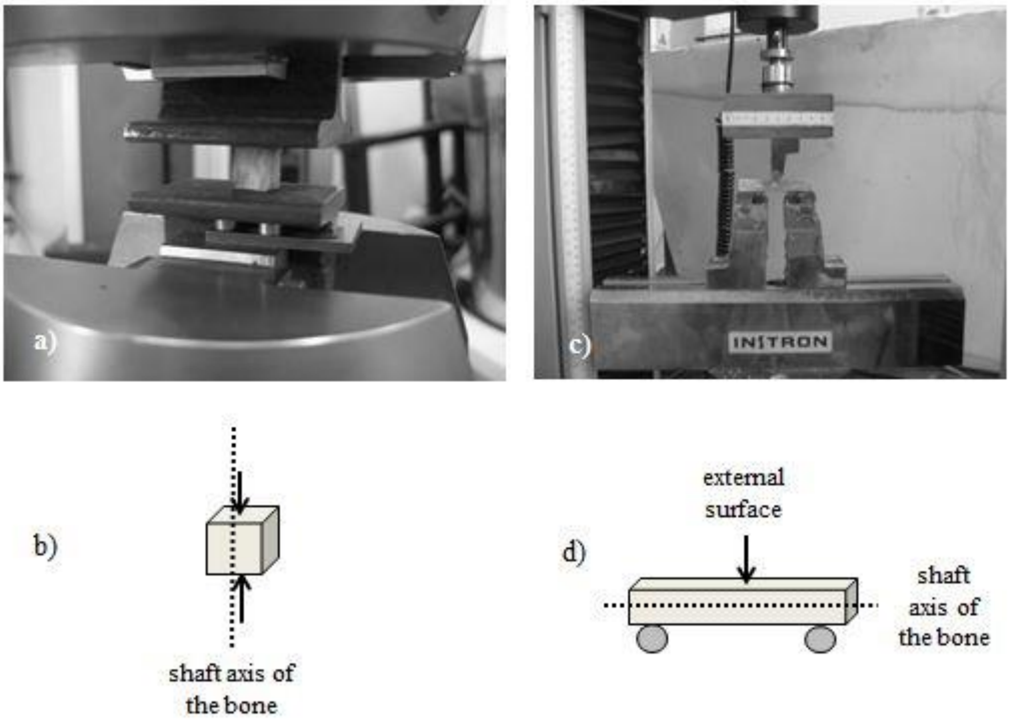

Figure 5.4 – Photographs and diagrams of compression and bending tests. a) Axial compression test on a cortical bone sample; b) Orientation of the cubes for compression tests; the dashed line represents the shaft axis of the bone; solid arrows indicate the compressive forces that the sample was subjected to; c) Three point bending test on a cortical bone sample; d) Orientation of the samples for bending tests; the dashed line represents the shaft axis of the bone; the solid arrow indicates the point of load application.

5.3.4. Statistical analysis

Data analysis was conducted with SAS 9.3 software (SAS Institute Inc., Cary, North Carolina, USA). The UNIVARIATE procedure was used to test the adjustment of variables to normal distribution. Differences between SOS measurements were analysed with a mixed model including the animal as a random effect. In order to evaluate the regional distribution of the mechanical properties within the McIII, a mixed model was also used. Independent but identical statistical analysis was conducted for data obtained in compression and bending tests. Bone region (dorsal or lateral), section level and their interaction were considered as fixed effects and the animal as a random effect. When significant differences were detected, differences among means were evaluated using the Tukey-Kramer test.

The average value of data from the compression tests performed on sections +1 and 0, and 0 and -1 of each right McIII from all mares was calculated (for both, lateral and dorsal regions) to evaluate the relationship with data obtained from the SOS measurements. These sections corresponded to the ROI in which the probe of the QUS device was applied for SOS measurements *in vivo*. Regression analysis was performed between SOS measurements and mechanical tests data. Pearson's correlation coefficients between variables were also calculated. Statistical significance was assumed when $P<0.05$.

5.4. Results

5.4.1. Quantitative ultrasound measurements

Speed of sound results obtained on the dorsal and lateral regions of mid-section of the right McIII were, respectively, 3940.1±38.2 m/s and 4296.0±38.2 m/s (lsmeans±sem). Values of SOS found for the dorsal region were significantly lower than those of the lateral region ($P<0.0001$), but a positive correlation was observed between them ($r=0.803$; $P<0.05$).

5.4.2. Mechanical tests

A total of 136 cortical bone samples from the right McIII were used for compression tests. Twelve samples were excluded from the analysis due to some experimental problems, which led to sample early breakage. In the bending tests, a total of 48 cortical bone samples from the left McIII were used.

The maximum stress was influenced by bone region (BR) and bone section (BS), and a significant interaction ($P<0.01$) was found between these two effects (Table 5.1). The maximum/yield stress presented higher values in the dorsal region in comparison with the lateral zone ($P<0.0001$). In the dorsal region, σ_{max} increased from the proximal section (4) to the mid-section of the diaphysis (sections 0 and -1) ($P<0.01$), decreasing thereafter to the distal sections (-3 and -4) ($P<0.001$). The same pattern was found for the lateral region, with higher values of σ_{max} observed in the mid-section (0) ($P<0.001$) (Table 5.1).

The Young's modulus was also influenced by BR and by BS (Table 5.1). Samples from the dorsal region were stiffer than samples from the lateral region ($P<0.0001$), and lower values were observed on the distal section (-4) of the McIII ($P<0.05$) (Table 5.1).

Table 5.1 – Proximo-distal variation of mean maximum stress (σ_{max}) and Young's modulus (E) on the dorsal and lateral regions of the right equine McIII, obtained from the compression tests.

Variable	Bone region (BR)	Bone Section (BS)	lsmean±SEM	Fixed effects (P value) BR	BS	BR*BS
σ_{max} (MPa)	Dorsal	5	129.3±5.6 abcd	<0.0001	<0.0001	0.006
		4	120.9±6.0 abc			
		3	144.5±6.0 cd			
		1	139.3±6.0 bcd			
		0	154.5±5.6 d			
		-1	141.1±6.0 cd			
		-3	104.6±5.6 ab			
		-4	98.9±5.6 a			
	Lateral	5	96.5±6.5 a			
		4	109.9±5.6 a			
		3	113.9±5.6 a			
		1	110.3±5.6 a			
		0	154.5±5.6 b			
		-1	108.5±5.6 a			
		-3	95.5±6.5 a			
		-4	94.2±5.6 a			
E (GPa)	Dorsal	5	28.8±2.1 ab	<0.0001	<0.0001	0.726
		4	33.3±2.1 b			
		3	32.4±2.1 ab			
		1	31.0±2.2 ab			
		0	33.6±2.1 b			
		-1	32.0±2.2 ab			
		-3	28.3±2.1 ab			
		-4	25.8±2.1 ab			
	Lateral	5	26.3±2.3 ab			
		4	27.6±2.1 b			
		3	26.0±2.1 ab			
		1	26.3±2.1 ab			
		0	25.3±2.1 ab			
		-1	25.4±2.1 ab			
		-3	20.9±2.1 ab			
		-4	20.0±2.1 a			

Abbreviations: McIII, third metacarpal bone; lsmeans, least square means; SEM, standard error of the mean.

Lower case letters indicate significant differences (Tukey-Kramer adjustment, P<0.05).

From the bending results, we may state that the lateral region had, on average, a lower strength than the dorsal one. The distal section of both regions (denote by III) had, in general, lower bending strength than I and II sections (*P*<0.01) (Table 5.2).

Table 5.2 – Failure stress (σ_f) (lsmeans±SEM) of samples from the dorsal and lateral regions of the left McIII, obtained from the three-point bending tests.

Bone region	Bone section	σ_f (MPa)	Fixed effects (*P* value)		
			Bone region (BR)	Bone section (BS)	BR*BS
Dorsal	I	190.8±10.6 a	0.0667	< 0.0001	0.0021
	II	199.0±10.6 a			
	III	104.8±10.6 b			
Lateral	I	161.3±10.6 a			
	II	175.7±10.6 a			
	III	125.0±10.6 b			

Abbreviation: McIII, third metacarpal bone; lsmeans, least squared means; SEM, standard error of the mean.

Lower case letters indicate significant differences (Tukey-Kramer adjustment, *P* < 0.05).

5.4.3. Comparison between in vivo and ex vivo assessment of the mechanical properties

The values of *E* determined from the compressive stress-strain curves made on samples of the mid-section (sections 1, 0, -1) of the right McIII (dorsal region) revealed to have good correlations with SOS measurements performed on the same region of the bone. The best relation was found for section 0 *per se* (r^2=0.92; *P*=0.0002) (Figure 5.5). There was no relationship between *E* of samples from the mid-section of the lateral region and SOS measurements on the same region.

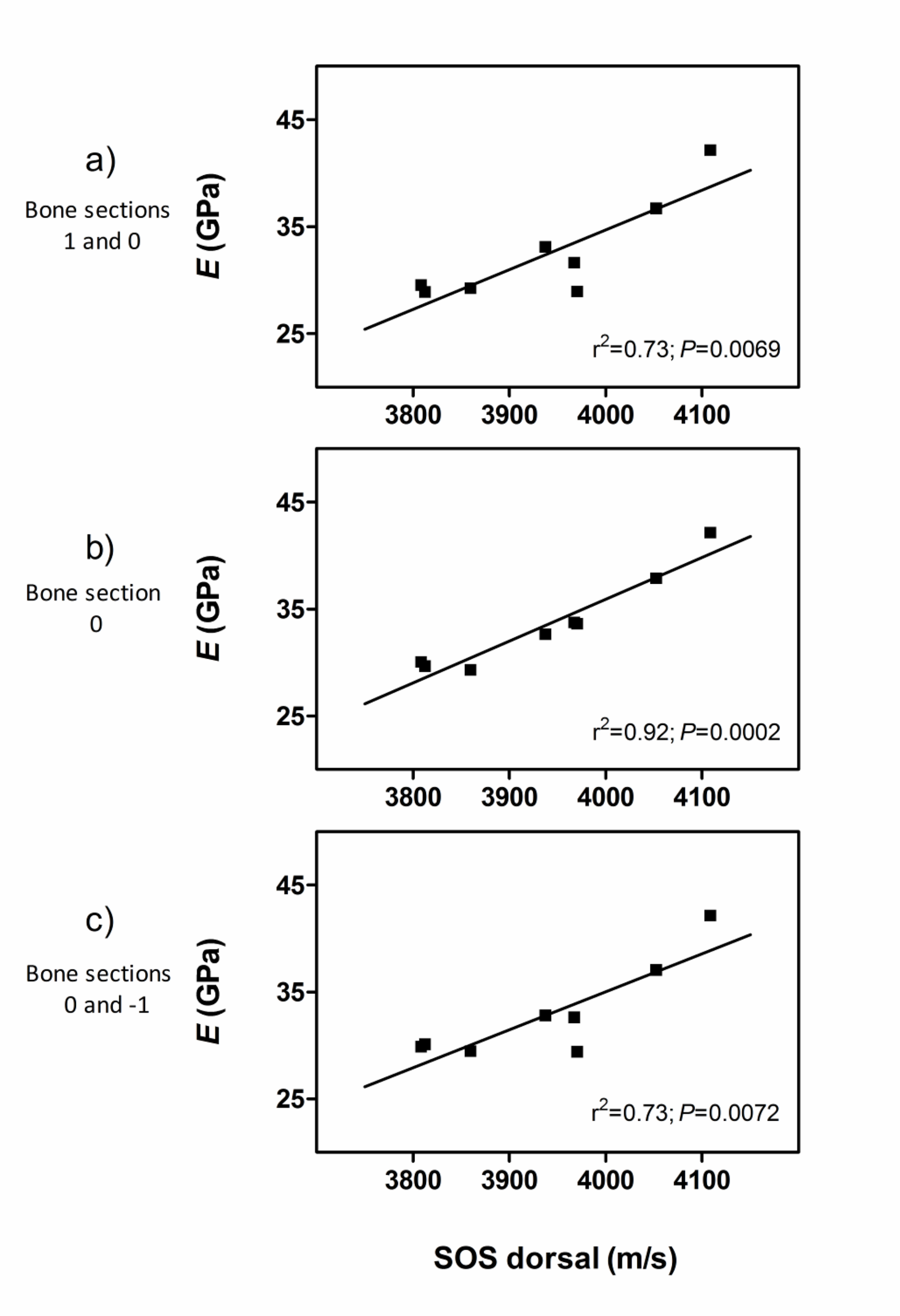

Figure 5.5 – Relationships between *in vivo* speed of sound (SOS) measurements on the dorsal region of the McIII and Young's modulus (*E*) obtained from stress-strain curves of compression tests performed on *ex vivo* cortical samples of the correspondent dorsal region: a) average *E* of 1 and 0 sections, b) *E* of section 0 and c) average *E* of 0 and -1 sections).

5.5. Discussion

Quantitative ultrasound is a well-tolerated and radiation free method for measuring bone properties in horses. The interest of researchers in ultrasound methods has grown in the last years, as this technique has the potential to provide information, not only, on bone density, but also, on the elasticity (Young's modulus) of the analysed segment of bone (Cavani et al., 2008).

The results regarding SOS values were in the range reported in literature for clinically sound Warmbloods and Thoroughbreds with similar age (Carstanjen et al., 2002; Lepage et al., 1998b). Furthermore, the dorsal mean value was lower in comparison with the lateral mean value, which is in accordance with the findings of Carstanjen et al. (2002; 2003a; 2003b). The fundamental relationship of the ultrasound method relates the ultrasound velocity to the square root of the Young's modulus divided by material density. But, besides bone mass density, other material properties like bone strength were also found to influence SOS measurements in cortical bone (Prevrhal et al., 2001; Lee et al., 1997). It was shown that geometry, porosity and structural features in the McIII change according to the section and the anatomic region (Skedros et al., 1996). Lower porosity (determined by histomorphometric analysis) was observed in the lateral region when compared with the dorsal region of the equine McIII cortex (Skedros et al., 1996; Martin et al., 1996b). In addition, osteon size and structure and collagen fibre orientation along the bone revealed to be also regionally dependent (Martin et al., 1996a; 1996c). Therefore, as previous hypothesised by Carstanjen et al. (2003a; 2003b), differences in SOS values obtained in the dorsal and lateral regions of the McIII could be related to the inherent regional microstructure and material properties.

Several mechanical tests may be used to evaluate the bone properties. While compressive tests may be more precise in representing the stress and strain existing in the cortex of a complete bone, bending tests are considered to be a standard measurement of the bending moment that causes failure, in similar size specimens (Bigot et al., 1996). A validation of the ultrasound method could be performed with mechanical tests.

Results of our study showed that the mechanical properties of bone varied according to the anatomic location. For example, bone strength, determined either by axial compression or by bending, exhibited bone region (BR) and bone section (BS) dependence. With respect to bone section, the regional dependence of the mechanical properties from proximal to distal metaphysis was in agreement with previous research. Cortical specimens taken from the diaphysis were generally stiffer and stronger than specimens taken from the proximal and distal metaphyseal levels of the McIII (Les et al., 1997). Although another mechanical device was used, significant spatial variation in the elastic properties of the equine cortical bone was also found in sample cubes located along the longitudinal axis of the McIII (Shahar et al., 2007). It is well known that the mechanical properties within cortical bone are greatly influenced by porosity, mineralization level and organization of the solid matrix (Rho et al., 1998). In fact, secondary osteons are differently organized along the axis of the bone and, although small, differences were also found in the mineral content from proximal to distal sections in the equine radius (Mason et al., 1995).

The bending strength was higher in the proximal and medial sections (I and II) than in the distal one (section III), which is in accordance with the study of Bigot et al. (1996), who observed similar variations in this mechanical property according to the longitudinal location of samples, along compact bones. Although not considered in our study, a possible explanation could be a lower mineral content in the distal section of the bone, as observed by Mason et al. (1995). In fact, Bigot et al. (1996) showed that the bending strength is positively correlated with the mineral content of cortical bone.

With regards to bone region, both compressive (σ_{max}) and flexural (σ_f) resistance seem to be higher in the dorsal sections in comparison to the lateral ones. The same is valid for the stiffness in compression, which presents higher values at the dorsal region compared to the lateral region. These results are not entirely consistent with others reported in the literature. Previous research aimed at studying the distribution of material properties in the equine McIII referred to no differences in the compressive strength between dorsal and lateral regions at diaphyseal level of the bone (Les et al., 1997). However, in this experiment the cortical samples from the lateral region were slightly stiffer than the dorsal samples tested. In contrast, a later experiment reported that the compressive resistance of samples obtained at the dorso-lateral region was higher than the resistance found for the lateral region, in spite of the similarity of the elastic modulus observed between the same samples (Skedros et al., 2006).

With reference to the flexural resistance, samples from the lateral region of the McIII tested in four-point bending to failure, were shown to be stronger and stiffer than the dorsal region when monotonically loaded, but the latter had a longer time to fatigue (Gibson et al., 1995). Nevertheless, another study aimed at evaluating the effect of exercise on the mechanical properties of bone, showed no differences in the bending strength of specimens obtained at different regions (dorsal, lateral, palmar and medial quadrants) of the McIII mid-diaphysis, on exercised horses (Reilly et al., 1997). However, in the control group of this study (non-exercised horses), the bending strength and Young's modulus were slightly higher in the specimens obtained in the outer part of the lateral cortex. In our experiment, the former work

load of the mares was not known, but because they came from a free ranging system, we assume they had a similar type of exercise.
It is commonly accepted that bones adapt to different types of loading (Yang et al., 2011). Mechanical stress produced by functional loading, influences bone cellular activities and it is recognized that the modelling and remodelling processes mediate stress-related structural and material adaptations produced during normal bone development (Skedros et al., 2003). Therefore, differences in strength within the regions (dorsal *vs.* lateral) and along the longitudinal axis (proximal, medial and distal sections) of the equine McIII may partially reflect the adaptation of bone tissue to the applied load. In the particular case of the horse, in which different movements are used during locomotion, orientation and loading modes are expected to vary, leading to different stress states along the bone (Bigot et al., 1996).
The other factor that has been extensively reported as influencing the mechanical properties of equine cortical bone is the collagen fibre organization in the lamellar microstructure (Martin et al., 1996a; Skedros et al., 2006; Riggs et al., 1993). In the study of Riggs et al. (1993) there was a predominance of longitudinal collagen fibre orientation in the cranial cortex of the equine radius when compared to the caudal one. However, later studies of the McIII concluded that collagen could be more longitudinally oriented in the lateral cortex than in the dorsal and medial regions, and that disposition of collagen fibres was correlated with greater *E* and monotonic strength (Martin et al., 1996a). Skedros et al. (1996; 2006) also confirmed a more oblique-to-transverse collagen fibre orientation in the samples obtained from the dorso-lateral and dorso-medial regions of the McIII, than in the lateral cortex.
Additionally, other features like the osteonal structure and size, namely the secondary osteon population, have been suggested to be useful in the interpretation of the mechanical behaviour and load history of the cortical bone. Smaller osteons (osteons with smaller diameter) were observed in the dorsal region of the McIII when compared with the lateral one (Martin et al., 1996c). These authors postulated that these regional variations in the osteon size, which are produced by variations in the remodelling process, have important mechanical implications. However, a more recent study presented a new classification score for osteon morphotypes and demonstrated that the new classification does not correlate well with more complexly loaded bones like the equine McIII (Skedros et al., 2009). A higher number of different osteon morphotypes was found in the dorsal region when compared with the lateral one, which might reveal a dorsal/lateral compression/tension adaptation. Because there were no differences between osteon morphotype scores in the dorsal and medial regions, the authors hypothesized that this observation could more accurately reflect the prevalent/predominant compression environment in both of these locations.

All these factors and regional variations in the structure of the cortical bone may partially explain some inconsistences of the dorsal *vs.* lateral mechanical results described in the present paper. Horse breed, age, exercise and the conditions of sample cutting, storing and testing (rate of load of the test specimen, dimensions of samples or humidity conditions) were suggested as having influence on mechanical tests results (Bigot et al., 1996). During the preparation of samples, it was observed that dorsal specimens consisted mainly of cortical bone, while the proximal and distal sections of lateral samples, had a few areas of trabecular bone in the inner side (endosteal surface). As trabecular bone is less resistant than cortical, this may in part explain the lower compression and bending strength results obtained on the lateral region.

The higher SOS values obtained laterally would support a higher stiffness and strength of the lateral cortex. In this sense, the inconsistences found in our study between the ultrasound results and the mechanical test results on the lateral region could be partially explained. Nevertheless, the good relations obtained between the speed of sound measurements on the dorsal region and the respective Young's modulus evaluated by compression tests, clearly demonstrated that the QUS could be a practical and non-invasive method for the *in vivo* assessment of cortical bone stiffness. However, given the complexity of the non-uniform strain environment experienced by the equine McIII under normal or athletic loading conditions, further research should include sample evaluation in tension and torsion mechanical tests, in order to reinforce the validity of the QUS method.

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CHAPTER VI – *GROWTH PATTERNS AND BONE STATUS*

6. Growth patterns, metabolic indicators and osteoarticular status in the Lusitano horse: a longitudinal field study

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Contribution of Maria J. Fradinho to this article:
Maria J. Fradinho performed the field and most of the laboratory work, analyzed the data, interpreted the results and wrote the manuscript.

(In preparation for submission)

6.1. Abstract

A longitudinal field study was performed in order to: (1) evaluate growth patterns and long-term changes on bone quality, bone metabolism, growth factors and metabolic related variables in the Lusitano horse; and (2) assess whether these long-term changes are related with radiographic findings regarding osteochondrosis-like lesions (OC) at the onset of training. Thirty-four Lusitano foals (12 colts and 22 fillies), born and raised at four stud-farms, were periodically weighed (BW) and withers height (WH) was measured from birth to 36 months of age. On the same days, blood samples were collected for determination of osteocalcin, bone alkaline phosphatase, insulin-like growth factor I (IGF-I), leptin, insulin, glucose, parathyroid hormone (PTH), calcium, phosphorus and magnesium plasma concentrations, and quantitative ultrasound measurements were performed on the right third metacarpal bone. At the end of the study and matching with the onset of training, foals underwent radiographic examination of the four fetlocks, hocks and stifles. Speed of sound measurements, bone markers, growth factors and other metabolic variables change markedly with age ($P<0.01$). The presence of radiographic findings compatible with OC at the onset of training (13 positive foals) was associated with changes in BW and WH growth rates. When compared to healthy foals, OC positive foals seem to be early maturing individuals as regards to BW. OC positive foals tended to have worse cortical bone quality, lower IGF-I and higher insulin and PTH concentrations ($P<0.1$). Besides the global set of information, this study underlines the importance of an early monitoring of foals' growth during the first year of life. In particular, sudden changes to the average growth rates observed for the breed should be avoided, in order to promote a better quality of the osteoarticular status of the Lusitano horse.

Keywords: Lusitano horse, growth patterns, quantitative ultrasonography, bone markers, growth factors, hormones, plasma mineral concentrations, osteochondrosis.

6.2. Introduction

In the equine athlete, development of a healthy and sound locomotor system is fundamental for the success and longevity of sports performance. Thus, one of the main concerns of horse breeding industry is the knowledge of the most adequate growth pattern for each breed and purpose, as well as the factors that influence the development of the skeleton.

Growth and development in the sport horse is particularly high during the first year of life (Martin-Rosset, 2005). Depending on the breed, 58% to 66.7% of adult body weight and 87 to 90% of adult withers height are reached at 12 months of age, pointing out to an early development of bone tissue (Valette et al., 2008; Kocher and Staniar, 2013; Fradinho et al., 2015b). However, body weight gain and skeletal growth are closely related with feed intake level (Thompson et al., 1988a; Cymbaluk et al., 1990; Trillaud-Geyl et al., 2004).

A rapid growth in the first stage of horse life was identified as one of the factors that is associated with the occurrence of skeletal development disorders (Sandgreen et al., 1993; Pagan and Jackson, 1996; van Weeren et al., 1999). Among these disorders, those affecting articular cartilage, in particular osteochondrosis (OC), have been recognized as a major problem in the horse industry (van Weeren and Brama, 2005; van Weeren and Jeffcott, 2013). Osteochondrosis is a dynamic disturbance in the natural process of endochondral ossification during growth, with a complex multifactorial etiology (Dik et al., 1999; van Weeren and Brama, 2001; Denoix et al., 2013). Genetic predisposition and environmental factors such as exercise/biomechanical stress, nutrition, growth rate and local/endocrine disturbances in the cartilage have been generally implicated in this condition (Barneveld and van Weeren, 1999; Donabédian et al., 2006; Ytrehus et al., 2007; Distl, 2013).

In order to assess bone quality, bone metabolism and the adaptive response of the skeleton to development or mechanical stimuli, some non-invasive techniques have been developed (Jeffcott et al., 1988; Lepage et al., 2001). Among them, quantitative ultrasonography (QUS) is a nonionizing radiation methodology that can provide an indication of bone quality, as it reflects mineral density and the mechanical properties of cortical bone (Carstanjen *et al*., 2002; Fradinho et al., 2015a). In humans, QUS is commonly used in the assessment of bone mineral status and risk fracture (Baroncelli, 2008; Olszynski et al., 2013). In the horse, the use of QUS to measure superficial cortical bone properties has been reported (Carstanjen *et al*., 2002; 2003a), and the workload effect on third metacarpal bone (McIII) failure in Thoroughbreds was evaluated with this technique (Tabar-Rodriguez et al., 2009).

The assessment of biochemical markers in body fluids is another non-invasive technique widely used to monitor changes of bone metabolism in the horse (Price, 1998; Lepage et al., 2001). Bone turnover occurs through two different processes, modeling and remodeling, both involving the action of osteoclasts and osteoblasts. In skeletally mature animals bone resorption and bone formation are balanced, while during growth the rate of bone formation tends to exceed that of resorption (Fraher, 1993; Allen, 2003). Osteocalcin (Oc) and bone alkaline phosphatase (BALP) are two proteins associated with osteoblastic activity, which use as bone formation markers in the horse has been investigated (Price et al., 1995a; 2001; Reller et al., 2003). Additionally, most recent findings in rodents and humans highlighted the hormonal action of Oc as a mediator between bone and energy metabolism. In this process, Oc is regulated by leptin and insulin, two hormones involved in energy homeostasis (Ducy, 2011; Ferron and Lacombe, 2014). Although not yet confirmed in the equine species, the role of leptin as a possible modulator of bone mass through a central relay has been emphasized (Confraveux et al., 2009).

Insulin-like growth factor I (IGF-I) is another hormone implicated in the regulation of longitudinal bone growth, acting locally in the growth plate as a stimulator of chondrocyte proliferation (van der Erden et al., 2003; Mackie et al., 2011). In the horse, IGF-I has been associated with bone turnover, cartilage maturation and average daily gain (Jackson et al., 2003a; Fortier et al., 2005; Staniar et al., 2007b). In the context of the nutritional imbalances that were implicated in skeletal development disorders, plasma mineral concentrations and some associated regulatory factors, such as parathyroid hormone (PTH), have been also investigated (Savage et al., 1993b; Sloet van Oldruitenborgh-Oosterbaan et al., 1999).

Nowadays, the reputation of the Lusitano as a sport and leisure horse is increasing worldwide. Thus, a better knowledge of the growing process, in relation to bone quality and osteoarticular status is pivotal in order to improve the efficiency of the production system. Therefore, the aims of the present study were: (1) to evaluate growth patterns and long-term changes on bone quality, bone metabolism, growth factors and metabolic related variables in the Lusitano horse; and (2) to assess whether these long-term changes are related with radiographic findings regarding OC-like lesions at the onset of training.

6.3. Materials and Methods

6.3.1. Animals and management

The protocol of this study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Lisbon, Portugal. All the animals were handled with care during the experimental procedures.

This investigation was conducted over a period of 5 years (2006 to 2010) in four stud-farms located at the main region of Lusitano breeding in Portugal, and was part of a longitudinal study aiming to investigate growth and development of the Lusitano horse. Thirty-four foals (12 colts and 22 fillies) born in 2006 (n=19), in 2007 (n=9) and in 2008 (n=6) were monitored from birth to 36 months of age. The foals were born between February and May (Feb, n=13; Mar, n=9; Apr, n=8; May, n=4).

All the animals, mares and foals, were kept on pasture throughout the study and had *ad libitum* access to water. The floristic composition of the pastures was typical of the permanent rainfed pasturelands of Mediterranean areas, with a dry matter production along the year quite dependent on climate conditions. A detailed characterization and composition of these pastures was previously described (Fradinho et al., 2013).

From foaling until weaning, mares of two stud-farms were supplemented once a day with commercial compound feeds and with grass hay or cereal straw, according to animals' lactation stage and pasture availability. On the other two stud-farms mares were only supplemented with grass hay in periods when pasture was scarce. Foals were not creep fed, although some of them had access to their dam´s concentrate. The weaning occurred on average at seven and half months of age (222±33d, mean±SD). During the post-weaning period, foals were kept in paddocks with different areas depending on the stud-farm, and were group-fed with compound feeds and grass hay. After the post-weaning, foals returned to pasture (between winter and the beginning of spring), and, according to pasture availability, continued to be supplemented along the study, also with compound feeds and/or grass hay. Routine vaccination and deworming programs were practiced in the four stud-farms.

6.3.2. Data collection

6.3.2.1. Body measurements

Foals' body weight (BW) and withers height (WH) were assessed monthly on the first year after birth, every two months on the second year, and every three months from 24 to 36 months of age. BW was determined using a portable electronic scale (Iconix, FX15, New Zealand), which accuracy was regularly checked. In the same days, WH was measured with a standard measuring stick from the ground to the highest point of the withers. All measurements were taken at a similar time of day and by the same operator.

6.3.2.2. Blood sampling and analysis

On the days of BW and WH assessment, between 8.00h and 11.00h and before any compound feed was distributed, blood samples (≈ 18 mL) were collected from 27 foals (11 colts and 16 fillies), by jugular venipuncture into heparinized tubes (Monovette® Li-Heparin, SARSTEDT AG & Co., Nümbrecht, Germany) for determination of Oc, BALP, IGF-I, leptin, insulin, glucose, PTH, calcium (Ca), phosphorus (P_i) and magnesium (Mg) plasma concentrations. All blood samples were transported to the laboratory on ice and were centrifuged at 2,000 x g, at 4ºC, for 15 minutes. Plasma samples were stored at –20ºC until analysis.

The concentrations of Oc (METRA Osteocalcin EIA kit, QUIDEL Corporation, San Diego, USA) and BALP (METRA BAP EIA kit, QUIDEL Corporation, San Diego, USA) were measured by using two specific competitive immunoassays (ELISA), previously used and validated for the horse (Hoyt and Siciliano, 1999; Jackson et al., 2003b; Trumble et al., 2008). For determination of IGF-I, a commercial IGF-I ELISA kit (DSL 10-5600 Active® IGF-I ELISA kit, Webster, Texas, USA) was used based on its validation for equine plasma (Cosden, 2007). This IGF-I ELISA is an enzymatically amplified "one-step" sandwich-type immunoassay. The assay includes a previous extraction step in which IGF-I was separated from its binding protein. Plasma leptin concentrations were determined by radioimmunoassay using a Multi-Species Leptin RIA kit (Multi-Species Leptin RIA, Linco, Millipore Corporation, Billerica, USA), widely used and validated for the horse (McManus and Fitzgerald, 2000; Cartmill et al., 2003). Insulin concentrations were determined with a commercial specific immunoassay kit (Mercodia Equine Insulin ELISA, Mercodia AB, Uppsala, Sweden) validated for the measurement of equine insulin (Tinworth et al., 2011).

The PTH concentrations were determined with a commercial kit (Intact PTH ELISA, Immunodiagnostic Systems Ltd, Boldon, UK). This two-site immunoenzymo-metric assay that was developed for the quantification of the intact parathyroid hormone (1-84), uses an N-terminal specific mouse monoclonal anti-PTH (1-34) in the solid phase and a purified goat anti-PTH (39-84) coupled to the enzyme horseradish peroxidase. Glucose and total Ca, P_i and Mg concentrations were measured by colorimetric methods in an auto-analyzer Kone Optima (Kone Optima Analyzer, Therma Clinical Labosystems, Vantaa, Finland) with appropriate commercial kits (Glucose HK, Calcium Arsenazo III and Inorganic Phosphorus UV - Bradford, Kemia Cientifica S.A., Madrid, Spain; and Konelab Magnesium - Thermo Electron Oy, Clinical Chemistry and Automation Systems, Vantaa, Finland). All the assays were performed according to the manufacturer's protocols.

6.3.2.3. Bone assessment by quantitative ultrasonography

Quantitative ultrasound measurements were performed on the foals which blood was collected (n=27), every two months from birth to 24 months of age, and every three months from 24 to 36 months of age. A final measurement was made on the same day of the radiographic examination. Measurements were performed with a quantitative ultrasound multisite device (Sunlight EQUS, BeamMed, Ltd., Petah Tikva, Israel) developed to measure the speed of sound (SOS) in axial transmission mode through bone (Carstanjen et al., 2002). Before any SOS measurements were made, the accuracy of the system was calibrated using a phantom (provided by the manufacturer), to obtain temperature corrected SOS values. Static measurements with the probe in axial position were performed on the mid-section of the right McIII (dorsal and lateral regions) of each foal, according to the methodology previously described (Carstanjen et al., 2003a; Fradinho et al., 2015a). Silicon oil was used as a coupling medium, without shaving. Foals were squared-up, standing still on the four limbs, with no sedation. All measurements were performed by the same operator.

6.3.2.4. Radiographic evaluation

At the end of the study and matching with the onset of training, 31 animals (10 males and 21 females) were radiographed with a portable unit (three of the initial 34 foals were sold before the radiographic examination). Radiographic projections were made using a portable high frequency x-ray generator with 2.4 kW allowing for 100 kV and 40 mAs and a Digital

Radiography (DR) Flat Panel of Amorphous Selenium receptor connected to a laptop with appropriate software. Radiographic examinations were performed to every fetlock (front and hindlimbs), hocks (tarsus) and stifles. This examination included 12 views: lateromedial views of the four fetlocks; lateromedial, dorsolateral-plantaromedial oblique and dorsomedial-plantarolateral oblique views of both tarsus and caudolateral-craniomedial views of both stifles. The radiographic findings were evaluated by an experienced veterinarian in order to classify the presence or the absence of osteochondrosis-like lesions (OC), based on the scoring system proposed by Dik et al. (1999).

6.3.3. Data analysis

In order to assess growth and development of the foals, individual adjustments were made to BW and WH data using Richards function, $y = A(1 - b.\exp(-kt))^M$, where the variable y is described as a function of age t. The adjustments were made using the NLIN procedures of SAS (SAS 9.3 Institute Inc., Cary, NC, USA) with the Marquardt iterative method. The parameters of Richards' equation can be biologically interpreted. Thus, A is the asymptotic value of y as age (t) approached infinity, and is commonly interpreted as the mean mature size; b is a scaling parameter that adjusts for situations where y_0 and/or t_0 do not equal to 0 (for example, when only postnatal observations are available and t_0 is taken as birth); k is a maturing index, establishing the earliness with which y approaches A (large k values indicate early maturing individuals and small k values indicate late maturing individuals); M determines the point of inflection where the estimate growth rate changes from an increasing to a decreasing function (for $0 < M < 1$, M is undefined) (Brown et al., 1976; Fitzhugh, 1976; Perotto et al., 1992; Richards, 1959; Staniar et al., 2004a).

On a first approach, a mixed model considering repeated measures on time was used to evaluate general changes of SOS and blood variables with age. The effects of foaling season (February-March or April-May), gender, time (expressed in days of age), time×time and their interactions were included in the model and an autoregressive covariance matrix was used. For SOS measurements, the region of the McIII (dorsal or lateral) was also included as a fixed effect.

Based on radiographic status of the foals (negative vs. positive OC) two groups were formed and growth models for BW and WH were adjusted for each group. In order to evaluate differences between the two models, a sum of squares reduction test was performed. The instantaneous rate of gain (BW instantaneous average daily gain (BW IADG), kg/d or WH instantaneous average daily gain (WH IADG), cm/d) at time *t* (*t* = days of age) was calculated from the resolution of the first derivative of Richards equation with respect to time ($\delta y/\delta t$): $y' = MAkb.\exp^{(-kt)} (1 - b.\exp^{-kt})^{M} (1 - b.\exp^{-kt})^{-1}$. Seven age-classes were considered from birth to 36 months: <45 days, 3mo [60-120 d], 6mo [150-210d], 12mo [335-395d], 18mo [480-600d], 24mo [670-790d] and 36 mo [1035-1155d]. For each foal and for each age-class, the first derivative was calculated for the average of age (days) in which measurements were performed because most of the age-classes encompass more than one measurement.

Body weight IADG (kg/d) and WH IADG (cm/d) from birth to 36 months of age were analyzed with a mixed model considering repeated measures on time (<45days, 3mo, 6mo, 12mo, 18mo, 24mo and 36 mo). Age-class, group and its interaction were included as fixed effects in the model and an autoregressive covariance matrix was used. The same methodology was used for the analysis of SOS and blood variables, considering the two groups of foals based on their radiographic OC status.

When significant differences were detected, the Tukey-Kramer test was used to evaluate the differences among means. Statistical significance was considered when $P < 0.05$. To evaluate the relationships among variables, Spearman's correlation coefficients were calculated. All results are presented as lsmeans±SEM, unless stated otherwise.

6.4. Results

6.4.1. General growth and development

The mean and the range of parameter estimates obtained for the individual growth models that were fitted to foals' BW and WH are presented in Table 6.1. Convergence criteria were met for all the models, and the mean of the calculated coefficient of determination (R^2) showed good fit adjustments for both, BW (0.981) and WH data (0.994). However, the range of values observed for each parameter presented a wide individual variability. In particular, the values obtained for the *k* parameter, both for BW and WH, showed a large range in the maturity index even within the four stud-farms (supplementary Tables A and B).

Table 6.1 – Descriptive statistic of the parameter estimates of the individual growth models fitted to body weight and withers height of Lusitano foals (n=34).

Measure [a]	Individual parameter estimates [b] Mean (range)				R^{2} [c] Mean (range)	RSD [d] Mean (range)
	A	*b*	*k*	*M*		
BW (kg)	544.0 (427.0 – 721.9)	0.9635 (0.7580-0.9998)	0.00135 (0.00060-0.00266)	0.671 (0.428-1.580)	0.981 (0.951-0.997)	16.5 (7.4-30.1)
WH (cm)	160.3 (152.1-173.1)	0.9472 (0.7630-0.9990)	0.00166 (0.00041-0.00345)	0.163 (0.101-0.295)	0.994 (0.985-0.998)	1.2 (0.9-1.9)

[a] BW – body weight; WH – withers height.

[b] *A* – asymptotic value for BW / WH as age approaches infinity (interpreted as mean BW / WH at maturity); *b* – scaling parameter that defines the degree of maturity when age = 0 d (intercept on y axis); *k* – maturing index (rate that establishes the spread of the curve along time axis); *M* – determines the point of inflexion of the curve (for $0 < M < 1$, M is undefined).

[c] R^2 correspond to a pseudo R^2, calculated as 1 – (SS(Residual) / SS(Total $_{corrected}$)).

[d] RSD – residual standard deviation.

6.4.2. Longitudinal changes of SOS measurements and blood variables

The results regarding SOS measurements on dorsal and lateral regions of the McIII from birth to 36 months of age are presented in Figure 6.1. SOS values obtained on both dorsal and lateral regions increased with age (P<0.0001), but this increase was influenced by an interaction between age and gender (P<0.01), with higher values observed for colts. Values of SOS found for the dorsal region were significantly lower than those of the lateral region (P<0.0001), and a positive correlation was observed between them (r=0.178; P<0.001; n=353).

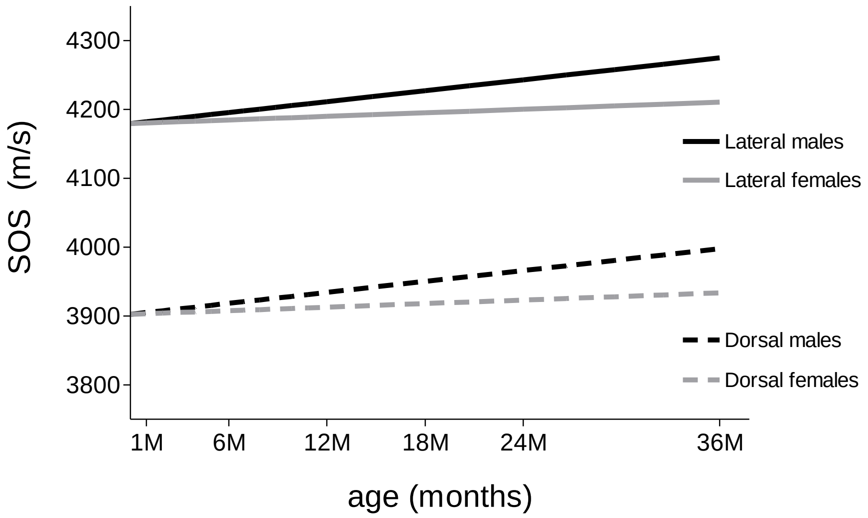

Figure 6.1 – Speed of sound measurements (SOS) obtained at the lateral (L) and dorsal (D) regions of the right third metacarpal bone in Lusitano foals (n=27) from birth to 36 months of age. Solid lines represent SOS values obtained at the lateral region and dotted lines represent values of the dorsal region. Dark lines represent male (♂) and light lines represent female (♀) SOS values. Equations:

yL♂ = 4180 (±13.6) + 0.087 (±0.021) t;   yL♀ = 4180 (±13.6) + 0.028 (±0.014) t;

yD♂ = 3902 (±11.8) + 0.087 (±0.021) t;   yD♀ = 3902 (±11.8) + 0.028 (±0.014) t;

t = age (days).

Plasma concentrations of bone biochemical markers decreased markedly with age (P<0.0001) (Figure 6.2, A and B). For both markers, Oc and BALP, the quadratic effect of age was also significant (P<0.0001) and a slight increase was observed after the 24 months. Oc and BALP values were highly correlated (*r*=0.534; *P*<0.0001; n=439). Similarly, IGF-I values also decreased with age (P<0.0001), but plasma concentrations of this growth factor were influenced by foaling season and by foal gender, with a significant interaction between those effects (P<0.01) (Figure 6.2, C). Positive correlations were observed between IGF-I and Oc plasma concentrations (*r*=0.371; *P*<0.0001; n=455) and BALP plasma concentrations (*r*=0.295; *P*<0.0001; n=443). After a slight decrease observed during the first months of life, leptin concentrations followed a quadratic pattern, increasing until the 36 months of age (P<0.05) (Figure 6.2, D). This increase was influenced by an interaction with gender (P<0.01), with higher leptin values observed in females. Leptin and Oc concentrations were negatively correlated (*r*=-0.098; *P*<0.05; n=413).

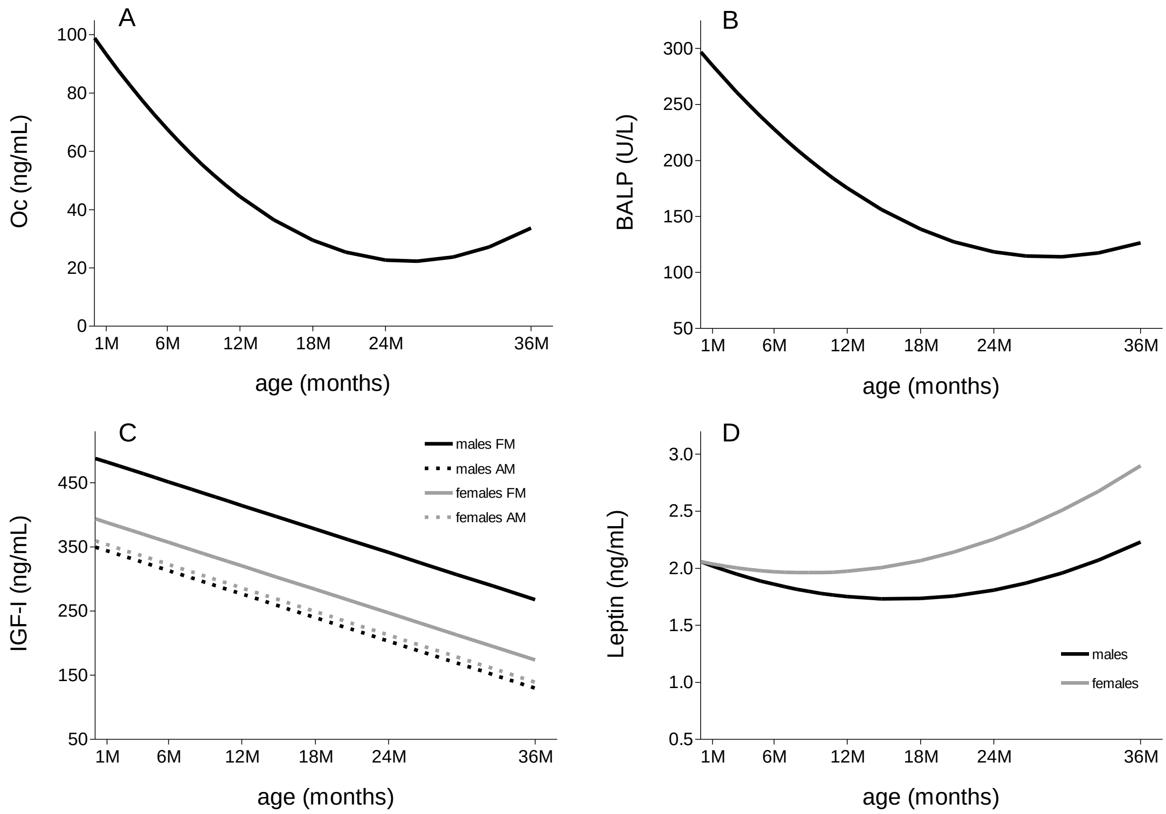

Figure 6.2 – Changes in plasma concentrations of osteocalcin (Oc) (A), bone alkaline phosphatase (BALP) (B), insulin-like growth factor I (IGF-I) (C) and leptin (D) in Lusitano foals (n=27) from birth to 36 months of age. On (C) panel, the dark lines represent male (♂) and light lines represent female (♀) IGF-I values. FM: foals born in February-March; AM: foals born in April-May. On (D) panel, the dark line represent male (♂) and light line represent female (♀) leptin values. Equations:

$$y\ Oc = 99.1\ (\pm 3.4) - 0.19\ (\pm 0.02)\ t + 0.0001\ (\pm 0.00001)\ t^2;$$

$$y\ BALP = 297.3\ (\pm 13.6) - 0.42\ (\pm 0.06)\ t + 0.0002\ (\pm 0.00005)\ t^2;$$

$$y\ \text{IGF-I} ♂\ FM = 487.8\ (\pm 26.9) - 0.2\ (\pm 0.03)\ t; \quad y\ \text{IGF-I} ♀\ FM = 393.8\ (\pm 27.3) - 0.2\ (\pm 0.03)\ t;$$

$$y\ \text{IGF-I} ♂\ AM = 349.8\ (\pm 38.1) - 0.2\ (\pm 0.03)\ t; \quad y\ \text{IGF-I} ♀\ AM = 359.2\ (\pm 22.6) - 0.2\ (\pm 0.03)\ t;$$

$$y\ \text{leptin} ♂ = 2.1\ (\pm 0.1) - 0.0013\ (\pm 0.0002)\ t + 0.000001\ t^2;$$

$$y\ \text{leptin} ♀ = 2.1\ (\pm 0.1) - 0.0007\ (\pm 0.0005)\ t + 0.000001\ t^2;$$

$$t = \text{age (days)}.$$

Glucose concentrations decreased with age (P<0.0001), and higher values were observed during the first six months after birth (Figure 6.3).

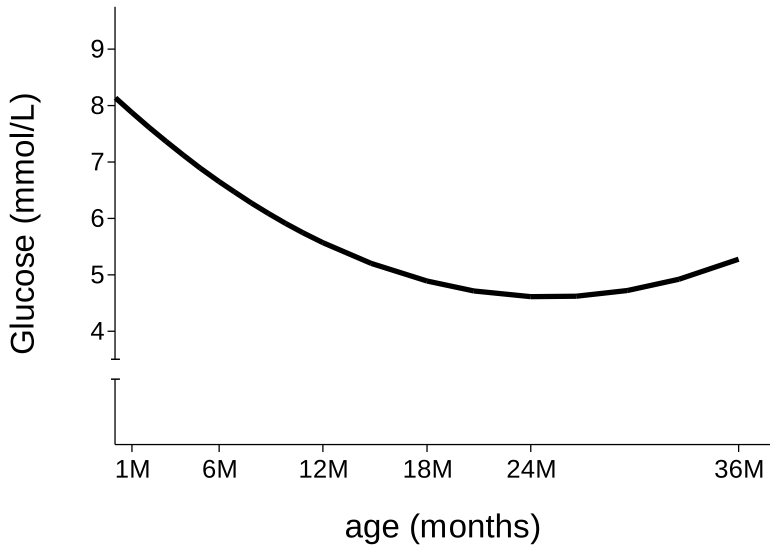

Figure 6.3 – Changes in plasma concentrations of glucose in Lusitano foals from birth to 36 months of age (n=27). Equation: $y = 8.15 (\pm 0.16) - 0.009 (\pm 0.001)\, t + 0.00001\, t^2$; t = age (days).

In this longitudinal analysis, insulin concentrations were not influenced by age, gender or foaling season but the observed values were correlated with glucose (r=0.279; P<0.0001; n=378), IGF-I (r=0.238; P<0.0001; n=367) and leptin concentrations (r=0.113; P<0.05; n=331).

Plasma total Ca concentrations increased with age (P<0.05) and an effect of foaling season (P<0.05) was observed (Figure 6.4 A). On the opposite, a significant decrease with age (P<0.0001) was observed on P_i plasma concentrations. Higher values were found in foals born in April-May, in particular after the 12 months of age (P<0.05) (Figure 6.4 B). Small changes were observed on plasma total Mg concentrations. Magnesium slightly decrease until 24 months of age, increasing thereafter until the end of the study (P<0.01) (Figure 6.4 C). In this longitudinal analysis, the concentrations of PTH were not specifically influenced by age, gender or foaling season, but negative correlations were observed between this hormone and total Ca (r=-0.129; P<0.01; n=405) and Mg (r=-0.177; P<0.001; n=399) plasma concentrations.

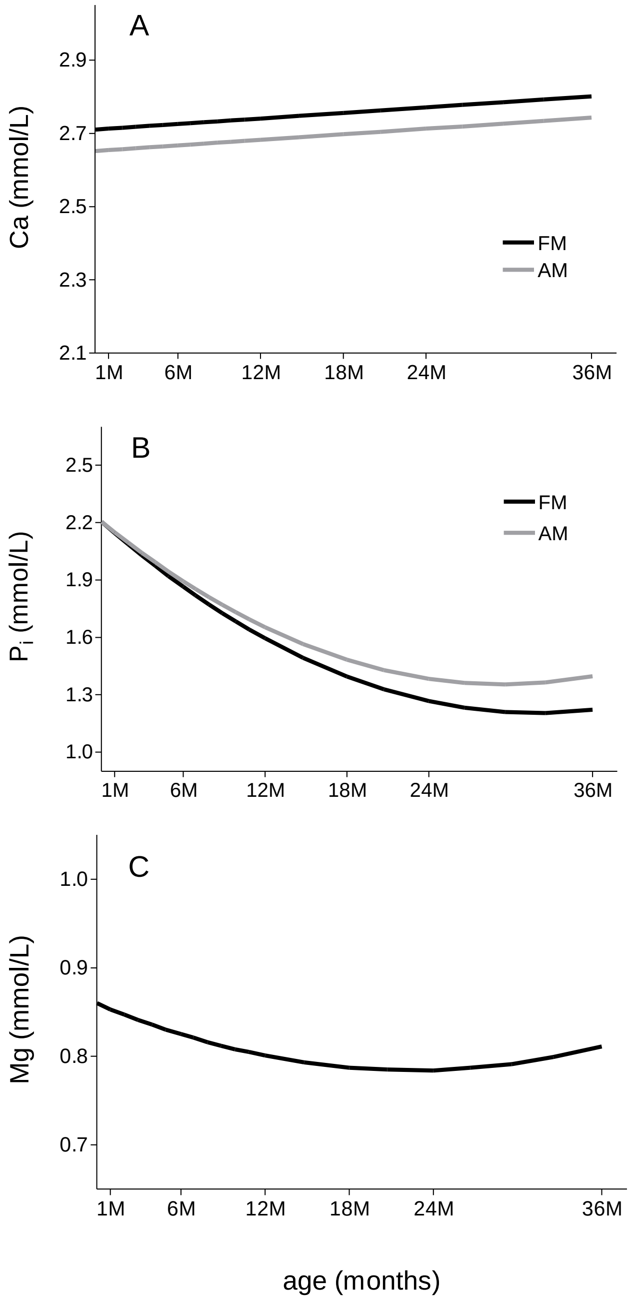

Figure 6.4 - Changes in plasma concentrations of total calcium (Ca) (A), phosphorus (P_i) (B) and magnesium (Mg) (C) in Lusitano foals (n=27) from birth to 36 months of age. On (A) and (B) panels, dark lines represent foals born in February-March (FM) and light lines represent foals born in April-May (AM). Equations:

$$y\ Ca\ FM = 2.71\ (\pm 0.03) + 0.00008\ (\pm 0.00004)\ t;$$

$$y\ Ca\ AM = 2.65\ (\pm 0.03) + 0.00008\ (\pm 0.00004)\ t;$$

$$y\ P_i\ FM = 2.21\ (\pm 0.03) - 0.021\ (\pm 0.00001)\ t - 0.000001\ t^2;$$

$$y\ P_i\ AM = 2.21\ (\pm 0.03) - 0.019\ (\pm 0.0002)\ t - 0.000001\ t^2;$$

$$y\ Mg = 0.86\ (\pm 0.01) - 0.0002\ (\pm 0.00006)\ t - 0.0000002\ t^2;$$

$$t = \text{age (days)}.$$

6.4.3. Radiographic status and growth patterns

The radiographic status and the radiographic findings by site of the 31 foals that underwent radiographic evaluation are presented in Table 6.2. Thirteen (41.9%) of the examined animals presented radiographic findings compatible with OC lesions. Seven of the positive foals presented lesions in more than one site.

Table 6.2 – Radiographic status and radiographic findings by site in 31 Lusitano foals

Number of foals			Site and number of lesions [c] (n=number animals)
Total	Negative [a]	Positive [b]	
31	18	13	Metacarpophalangeal joint: 3 Metatarsophalangeal joint: 11 (n=10) Tibiotarsal joint: 6 (n=5) Femorotibial joint: 1 (n=1)

[a] Negative – Without any radiographic findings compatible with OC lesions;

[b] Positive – With radiographic findings compatible with OC lesions;

[c] Some foals (n=7) presented more than one site with lesions.

The growth models that were adjusted to BW and WH data according to the radiographic status of the foals (group negative, n=18 *vs.* group positive, n=13) are presented in Table 6.3. Based on the adjustment of Richards function, the models obtained for the two groups were significantly different for both measurements, showing different patterns of growth in what concerns BW (P<0.001) and WH (P<0.01). The estimated maturing index (*k* parameter value) of the BW model was clearly superior in the OC positive group of foals.

Table 6.3 – Body weight and withers height growth models according to radiographic status (negative vs. positive OC) of Lusitano foals (n=31).

Measure [a]	RX OC status	Parameters [b]				R^{2} [d]	RSD [e]	*P* value [f]
		A (± SE[c])	*b* (± SE[c])	*k* (± SE[c])	*M* (± SE[c])			
BW (kg)	Negative	497.8 ± 16.8	0.984 ± 0.012	0.00137 ± 0.0002	0.569 ± 0.049	0.927	31.1	< 0.001
	Positive	493.5 ± 17.3	0.906 ± 0.060	0.00200 ± 0.0004	0.893 ± 0.177	0.938	30.6	
WH (cm)	Negative	157.8 ± 0.9	0.956 ± 0.014	0.00175 ± 0.0002	0.153 ± 0.011	0.966	2.7	< 0.01
	Positive	159.4 ± 1.1	0.923 ± 0.027	0.00184 ± 0.0002	0.185 ± 0.020	0.953	3.3	

[a] BW – body weight; WH – withers height.

[b] *A* – asymptotic value for BW / WH as age approaches infinity (interpreted as mean BW / WH at maturity); *b* – scaling parameter that defines the degree of maturity when age = 0 d (intercept on y axis); *k* – maturing index (rate that establishes the spread of the curve along time axis); *M* – determines the point of inflexion of the curve (for $0 < M < 1$, M is undefined).

[c] SE – approximate standard error.

[d] R^2 correspond to a pseudo R^2, calculated as $1 - (SS(Residual) / SS(Total_{corrected}))$.

[e] RSD – residual standard deviation.

[f] *P* value obtained under an *F* distribution, using the result of a sum of squares reduction test in order to compare differences between the two models (Negative *vs.* Positive).

Table 6.4 represents BW IADG and WH IADG for seven age-classes, which were calculated from the models presented in Table 6.3. An interaction between age-class and group was found for both variables (P<0.0001). Positive foals presented lower BW IADG in the first age-class, but the opposite was observed from the six to 18 months, indicating a higher rate of gain during this period, when compared to the OC negative foals (P<0.001). The WH IADG of positive foals were only lower than that of negative foals in the class <45 days (P<0.001).

Table 6.4 – Body weight and withers height instantaneous average daily gain of Lusitano foals (n=31) from birth to 36 months of age according to radiographic status (negative *vs.* positive OC).

Variable	OC group status	Age-class							Significance of fixed effects		
		<45d	3mo	6mo	12mo	18mo	24mo	36mo	Age	Group	Age × Group
BW IADG (kg)	Negative	1.436±0.009 A	0.811±0.008	0.561±0.008 A	0.343±0.008 A	0.238±0.008 Y	0.172±0.008	0.092±0.008	<.0001	<.0001	<.0001
	Positive	0.948±0.010 B	0.779±0.010	0.625±0.009 B	0.411±0.009 B	0.291±0.009 Z	0.193±0.010	0.092±0.010			
WH IADG (cm)	Negative	0.343±0.003 A	0.145±0.003	0.081±0.003	0.039±0.003	0.023±0.003	0.015±0.003	0.006±0.003	<.0001	0.0064	<.0001
	Positive	0.284±0.004 B	0.149±0.003	0.088±0.003	0.043±0.003	0.027±0.003	0.017±0.003	0.008±0.003			

Values represent lsmeans±SEM;

Capital letters indicate significant differences between groups in the same age-class (Tukey-Kramer adjustment, A, B: P<0.0001; Y, Z: P<0.01).

6.4.4. Changes in SOS measurements and blood variables according to radiographic status

SOS measurements on the dorsal region of the McIII were only influenced by age ($P<0.01$), but there was a trend for a difference between groups in what concerns SOS measurements on the lateral region, with lower values for the OC positive group (positive, 4160±20 m/s *vs.* negative, 4212±20 m/s ; $P=0.077$) (Table 6.5).

Plasma concentrations of bone markers were not different between OC positive and negative foals (Table 6.5). A significant effect of age was observed for both, Oc and BALP ($P<0.0001$), following a similar decrease already observed in Figure 6.2 (A and B). IGF-I plasma concentrations were influenced by age and there was also a trend for lower values in the OC positive group (positive, 299.9±24.5 ng/mL *vs.* negative, 362.1±24.1 ng/mL; $P=0.084$). A sharp decrease in IGF-I concentrations was observed from 12 to 18 months of age (Table 6.5). In what concerns insulin, there was a significant effect of age ($P<0.0001$). Insulin increased, from three to 12 months of age, and decreased on the following months ($P<0.05$). A trend for higher values of insulin concentrations was observed in the OC positive group (positive, 4.29±0.67 μIU/L vs. negative, 2.62±0.69 μIU/L; $P=0.099$) (Table 6.5). Plasma glucose concentrations were not different between OC positive and negative foals. Glucose decreased from <45days to 12 months of age, maintaining these steady levels until the end of the study (Table 6.5). Leptin plasma concentrations were only influenced by age ($P=0.0002$). Values decreased from <45days to 6 months of age and increased in the last two age-classes (Table 6.5).

On Table 6.6, a great variation was observed on PTH plasma concentrations. Parathyroid hormone concentrations were influenced by age ($P<0.05$) and a trend for higher values was observed in the OC positive group (positive, 33.7±4.1 pg/mL *vs.* 22.4±4.1 pg/mL; $P=0.063$) (Table 6.6). Concerning Ca plasma concentrations, there was a trend for an interaction between age and OC group status ($P=0.078$), but differences between age-classes were only observed on the negative group ($P<0.05$). Phosphorus and magnesium plasma concentrations were only influenced by age ($P<0.0001$) with higher values observed at the youngest age-classes (Table 6.6).

Table 6.5 – Speed of sound measurements and plasmatic concentrations of osteocalcin, bone alkaline phosphatase, insulin-like growth factor I, leptin, insulin and glucose of Lusitano foals (n=24*) from birth to 36 months of age according to the radiographic status.

Variable	Age-class							Significance of fixed effects		
	<45d	3mo	6mo	12mo	18mo	24mo	36mo	Age	Group	Age × Group
SOSD (m/s)	3833±31 a	3911±24 ab	3962±25 b	3958±24 b	3914±24 ab	3895±24 ab	3921±23 ab	0.0057	ns	ns
SOSL (m/s)	4026±36 a	4160±24 b	4222±25 bc	4259±25 c	4215±23 bc	4209±24 bc	4212±24 bc	<.0001	0.0767	ns
Oc (ng/mL)	98.6±5.5 c	85.4±5.0 c	59.9±4.9 b	39.5±5.1 a	29.2±4.9 a	35.4±5.0 a	26.2±5.7 a	<.0001	ns	ns
BALP (U/L)	298.9±17.7 d	229.8±16.4 c	199.6±16.1 bc	201.2±15.9 bc	128.9±16.0 a	150.1±16.1 ab	126.8±17.5 a	<.0001	ns	ns
IGF-I (ng/mL)	406.1±32.8 cd	441.8±30.3 d	359.2±29.7 bcd	353.6±30.2 bcd	228.0±29.7 a	289.4±30.2 abc	238.7±33.5 ab	<.0001	0.0838	ns
Insulin (μIU/L)	3.11±1.78 ab	1.73±1.28 a	5.29±1.33 ab	9.71±1.43 b	1.24±1.33 a	1.70±1.33 a	1.38±1.44 a	0.0001	0.0998	ns
Glucose (mmol/L)	9.42±0.19 d	7.46±0.18 c	6.17±0.17 b	5.37±0.18 a	4.99±0.18 a	5.16±0.18 a	5.00±0.20 a	<.0001	ns	ns
Leptin (ng/mL)	2.35±0.19 bc	2.04±0.16 abc	1.57±0.16 a	1.82±0.15 ab	1.88±0.16 ab	2.44±0.19 bc	2.69±0.18 c	0.0002	ns	ns

* Three of the 27 foals which SOS measurements were made and blood samples collected, were sold before the RX examination;
OC – osteochondrosis; SOSD – speed of sound on dorsal region of the third metacarpal bone; SOSL – speed of sound on lateral region of the third metacarpal bone; Oc – osteocalcin; BALP – bone alkaline phosphatase; IGF-I – insulin-like growth factor I;
Values represent lsmeans±SEM; Lowercase letters indicate significant differences within a row (Tukey-Kramer adjustment, P<0.05).

Table 6.6 – Plasmatic concentrations of parathyroid hormone, calcium, phosphorus and magnesium of Lusitano foals (n=24[*]) from birth to 36 months of age according to the radiographic status.

Variable	OC group status	Age-class							Significance of fixed effects		
		<45d	3mo	6mo	12mo	18mo	24mo	36mo	Age	Group	Age × Group
PTH (pg/mL)		38.9±6.1	29.6±5.5	17.4±5.4	26.9±5.6	20.7±5.3	35.2±5.5	27.6±6.0	0.0292	0.0626	ns
Ca (mmol/L)	Negative	2.57±0.06 a	2.70±0.05 ab	2.76±0.05 ab	2.67±0.06 ab	2.73±0.05 ab	2.90±0.05 b	2.74±0.06 ab	0.0334	ns	0.0783
	Positive	2.74±0.06	2.82±0.06	2.65±0.05	2.69±0.05	2.69±0.05	2.77±0.06	2.76±0.07			
P_i (mmol/L)		2.31±0.06 d	2.12±0.05 d	1.80±0.05 c	1.59±0.05 b	1.47±0.05 ab	1.62±0.05 bc	1.29±0.06 a	<.0001	ns	ns
Mg (mmol/L)		0.85±0.02 cd	0.86±0.02 d	0.80±0.02 abc	0.77±0.02 ab	0.82±0.02 bcd	0.76±0.02 a	0.79±0.02 abc	<.0001	ns	ns

[*] Three of the 27 foals which blood samples were collected, were sold before the RX examination;
OC – osteochondrosis; PTH – parathyroid hormone; Ca – calcium; P_i – phosphorus; Mg – magnesium; Values represent lsmeans±SEM;
Lowercase letters indicate significant differences within a row (Tukey-Kramer adjustment, P<0.05).

6.5. Discussion

The present field study was conducted on four stud-farms with similar environmental conditions (e.g. climate, pasture type). Besides some differences in management practices, they can be considered as representative of the Lusitano horse breeding systems. The great asset of this longitudinal study was to provide information on a sequence of events during the first 3 years of life on the same foals.

The mean values found for each parameter of the growth models are consistent with the parameter estimates of BW and WH growth functions previously reported for the Lusitano horse (Fradinho et al., 2015b). According to this last study, which involved a higher number of animals (n=121) including the present sub-sample, Lusitano foals showed a slower growth rate for BW, when compared with other sport breeds. In contrast, the WH growth rate was similar to those presented by early maturing breeds.

The results regarding the general increase of SOS values with age are in agreement with previous studies reported for young Thoroughbreds and Lusitano foals, in particular, in what concerns values obtained at the lateral region of the McIII (Jeffcott and McCartney, 1985; Carstajen et al., 2003; Fradinho et al., 2009). It was shown that the ash content and failure stress resistance of the equine McIII increase with age reaching its maximum values at 4 to 7 years (El Shorafa et al., 1979). In addition, other mechanical properties were positively correlated with age, during the growth period (Bigot et al., 1996). In the horse, the velocity of sound was previously correlated with bone mass (Jeffcott and McCartney, 1985) and a recent study of ours shows also a good correlation between SOS measurements and cortical bone stiffness (Fradinho et al., 2015a). Therefore, the SOS results of the present study could reflect the inherent increase of material and mechanical bone properties associated with growth.

The higher SOS values observed at the lateral region when compared with values of the dorsal region are consistent with other studies in foals and older horses (Carstanjen et al., 2002; 2003a; Fradinho et al., 2009; 2015a). However, in our study, SOS values obtained for males were higher than for females, both for lateral and dorsal regions, contrasting with the results found by Carstanjen et al. (2003a) in young exercised Thoroughbred racehorses between 2 and 4 years of age. The differences between lateral and dorsal measurements could be related to the regional microstructure and material properties since geometry, porosity and structural features of the McIII change according to its section and anatomic region (Skedros et al., 1996). Lower porosity was observed in the lateral region when compared with the dorsal region of the equine McIII cortex (Skedros et al., 1996) and osteon size and structure, and collagen fibre orientation along the bone revealed to be also regionally dependent (Martin et al., 1996a; 1996b). It was observed that mechanical properties of McIII were highly related with total body weight in the horse (Bigot et al., 1996). The effect of gender observed in SOS values could be partially explained by management differences and by the sexual dimorphism found in the Lusitano horse in what concerns conformation, as showed in a previous study (Fradinho et al., 2015b).

The age-related decrease observed for Oc and BALP plasma concentrations is in agreement with the reported in literature for other breeds, either in the first months (Lepage et al., 1990; Price et al., 2001; Vervuert et al., 2007a) or at later stages of horse life (Price et al., 1995a; 1995b; Lepage et al., 1998a). These characteristic age-related changes, reflects bone turnover and, in particular, bone cell activity associated with skeletal modelling during growth. The slight increase observed in both markers in the last year of the study was probably related with seasonal and reproductive status influence as reported before for Thoroughbreds (Price et al., 2001; Jackson et al., 2006).

From birth to 36 months old, IGF-I concentrations followed a decreasing pattern similar to the observed in some cross-sectional studies that involved horses with comparable age-groups (Champion et al., 2002; Noble et al., 2007; Munoz et al., 2011). In these studies, higher IGF-I values were reported for stallions when compared with mares or geldings. Our results showed also a significant effect of gender, but IGF-I plasma concentrations were simultaneously influenced by foaling season, with higher values observed in males and females born in February-March. This interaction with foaling season may be associated to environmental factors, such as pasture quality and availability. In fact, previous studies reported a possible relation between IGF-I concentrations and nutritional factors based on local pasture conditions (Champion et al., 2002; Staniar et al., 2007b). It was also shown that in extensive grazing systems, Lusitano foals born in February-March have better growth performances compared with foals born in April-May (Fradinho et al., 2012). Results of our study indicated a positive correlation between IGF-I and bone markers. IGF-I is one of the growth factors implicated on the regulation of endochondral ossification process that occurs in long bones, through its action on the proliferation and hypertrophy of chondrocytes. Besides, chondrocytes will release growth factors that promote the invasion of the growth plate cartilage by osteoblasts and osteoclasts (see review by Mackie et al., 2011). Other investigators have also observed significant correlations between IGF-I concentrations and bone formation markers in the horse (Davicco et al., 1994; Jackson et al., 2003a).

Plasma leptin concentrations throughout the first months of life were in the range reported for suckling foals (Kearns et al., 2006; Berg et al., 2007). One of the first studies describing peripheral concentrations of leptin in the horse, reported a trend for an increase with age based on single blood samples collected in horses from 8 days until 24 years of age and divided in four age-classes (< 2years; 2-4 years; 5-12 years and >12years) (Buff et al., 2002). Also Kearns et al. (2006) referred higher leptin concentrations in ten year mature mares when compared with weanling foals based on a single blood sampling. In the growing horse, the study of Cebulj-Kadunc and Cestnik (2008) reported higher leptin concentrations in three-year old Lipizzan when compared with yearlings. To the best of our knowledge there is no references in literature regarding leptin concentration changes on the same growing foals during such a long period (from birth to 36 months) and with a high number of blood samples. Because leptin concentrations are proportional to adiposity in horses and because adipose tissue is generally higher in mature horses (Buff et al., 2002), the quadratic model adjusted to leptin concentrations changes in our study, confirms the increase with age after the first year of life. The effect of gender, with higher leptin concentrations in fillies is in agreement with the results of Cartmill et al. (2003; 2006), who reported higher leptin concentrations in mares when compared with geldings and stallions. Although with a slight decrease during the experimental period, Kedzierski and Kapica (2008) also found higher leptin concentrations in Standardbred trotter fillies from 1.5 to 3 years of age, in exercise conditions. It was recognized that leptin could have a permissive role on the onset of puberty and circulating leptin increases during pubertal development in rodents, human females and heifers (Hall, 2003; Zieba et al., 2005). In our study, leptin concentrations in female increases mainly after 12 months of age, matching with the onset of puberty observed in the Lusitano and other horse breeds (Brown-Douglas et al., 2004; Cebulj-Kadunc et al., 2006; Fernandes, 2009). The change-pattern observed in males could be in part ascribed to the results reported by Fernandes (2009), who observed a later onset of puberty in Lusitano colts, when compared with fillies of the same study. Curiously, the negative correlation found between leptin and Oc concentrations seems to support the new findings in humans and rodents, which pointed out to an inhibitory effect of leptin on Oc function, as regards to the endocrine cross-talk between energy metabolism and bone (see reviews Ducy, 2011; Ferron and Lacombe, 2014).

Blood glucose is under a strong homeostatic system that keeps it within narrow limits. In the present study, baseline plasma glucose concentrations steadily decreased from birth until two years of age, although with small changes between 15 and 36 months of age. The high and decreasing glucose values observed in the first months of our study were in agreement with the reported for pre-weaning Thoroughbred foals (George et al., 2009) and in the range of values found in other studies with foals of similar ages (Rumbaugh and Adamson, 1983; Bauer et al., 1984). As hypothesized by George et al. (2009), this decrease in glucose concentrations during the first months after birth may reflect the progressive transition in dietary energy substrate, as the foal begins to consume more forage and less lactose-rich milk, which is primarily digested and absorbed in the small intestine. Although not influenced by age, gender or foaling season, the positive correlations observed between insulin and glucose, and insulin and IGF-I concentrations reflects the known role of insulin in the carbohydrate metabolism and cell growth (Satiel and Khan, 2001; Clemmons, 2004). In addition, a positive correlation was observed between insulin and leptin. Despite the differences between signalling pathways and locals of secretion, these two hormones are important regulators of energy homeostasis (Benoit et al., 2004). In an exploratory study with a large population of horses older than 3 years, a positive correlation between insulin and leptin concentrations was also described (Pratt-Phillips et al., 2010).

The present study provides information regarding total plasma Ca, P_i and Mg changes during a comprehensive period of the Lusitano horse growth. In blood, Ca is under a well-known and powerful homeostatic control that keeps it within a narrow range (Breslau, 1996; Toribio, 2011). Nevertheless, the increase in total Ca concentrations from birth to three years of age is consistent with the findings of Berlin and Aroch (2009), who observed lower plasma total Ca concentrations in neonate foals, when compared with adult horses. The regulation of P_i plasma concentrations is not as precise as that of Ca. Therefore, concentrations of P_i in blood are much more influenced by factors like diet, age and physical activity among others (Toribio, 2011; van Doorn et al., 2011). The decrease of P_i concentrations with age that was found in our study is in agreement with the reported in literature for foals and adult horses (Lumsden et al., 1980; Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Toribio, 2011). Also higher plasma total Mg concentrations were found in neonate foals when compared with the values of adult horses (Berlin and Aroch, 2009), and a decrease during the first year of age was reported in the experiment of Sloet van Oldruitenborgh-Oosterbaan et al. (1999), which is in accordance with the results of our study. Because Mg digestibility is higher in foals (Harrington and Walsh, 1980), it is possible that higher blood concentrations may be found in younger animals. The effect of foaling season that was observed in total Ca and P_i plasma concentrations could probably be explained by dietary factors, affecting both, lactating mares and indirectly nursing foals in a first stage, and growing foals thereafter.

Although not specifically influenced by age, gender or foaling season in this first analysis, an inverse relationship between PTH concentrations and plasma total Ca and Mg was observed. The synthesis and secretion of PTH is highly sensitive to acute and small decreases of ionized calcium (Ca^{2+}) in the blood, which is the biologically active form of Ca. But the calcium-sensing receptors in parathyroid cells also recognize other divalent cations such as magnesium

(Mg^{2+}) (Breslau, 1996). Several studies have shown a negative relation between PTH and (Ca^{2+}) in exercised horses (Aguilera-Tejero et al., 2001; Vervuert et al., 2002). Despite the concentrations of (Ca^{2+}) or (Mg^{2+}) have not be specifically determined in our study, Berlin and Aroch (2009) observed that, in horses of different ages and physiological stages, the ionized to total Ca ratio was above 50.9% and the ionized to total Mg ratio was above 63.0%, which can support our results.

In the present study, the assessment of the osteoarticular status was performed by radiographic examinations at a mature stage of foals' life, evaluating the presence or absence of radiographic findings compatible with OC-like lesions. Thus, considering the dynamic character of this condition and the fact that after 18 months most of the OC lesions became stable, the present results can be considered quite reliable (Dik et al., 1999; Jacquet et al., 2013; van Weeren and Jeffcott, 2013). However, taking into account the small number of animals involved in this field study, any extrapolations for the Lusitano breed should be taken with care. Furthermore, comparisons within the breed or between populations of other breeds in what concerns estimates of OC prevalence based in radiographic studies, can only be made when factors like age and the number of joints are properly standardized (van Weeren and Jeffcott, 2013). In the Lusitano horse there is a lack of radiographic surveys with a high number of animals, in standardized conditions. Although an in depth discussion regarding OC prevalence in this breed is beyond the scope of the present paper, our results regarding the lesions found in the tibiotarsal joint are in agreement with the findings of Baccarin et al. (2012), who observed a persistence of 16.2% of lesions in the same joint, in 18 months Lusitano foals. Considering the global radiographic results in our study, also a higher number of lesions in the metacarpal and metatarsal phalangeal joints were found, confirming the observations previously reported for Lusitano stallions (Bernardes, 2008; Teixeira, 2009).

Recently, a multifactorial approach was used in some field studies, in order to investigate the etiologic factors of equine OC (Vander Heyden et al., 2013; Robert et al., 2013). However, only the study reported by Robert et al. (2013) has explored growth parameters as a potential risk factor. In the present study different growth patterns were observed for OC positive and negative foals. The larger maturing index obtained in the BW growth model of positive foals indicated that these animals were earlier maturing individuals when compared to negative OC foals. In fact, this BW maturing index was clearly superior to the observed in a previous study with a higher number of Lusitano foals (Fradinho et al., 2015b). Furthermore, the results concerning instantaneous growth rates of both measures showed an interaction between age and OC group status. Lower IADG were observed for both measures in the positive group before the 45 days of age, but this differences disappear after the 3 months of age, showing an early change in the growth rates. The higher BW IADG observed in the OC positive group between the six and 18 months, reflects an acceleration in the growth rate, which is probably related with feeding practices introduced during the weaning and post-weaning period. In a controlled experiment with different nutritional levels Donabédian et al. (2006) showed an association between OC and fast development of some skeletal segments, being WH at early ages, the variable most frequently implicated. Also a cohort study with sport breed weanlings identified WH at 30 days of age and the slope of WH in the first six months after birth, as one of the risk factors for the presence of juvenile osteochondral conditions (which includes OC) (Lepeule et al., 2009). Additionally, the same group of researchers concluded that a rapid growth in girth perimeter together with irregular exercise conditions were associated to a poor osteoarticular status in the young foal (Lepeule et al., 2013). In that study, foals were not weighed, but a close and well known relation between girth and BW measurements in the horse has been often described, making girth as one of the linear measurements that are commonly used in weight prediction equations (Carrol and Huntington, 1988; Staniar et al., 2004b). In our study a high correlation was also found between BW and girth measurements (data not shown). Therefore the higher BW maturing index of the positive OC foals could also reflect a higher maturing index of girth.

The concept of bone quality as assessed by QUS includes, in a general way, bone density and bone mechanical properties. To the best of our knowledge there are no reports regarding a possible relationship between OC radiographic findings and SOS measurements on the same animals, although the potential applications of this technique on longitudinal studies concerning growth and development of the equine bone (Carstanjen et al., 2002; 2003b). Bone mineral density as assessed by dual-X ray absorptiometry was found to be lower in foals with high OC scores (Firth et al., 1999). Furthermore, cannon bone width rate gain was positively correlated with a higher incidence of developmental orthopaedic disease (DOD) (Donabédian et al., 2006). This group of researchers suggested that a faster intramembranous ossification rate (responsible for the periosteal apposition and widening of long bones), could be a predisposing factor to DOD, including OC. In fact, during the periosteal apposition of primary or new bone or in situations of high bone formation, the collagen fibrils could be laid down in a disorganized manner different from the lamellar pattern commonly found in cortical bone, assuming a different microstructure called woven bone (Rho et al., 1998; Clarke, 2008). Decreased SOS values on the dorsal region of the McIII were also associated to a high periosteal fibre apposition related to an increase in bone modelling process in young exercised Thoroughbreds (Carstanjen et al., 2003a). Because mechanical properties of cortical bone are greatly influenced by mineral density and by microstructural organisation of the solid matrix (Rho et al., 1998), the trend for lower SOS values in the lateral region of the McIII found in our study, could reflect a general low quality of the cortical bone in the positive OC foals.

In the present study, plasma concentrations of Oc and BALP were not different between groups regarding the OC radiographic status, which is agreement with the results obtained by Vervuert et al. (2007a) in healthy and OC positive Hanoverian foals. Our results contrast however, with those reported in other studies (Billinghurst et al., 2004; Donabédian et al., 2008), where some positive correlations were found between Oc concentrations and the occurrence of OC. Nevertheless, the use of different methodologies and data analysis in those studies should be taken into account.

For example, the study performed by Billinghurst et al. (2004) did not included unaffected foals. Therefore the analysis of serum bone markers was achieved in reference to the severity of OC in euthanized foals, which included three score methods (macroscopic OC severity score, total OC lesion count, and total OC radiographic scores in the hock plus stifle joints). Also the positive correlations between Oc levels at 2 weeks of age and the occurrence of OC found in the study of Donabédian et al. (2008) included the analysis of the total number of lesions detected radiographically and the necropsy score in a subsample of foals. The study of Valette et al. (2007) reported lower levels of Oc and BALP during the first six months of life in severely OC affected foals, based on radiographic findings obtained at 18 months of age. But in the correlation analysis between biochemical parameters and osteoarticular status, only the group of healthy foals (with no radiographic findings) and the group of severely affected foals were considered, being excluded from the analysis, the animals that were moderately affected. Lower levels of serum BALP were also found in Thoroughbreds that underwent arthroscopic surgery for the removal of OC fragments when compared with healthy horses in a similar median age (Trumble et al., 2008).

The trend for lower IGF-I values found in the positive OC group of foals in our study is in accordance with previous results reported in literature (Sloet van Oldruitenborgh-Oosterban et al., 1999; Baccarin et al., 2011). In a cross-sectional study with a single point blood sampling, which included radiographic examinations as a part of the protocol for stallion admission to Belgian Warmblood Studbook, also lower values of IGF-I were observed in the horses of the two year old group, which showed higher radiographic severity scores in what concerns osteoarticular status (Verwilghen et al., 2009a). Furthermore, lower IGF-I plasma concentrations were observed in pathological horses when compared to healthy ones, in a sequential study aiming at studying juvenile digital degenerative osteoarthropathy in the Ardenner breed (Lejeune et al., 2007).These results contrast however, with the reported in another similar study where higher IGF-I plasma levels per body weight unit were observed in OC affect horses (Verwilghen et al., 2009b). IGF-I is one of the most important anabolic peptides involved in the normal cartilage homeostatic balance, being a crucial regulator of chondrocyte proliferation and differentiation (Fortier et al., 2001; van der Eerden et al., 2003). In addition, IGF-I concentrations can be influenced by a number of endogenous (genetic and hormonal) and exogenous (nutritional and physical activity) factors (Zofková, 2003). Therefore, as suggested by Verwilghen et al. (2009a) longitudinal studies involving repeated blood sampling and measurements of markers may be more helpful for a better interpretation of the provided information. The trend for lower IFG-I values in the OC positive group foals of our study may reflect a dysregulation of the mechanisms involved in the local control of joint and bone tissue development. Furthermore, as IGF-I appears to function as an anabolic growth factor, the trend for lower values during the first months may have also impaired the natural regression process of some OC lesions. The sharp decrease in IGF-I concentrations from 12 to 18 months of age was probably associated to the decrease in pasture quality and availability during fall, in Mediterranean conditions. These seasonal-related changes confirm the findings of Staniar et al. (2007b), although in this last study, circulating IGF-I changes were more significant during the spring months in northern Virginia.

A trend for higher plasma insulin concentrations was observed in the OC positive group. In fact, the hypothesis that relative hyperinsulinaeamia may be a contributory factor to equine OC was suggested by Henson et al. (1997) from *in vitro* studies with fetal and neonate equine chondrocytes. Altered levels of circulating insulin could have therefore a direct endocrine effect on cartilage maturation through an increase on chondrocyte survival (Henson et al., 1997; Jeffcott and Henson, 1998). In the present study, variable amounts of concentrate feeds were introduced in foals' diet during the weaning and post-weaning periods, which occurred from the six months onwards. The higher insulin concentrations observed during this period could suggest a higher energy intake from diet, which may have led to alterations in the process of cartilage maturation and endochondral ossification and/or impaired the regression of some OC lesions. Furthermore, it was observed that positive OC foals had higher postprandial glucose and insulin responses to feeding than healthy foals (Ralston, 1996). In the study of Baccarin et al. (2011), where concentrates feeds were introduced in foals' diet after weaning, a transient increase in insulin concentrations was also observed in the positive OC foals between the seven and 10 months of age.

There were no differences between OC group status concerning basal glucose concentrations. Similar results were observed in Lusitano foals between birth and 18 months of age (Baccarin et al., 2011). Also no differences were found for plasma leptin concentrations between OC positive and OC negative foals. Like for the SOS measurements we could not found any reports relating plasma leptin concentrations and OC status in the horse. The increased values observed in last two age-classes are in agreement with the change pattern discussed above in the first analysis.

Very few studies have investigated horse PTH concentrations under field conditions and none during such a long period in the growing foal. The values found in the present study were in the range of values obtained in the studies of Estepa et al. (1998; 2003) for healthy adult horses and young foals. But our results were quite under the values observed by Sloet van Oldruitenborgh-Oosterban et al. (1999), which can be clearly explained by the different type of assay used in this last study. In addition to the intact PTH molecule, the commercial kit used by this group of researchers, also measured the C-terminal/mid molecule fragments. Despite the different unit scale, our results regarding the trend for higher PTH values in the OC positive foals were in agreement with the findings of Sloet van Oldruitenborgh-Oosterban et al. (1999), who observed higher PTH concentrations in OC positive foals at four months of age. In the same study, also total Ca concentrations tended to be lower during the first seven months in OC positive foals. The results of our study regarding total Ca plasma concentrations were not very clear in what concerns foals OC status. The small changes in total Ca concentrations between age-classes observed in the OC negative group seems to be followed by the expected physiologic changes of PTH until the 18 months of age. Despite Ca^{2+} concentrations were not precisely determined the results from this age-class onwards could be partially explained by the hysteresis phenomenon of PTH secretion, as total Ca concentrations reflect also its ionized active form in the blood. Hysteresis for PTH secretion, reported in several studies with normal human, dog and horse subjects, is defined as a higher PTH value for the same Ca^{2+} concentration during the induction of hypocalcemia than during the recovery from hypocalcemia (Toribio et al., 2003; Felsenfeld et al., 2007). The study of Toribio et al. (2003) showed that horses in which hypocalcemia was followed by hypercalcemia secreted more PTH than horses in which hypercalcemia was followed by hypocalcemia.

The P_i and Mg concentrations were not influenced by OC status and followed the age-change pattern found in our first analysis. Very few studies have investigated basal P_i and Mg plasma concentrations in relation to OC, but the lack of differences in P_i and Mg blood concentrations between positive and negative OC foals was also reported in the study of Sloet van Oldruitenborgh-Oosterban et al. (1999).

In the present study, the regularity of exercise conditions and the type of pasture surface, which were previously considered as risk factors for a poor osteoarticular status (Lepeule et al., 2009) were not investigated. Regarding exercise, and because all the foals had been exposed to similar conditions during the first months of life, we can only speculate that the different size of the paddocks during the post-weaning period may have provided different free exercise regimen, which may have had an additional effect on the latter OC status. In order to consolidate the body of evidences found in our research, a higher number of animals should be included in further longitudinal studies.

6.6. Conclusions

The present study comprehends a large characterization of a wide set of variables involved in the skeletal development of the horse, based on data collected under field conditions. Because the stud-farms included in the study have a profile that reflects most of the current management practices in the Lusitano production systems, some results regarding blood variables may be faced as reference values for the breed, and become useful for further investigation. The presence of radiographic findings compatible with OC lesions at the onset of training was associated with changes in BW and WH growth patterns. In addition, OC positive foals presented a higher maturing index than healthy foals concerning BW growth curve. Bone quality, bone markers, growth factors and other metabolic variables change markedly with age, and some trends were identified as having a relation with OC status. In particular, OC positive foals tended to have worse cortical bone quality, lower IGF-I and higher insulin and PTH concentrations, which may reflect some alterations at bone and energy metabolism levels. Besides the global set of information, this study underlines the importance of an early monitoring of foals' growth during the first year of life. In particular, sudden changes to the average growth rates observed for the breed should be avoided. Thus, the present results may complement the knowledge regarding some management practices, in order to prevent an inadequate feed intake of the young foal, and to promote a better quality of the osteoarticular status of the Lusitano horse.

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Suporting Information

Table 6.S1 – Parameter estimates of the individual growth models fitted to body weight-age data set of the Lusitano foals included in the study (n=34).

Measure [a]	Stud	Parameters [b]				R^2 [c]	RSD [d]
		A (± SE[e])	*b* (± SE[e])	*k* (± SE[e])	*M* (± SE[e])		
BW (kg)	A (n=10)	584.3	0.9866	0.00086	0.519	0.990	13.2
		533.4	0.9484	0.00121	0.712	0.987	15.2
		587.9	0.9829	0.00090	0.573	0.993	11.2
		558.7	0.9970	0.00062	0.428	0.977	17.2
		456.9	0.9965	0.00103	0.429	0.967	19.4
		470.3	0.9926	0.00139	0.448	0.979	16.8
		548.2	0.9879	0.00062	0.499	0.981	15.3
		427.7	0.9669	0.00148	0.580	0.983	13.5
		448.7	0.9944	0.00132	0.462	0.986	12.5
		571.2	0.9990	0.00060	0.452	0.979	16.3
	B (n=9)	587.1	0.8836	0.00212	1.053	0.990	16.2
		510.0	0.9176	0.00222	0.932	0.951	30.1
		631.5	0.9676	0.00110	0.731	0.983	20.0
		665.0	0.9658	0.00104	0.709	0.976	23.7
		628.9	0.9700	0.00119	0.673	0.992	14.2
		516.2	0.9063	0.00166	1.023	0.989	15.2
		472.8	0.7580	0.00266	1.580	0.995	10.2
		507.6	0.9708	0.00149	0.731	0.989	14.3
		500.1	0.8672	0.00222	1.044	0.970	23.3
	C (n=6)	554.4	0.9823	0.00168	0.640	0.981	17.1
		538.2	0.8896	0.00193	0.922	0.997	8.5
		600.3	0.9812	0.00145	0.681	0.997	9.1
		598.7	0.9555	0.00151	0.794	0.997	7.4
		721.9	0.9896	0.00071	0.634	0.997	8.2
		689.1	0.9688	0.00094	0.730	0.991	13.5
	D (n=9)	592.4	0.9958	0.00091	0.546	0.988	15.4
		544.0	0.9940	0.00119	0.559	0.977	19.6
		617.8	0.9573	0.00083	0.558	0.958	22.6
		454.6	0.9957	0.00176	0.553	0.980	17.0
		447.8	0.9998	0.00191	0.540	0.969	21.5
		548.5	0.9988	0.00072	0.465	0.967	21.2
		518.8	0.9986	0.00171	0.597	0.971	25.7
		434.9	0.9952	0.00157	0.523	0.971	19.6
		427.0	0.9982	0.00145	0.495	0.966	19.2
	Mean	**544.0 ± 74.5**	**0.9635±0.0675**	**0.00135±0.00069**	**0.671±0.236**	**0.981**	**16.6**

[a] BW – body weight.

[b] *A* – asymptotic value for BW as age approaches infinity (interpreted as mean BW at maturity); *b* – scaling parameter that defines the degree of maturity when age = 0 d (intercept on y axis); *k* – maturing index (rate that establishes the spread of the curve along time axis); *M* – determines the point of inflexion of the curve (for $0 < M < 1$. M is undefined).

[c] R^2 correspond to a pseudo R^2. calculated as 1 – (SS(Residual) / SS(Total $_{corrected}$)).

[d] RSD – residual standard deviation.

[e] SE – approximate standard error.

Table 6.S2 – Parameter estimates of the individual growth models fitted to withers height-age data set of the Lusitano foals included in the study (n=34).

Measure [a]	Stud	Parameters [b]				$R^{2\ c}$	RSD [d]
		A (± SE[e])	*b* (± SE[e])	*k* (± SE[e])	*M* (± SE[e])		
WH (cm)	A (n=10)	163.4	0.9832	0.00098	0.128	0.995	1.2
		162.4	0.9604	0.00130	0.146	0.995	1.1
		159.2	0.9009	0.00206	0.205	0.997	0.9
		158.6	0.8999	0.00214	0.198	0.993	1.4
		157.0	0.9767	0.00180	0.132	0.995	1.0
		157.6	0.9242	0.00236	0.165	0.988	1.8
		163.9	0.9754	0.00078	0.126	0.997	1.0
		160.4	0.9859	0.00079	0.114	0.985	1.9
		157.9	0.9716	0.00130	0.132	0.995	1.1
		173.1	0.9956	0.00041	0.107	0.988	1.7
	B (n=9)	160.3	0.9291	0.00168	0.180	0.992	1.5
		162.7	0.9507	0.00161	0.170	0.989	1.6
		161.3	0.8165	0.00264	0.281	0.991	1.7
		156.3	0.9454	0.00190	0.159	0.995	1.2
		166.8	0.9674	0.00123	0.133	0.994	1.3
		155.6	0.9182	0.00175	0.190	0.993	1.5
		159.2	0.9627	0.00145	0.168	0.995	1.3
		155.5	0.9473	0.00191	0.184	0.997	1.0
		155.8	0.7630	0.00345	0.295	0.993	1.3
	C (n=6)	161.7	0.9072	0.00213	0.196	0.996	0.9
		167.6	0.9762	0.00126	0.150	0.997	0.9
		167.9	0.9479	0.00190	0.192	0.998	1.0
		162.7	0.9526	0.00146	0.178	0.994	1.2
		158.0	0.9137	0.00194	0.215	0.997	0.9
		161.3	0.9403	0.00159	0.183	0.996	0.9
	D (n=9)	162.1	0.9870	0.00139	0.133	0.995	1.2
		163.1	0.9856	0.00157	0.140	0.993	1.5
		156.0	0.9812	0.00215	0.138	0.997	0.9
		155.1	0.9852	0.00166	0.126	0.995	1.1
		154.8	0.9471	0.00208	0.162	0.994	1.2
		159.0	0.9764	0.00148	0.138	0.997	0.9
		162.9	0.9758	0.00154	0.150	0.995	1.3
		159.2	0.9990	0.00088	0.101	0.995	1.0
		152.1	0.9544	0.00181	0.146	0.994	1.2
	Mean	**160.3 ± 2.7**	**0.9472±0.0360**	**0.00166±0.0004**	**0.163±0.029**	**0.994**	**1.2**

[a] WH – withers height.

[b] *A* – asymptotic value for BW as age approaches infinity (interpreted as mean BW at maturity); *b* – scaling parameter that defines the degree of maturity when age = 0 d (intercept on y axis); *k* – maturing index (rate that establishes the spread of the curve along time axis); *M* – determines the point of inflexion of the curve (for 0 < *M* < 1. M is undefined).

[c] R^2 correspond to a pseudo R^2, calculated as 1 – (SS(Residual) / SS($\text{Total}_{\text{corrected}}$)).

[d] RSD – residual standard deviation.

[e] SE – approximate standard error.

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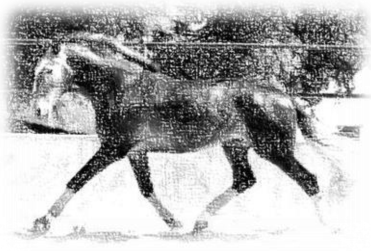
  

CHAPTER VII – ***GENERAL DISCUSSION***

The potential competitiveness of the Lusitano with other breeds, as a sport and leisure horse, is nowadays unquestionable. However, although the general concern to produce competitive and sound animals, some important features regarding the Lusitano production systems remain to be characterized and studied. In the scope of this thesis, the study and characterization of the broodmare productive cycle allowed for the integration of important information into the global evaluation of growth and development of the Lusitano foal from pregnancy to weaning. In addition, the results concerning the nutritional status and body condition changes of the mares along the year provided new insights about the appropriate scores in order to increase the productive efficiency of the system. Furthermore, the application of non-linear models to Lusitano foals' growth data enabled a comprehensive overview on growth patterns of this horse when managed in extensive systems, which constitutes innovative information for the breed. Considering the osteoarticular status at the onset of training and based on a long monitoring period under farm conditions it was also possible to characterize the changes of a large set of variables involved in skeletal development. Besides, some relations between OC status and BW and WH growth patterns, bone quality and some blood parameters related with bone and energy metabolism were identified.

The first study (Chapter III, 3.1) was designed in order to investigate the effects of feeding management and foaling season on nutritional status of Lusitano broodmares throughout the gestation/lactation cycle, by the assessment of BC, BW and blood metabolic indicators. Regardless the broodmare' stage of gestation/lactation, BW and BC changes along the year are usually influenced by seasonal and management factors (Martin-Rosset et al., 2006a; Pagan et al., 2006). In fact, our results showed that changes in BW and BC in the Lusitano broodmare, when managed on extensive grazing systems under Mediterranean climate conditions, were mainly influenced by pasture availability and quality and the time when foaling season occurs in the year. Corresponding to pasture cycle, a general increase in body reserves was observed during spring followed by a mobilization until winter, although this change does not represent more than half point of BC. Besides the availability of pasture, its nutritional value and the quality of supplementary feeds among stud-farms, appeared to have a strong effect on the mean annual BC, determining almost one point of BC variance, which was somewhat expected, attending the different feeding practices. Another important observation drawn from this study was the marked effect of the early foaling on mares' BC, in particular those that were mainly dependent on grazing resources. Mares that foaled early in the season showed a low or even no recovery of BC during the spring and a decreased level of BC throughout the whole cycle. Nevertheless, higher growth performances through the first three months of life were observed in early born foals, which may indicate a higher milk production. In fact, the shift of nutrients for milk production at this stage, would justify a less effective recovery of mares' BC during the spring in comparison with mares that were supplemented and foaled later in the year. Considering global BC and BW changes and, in particular, the results concerning blood indicators, it can be concluded that Lusitano mares managed in extensive systems showed an overall balanced nutritional status and an apparent metabolic welfare. Since there were no evident signs of under or over nutrition, Lusitano mares' appear to be well adapted to feed availability and climate.

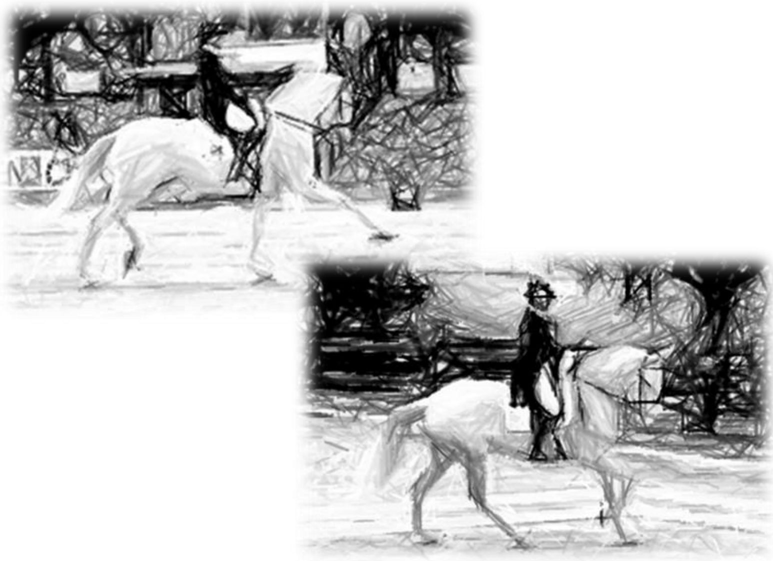
In the second study (Chapter III, 3.2) we aimed to investigate the effects of BC, BC changes, and plasma leptin concentrations on the reproductive performance of Lusitano broodmares. The effect of nutritional status and energy balance (reflected by BC and its changes) on reproductive performance of the mare has been recognized (Henneke et al., 1984; Guillaume et al., 2006). Nevertheless, the most appropriate scores in order to improve the productive efficiency were not definitely established for all systems and breeds. Our study clearly indicates that reproductive performance of the Lusitano broodmare on extensive systems is highly influenced by the nutritional status in the early postpartum period. The results showed that ΔBCScon had a strong effect on fertility outcome of the first two estrous cycles after foaling, being highly impaired by BCS negative changes, whatever the BCS. On the contrary, best fertility results were obtained with positive and greater BCScon changes. According to our model, the best predictive results regarding the probability to conceive during the two first estrous cycles and foaling (above 85%) were obtained for a BCScon between 3.0 and 3.75 and with a ΔBCScon of 0.25. The best predictive value (91%) was achieved at BCScon 3.0 and ΔBCScon 0.375. Because leptin has been suggested to be an important link between adipose tissue and the reproductive system, signaling the adequacy of energy reserves for a normal reproductive function (Gentry et al., 2002; Gastal et al., 2004; Ferreira-Dias et al., 2005), a possible relationship with reproductive performance was also investigated. In our study, leptin peripartum concentrations were influenced by foaling season, with lower levels in mares that foaled early in the year. However, the values recorded seem to be sufficient to allow for a normal reproductive activity, since there was no effect of leptin concentrations on fertility, regarding the first two estrous cycles. Another relevant observation of this work was the effect of mares' nutritional status on the growth of suckling foals. Foal growth performance appears to be influenced by mares BCS changes during the first three months of lactation with lower growth rates observed in foals which dams presented negative ΔBCS throughout this period. Together, the results of this study suggest that foaled mares should be gaining body reserves throughout the early postpartum period in order to enhance fertility and to support an adequate milk production for the growth of the suckling foal.

The next important step was the study and characterization of growth patterns of the Lusitano horse managed on grazing systems, from birth to 42 months of age, stage at which foals began to be ridden (Chapter IV). In long term growth studies with large volume of data, models which are nonlinear in their parameters adjust better to data and, usually, have an easier biological interpretation (Fitzhugh, 1976). Similar to other breeds, in which nonlinear growth models were applied (Staniar et al., 2004a; Heugebaert et al., 2010; Kocher & Staniar, 2013), the Richards function was also the best fit equation to describe BW, WH, G and CC growth of the Lusitano. The apparent late maturity of this breed, concerning BW, was confirmed by a lower maturing index and lower ADG, which were comparable with the moderate growth levels proposed for other sport horses. In contrast, the maturing index and the pattern of WH growth observed for the Lusitano was similar to those found in earlier maturing breeds like the Thoroughbred. These results showed that skeletal development may follow a between-breed similar pattern and confirms the recognized precocity of bone tissue. Nevertheless, and despite the continuous shape of the proposed growth models, it is expected that some deviations related with environmental influences and, in particular those associated with pasture seasonal availability, may occur (Staniar et al., 2004a; Kocher & Staniar, 2013). In our study, ADG changes of foals' BW and G were mainly associated with winter and spring coinciding with pasture productive cycle. As observed in other horse breeds, also an influence of gender was reflected in higher mature values for males, for all the studied variables. However, our results suggested that mature WH values may be slightly above those defined for the Lusitano breed standard, both for males and females. The great asset of this study was the comprehensive and updated overview of the Lusitano growth patterns, providing innovative information for the breed in what concerns reference growth curves for BW, WH, G and CC, based on local management conditions.

Besides the knowledge of the most adequate growth model for each breed and purpose, the development of a healthy and sound locomotor system is one of the main concerns of the sport horse industry. The use of non-invasive techniques that allowed the assessment of bone quality is therefore of vital importance for breeders and users. In Chapter V of this thesis the biomechanical properties of the equine McIII were studied using two different approaches: *in* *vivo* QUS and *ex vivo* destructive mechanical tests. The ultimate objective was to compare the results obtained by both techniques, on the same bone. The interest of researchers on QUS has grown in last years, as this non-invasive and radiation free method has the potential to provide information on both, bone density and elasticity (*E*) (Cavani et al., 2008). The results obtained in our study regarding SOS were in the range of the reported in literature for clinically sound horses, with differences between regions being ascribed to the inherent regional microstructure and material properties of the bone. Also the results obtained from the mechanical tests confirm that the mechanical properties of bone varied according to the anatomic location. Bone strength, determined either by axial compression or by bending, exhibited BR and BS dependence, with cortical specimens taken from the diaphysis being generally stiffer and stronger than the specimens taken from the proximal and distal metaphyseal levels of the McIII. The major outcome of this study was the good relation obtained between the SOS measurements on the dorsal region and the respective Young's modulus evaluated by compression tests on the same bone, which constitutes innovative results. This observation clearly reinforces the validity of QUS as a practical and non-invasive method for the *in vivo* assessment of equine cortical bone mechanical properties and overall bone quality.

Finally, considering that a better knowledge of the growing process, in relation to bone quality and osteoarticular status, is pivotal in order to improve the efficiency of the Lusitano production system, a last experimental work was performed in the scope of this thesis (Chapter VI). In this study we aimed at evaluating growth patterns and long-term changes on bone quality, bone metabolism, growth factors and metabolic related variables, and assessing whether these changes were related with radiographic findings regarding OC-like lesions at the onset of training. One of the important assets of this longitudinal study was to provide information on a sequence of events during the first three years of life on the same foals, under field conditions. Therefore, the in-depth characterization of age-related changes of a wide set of variables involved in skeletal growth and development of the foal, during such a comprehensive period, may become useful for further investigation, even in other breeds. In addition, as the stud-farms included in this work have a profile that reflects most of the current management practices in the Lusitano production systems, some results regarding blood variables may be faced as reference values for the breed.

Although being one of the main subjects of research in the growing horse, the complete mechanisms that lead to OC and its complex etiology remains to be fully elucidated. However, very few field studies under real-life farm conditions were conducted in order to investigate and define some risk factors of this multifactorial condition (Robert et al., 2013; Vander Heyden et al., 2013). Among these studies, only one group of researchers has explored growth parameters as being potentially implicated (Lepaule et al., 2009; 2013). Our results showed that changes in BW and WH growth patterns were associated with the presence of radiographic findings compatible with OC-like lesions at the onset of training. Taken together, the lower BW IADG and WH IADG under 45 days of age observed in positive foals, and the higher BW maturing index of this group, reflected by higher growth rates between the six and 18 months of age, confirms the involvement of growth characteristics as a risk factor for a poor osteoarticular status. Under the scope of this longitudinal study, some other observations were identified as being related to OC. The trend for lower SOS values observed in OC positive foals, which was reported for the first time, suggested that animals with this condition may have worse cortical bone quality than healthy foals. Also the trends for lower IGF-I and higher insulin and PTH concentrations, reinforce some previous observations, indicating that some alterations at bone and energy metabolism levels may be involved in the etiology of OC. Because IGF-I is one of the most important peptides involved in the normal cartilage homeostatic balance (Fortier et al., 2001; van Eerden et al., 2003), the trend for lower IGF-I in OC positive foals may reflect a dysregulation of the mechanisms involved in local control of joint and bone tissue development. Also, altered levels of circulating insulin have been implicated as a contributory factor to equine OC, having a direct endocrinal effect on cartilage maturation (Henson et al., 1997). In fact, higher insulin concentrations were observed from six months onwards when variable amounts of concentrate feeds were included in foals' diet after weaning. These higher insulin levels, which were probably induced by a higher energy intake, may have led to alterations in the process of cartilage maturation and endochondral ossification and/or impaired the regression of some OC lesions. Considering the overall body of evidences drawn from this study, the importance of an early monitoring of foals' growth, was clearly highlighted. In particular, sudden changes to the average growth rates should be avoided. These results may complement the current knowledge regarding some management practices, including the prevention of inadequate diets, in order to promote a sound skeletal development and a better osteoarticular quality of the Lusitano horse.



CHAPTER VIII – *CONCLUSIONS, IMPLICATIONS AND FUTURE PERSPECTIVES*

Taken together, and looking at the "whole picture" from pregnancy to the onset of training, the results presented in this work brought relevant insights into the characterization of the Lusitano production systems, both for the broodmare productive cycle and further foals´ growth and development. Bearing in mind that the results were mainly based on real field conditions and giving the limited information regarding some data in the sport horse, the conclusions drawn from the present research may be useful even for other breeds managed in similar pasture based systems.

Therefore, from the following conclusions, some practical implications and further research studies are suggested:

1. Changes in BW and BC in the Lusitano broodmare are mainly influenced by pasture availability and quality, as well as the time when foaling season occurs in the year. Thus, mares that foaled early in the year and depend mainly on grazing resources have a less effective recovery of body reserves after foaling and maintains a decreased level of BC throughout the whole cycle.
2. The reproductive performance of the broodmare is clearly influenced by the nutritional status. Foaled mares should be gaining body reserves throughout the early postpartum period in order to enhance fertility in the following breeding cycle and to support an adequate milk production for the growth of the suckling foal.

Considering the herbage productive cycle under Mediterranean climate conditions, it is advisable that the foaling season be centered in early spring in order to take advantage of pasture quantity and quality, as a main feed resource. Additionally, the introduction of some supplementary feed may be needed and should be timely provided. In this respect, body condition scoring should be an important tool to help breeders in the assessment of broodmare nutritional status and subsequent decisions regarding the most suitable feeding strategies.

As Lusitano horse production is mainly based on extensive grazing systems, it would be important that further studies determine the contribution of native pastures to meet the nutritional requirements of mares and foals. The assessment of voluntary feed intake of animals kept in pasture would be of great interest for the definition of feeding strategies. Also, the identification of broodmares appropriate BCS along the whole productive cycle should be an important contribution for economic efficiency improvement in the Lusitano production system.

3. The detailed characterization of BW, WH, G and CC growth patterns provided innovative information for the Lusitano breed.
4. Compared with other sport breeds, the Lusitano foal managed on grazing systems presented moderate BW growth rates. In contrast, it appeared to have similar WH growth patterns of earlier maturing breeds.
5. BW and G growth rates were prone to short-term deviations, with ADG changes generally observed during winter and spring time. These ADG changes showed the known seasonal compensatory growth ability related with pasture availability and quality.

The objective identification of periods where growth rates could be negatively affected may help breeders to develop feeding strategies in order to minimize those effects. Despite the in-depth characterization of the Lusitano foal growth patterns that was performed under the scope of this thesis, further research with controlled feeding levels should be done in order to verify the potential growth response of this breed.

6. Changes in foals BW and WH growth patterns were associated with the presence of radiographic findings compatible with OC-like lesions at the onset of training. In addition, OC positive foals seem to be early maturing animals as regards to BW.
7. OC positive foals showed a tendency for some alterations at bone and energy metabolism levels, reflected by changes in bone quality, growth factors and metabolic variables. In particular, insulin concentrations, which tended to be higher in OC positive foals, were probably the consequence of the inclusion of variable amounts of high energy concentrate feeds in the diet during the weaning and post-weaning periods.

Foal’s growth should be carefully monitored early after birth and in particular during the first year of life, in order to prevent the effects associated to sudden changes to average growth rates.

Because taller horses are often preferred for some equestrian disciplines, it is natural that Lusitano breeders will pursue this goal. Recently, WH was identified as being an objective trait for selection in the Lusitano horse, due to its high h^2 value (Vicente et al., 2014). As regards to this subject, and considering the environmental influence, two important observations drawn from our research are of concern: (1) the high maturity index of WH, also observed in the Lusitano foal; and (2) the evidence of a relationship between WH growth pattern changes and OC findings at the onset of training. Thus, early implementation of adequate management practices, including prevention of inadequate feed intake is vital for breeders, in order to promote a sustained growth and a better osteoarticular quality of the foals.

In the light of the current knowledge regarding the multifactorial etiology of OC, the implication of growth characteristics as a risk factor for a poor osteoarticular status was shown. However, in order to consolidate the body of evidences found in our research, further longitudinal studies should include a high number of animals and should monitor other environmental factors that were previously pointed out as being potentially involved in the etiology of this condition.

Finally, an obvious and practical implication that should be emphasized is the advantage of an integrated approach in what concerns mares and foal management decisions. In particular, the use of adequate feeding strategies in key periods will be determinant for the improvement of efficiency and profitability of the Lusitano production system.

The results obtained in this work encompasses a wide set of relevant features that contributes for a better knowledge of the Lusitano production cycle, in particular, concerning the characterization of growth and development process of the Lusitano foal. In this sense, our study might be considered as a sound basis for future research that will strengthen the present findings.

References

Abd-Elnaeim, M.M.M., Leiser, R., Wilsher, S. & Allen, W.R. (2006). Structural and haemovascular aspects of placental growth throughout gestation in young and aged mares. *Placenta,* 27, 1103-1113.

Adams, A.A., Katepalli, M.P., Kohler, K., Reedy, S.E., Stilz, J.P., Vick, M.M., Fitzgerald, B.P., Lawrence, L.M. & Horohov, D.W. (2009). Effect of body condition, body weight and adiposity on inflammatory cytokine responses in old horses. *Veterinary Immunology and Immunopathology,* 127, 286-294.

Aguilera-Tejero, A., Estepa, J., Lopez, I., Bas, S., Garfia, B. & Rodriguez, M. (2001). Plasma ionised calcium and parathyroid hormone concentrations in horses after endurance raids. *Journal of the American Veterinary Medical Association,* 219, 488– 490.

Allen, M. (2003). Biochemical markers of bone metabolism in animals: uses and limitations. *Veterinary Clinical Pathology,* 32, 101– 113.

Allen, V.G., Batello, C., Berretta, E.J., Hodgson, J., Kothmann, M., Li, X., McIvor, J., Milne, J., Morris, C., Peeters, A. & Sanderson, M. 2011. An international terminology for grazing lands and grazing animals. *Grass and Forage Science*, 66, 2-28.

Allen, W.R., Wilsher, S., Turnbull, C., Stewart, F., Ousey, J., Rossdale, P.D. & Fowden, A.L. (2002). Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development *in utero*. *Reproduction,* 123, 445-453.

Andrade, F.S. d'. (1988). O cavalo Lusitano. *Vida Rural,* 17, 16-17.

Andrade, R. d'. (1954). *Alrededor del caballo español*: colección de estudios. Sociedade Astória: Lisboa.

APSL (2010). Studbook Regulation. Associação Portuguesa de Criadores do Cavalo Puro-sangue Lusitano. Cascais, Portugal.

Ashworth, C.J., Hoggard, N., Thomas, L., Mercer, J.G., Wallace, J.M. & Lea, R.G. (2000). Placental leptin. *Reviews of Reproduction,* 5, 18–24.

Baccarin, R.Y.A., Pereira, M.A., Roncati, N.V., Furtado, P.V., Oliveira, C.A. & Hagen, S.C.F. (2011). Identificação dos níveis séricos do fator de crescimento tipo insulina 1 em potros com osteocondrose. *Pesquisa Veterinária Brasileira,* 31, 677-682.

Baccarin, R.Y., Pereira, M.A., Roncati, N.V., Bergamaschi, R.R. & Hagen S.C. (2012). Development of osteochondrosis in Lusitano foals: a radiographic study. *Canadian Veterinary Journal,* 53, 1079-1084.

Barbosa, M. & Vidal Abreu, J. (1986). Some reproductive parameters in Lusitanian and Arabian horses. *Revista Portuguesa de Ciências Veterinárias,* 478, 147-169.

Barneveld, A. & van Weeren, P.R. (1999). Conclusions regarding the influence of exercise on the development of the equine musculoskeletal system with special reference to osteochondrosis. *Equine Veterinary Journal Suppl*., 31, 112–119.

Baroncelli, G.I. (2008). Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. *Pediatric Research,* 63, 220–228.

Barrey, E., Desliens, F., Poirel, D., Biau, S., Lemaire, S., Rivero, J.–L.L. & Langlois, B. (2002). Early evaluation of dressage ability in different breeds. *Equine Veterinary Journal,* 34, 319–324.

Bauer, J.E., Harvey, J.W., Asquith, R.L., McNulty, P. & Kivipelto, J. (1984). Clinical chemistry reference values of foals during the first year of life. *Equine Veterinary Journal*, 16, 361-363.

Bell, A.W., Hay Jr, W.W. & Ehrhardt, R.A. (1999). Placental transport of nutrients and its implications for fetal growth. *Journal of Reproduction and Fertility Suppl.*, 54, 401-410.

Bell, R., Nielsen, B.,Waite, K., Rosenstein, D. & Orth, M. (2001). Daily access to pasture turnout prevents loss of mineral in the third metacarpus of Arabian weanlings. *Journal of Animal Science*, 79, 1142–1150.

Benoit, S.C., Clegg, D.J., Seeley, R.J. & Woods, S.C. (2004). Insulin and leptin as adiposity signals. *Recent Progress in Hormone Research*, 59, 267-285.

Berg, E.L., McNamara, D.L., Keisler, D.H. (2007). Endocrine profiles of periparturient mares and their foals. *Journal of Animal Science*, 85,1660-1668.

Bergero, D., Ventorp, M., Vervuert, I., Miraglia, N., Trillaud-Geyl, C. & Valle, E. (2006). Overview practices in the field in Europe: rationing and prevention of nutritional related problems in the mares. In N. Miraglia & W. Martin-Rosset (Eds.), *Nutrition and feeding of the broodmare*. (pp. 263–277). EAAP Publ. Nº 120. Wageningen, the Netherlands: Wageningen Academic Publishers.

Berlin, D. & Aroch, I. (2009). Concentrations of ionized and total magnesium and calcium in healthy horses: Effects of age, pregnancy, lactation, pH and sample type. *The Veterinary Journal*, 181, 305–311.

Bernardes, N.F.G. (2008). *Estudo da influência do exercício, da idade e da presença de lesões de osteocondrose nos níveis séricos de biomarcadores ósseos no cavalo Lusitano linha Alter Real*. Dissertação de Mestrado em Produção Animal. Faculdade de Medicina Veterinária/Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Lisboa.

Berndt, T. & Kumar, R. (2009). Novel mechanisms in the regulation of phosphorus homeostasis. *Physiology (Bethesda)*. 24, 17–25.

Biewener, A.A., Thomason, J., Goodship, A. & Lanyon, L.E. (1983). Bone stress in the horse forelimb during locomotion at different gaits: a comparison of two experimental methods. *Journal of Biomechanics*, 16, 565-576.

Bigot, G., Trillaud-Geyl, C., Jussiaux, M. & Martin-Rosset, W. (1987). Elevage du cheval de selle du sevrage au debourrage: alimentation hivernale, croissance et developpement. *Bulletin Technique* CRZV Theix, INRA. 69, 45-53.

Bigot, G., Martin-Rosset, W. & Dubroeucq, H. (1988). Evolution du format du cheval de selle de la naissance à 18 mois: critères et méthods d'appréciation. *Proceedings 14éme Journée de la Recherche Chevaline*, pp. 87-101. Paris: CEREOPA.

Bigot, G., Bouzidi, A., Rumelhart, R., Roux, R., Vantome, Y., Collobert-Laugier, C. & Martin-Rosset, W. (1990). Evolution au cours de la croissance des propriétés biomécaniques de l'os canon du cheval. *Proceedings 16e Journée de la Recherche Équine*, pp. 64-76. Paris: CEREOPA.

Bigot, G., Bouzidi, A., Rumelhart, C. & Martin-Rosset, W. (1996). Evolution during growth of the mechanical properties of the cortical bone in equine cannon-bones. *Medical Engineering and Physics*, 18, 79-87.

Billinghurst, R.C., Brama, P.A.J., van Weeren, P.R., Knowlton, M.S. & McIlwraith, C.W. (2004). Evaluation of serum concentrations of biomarkers of skeletal metabolism and results of radiography as indicators of severity of osteochondrosis in foals. *American Journal of Veterinary Research*, 65, 143–150.

Bjorbaek, C. (2009). Central leptin receptor action and resistance in obesity. *Journal of Investigative Medicine*, 57, 789-794.

Bjorbaek, C. & Kahn, B.B. (2004). Leptin signalling in the central nervous system and the periphery. *Recent Progress in Hormonal Research*, 59, 305-331.

Black, A., Schoknecht, P.A., Ralston, S.L. & Shapses, S.A. (1999). Diurnal variation and age differences in the biochemical markers of bone turnover in horses. *Journal of Animal Science*, 77, 75-83.

Blanchard, T.L., Thompson, J.A., Love, C.C., Brinsko, S.P., Ramsey, J., O'Meara, A. & Varner, D.D. (2012). Influence of day of postpartum breeding on pregnancy rate, pregnancy loss rate, and foaling rate in Thoroughbred mares. *Theriogenology*, 77, 1290-1296.

Block, S.S., Butler, W.R., Ehrhardt, R.A., Bell, A.W., Van Amburgh, M.E. & Boisclair, Y.R. (2001). Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. *Journal of Endocrinology*, 171, 339–348.

Boden, G. & Laakso, M. (2004). Lipids and glucose in type 2 diabetes: what is the cause and effect? *Diabetes Care*, 27, 2253–2259.

Bonewald, L.F. (2011). The amazing osteocyte. *Journal of Bone and Mineral Research*, 26, 229–238.

Bonucci, E. (2012). Bone mineralization. *Frontiers in Bioscience*, 17, 100-128.

Boskey, A.L. (2007). Mineralization of bones and teeth. *Elements*, 3, 387–393.

Boskey, A.L. (2013). Bone composition: relationship to bone fragility and antiosteoporotic drug effects. *BoneKEy Reports*, 2, Article, 447.

Boskey, A.L. & Coleman, R. (2010). Aging and bone. *Journal of Dental Research*, 89, 1333–1348.

Bosy-Westfal, A., Later, W., Hitze, B., Sato, T., Kossel, E., Glüer, C., Heller, M. & Müller, M.J. (2008). Accuracy of Bioelectrical Impedance consumer devices for measurement of body composition in comparison to whole body magnetic resonance imaging and Dual-X ray absorptiometry. *Obesity facts*, 1, 319-324.

Boyce, B.F., Yao, Z. & Xing, L. (2009). Osteoclasts have multiple roles in bone in addition to bone resorption. *Critical Reviews of Eukaryotic Gene Expression*, 19, 171–180.

Bragança, D. (1997). *Arte Equestre, Picaria Antiga, Equitação Moderna*. Edições Inapa: Lisboa.

Breslau, N. (1996). Calcium homeostasis. In J. Griffin & S. Ojeda (Eds.), *Textbook of Endocrine Physiology*. (3rd ed.). (pp. 314–348). New York: Oxford University Press.

Bridges, C.H. & Moffitt, P.G. (1990). Influence of variable content of dietary zinc on copper metabolism of weaning foals. *American Journal of Veterinary Research*, 51, 275-280.

Brown, E.M. & Jüppner, H. (2006). Parathyroid Hormone: Synthesis, Secretion, and Action. In M.J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism*. (6th ed.). (pp. 90-99). Washington, DC: American Society for Bone and Mineral Research.

Brown, J.E., Fitzhugh Jr, H.A. & Cartwright, T.C. (1976). A comparison of nonlinear models for describing weight-age relationship in cattle. *Journal of Animal Science*, 42, 810-818.

Brown-Douglas, C.G., Firth, E.C., Parkinson, T.J. & Fennessy, P.F. (2004). Onset of puberty in pasture-raised Thoroughbreds born in southern hemisphere spring and autumn. *Equine Veterinary Journal*, 36, 499-504.

Brown-Douglas, C.G., Parkinson, T.J., Firth, E.C. & Fennessy, P.F. (2005). Body weights and growth rates of spring and autumm-born thoroughbreds horses raised on pasture. *New Zealand Veterinary Journal,* 53, 326-331.

Brown-Douglas, C.G. & Pagan, J.D. (2006). Body weight, wither height and growth rates in thoroughbreds raised in America, England, Australia, NewZealand, and India. *Proceedings of the 15th Annual KER Conference*, pp.15–22. Kentucky, Lexington: Kentucky Equine Research, Inc.

Buckingham, S.H., Jeffcott, L.B., Anderson, G.A. & McCartney, R.N. (1992). In vivo measurement of bone quality in the horse: estimates of precision for ultrasound velocity measurement and single photon absorptiometry. *Medical and Biological Engineering and Computing,* 30, 41-45.

Buff, P.R., Dodds, A.C., Morrison, C.D., Whitley, N.C., McFadin, E.L., Daniel, J.A., Djiane, J. & Keisler, D.H. (2002). Leptin in horses: tissue localization and relationship between peripheral concentrations of leptin and body condition. *Journal of Animal Science,* 80, 2942–2948.

Buff, P.R., Morrison, C.D., Ganjam, V.K. & Keisler, D.L. (2005). Effects of short-term feed deprivation and melatonin implants on circadian patterns of leptin in the horse. *Journal of Animal Science,* 83, 1023-1032.

Buff, P.R., Spader, B.R., Morrison, C.D. & Keisler, D.H. (2006). Endocrine responses in mares undergoing abrupt changes in nutritional management. *Journal of Animal Science,* 84, 2700-2707.

Buff, P.R., Messer IV, N.T., Cogswell, A.M., Johnson, P.J., Keisler, D.H. & Ganjam, V.K. (2007). Seasonal and pulsatile dynamics of thyrotropin and leptin in mares maintained under a constant energy balance. *Domestic Animal Endocrinology,* 33, 430-436.

Bühler, M., Fürst, A., Lewis, F.I., Kummer, M. & Ohlerth, S. (2014). Computed tomographic features of apical infection of equine maxillary cheek teeth: a retrospective study of 49 horses. *Equine Veterinary Journal,* 46, 468–473.

Butler, A.A. & Le Roith, D. (2001). Control of growth by the somatropic axis: growth hormone and the insulin-like growth factors have related and independent roles. *Annual Review Physiology,* 63, 41–64.

Cabral, G.C., Almeida, F.Q., Quirino, C.R., Azevedo, P.C.N., Pinto, L.F. B. & Santos, E.M. (2004a). Avaliação morfométrica de equinos da raça Mangalarga Marchador: índices de conformação e proporções corporais. *Revista Brasileira de Zootecnia,* 33 (Supl. 1), 1798-1805.

Cabral, G.C., Almeida, F.Q., Quirino, C.R., Pinto, L.F., Santos, E.M. & Corassa, A. (2004b). Avaliação morfométrica de equinos da raça Mangalarga Marchador: medidas lineares. *Revista Brasileira de Zootecnia,* 33, 989-1000.

Caldeira, R.M.V.H. (1995). *Contributo para a interpretação metabólica da condição corporal e da sua variação na fêmea ovina*. Tese de Doutoramento. Lisboa: Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa.

Caldeira, R.M. (2005). Monitorização da adequação do plano alimentar e do estado nutricional em ovelhas. *Revista Portuguesa de Ciências Veterinárias,* 100 (555-556), 125-139.

Caldeira, R.M. & Vaz Portugal, A. (1991). Interrelationship between body condition and metabolic status in ewes. *Small Ruminant Research*, 6, 15-24.

Caldeira, R.M. & Vaz Portugal, A. (1998). Condição corporal: conceitos, métodos de avaliação e interesse da sua utilização como indicador na exploração de ovinos. *Revista Portuguesa de Ciências Veterinárias*, Vol. XCIII. 526, 95-102.

Caldeira, R.M., Belo, A.T., Santos, C.C., Vazques, M.I. & Portugal, A.V. (2007a). The effect of body condition score on blood metabolites and hormonal profiles in ewes. *Small Ruminant Research*, 68, 233-241.

Caldeira, R.M., Belo, A.T., Santos, C.C., Vazques, M.I. & Portugal, A.V. (2007b). The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. *Small Ruminant Research,* 68, 242-255.

Cameron, E.Z., Linklater, W.L., Stafford, K.J. & Veltman, C.J. (1999). Birth sex ratios relate to mare condition at conception in Kaimanawa horses. *Behavioral Ecology*, 10, 472-475.

Cameron, N.D. & Cienfuegos-Rivas, E. (1994). Genetic and phenotypic relations between physiological traits and performance test traits in sheep. *Genetics Selection Evolution*, 26, 137-150.

Camillo, F., Marmorini, P., Romagnoli, S., Vannozzi, I. & Bagliacca, M. (1997). Fertility at the first post partum estrous compared with fertility at the following estrous cycles in foaling mares and with fertility in nonfoaling mares. *Journal of Equine Veterinary Science*, 17, 612–616.

Carroll, C.L. & Huntington, P.J. (1988). Body condition scoring and weight estimation of horses. *Equine Veterinary Journal*, 20, 41-45.

Carstanjen, B., Lepage, O., Detilleux, J., Duboeuf, F. & Amory, H. (2002). Use of multisite quantitative ultrasonography for non-invasive assessment of bone in horses. *American Journal of Veterinary Research*, 63, 1464-1469.

Carstanjen, B., Duboeuf, F., Detilleux, J. & Lepage, O. (2003a). Equine third metacarpal bone assessment by quantitative ultrasound and dual energy X-Ray absorptiometry: an *ex vivo* study. *Journal of Veterinary Medicine Series A*, 50, 42-47.

Carstanjen, B., Lepage, O., Hars, O., Langlois, P., Duboeuf, F. & Amory, H. (2003b). Speed of sound measurements of the third metacarpal bone in young exercising Thoroughbred racehorses. *Bone*, 33, 411-418.

Carter, R.A., Geor, R.J., Staniar, W.B., Cubitt, T,A, & Harris, P.A. (2009). Apparent adiposity assessed by standardised scoring systems and morphometric measurements in horses and ponies. *The Veterinary Journal*, 179, 204-210.

Carter, R.A., McCutcheon, L.J., Valle, E., Meilahn, E.N. & Geor, R.J. (2010). Effects of exercise training on adiposity, insulin sensitivity, and plasma hormone and lipid concentrations in overweight or obese, insulin-resistent horses. *American Journal of Veterinary Research*, 71, 314-321.

Cartmill, J.A. (2004). *Leptin in horses: Influences of body condition, gender, insulin insensitivity, feeding, and dexamethasone.* Ph.D. Thesis. Louisiana State University. Baton Rouge.

Cartmill, J.A., Thompson, D.L., Gentry, L.R., Pruett, H.E. & Johnson, C.A. (2003). Effects of dexamethasone, glucose infusion, adrenocorticotropin, and propylthiouracil on plasma leptin concentrations in horses. *Domestic Animal Endocrinology*, 24, 1-14.

Cartmill, J.A., Thompson Jr., D.L., Storer, W.A., Crowley, J.C., Huff, N.K. & Waller, C.A. (2005). Effect of dexamethasone, feeding time, and insulin infusion on leptin concentrations in stallions. *Journal of Animal Science*, 83, 1875-1881.

Cartmill, J.A., Thompson Jr., D.L., Del Vecchio, R.P., Storer, W.A. & Crowley, J.C. (2006). Leptin secretion in horses: Effects of dexamethasone, gender, and testosterone. *Domestic Animal Endrocrinology,* 31, 197-210.

Cassill, B., Jackson, S. & Lawrence, L. (2009). Body weight changes in pregnant mares. *Journal of Equine Veterinary Science*, 29, 400-401.

Cavani, F., Giavaresi, G., Fini, M., Bertoni, L., de Terlizzi, F., Barkmann, R. & Cane, V. (2008). Influence of density, elasticity, and structure on ultrasound transmission through trabecular bone cylinders. *IEEE Transactions Ultrasonics, Ferroelectrics and Frequency Control Society*, 55, 1465-1472.

Cavinder, C.A. (2006). Reproductive and endocrine parameters of fat versus moderately conditioned mares following parturition. Ph.D. Thesis. Texas A&M University.

Cavinder, C.A., Vogelsang, M.M., Gibbs, P.G., Forrest, D.W. & Schmitz, D.G. (2009). Variances in reproductive efficiency of mares in fat and moderate body condition following parturition. *Professional Animal Scientist*, 25, 250-225.

Cebulj-Kadunc, N. & Chestnik, V. (2005). Circulating leptin concentrations in Lipizzan horses and Jezersko-Solchava sheep. *Slovenian Veterinary Research,* 42, 11-14.

Cebulj-Kadunc, N., Cestnik, V. & Kosec, M. (2006). Onset of puberty and duration of seasonal cyclicity in Lipizzan fillies. *Equine Veterinary Journal,* 38**,** 350-353.

Cebulj-Kadunc, N. & Cestnik, V. (2008). The influence of season and age on circulating melatonin and leptin concentrations in Lipizzan fillies. *Acta Veterinaria (Beograd),* 58, 25-31.

Champion, Z.J., Breier, B.H., Ewen, W.E., Tobin, T.T. & Casey, P.J. (2002). Blood plasma concentrations of insulin-like growth factor-I (IGF-I) in resting Standardbred horses. *The Veterinary Journal* 163, 45–50.

Chen, J.-H., Liu, C., You, L. & Simmons, C.A. (2010). Boning up on Wolff's law: mechanical regulation of the cells that make and maintain bone. *Journal of Biomechanics,* 43, 108–118.

Christensen, R.A., Malinowski, K., Massenzio, A.M., Hafs, H.D. & Scanes, C.G. (1997). Acute effects of short-term feed deprivation and refeeding on circulating concentrations of metabolites, insulin-like growth factor-1, insulin-like growth binding proteins, somatotropin, and thyroid hormones in adult geldings. *Journal of Animal Science,* 75, 1351-1358.

Christenson, R.H. (1997). Biochemical markers of bone metabolism: an overview. *Clinical Biochemistry,* 30, 573-593.

Christie, J.L., Hewson, C.J., Riley, C.B., McNiven, M.A., Dohoo, I.R. & Bate, L.A. (2006). Management factors affecting stereotypies and body condition score in nonracing horses in Prince Edward Island. *Canadian Veterinary Journal,* 47, 136-143.

Christou, H., Serdy, S. & Mantzoros, C.S. (2002). Leptin in relation to growth and developmental processes in the fetus. *Seminars in Reproductive Medicine,* 20, 123-130.

Çilek, S. (2009). Environmental factors affecting growth characteristics in Purebred Arabian foals reared at Anadolu State Farm in Turkey. *Journal of Animal and Veterinary Advances,* 8, 148-154.

Clarke, B. (2008). Normal Bone Anatomy and Physiology. *Clinical Journal of the American Society of Nephrology*, 3, S131–S139.

Clemmons, D.R. (2004). The relative roles of growth hormone and IGF-1 in controlling insulin sensitivity. *Journal of Clinical Investigation,* 113, 25–27.

Coenen, M. (2001). German Feeding Standards. In Pagan, J.D. & Geor. R.J. (Eds) *Advances in equine nutrition II.* (pp. 365-378). Nottingham, UK: Nottingham University Press.

Coenen, M., Kienzle, E., Vervuert, I. & Zeyner, A. (2011). Recent German developments in the formulation of energy and nutrient requirements in horses and the resulting feeding recommendations. *Journal of Equine Veterinary Science*. 31, 219-229.

Coleman, D.L. (1973). Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia*. 9, 294–298.

Coleman, R.J., Mathison, G.W. & Burwash, L. (1999). Growth and condition at weaning of extensively managed creep-feed foals. *Journal of Equine Veterinary Science,* 19, 45-50.

Collas, C., Dumont, B., Delagarde, R., Martin-Rosset, W. & Fleurance, G. (2015). Energy supplementation and herbage allowance effects on daily intake in lactating mares. *Journal of Animal Science,* 93, 2520-2529.

Confavreux, C.B., Levine, R.L. & Karsenty, G. (2009). A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms. *Molecular and Cellular Endocrinology*, 310, 21–29.

Cordeiro, A. (1997). *Cavalo Lusitano – O filho do vento*. 4ª edição. Edições Inapa: Lisboa.

Cosden, R.S. (2007). *Insulin-like growth factor-I in growing horses and RNA isolation from small articular cartilage samples.* Master Science Dissertation. Virginia Polytechnic Institute and State University. Blacksburg, VA, USA.

Currey, J. (2009). Measurement of the mechanical properties of bone: a recent history. *Clinical Orthopaedics and Related Research,* 467, 1948–1954.

Cymbaluk, N.F., Christison, G.I. & Leach, D.H. (1990). Longitudinal growth analysis of horses following limited and ad libitum feeding. *Equine Veterinary Journal,* 22, 198–204.

Dalamaga, M., Chou, S.H., Shields, K., Papageorgiou, P., Polyzos, S.A. & Mantzoros, C.S. (2013). Leptin at the Intersection of neuroendocrinology and Metabolism: Current Evidence and Therapeutic Perspectives. *Cell Metabolism,* 18, 29-42.

Dario, C., Carnicela, D., Dario, M. & Bufano, G. (2006). Morphological evaluation and heritability estimates for some biometric traits in the Murguese horse breed. *Genetics and Molecular Research,* 5, 309-314.

Davicco, M.J., Faulconnier, Y., Coxam, V., Dubroeucq, H., Martin-Rosset, W. & Barlet, J.P. (1994). Systemic bone growth factors in light breed mares and their foals. *Archives Internationales de Physiology, de Biochimie et de Biophysique,* 102, 115-119.

Davies, H.M. & McCarthy, R.N. (1994). Strain in the yearling equine metacarpus during locomotion. *Equine Veterinary Journal Suppl.* 17, 25-28.

Davies, H.M., McCarthy, R.N., Jeffcott, L.B. (1993). Surface strain on the dorsal metacarpus of Thoroughbreds at different speeds and gaits. *Acta Anatomica,* 146, 148-153.

Davies Morel, M.C.G., Newcombe, J.R. & Holland, S.J. (2002). Factors affecting gestation length in the Thoroughbred mare. *Animal Reproduction Science,* 74, 175-185.

Davies Morel, M.C.G., Newcombe, J.R. & Hayward, K. (2010). Factors affecting pre-ovulatory follicle diameter in the mare: the effect of mare age, season and presence of other ovulatory follicles (multiple ovulation). *Theriogenology,* 74, 1241-1247.

Deftos, L.J. (2006). Calcitonin. In M.J. Favus (Ed.) *Primer on the metabolic bone diseases and disorders of mineral metabolism,* 6th Edition (pp. 115-117). Washington, DC: American Society for Bone and Mineral Research.

DeLuca, H.F. (2004). Overview of general physiologic features and functions of vitamin D. *The American Journal of Clinical Nutrition,* 80, 1689S–196S.

Denoix, J.-M., Jeffcott, L.B., McIlwraith, C.W. & van Weeren, P.R. (2013). A review of terminology for equine juvenile osteochondral conditions (JOCC) based on anatomical and functional considerations. *The Veterinary Journal,* 197, 29–35.

DePew, C.L., Thompson Jr., D.L., Fernandez, J.M., Stiker, L.S. & Burleigh, D.W. (1994). Changes in concentrations of hormones, metabolites amino acids in plasma of adult horses relative to overnight feed deprivation followed by a pellet-hay meal fed at noon. *Journal of Animal Science,* 72, 1530-1539.

Dik, K.J., Enzerink, E. & van Weeren, P.R. (1999). Radiographic development of osteochondral abnormalities in the hock and stifle of Dutch Warmblood foals, from age 1 to 11 months. *Equine Veterinary Journal,* 31, 9-15.

Distl, O. (2013). The genetics of equine osteochondrosis. *The Veterinary Journal,* 197, 13–18.

Donabédian, M., Delguste, C., Perona, G., Lebecque, P., Duboeuf, F., Lepage, O. & Martin-Rosset, W. (2005). Third metacarpal bone mineral density assessment in the standing horse by dual X-ray absorptiometry: suitability, precision and accuracy. *Veterinary and Comparative Orthopaedic Traumatology,* 18, 26-30.

Donabédian, M., Fleurance, G., Perona, G., Robert, C., Lepage, O., Trillaud-Geyl, C., Léger, S., Ricard, A., Bergero, D. & Martin-Rosset, W. (2006). Effect of fast vs. moderate growth rate related to nutrient intake on developmental orthopaedic disease in the horse. *Animal Research,* 55, 471–486.

Donabédian, M., van Weeren, P.R., Perona, G., Fleurance, G., Robert, C., Léger, S., Bergero, D., Lepage, O. & Martin-Rosset, W. (2008). Early changes in biomarkers of skeletal metabolism and their association to the occurrence of osteochondrosis (OC) in the horse. *Equine Veterinary Journal,* 40, 253-259.

Donnely, E. (2011). Methods for assessing bone quality, a review. *Clinical Orthopaedics and Related Research,* 469, 2128–2138.

Doreau, M., Martin-Rosset W. & Barlet, J. (1981). Variations de quelques constituants plasmatiques chez la jument allaitante en fin de gestation et début de lactation. *Annale de Recherches Véterinaires*, 12, 219-225.

Doreau, M., Boulot, S., Martin-Rosset, W. & Robelin, J. (1986). Relationship between nutrient intake, growth and body composition of the nursing foal. *Reproduction Nutrition Development,* 26, 683-690.

Doreau, M., Moretti, C. & Martin-Rosset, W. (1990). Effect of quality of hay given to mares around foaling on their voluntary intake and foal growth. *Annales de Zootechnie,* 39, 125-131.

Doreau, M., Boulot, S., Bauchard, D., Barlet, J.P. & Martin-Rosset, W. (1992). Voluntary intake, milk production and plasma metabolites in nursing mares fed two different diets. *Journal of Nutrition,* 122, 992-999.

Doreau, M., Boulot, S. & Chiliard, Y. (1993). Yield and composition of milk from lactating mares: effect of body condition at foaling. *Journal of Dairy Research,* 60, 457-466.

Douglas Wilson, A., Badnell-Waters, A.J., Bice, R., Kelland, A., Harris, P.A. & Nicol, C.J. (2007). The effects of diet on blood glucose, insulin, gastrin and the serum tryptophan: Large neutral amino acid ratio in foals. *The Veterinary Journal,* 174, 139-149.

Druml, T., Baumung, R. & Sölkner, J. (2008). Morphological analysis and effect of selection for conformation in the Noriker draught horse population. *Livestock Science,* 115, 118-128.

Ducy, P. (2011). The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. *Diabetologia,* 54, 1291–1297.

Dugdale, A.H., Curtis, G.C., Cripps P., Harris, P.A. & Argo, C.M. (2010a). Effect of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. *Equine Veterinary Journal,* 42, 600-610.

Dugdale A.H., Curtis, G.C., Cripps, P., Harris, P.A. & Argo, C.M. (2010b). Effects of season and body condition on appetite, body mass and body composition in ad libitum fed pony mares. *The Veterinary Journal*. 190, 329-337.

Dyson, S., Murray, R., Schramme, M. & Branch, M. (2003). Magnetic resonance imaging of the equine foot: 15 horses. *Equine Veterinary Journal,* 35, 18-26.

Ekman, S., Carlson, C.S. & van Weeren, P.R. (2009). Third international workshop on equine osteochondrosis, Stockholm, 29–30th May 2008. *Equine Veterinary Journal,* 41, 504–507.

E1 Shorafa, W.M., Feaster, J.P. & Ott, E.A. (1979). Horse metacarpal bone: age, ash content, cortical area and failure stress interrelationships. *Journal of Animal Science,* 49, 979-982.

Elliot, C., Morton, J. & Chopin, J. (2009). Factors affecting foal birth weight in Thoroughbred horses. *Theriogenology*, 71, 683-689.

Ellis, P. (2002) Condition scoring and weight estimation of horses. *Agriculture Notes*. Dep. of Natural Resources and Environment. Australia. Retrieved Jan. 21st, 2006, http://saveequus.org/wp-content/uploads/2008/10/condition-scoring-and-weight-estimation-for-horses1.pdf

EN ISO 6865 (2000). *Animal feeding stuffs. Determination of crude fibre content – Method with intermediate filtration.* International Organization for Standardization.

Essén-Gustavsson, B. (2008). Triglyceride storage in skeletal muscle. In M. Saastamoinan and W. Martin-Rosset (Eds.), *Nutrition of the exercising horse*. EAAP Publication Nº125. (pp. 31-42). Wageningen, the Netherlands: Wageningen Academic Publishers.

Estepa, J.C., Aguilera-Tejero, E., Mayer-Valor, R., Almaden, Y., Felsenfeld, A.J. & Rodriguez, M. (1998). Measurement of parathyroid hormone in horses. *Equine Veterinary Journal,* 30, 476–481.

Estepa, J.C., Garfia, B., Gao, P., Cantor, T., Rodriguez, M. & Aguilera-Tejero, E. (2003). Validation and clinical utility of a novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone in the horse. *Equine Veterinary Journal,* 35, 291–295.

Evans, P. (2005) Body Condition Scoring: a management tool for evaluating all horses. Utah State University Extension. Retrieved Jan. 18th, 2006, http://extension.usu.edu/files/publications/equine1.6.02.pdf

FAO/IIASA/ISRIC/ISSCAS/JRC (2009). *Harmonized World Soil Database (version 1.1)*. FAO, Rome, Italy and IIASA, Laxenburg, Austria. Available from: http://www.fao.org/fileadmin/templates/nr/documents/HWSD/HWSD_Documentation.pdf

Favus, M.J., Bushinsky, D.A. & Lemann Jr., J. (2006). Regulation of calcium, magnesium, and phosphate metabolism. In M.J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism.* (6th ed.). (pp. 76-83). Washington, DC: American Society for Bone and Mineral Research.

Felsenberg, D. & Boonen, S., (2005). The bone quality framework: determinants of bone strength and their interrelationships, and implications for osteoporosis management. *Clinical Therapeutics,* 27, 1-11.

Felsenfeld, A.J., Rodriguez, M. & Aguilera-Tejero, E. (2007). Dynamics of parathyroid hormone secretion in health and secondary hyperparathyroidism. *Clinical Journal of the American Society of Nephrology,* 2, 1283–1305.

Fernandes, R. (2009). *Study of the onset of puberty in the Lusitano horse in extensive systems*. Master Thesis in Animal Science. Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Lisboa.

Ferreira-Dias, G., Claudino, F., Carvalho, H., Agrícola, R., Alpoim-Moreira, J. & Robalo-Silva, J. (2005). Seasonal reproduction in the mare: possible role of plasma leptin, body weight and immune status. *Domestic Animal Endocrinology*, 29, 203-213.

Ferris, R.A. & McCue, P.M. (2011). Gestation length, duration of labor and foal survival. *Journal of Equine Veterinary Science,* 31, 318.

Ferron, M. & Lacombe, J. (2014). Regulation of energy metabolism by the skeleton: osteocalcin and beyond. *Archives of Biochemistry and Biophysics*. 561, 137–146.

Fielding, C.L., Magdesian, K.G., Elliot, D.A., Cowgill, L.D. & Carlson, G.P. (2004). Use of multifrequency bioelectrical impedance analysis for estimation of total body water and extracellular and intracellular fluid volumes in horses. *American Journal of Veterinary Research*, 65, 320-326.

Filipović, N., Stojević, Z. & Prvanović, N. (2010). Serum fructosamine concentrations in relation to metabolic changes during late pregnancy and early lactation in mares. *Berliner und Münchener tierärztliche Wochenschrift,* 123, 169-173.

Firshman, A.M. & Valberg, S.J. (2007). Factors affecting clinical assessment of insulin sensitivity in horses. *Equine Veterinary Journal,* 39, 567-575.

Firth, E.C. (2006). The response of bone, articular cartilage and tendon to exercise in the horse. *Journal of Anatomy,* 208, 513–526.

Firth, E.C. & Rogers, C.W. (2005). Musculoskeletal responses of 2-year-old thoroughbred horses to early training. 7. Bone and articular cartilage response in the carpus. *New Zealand Veterinary Journal*, 53, 113-122.

Firth, E.C., van Weeren, P.R., Pfeiffer, D.U., Delahunt, J. & Barneveld, A. (1999). Effect of age, exercise and growth rate on bone mineral density (BMD) in third carpal bone and distal radius of Dutch Warmblood foals with osteochondrosis. *Equine Veterinary Journal Suppl.,* 31, 74-78.

Fitzgerald, B.P. & McManus, C.J. (2000). Photoperiodic versus metabolic signals as determinants of seasonal anestrus in the mare. *Biology of Reproduction,* 63, 335-340.

Fitzhugh, Jr., H.A. (1976). Analysis of growth curves and strategies for altering their shape. *Journal of Animal Science*, 42, 1036-1051.

Fonseca, R.G., Kenny, D.A., Hill, E.W. & Katz, L.M. (2013). The relationship between body composition, training and race performance in a group of Thoroughbred flat racehorses. *Equine Veterinary Journal*, 45, 552–557.

Fontes, M.A. & Jorge, M.N. (2013). *Produção equina: uma abordagem económica*. Formação Pós-Graduada. Faculdade de Medicina Veterinária. Universidade Técnica de Lisboa.

Fortier, L.A., Balkman, C.E., Sandell, L.J., Ratcliffe, A. & Nixon, A.J. (2001). Insulin-like growth factor-I gene expression patterns during spontaneous repair of acute articular cartilage injury. *Journal of Orthopaedic Surgery*, 19, 720-728.

Fortier, L.A., Kornatowski, M.A., Mohammed, H.O., Jordan, M.T., O'Cain, L.C. & Stevens W.B. (2005). Age-related changes in serum insulin-like growth factor-I, insulin-like growth factor-I binding protein-3 and articular cartilage structure in Thoroughbred horses. *Equine Veterinary Journal*, 37, 37-42.

Fowden, A.L., Comline, R.S. & Silver, M. (1984). Insulin secretion and carbohydrate metabolism during pregnancy in the mare. *Equine Veterinary Journal*, 16, 239-246.

Fowden, A.L., Gardner, D.S., Ousey, J.C., Giussani, D.A. & Forhead, A.J. (2005). Maturation of pancreatic-cell function in the fetal horse during late gestation. *Journal of Endocrinology*, 186**,** 467–473.

Fradinho, M.J. & Abreu, J.M. **(**2006). Aspectos biométricos e ponderais avaliados em equinos de raça Lusitana: estimativa do peso vivo em animais adultos [Abstract]. In *Proceedings of the XVI Congresso de Zootecnia*, Castelo Branco, Portugal, pp. 28.

Fradinho, M.J., Fernandes, R., Gomes, T., Correia, M.J., Vila-Viçosa, M.J.C., Silva, M.F., Ferreira-Dias, G. & Caldeira, R.M. (2008). Growth and conformation changes in the young Lusitano horse: evaluation from birth to one year of age. In M.T. Saastamoinen & W. Martin-Rosset (Eds.), *Nutrition of the exercising horse.* (pp. 367-370). EAAP Publ Nº 125, Wageningen, the Netherlands: Wageningen Academic Publishers.

Fradinho, M.J., Correia, M.J., Vila-Viçosa, M.J.C., Farrim, A., Harris, P.A., Bessa, R.J.B., Caldeira, R.M. & Ferreira-Dias, G. (2009). Non-invasive bone assessment by quantitative ultrasonography in Lusitano suckling foals: A preliminary study. In A. Lindner (Ed.), *Applied equine nutrition and training.* (pp. 191-196). ENUTRACO 2009, Wageningen, the Netherlands: Wageningen Academic Publishers.

Fradinho, M.J., Correia, M.J., Beja, F., Rosa, A., Perestrello, F., Bessa, R.J.B., Ferreira-Dias, G. & Caldeira, R.M. (2012). Effects of foaling season on growth and development of *Lusitano* suckling foals raised on extensive grazing systems. In M. Saastamoinen, M.J. Fradinho, A.S. Santos & N. Miraglia (Eds.), *Forages and grazing in horse nutrition*. (pp. 315–318). EAAP Publ Nº132, Wagenningen, The Netherlands: Wageningen Academic Publishers.

Fradinho, M.J., Bessa, R.J.B., Martin-Rosset, W., Ferreira-Dias, G. & Caldeira, R.M. (2013) Nutritional status of Lusitano broodmares on extensive feeding systems: body condition, live weight and metabolic indicators. *Italian Journal of Animal Science*, 12e71, 436-443.

Fradinho, M.J., Correia, M.J., Grácio, V., Bliebernicht, M., Farrim, A., Mateus, L., Martin-Rosset, W., Bessa, R.J.B., Caldeira, R.M. & Ferreira-Dias, G. (2014). Effects of body condition and leptin on the reproductive performance of Lusitano mares on extensive systems. *Theriogenology*. 81, 1214-1222.

Fradinho, M.J., A.C. Vale, N. Bernardes, R.M. Caldeira, M.F. Vaz & G. Ferreira-Dias. (2015a). Biomechanical properties of the equine third metacarpal bone: *in vivo* quantitative ultrasonography *vs ex vivo* compression and bending techniques. *Journal of Equine Veterinary Science.* 35, 198-205.

Fradinho, M.J., Bessa, R.J.B., Ferreira-Dias, G. & Caldeira, R.M. (2015b). Growth and development of the Lusitano horse managed on grazing systems. *Livestock Science*. DOI: 10.1016/j.livsci.2015.06.006.

Fraher, L. (1993). Biochemical markers of bone turnover. *Clinical Biochemestry,* 26, 431–432.

Frank, N., Elliott, S.B., Brandt, L.E. & Keisler, D.H. (2006). Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *Journal of the American Veterinary Medical Association,* 228, 1383–1390.

Fürst, A., Meier, D., Michel, S., Schmidlin, A., Held, L. & Laib, A. (2008). Effect of age on bone mineral density and micro architecture in the radius and tibia of horses: an Xtreme computed tomographic study. *BMC Veterinary Research*, 4, 3.

Gannong, W.F. (2009). Blood as a circulatory fluid and the dynamics of blood and lymph flow. In K. Barrett, H. Brooks, S. Boiano & S. Barman (Eds.), *Review of Medical Physiology*. (23rd ed.). (pp.521-553). McGraw Hill Medical U.S.A.

Garcia, M.C., Casanueva, F.F., Diéguez, C. & Señaris, R.M. (2000). Gestational Profile of Leptin Messenger Ribonucleic Acid (mRNA) Content in the Placenta and Adipose Tissue in the Rat, and Regulation of the mRNA Levels of the Leptin Receptor Subtypes in the Hypothalamus During Pregnancy and Lactation. *Biology of Reproduction,* 62, 698–703.

Gastal, M.O., Gastal, E.L., Spinelli, V. & Ginther O.J. (2000). Body condition influences diameter of the ovulatory follicle in mares [Abstract]. *Biology of Reproduction,* 62, 222.

Gastal, M.O., Gastal, E.L., Spinelli, V. & Ginther, O.J. (2004). Relationships between body condition and follicle development in mares. *Animal Reproduction,* 1, 115-121.

Gee, E.K., Fennessy, P.F., Morel, P.C., Grace, N.D., Firth, E C. & Mogg, T.D. (2003). Chemical composition of 20 Thoroughbred foals at 160 days of age, and preliminary investigation of techniques used to predict body fatness. *New Zealand Veterinary Journal*, 51, 125-131.

Gee, E.K., Firth, E.C., Morel, P.C.H., Fennessy, P.F., Grace, N.D. & Mogg, T.D. (2005). Articular/epiphyseal osteochondrosis in Thoroughbred foals at 5 months of age: Influences of growth of the foal and prenatal copper supplementation of the dam. *New Zealand Veterinary Journal,* 53, 448–456.

Gentry, L.R., Thompson Jr., D.L., Gentry, G.T., Davies, K.A., Godke, R.A. & Cartmill, J.A. (2002). The relationship between body condition, leptin and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. *Journal of Animal Science,* 80, 2695-2703.

Geor, R.J. (2008). Metabolic predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. *Journal of Equine Veterinary Science,* 28, 753-758.

George, L.A., Staniar, W.B., Treiber, K.H., Harris, P.A. & Geor, R.J. (2009). Insulin sensitivity and glucose dynamics during pre-weaning foal development and in response to maternal diet composition. *Domestic Animal Endrocrinology,* 37, 23-29.

Giannetto, C., Casella, S., Fazio, F., Messina, V. & Piccione, G. (2010). Circadian variations in biochemical markers of bone metabolism in horse of different age. *Journal of Applied Biomedicine,* 8, 73-79.

Gibbs, P.G. & Davison, K.E. (1992). A field-study on reproductive efficiency of mares maintained predominately on native pasture. *Journal of Equine Veterinary Science,* 12, 219-222.

Gibbs, P., Potter, G.D. & Vogelgang, M. (2005). Nutrition and feed management of broodmares. Texas A&M University. Texas Cooperative Extension. Retrieved Oct. 15th, 2010, http://animalscience.tamu.edu/images/pdf/equine/equine-nutrition-feeding-mgmt-broodmares.pdf

Gibson, V.A., Stover, S.M., Martin, R.B., Gibeling, J.C., Willits, N.H., Gustafson, M.B. & Griffin, L.V. (1995). Fatigue behavior of the equine third metacarpus: Mechanical property analysis. *Journal of Orthopaedic Research,* 13, 861–868.

Ginther, O.J. (1974). Occurrence of anestrous, estrus, diestrus, and ovulation over a twelve-months period in mares. *American Veterinary Research,* 35, 1173-1179.

Ginther, O.J. (1992). Characteristics of the ovulatory season. In *Reproductive Biology of the mare: Basic and Applied Concepts*. (pp.173-232). (2nd Ed.) Cross Plains, Wisconsin, USA: Equiservices.

Giussani, D.A., Forhead, A.J. & Fowden, A.L. (2005). Development of cardiovascular function in the horse fetus. *Journal of Physiology,* 565, 1019–1030.

Glade, M.J. (1984). The influence of dietary fiber digestibility on the nitrogen requirements of mature horses. *Journal of Animal Science.* 58, 638-646.

Glade, M.J., Gupta, S. & Reimers, T.J. (1984). Hormonal responses to high and low planes of nutrition in weanling Thoroughbreds. *Journal of Animal Science,* 59, 658-665.

Glade, M.J., Luba, N.K. & Schryver, H.F. (1986). Effects of age and diet on the development of mechanical strength by the third metacarpal and metatarsal bones of young horses. *Journal of Animal Science,* 63, 1432-1444.

Godoi, D.B., Gastal, E.L. & Gastal, M.O. (2002). A comparative study of follicular dynamics between lactating and non-lactating mares: effect of the body condition. *Theriogenology,* 58, 553-556.

Gomes, L.G., Gastal, M.O. & Gastal, E.L. (2002). Follicular activity in fillies during the prepubertal phase: effects of season, body condition and hair coat. *Theriogenology*, 58, 615-618.

Gomez, M.D., Goyache, F., Molina, A. & Valera, M. (2009). Sire x stud interaction for body measurement traits in Spanish Purebred horses. *Journal of Animal Science,* 87, 2502–2509.

Goody, P.C. (1983). *Horse anatomy. A pictorial approach to equine structure*. London, UK: J.A. Allen.

Gordon, M.E., McKeever, K.H., Betros, C.L. & Manso Filho, H.C. (2007). Plasma leptin, ghrelin and adiponectin concentrations in young fit racehorses versus mature unfit standardbreds. *The Veterinary Journal,* 173, 91-100.

Grace, N.D., Rogers, C.W., Firth, E.C., Faram, T.L. & Shaw, H.L. (2002). Digestible energy intake, dry matter digestibility and effect of increased calcium intake on bone parameters of grazing Thoroughbred weanlings in New Zealand. *New Zealand Veterinary Journal*, 51, 165-173.

Gray, S.M. & Staniar, W.B. (2011). Growth hormone, glucose , insulin, and NEFA patterns in meal fed Quarter Horse weanlings. *Journal of Equine Veterinary Science*, 31, 250-260.

Greco, D. & Stabenfeldt, G.H. (1997). Glândulas endócrinas e suas funções. In J.G. Cunningham, *Tratado de Fisiologia Veterinária*. (pp. 404-439). 2ª Ed. Rio de Janeiro: Guanabara Koogan.

Griffith, J.F. & Genant, H.K. (2008). Bone mass and architecture determination: state of the art. *Best Practice & Research Clinical Endocrinology & Metabolism*. 22, 737–764.

Guglielmi, G., Adams, J. & Link, T.M. (2009). Quantitative ultrasound in the assessment of skeletal status. *European Radiology*, 19, 1837–1848.

Guillaume, D., Duchamp, G., Salazar-Ortiz, J. & Nagy, P. (2002). Nutrition influences the winter ovarian inactivity in mares. *Theriogenology*, 58, 593-597.

Guillaume, D., Salazar-Ortiz, J. & Martin-Rosset, W. (2006). Effects of nutrition level in mare´s ovarian activity and in equines' puberty. In N. Miraglia & W. Martin-Rosset (Eds.), *Nutrition and feeding of the broodmare*. (pp. 315–339). EAAP Publ. Nº 120, Wageningen, the Netherlands: Wageningen Academic Publishers.

Gunson, D.E., Kowalczyk, D.F., Shoop, C.R. & Ramberg, C.F. Jr.. (1982). Environmental zinc and cadmium pollution associated with generalized osteochondrosis, osteoporosis, and nephrocalcinosis in horses. *Journal of the American Veterinary Medical Association*, 180, 295-299.

Hall, C.M. (2003). Applied physiology: the control of puberty. *Current Paediatrics*, 13, 371-375.

Hallebeek, J.M. & Beynen, A.C. (2001). Effect of dietary medium chain triacylglycerols on plasma triacylglycerol levels in horses. *Archives of Animal Nutrition*, 54, 159-171.

Harker, I.J., Harris, P.A. & Barfoot, C.F. (2011). The body condition score of leisure horses competing at an unaffiliated championship in the UK. *Journal of Equine Veterinary Science*, 31, 253-254.

Harrington, D. & Walsh, J. (1980). Equine magnesium supplements: evaluation of magnesium oxide, magnesium sulphate and magnesium carbonate in foals purified diets. *Equine Veterinary Journal*, 12, 32-33.

Harvey, J.W., Pate, M.G., Kivipelto, J. & Asquith, R.L. (2005). Clinical biochemistry of pregnant and nursing mares. *Veterinary Clinical Pathology*, 34, 248-254.

Hauguel-de Mouzon, S., Lepercq, J. & Catalano, P. (2006). The known and unknown of leptin in pregnancy. *American Journal of Obstetrics and Gynecology*, 194, 1537–1545.

Hauschka, P.V., Lian, J.B., Cole, D.E. & Gundberg, C.M. (1989). Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiology Reviews*, 69, 990–1047.

Hayes, E.K., Lechowicz, A., Petrik, J.J., Storozhuk, Y., Paez-Parent, S., Dai, Q., Samjoo I.A., Mansell, M., Gruslin, A., Holloway, A.C. & Raha, S. (2012). Adverse fetal and neonatal outcomes associated with a life-long high fat diet: role of altered development of the placental vasculature. *PLoS ONE*, 7(3), e33370.

Heidler, B., Parvizi, N., Sauerwein, H., Bruckmaier, R.M., Heintges, U., Aurich, J.E. & Aurich, C. (2003). Effects of lactation on metabolic and reproductive hormones in Lipizzaner mares. *Domestic Animal Endocrinology*, 25, 47-59.

Heidler, B., Aurich, J.E., Pohl, W. & Aurich, C. (2004). Body weight of mares and foals, estrous cycles and plasma glucose concentration in lactating and non-lactating Lipizzaner mares. *Theriogenology*, 61, 883-893.

Hemberg, E., Lundeheim, N. & Einarsson, S. (2004). Reproductive performance of thoroughbred mares in Sweden. *Reproduction in Domestic Animals,* 39, 81-85.

Henneke, D.R., Potter, G.D., Kreider, J.L. & Yeates, B.F. (1983). Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Veterinary Journal*, 15, 371-372.

Henneke, D.R., Potter, G.D. & Kreider, J.L. (1984). Body condition during pregnancy and lactation and reproductive efficiency of mares. *Theriogenology,* 21, 897-909.

Henson, F.M.D., Davenport, C., Butler, L., Moran, I., Shingleton, W.D., Jeffcott, L.B. & Schofield, P.N. (1997). Effects of insulin and insulin-like growth factors I and II on the growth of equine fetal and neonatal chondrocytes. *Equine Veterinary Journal*, 29, 441-447.

Heugebaert, S., Garcia-Launay, F., Trillaud-Geyl, C., Dubroeucq, H., Arnaud, D., Bigot, G., Valette, J.-P., Fleurance, G., Hoch, T., Agabriel, J. & Martin-Rosset, W. (2010). Modélisation de la croissance des poulains: première étape vers de nouvelles recommandations alimentaires. In *Proceedings of 36ème Journée de la Recherche Equine*. Paris, France, pp. 61-70.

Hiney, K.N., Potter, G.D., Gibbs, P.G. & Bloomfield, S.M. (2000). Response on serum biochemical markers of bone metabolism to training in the juvenile race horse. *Journal of Equine Veterinary Science,* 20, 851-857.

Hiney, K.M., Nielsen, B.D. & Rosenstein, D. (2004). Short-duration exercise and confinement alters bone mineral content and shape in weanling horses. *Journal of Animal Science,* 82, 2313-2320.

Hintz, H.F., Hintz, R.L. & Van Vleck, L.D. (1979). Growth rate of Thoroughbreds: Effect of age of dam, year and month of birth, and sex of foal. *Journal of Animal Science,* 48, 480–487.

Hoaglund, C.M., Thomas, V.M., Petersen, M.K. & Kott, R.W. (1992). Effects of supplemental protein source and metabolizable energy intake on nutritional status in pregnant Ewes. *Journal of Animal Science*, 70, 273-280.

Hoekstra, K.E., Nielsen, B.D., Orth, M.W., Rosenstein, D.S., Scott, H.C. & Shelle, J.E. (1999). Comparison of bone mineral content and biochemical markers of bone metabolism in stall vs. pasture reared horses. *Equine Veterinary Journal Suppl.*, 30, 601-604.

Hoffman, P.C., Esser, N.M., Bauman, L.M., Denzine, S.L., Engstrom, M. & Chester-Jones H. (2001). Effect of Dietary Protein on Growth and Nitrogen Balance of Holstein Heifers. *Journal of Dairy Science,* 84, 843-847.

Hoffman, R.M., Lawrence, L.A., Kronfeld, D.S., Cooper, W.L., Sklan, D.J., Dascanio, J.J. & Harris, P.A. (1999). Dietary carbohydrates and fat influence radiographic bone mineral content of growing foals. *Journal of Animal Science*, 77, 3330-3338.

Hoffman, R.M., Kronfeld, D.S., Cooper, W.L. & Harris, P. (2003a). Glucose clearance in grazing mares is affected by diet, pregnancy, and lactation. *Journal of Animal Science,* 81, 1764-1771.

Hoffman, R.M., Bóston, R.C., Stefanovski, D., Kronfeld, D.S. & Harris, P. (2003b). Obesity and diet affect glucose dynamics and insulin sensivity in Thoroughbred geldings. *Journal of Animal Science,* 81, 2333-2342.

Holdstock, N.B., Allen, V.L., Bloomfield, M.R., Hales, C.N. & Fowden, A.L. (2004). Development of insulin and proinsulin secretion in newborn pony foals. *Journal of Endocrinology,* 181, 469–476.

Holick, M.F. & Garabedian, M. (2006). Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In M.J. Favus (Ed.) *Primer on the metabolic bone diseases and disorders of mineral metabolism*. (6th ed.). (pp. 106-114). Washington, DC: American Society for Bone and Mineral Research.

Houseknecht, K. L & Portocarrero, C. P. (1998). Leptin and its receptors: regulators of whole-body energy homeostasis. *Domestic Animal Endocrinology,* 15, 457-475.

Hoyt, S. & Siciliano, P. (1999). A comparison of ELISA and RIA techniques for the detection of serum osteocalcin in horses. In *Proceedings 16th Equine Nutrution and Physiology Symposium*. pp. 351–352.

Huff, N.K., Thompson Jr., D.L., Gentry, L.R. & Depew, C.G. (2008). Hyperleptinemia in Mares: Prevalence in Lactating Mares and Effect on Rebreeding Success. *Journal of Equine Veterinary Science,* 28, 579-586.

Huff, N.K., Thompson Jr., D.L. & Bondioli, K.R. (2009). Search for Polymorphism in Exon 2 of equine leptin gene. *Journal of Equine Veterinary Science,* 29(6), 519-526.

Hurtig, M., Green, S.L., Dobson, H., Mikuni-Tagaki, Y. & Choi, J. (1993). Correlative study of defective cartilage and bone growth in foals fed a low copper diet. *Equine Veterinary Journal Suppl.,* 16, 66-73.

Hyyppä, S. (2008). Blood glucose in horses at rest and exercise. In M. Saastamoinan and W. Martin-Rosset (Eds.), *Nutrition of the exercising horse*. EAAP Publication Nº 125. (pp. 17-29). Wageningen, the Netherlands: Wageningen Academic Publishers.

INRA (1990). *L'alimentation des chevaux*. W. Martin-Rosset (Ed.) Paris, France: INRA Editions.

INRA (2012). *Nutrition et alimentation des chevaux.* W. Martin-Rosset (Coord.) Versailles, France: Éditions Quae.

INRA. (2015). *Equine nutrition, INRA nutrient requirements, recommended allowances and feed tables*. Wageningen, the Netherlands: Wageningen Academic Publishers.

INRA-HN-IE. (1997). *Notation de l'état corporel des chevaux de selle et de sport. Guide Pratique*. Paris, France: Institut d'Elevage.

Instituto de Meteorologia, I. P. (2012). Accessed on February 2012, available on: http://www.meteo.pt/pt/oclima/normais.clima

ISO 5983-1. (2005). *Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content - Part 1: Kjeldahl method.* International Organization for Standardization.

Jackson, B.F., Goodship, A. E., Eastell, R. & Price, J.S. (2003a). Evaluation of serum concentrations of biochemical markers of bone metabolism and insulin-like growth factor I associated with treadmill exercise in young horses. *American Journal of Veterinary Research*, 64, 1549-1556.

Jackson, B.F., Lonnell, C., Verheyen, K., Wood, J.L., Pfeiffert, D.U. and Price, J.S. (2003b). Gender differences in bone turnover in 2-year-old Thoroughbreds. *Equine Veterinary Journal,* 35, 702-706.

Jackson, B.F., Lonnell, C., Verheyen, K., Dyson, P., Pfeiffert, D.U. & Price, J.S. (2005). Biochemical markers of bone metabolism and risk of dorsal metcarpal disease in 2-year-old Thoroughbreds. *Equine Veterinary Journal,* 37, 87-91.

Jackson, B.F., Dyson, P.K., Hattersley, R.D., Kelly, H.R., Pfeiffer, D.U. & Price, J.S. (2006). Relationship between stages of the estrous cycle and bone cell activity in Thoroughbreds. *American Journal of Veterinary Research*, 67, 1527-1532.

Jacquet, S., Robert, C., Valette, J.-P. & Denoix, J.-M. (2013). Evolution of radiological findings detected in the limbs of 321 young horses, between the age of 6 and 18 months. *The Veterinary Journal*, 197, 58–64.

Jeffcott, L.B. (1991). Osteochondrosis in the horse – Searching for the key to pathogenesis. *Equine Veterinary Journal,* 23, 331–338.

Jeffcott, L.B. (2005). Developmental diseases affecting growing horses. In V. Julliand & W. Martin-Rosset (Eds.). *The Growing Horse: Nutrition and Prevention of Growth Disorders*. (pp. 243–255). EAAP Publication Nº 114. Wageningen, the Netherlands: Wageningen Academic Publishers.

Jeffcott, L.B. & McCartney, R.N. (1985). Ultrasound as a tool for assessment of bone quality in the horse. *Veterinary Records,* 116, 337–342.

Jeffcott, L.B. & Davies, M.E. (1998). Copper status and skeletal development in horses: still a long way to go. *Equine Veterinary Journal,* 30, 183-185.

Jeffcott, L.B. & Henson, F.M.D. (1998). Studies on growth cartilage in the horse and their application to aetiopathogenesis of dyschondroplasia (osteochondrosis). *The Veterinary Journal*, 156, 177-192.

Jeffcott, L.B., Buckingham, S.H. & McCartney, R.N. (1987). Noninvasive measurements of bone quality in horses and changes associated with exercise. In J.R. Gillespie & N.E. Robinson (Eds.), *Equine exercise physiology*. (pp. 615-630). 2nd ed. Davis C.A.: ICCEP.

Jeffcott, L.B., Buckingham, S.H.W., McCarthy, R.N., Cleeland, J.C. & Scotti, E. (1988). Non-invasive measurement of bone: a review of clinical and research applications in the horse. *Equine Veterinary Journal Suppl.,* 6, 71-79.

Jelan, Z.A., Jeffcott, L.B., Lundeheim, N. & Osborne, M. (1996). Growth rates in Thoroughbred foals. *Pferdeheilkunde,* 12, 291-295.

Kaneko, J.J., Harvey, J.W. & Bruss, M.L. (1997). *Clinical biochemistry of domestic animals* (5th ed.). Academic Press, Inc., London, UK.

Karsenty, G. (2006). Convergence between bone and energy homeostases: Leptin regulation of bone mass. *Cell Metabolism,* 4, 341–348.

Karsenty, G. & Oury, F. (2010). The central regulation of bone mass, the first link between bone remodeling and energy metabolism. *Journal of Clinical Endocrinology and Metabolism*, 95, 4795–4801.

Katila, T., Reilas, T., Nivola, K., Peltonen, T. & Virtala, A-M. (2010). A 15-year survey of reproductive eficiency of Standardbred and Finnhorse trotters in Finland-descriptive results. *Acta Veterinaria Scandinavica*, 52, 40.

Kavazis, A.N. & Ott, E.A. (2003). Growth rates in Thoroughbreds horses raised in Florida. *Journa of Equine Veterinary Science*, 23**,** 353-357.

Kearns, C.F., McKeever, K.H. & Abe, T. (2002). Overview of horse body composition and muscle architecture: implications for performance. *The Veterinary Journal,* 164, 224-234.

Kearns, C.F., McKeever, K.H., Roegner, V., Brady, S.M. & Malinowski, K. (2006). Adiponectin and leptin are related to fat mass in horses. *The Veterinary Journal*, 172, 460–465.

Kedzierski, W. & Kapica M. (2008). Plasma concentration of leptin and ghrelin in Standardbred foals as related to the age, sex, exercise and training. *Animal*, 2, 582–587.

Kelesidis, T., Kelesidis, I., Chou, S. & Mantzoros, C.S. (2010). Narrative Review: The Role of Leptin in Human Physiology: Emerging Clinical Applications. *Annals of Internal Medicine*, 152, 93–100.

Kennedy, G.C. (1953). The role of depot fat in the hypothalamic control of food intake in the rat. *Proceedings of the Royal Society of London B: Biological Sciences*, 140, 578–596.

Kienzle, E. & Schramme, S.C. (2004). Scoring and prediction of body weight in adult warm blooded horses. *Pferdheilkunde*, 20, 517-524.

Kini, U. & Nandeesh, B.N. (2012). Physiology of Bone Formation, Remodeling, and Metabolism. In I. Fogelman, G. Gnanasegaran & H. van der Wall (Eds.) *Radionuclide and Hybrid Bone Imaging.* (pp.29-57). Heidelberg: Springer-Verlag.

Knight, D.A., Weisbrode, S.E., Schmall, L.M., Reed, S.M., Gabel, A.A., Bramlage, L.R. & Tyznik, W.I. (1990). The effects of copper supplementation on the prevalence of cartilage lesions in foals. *Equine Veterinary Journal*, 22, 426-432.

Knothe Tate, M.L., Adamson, J.R., Tami, A.E. & Bauer, T.W. (2004). The osteocyte. *The International Journal of Biochemistry & Cell Biology*, 36, 1-8.

Kocher, A. & Staniar, W.B. (2013). The pattern of thoroughbred growth is affected by foal's birth date. *Livestock Science*, 154, 204-214.

Komosa, M., Frąckowiak, H., Purzyc, H., Wojnowska, M., Gramacki, A. & Gramacki, J. (2013). Differences in exterior conformation between primitive, Half-bred, and Thoroughbred horses: Anatomic-breeding approach. *Journal of Animal Science*, 91, 1660–1668.

Kristjansson, T., Bjornsdottir, S., Sigurdsson, A., Crevier-Denoix, N., Pourcelot, P. & Arnason, T. (2013). Objective quantification of conformation of the Icelandic horse based on 3-D video morphometric measurements. *Livestock Science*, 158, 12–23.

Kronfeld, D.S., Treiber, K.H., Hess, T.M. & Boston, R.C. (2005). Insulin resistence in the horse: Definition, detection, and dietetics. *Journal of Animal Science,* 83(E. Suppl.), E22-E31.

Kubiak, J.R., Evans, J.W., Potter, G.D., Harms, P.G. & Jenkins, W.L. (1989). Postpartum reproductive performance in the multiparous mare fed to obesity. *Theriogenology*, 32, 27-36.

Landau, S., Madar, Z., Nitsan, Z. & Zoref, Z. (1992). Estimation of energy status in non - pregnant and pregnant prolific ewes according to plasma levels of non -esterified fatty acids. In A. Purroy (Ed.). *Etat corporel des brebis et chèvres.* Options Méditerranéennes: Série A. Séminaires Méditerranéens. (pp.57-62). Nº 13. Zaragoza: CIHEAM.

Langlois, B. & Blouin, C. (2004). Statistical analysis of some factors affecting the number of horse births in France. *Reproduction Nutrition Development,* 44, 583-595.

Lawrence, L.A., Ott, E.A., Miller, G.J., Poulos, P.W., Piotrowski, G. & Asquith, R.L. (1994). The mechanical properties of equine third metacarpals as affected by age. *Journal of Animal Science,* 72, 2617-2623.

Lawrence, L.M., DiPietro, J., Ewert, K. & Parrett, D. (1992). Changes in body weight and condition of gestating mares. *Journal of Equine Veterinary Science,* 12, 355-358.

Lawrence, L.M., Williams, J., Soderholm, L.V., Roberts, A.M. & Hintz, H.F. (1995). Effect of feeding state on the response of horses to repeated bouts of intense exercise. *Equine Veterinary Journal*, 27, 27-30.

Lawrence, T.L.J., Fowler, V.R. & Novakofski, J.E. (2012). *Growth of farm animals.* (3rd ed.) Cambridge, USA: Cabi International.

Lee, N.K., Sowa, H., Hinoi, E., Ferron, M., Ahn, J.D., Confavreux, C., Dacquin, R., Mee, P.J., McKee, M.D., Jung, D.Y., Zhang, Z., Kim, J.K., Mauvais-Jarvis, F., Ducy, P. & Karsenty, G. (2007). Endocrine Regulation of Energy Metabolism by the Skeleton. *Cell*, 130, 456–469.

Lee, S.C., Coan, B.S. & Bouxsein, M.L. (1997). Tibial ultrasound velocity measured *in situ* predicts the material properties of tibia1 cortical bone. *Bone*, 21, 119-125.

Leighton-Hardman, A.C. (1980). *Equine Nutrition*. Pelham Books, London.

Lejeune, J.-Ph., Franck, T., Gangl, M., Schneider, N., Michaux, C., Deby-Dupont, G. & Serteyn, D. (2007). Plasma concentration of Insulin-like Growth Factor I (IGF-I) in growing Ardenner horses suffering from Juvenile Digital Degenerative Osteoarthropathy. *Veterinary Research Commununications,* 31, 185–195.

Lemma, A., Bekana, M., Schwartz, H.J. & Hildebrandt, T. (2006). The effect of body condition on ovarian activity of free ranging tropical jennies (*Equus asinus*). *Journal of Veterinary Medicine Series A,* 53, 1-4.

Lepage, O., Marcoux, M. & Tremblay, Y. (1990). Serum osteocalcin or bone Gla-protein, a biochemical marker for bone metabolism in horses: differences in serum levels with ages. *Canadian Journal of Veterinary Reserch,* 54, 223–226.

Lepage, O.M., Descoteaux, L., Marcoux, M. & Tremblay, Y.A. (1991). Circadian rythms of osteocalcin in equine serum. Correlation with alkaline phosphatase, calcium, phosphate and total protein levels. *Canadian Journal of Veterinary Research*, 55, 5-10.

Lepage, O.M., Eicher, R., Uebelhart, B. & Tschudi, P. (1997). Influence of type and breed of horse on serum osteocalcin concentration, and evaluation of the applicability of a bovine radioimmunoassay and a human immunoradiometric assay. *American Journal of Veterinary Research,* 58, 574-578.

Lepage, O., Hartmann, D., Eicher, R., Uebelhart, B., Tschudi, P. & Uebelhart, D. (1998a). Biochemical markers of bone metabolism in Draught and Warmblood horses. *The Veterinary Journal*, 156, 169– 175.

Lepage, O.M., Whitton, C., Danieli, A. & Niv, Y. (1998b). Multi-site quantitative ultrasound measurements in horses: preliminary results for metacarpal bone assessment. *Journal of Bone and Mineral Research,* 13(S1), S461.

Lepage, O., Carstanjen, B. & Uebelhart, D. (2001). Non-invasive assessment of equine bone: an update. *The Veterinary Journal*, 161, 10– 23.

Lepeule, J., Bareille, N., Robert, C., Ezanno, P., Valette, J.P., Jacquet, S., Blanchard, G., Denoix, J.M. & Seegers, H. (2009). Association of growth, feeding practices and exercise conditions with the prevalence of developmental orthopaedic disease in limbs of foals. *Preventive Veterinary Medicine*, 89, 167–177.

Lepeule, J., Seegers, H., Rondeau, V., Robert, C., Denoix, J.M. & Bareille, N. (2013). Association of growth, feeding practices and exercise conditions with the severity of the osteo-articular status of limbs in French foals. *The Veterinary Journal*, 197, 65–71.

Lerner, U.H. (2012). Osteoblasts, osteoclasts, and osteocytes: unveiling their intimate-associated responses to applied orthodontic forces. *Seminars in Orthodontics*, 18, 237-248.

Les, C.M., Stover, S.M., Keyak, J.H., Taylor, K.T. & Willits, N.H. (1997). The distribution of material properties in the equine third metacarpal bone serves to enhance sagittal bending. *Journal of Biomechanics*, 30, 355-361.

Leturque, A., Hauguel, S., Ferré, P. & Girard. J. (1987). Glucose metabolism in pregnancy. *Biology of the Neonate*, 51, 64-69.

Lumsden, J. H., Rowe, R. & Mullen, K. (1980). Hematology and biochemistry reference values for the light horse. *Canadian Journal of Comparative Medicine*, 44, 32-42.

Łuszczyński, J., Pieszka, M. & Kosiniak-Kamysz, K. (2011). Effect of horse breed and sex on growth rate and radiographic closure time of distal radial metaphyseal growth plate. *Livestock Science*, 141, 252–258.

Lynch, G.P. e Jackson, C. Jr. (1983). Metabolic responses of ewes to different protein intakes during late gestation. *Canadian Journal of Animal Science*, 63, 595-601.

Mackie, E.J. (2003). Osteoblasts: novel roles in orchestration of skeletal architecture. *The International Journal of Biochemistry & Cell Biology*, 35, 1301–1305.

Mackie, E. J., Tatarczuch, L. & Mirams, M. (2011). The skeleton: a multi-functional complex organ. The growth plate chondrocyte and endochondral ossification. *Journal of Endocrinology*, 211, 109–121.

Mäenpää, P.H., Pirskanen, A. & Koskinen, E. (1988). Biochemical indicators of bone formation in foals after transfer from pasture to stables for the winter months. *Am J Vet Res.* 49, 1990-1992.

Manso Filho, H.C., Manso, H., Ferreira, L., Santiago, T., Wanderley, E., & Abreu, J. (2009). Percentagem de gordura de cavalos criados em região tropical. *Acta Scientae Veterinariae*, 37, 239-243.

Marin, F., Gonzalez-Macias, J., Diez-Perez, A., Palma, S. & Delgado-Rodriguez, M. (2006). Relationship between bone quantitative ultrasound and fractures: a meta-analysis. *Journal of Bone and Mineral Research*, 21, 1126-1135.

Martin, K.L., Hoffman, R.M., Kronfeld, D.S., Ley, W.B. & Warnick, L.D. (1996). Calcium decrease and Parathyroid Hormone increases in serum of periparturient mares. *Journal of Animal Science*, 74, 834-89.

Martin, R.B., Lau, S.T., Mathews, P.V., Gibson, V.A. & Stover, S.M. (1996a). Collagen fiber organization is related to mechanical properties and remodelling in equine bone. A comparison of two methods. *Journal of Biomechanics*, 29, 1515-1521.

Martin, R.B., Stover, S.M., Gibson, V.A., Gibeling, J.C. & Griffin, L.V. (1996b). In vitro fatigue behavior of the equine third metacarpus: remodeling and microcrack damage analysis. *Journal of Orthopaedic Research*, 14, 794-801.

Martin, R.B., Gibson, V.A., Stover, S.M., Gibeling, J.C. & Griffin, L.V. (1996c). Osteonal structure in the equine third metacarpus. *Bone*, 19, 165-171.

Martin-Rosset, W. (1983). Particularités de la croissance et du développement du cheval. *Annales de Zootechnie*, 32, 109-130.

Martin-Rosset, W. (2005). Growth and development in the equine. In V. Juliand & W. Martin-Rosset (Eds.), *The growing horse: nutrition and prevention of growth disorders*. EAAP series Nº 114. (pp. 15–50). Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W. (2008). Energy requirements and allowances of exercising horses. In M. Saastamoinan and W. Martin-Rosset (Eds.), *Nutrition of the exercising horse*. EAAP series Nº 125. (pp. 103-138). Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W. & Vermorel, M. (1991). Maintenance energy requirement variations determined by indirect calorimetry and feeding trials in light horses. *Journal of Equine Veterinary Science*, 11, 42-45.

Martin-Rosset, W. & Tisserand, J.L. (2004). Evaluation and expression of protein allowances and protein value of feeds in the MADC system for the performance. In V. Julliand and W. Martin-Rosset (Eds.), *Nutrition of the performance horse*. EAAP series Nº111. (pp. 103-140). Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W. & Young, B. (2006). Energy and protein requirements and feeding of the sucking foal. In Miraglia, N. & Martin-Rosset, W. (Eds). *Nutrition and feeding of the broodmare*. EAAP series Nº 120. (pp. 221–243). Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W., Doreau, M. & Cloix, J. (1978). Etude des activités d'un troupeau de poulinières de trait et de leurs poulains au pâturage. *Annales de Zootechnie*, 27, 33-45.

Martin-Rosset, W., Boccard, R., Jussiaux, M., Robelin, J. & Trillaud-Geyl, C. (1983). Croissance relative des différents tissues, organs et régions corporelles entre 12 et 30 mois chez le cheval de boucherie de différents races lourdes. *Annales de Zootechnie*, 32, 153-174.

Martin-Rosset, W., Doreau, M. & Espinasse, J. (1986). Alimentation de la jument lourde allaitante: évolutions du poids vif des juments et croissance des poulains. *Annales de Zootechnie*, 35, 21-36.

Martin-Rosset, W., Austbo, D. & Coenen, M. (2006a). Energy and protein requirements and recommended allowances in lactating mares. In N. Miraglia & W. Martin-Rosset (Eds.), *Nutrition and feeding of the broodmare*. (pp. 89–15). EAAP series Nº 120. Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W., Vervuert, I. & Austbo, D. (2006b). Energy and protein requirements and recommended allowances in pregnant mares. In N. Miraglia and W. Martin-Rosset (Eds.) *Nutrition and feeding of the broodmare*. EAAP series Nº 120. (pp. 15-39). Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W., Vernet, J., Dubroeucq, H., Arnaud, G., Picard, A. & Vermorel, M. (2008). Variations of fatness and energy content of the body with body condition score in sport horses and its prediction. In M. Saastamoinan & W. Martin-Rosset (Eds.), *Nutrition of* *the exercising horse.* (pp.167-176). EAAP series Nº 125. Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W., Tavernier, L., Trillaud-Geyl, C. & Cabaret, J. (2012). Bases du rationnement. In W. Martin-Rosset (Coord.), *Nutrition et alimentation des chevaux*. (pp. 81-109). Versailles, France: Éditions Quæ.

Martin-Rosset, W., Fleurence, G, Baumont, R., Cabaret, J., Carrere, P., Edouard, N., Dumont, B., Duncan, P., Lecomte, D., Morhain, B. & Trillaud-Geyl, C. (2015a). Pasture. In W. Martin-Rosset (Ed.), *Equine Nutrition: INRA nutrient requirements, recommended allowances and feed tables.* (pp 347-383). Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W., Trillaud-Geyl, C. & Agabriel, J. (2015b). The growing horse. In W. Martin-Rosset (Ed.), *Equine Nutrition: INRA nutrient requirements, recommended allowances and feed tables.* (pp 169-216). Wageningen, the Netherlands: Wageningen Academic Publishers.

Martin-Rosset, W., Doreau, M. & Guillaume, D. (2015c). The mare. In W. Martin-Rosset (Ed.), *Equine Nutrition: INRA nutrient requirements, recommended allowances and feed tables.* (pp 121-156). Wageningen, the Netherlands: Wageningen Academic Publishers.

Mason, M.W., Skedros, J.G. & Bloebaum, R.D. (1995). Evidence of strain-mode-related cortical adaptation in the diaphysis of the horse radius. *Bone*, 17, 229-237.

McCarthy, R.N. & Jeffcott, L.B. (1992). Effects of treadmill exercise on cortical bone in the third metacarpus of young horses. *Research in Veterinary Science,* 52, 28–37.

McDowell, L.R. (2003). *Minerals in animal and human nutrition.* (2nd ed.). Amsterdam, The Netherlands: Elsevier Science B.V.

McIlwraith, C.W. (2003). Advanced techniques in the diagnosis of bone disease. In J. Pagan (Ed.), *Advances in equine nutrition III*. (pp. 365-372). Nothingham, UK: Nothingham University Press.

McIlwraith, C.W. (2004). Developmental orthopedic disease: problems of limbs in young horses. *Journal of Equine Veterinary Science*, 24, 475-479.

McManus, C.J. & Fitzgerald, B.P. (2000). Effects of a single day of feed restriction on changes in serum leptin, gonadotropins, prolactin, and metabolites in aged and young mares. *Domestic Animal Endocrinology,* 19, 1–13.

McManus, C.M., Louvandini, H. & Campos, V.A.L. (2010). Non linear growth curves for weight and height in four genetic groups of horses. *Ciência Animal Brasileira,* 11, 80-89.

Meakim, D.W., Ott, E.A., Asquith, R.L. & Feaster, J.P. (1981). Estimation of mineral content of the equine third metacarpal by radiographic photometry. *Journal of Animal Science,* 53, 1019–1026.

Meneses, A.C.A., Costa, M.D., Maruch, S., Moreira, P.R. & Neto, T.M. (2014). Medidas lineares e angulares de animais da raça Quarto de Milha utilizados em uma prova de vaquejada. *Revista Brasileira de Ciência Veterinária,* 21, 256-261.

Merritt, J.S., Burvill, C.R., Pandy, M.G. & Davies, H.M. (2006). Determination of mechanical loading components of the equine metacarpus from measurements of strain during walking. *Equine Veterinary Journal Suppl.,* 36, 440-444.

Meyer, H. (1995). *Alimentação de cavalos. (Trad. Pferdefütering, 2nd ed.)* Ed. Livraria Varela, S. Paulo, Brasil.

Meyer, H. & Ahlswede, L. (1976). Über das intrauterine wachstum und die körperzusammensetzung von fohlen sowie den nährstoffbedarf tragender stuten. *Übersichten Tierernährung*. 4, 263-292.

Millán, J.L. (2013). The Role of phosphatases in the initiation of skeletal mineralization. *Calcified Tissue International*, 93, 299–306.

Miller-Graber, P., Lawrence, L., Foreman, J., Bump, K., Fisher, M. & Kurcz, E. (1991). Effect of dietary protein level on nitrogen metabolites in exercised Quarter Horse. In S.G.B. Persson, A. Lindholm & L.B. Jeffcott (Eds.), *Proceedings of the 3rd International Conference Equine Exercise Physiology*, Uppsala, Sweden. (pp. 305-314) Davis, California: ICEEP Publications.

Miraglia, N., Saastamoinen, M. & Martin-Rosset, W. (2006). Role of pastures in mares and foals management in Europe. In N. Miraglia & W. Martin-Rosset (Eds.), *Nutrition and feeding of the broodmare.* (pp 279-297). EAAP series Nº 120. Wageningen, the Netherlands: Wageningen Academic Publishers.

Miraglia, N., Costantini, M., Polidori, M., Meineri, G. & Peiretti, P.G. (2008). Exploitation of a natural pasture by wild horses: comparison between nutritive characteristics of the land and the nutrient requirements of the herds over a 2-year period. *Animal*, 2, 410-418.

Molina, A., Valera, M., dos Santos, R. & Rodero, A. (1999). Genetic parameters of morphofunctional traits in Andalusian horse. *Livestock Production Science*, 60, 295-303.

Monteiro, J. (1983). *O cavalo Lusitano – Contributo para o seu estudo*. Boletim Pecuário. Ano XLIX. Ministério da Agricultura e Pescas.

Morel, P.C.H., Bokor, A., Rogers, C.W. & Firth, E.C. (2007). Growth curves from birth to weaning for Thoroughbred foals raised on pasture. *New Zealand Veterinary Journal*, 55, 319-325.

Morgan, K., Eherlmark, K. & Sallvik, K. (1997). Dissipation of heat from standing horse exposed to ambient temperatures between -3C° and 37C°. *Journal of Thermal Biology*, 22,177-186.

Morgan, K. (1998). Thermoneutral zone and critical temperatures of horses. *Journal of Thermal Biology*, 23, 59-61.

Morris, L.H. & Allen, W.R. (2002). Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. *Equine Veterinary Journal*, 34, 51-60.

Muir, H. (1995). The chondrocyte, architect of cartilage. Biomechanics, structure, function and molecular biology of cartilage matrix macromolecules. *Bioessays*, 17, 1039-1048.

Muniyappa, R., Lee, S., Chen, H. & Quon, M.J. (2008). Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations and appropriate usage. *American Journal of Physiology - Endocrinology and Metabolism*, 294, E15–E26.

Munoz, A., Trigo, P., Riber, C., Malonda, V. & Castejon, F. (2001). A study of serum insulin-like growth factor type 1 (IGF-1) concentrations in resting untrained Andalusian horses: influence of age and gender. *Veterinarni Medicina*, 56, 231–242.

Murray, J.A. (1919). Meat production. *Journal of Agricultural Science*, 9, 174-181.

Nadal, M.R., Thompson, D.L. & Kincaid, L.A. (1997). Effect of feeding and feed deprivation on plasma concentrations of prolactin, insulin, growth hormone and metabolites in horses. *Journal of Animal Science,* 75, 736-744.

Nadeau, J.A., Frank, N., Valipe, S.R. & Elliot, S.B. (2006). Blood lipid, glucose, and insulin concentrations in Morgan horses and Thoroughbreds. *Journal of Equine Veterinary Science,* 26, 401-405.

Nagy, P., Huszenicza, G., Juhasz, J. Kulcsar, M., Solti, L., Reiczigel, J. & Abavary, K. (1998). Factors influencing ovarian activicty and sexual behavior of postpartum mares under farm conditions. *Theriogenology,* 50, 1109-1119.

Newcombe, J.R. & Wilson, M.C. (2005). Age, body weight and pregnancy loss. *Journal of Equine Veterinary Science,* 25, 188-194.

Ng, K.W. (2011). Regulation of glucose metabolism and the skeleton. *Clinical Endocrinology,* 75, 147–155.

Noble, G.K., Houghton, E., Roberts, C.J., Faustino-Kemp, J., de Kock, S.S., Swanepoel, B.C. & Sillence, M.N. (2007). Effect of exercise, training, circadian rhythm, age, and sex on insulin-like growth factor-1 in the horse. *Journal of Animal Science,* 85, 163-171.

NP 872 (1983). *Portuguese Standard for Animal feed. Determination of total ash content.* Direcção-Geral da Qualidade, Lisboa.

NRC (2007). Nutrient Requirements of Horses, sixth revised edition. Washington, DC, USA: National Academy Press.

Ohlsson, C., Bengtsson, B.-Å., Isaksson, O.G.P., Andreassen, T.T. & Slootweg, M.C. (2008). Growth hormone and bone. *Endocrine Reviews,* 19, 55–79.

Olive, J., d'Anjou, M.-A., Alexander, K., Beauchamp, G. & Theoret, C.L. (2010). Correlation of signal attenuation–based quantitative magnetic resonance imaging with quantitative computed tomographic measurements of subchondral bone mineral density in metacarpophalangeal joints of horses. *American Journal of Veterinary Research,* 71, 412–420.

Olstad, K., Cnudde, V., Masschaele, B., Thomassen, R. & Dolvik, N.I. (2008). Micro-computed tomography of early lesions of osteochondrosis in the tarsus of foals. *Bone,* 43, 574–583.

Olszynski, W.P., Brown, J.P., Adachi, J.D., Hanley, D.A., Ioannidis, G. & Davison, K.S. (2013). Multisite quantitative ultrasound for the prediction of fractures over five years of follow-up: the Canadian Multicenter Osteoporosis Study. *Journal of Bone and Mineral Research,* 28, 2027-2034.

Oom, M.M. (1992). *O Cavalo Lusitano. Uma raça em recuperação.* Ph.D. Tesis, Lisbon: University of Lisbon.

Oom, M.M. & Costa Ferreira, J. (1987). Estudo biométrico do cavalo Alter. *Revista Portuguesa de Ciências Veterinárias,* 482, 101-148.

Onoda, T., Yamamoto, R., Sawamura, K., Inoue, Y., Matsui, A., Miyake, T. & Hirai, N. (2011). Empirical growth curve estimation using sigmoid sub-functions that adjust seasonal compensatory growth for male body weight of thoroughbred horses. *Journal of Equine Science,* 22, 37–42.

Onoda, T., Yamamoto, R., Sawamura, K., Inoue, Y., Murase, H., Nambo, Y., Tozaki, T., Matsui, A., Miyake, T. & Hirai, N. (2013). Empirical growth curve estimation considering multiple seasonal compensatory growths of body weights in Japanese Thoroughbred colts and fillies. *Journal of Animal Science*, 91, 5599-5604.

Orme, C.E., Dunnett, M. & Harris, R.C. (1994). Variation in the concentration of long chain free fatty acids in equine plasma over 24 hours. *British Veterinary Journal*, 150, 339-347.

Ott, E.A. & Asquith, R.L. (1989). The influence of mineral supplementation on growth and skeletal development of yearling horses. *Journal of Animal Science,* 67, 2831-2840.

Ott, E.A. & Asquith, R.L. (1995). Trace mineral supplementation of yearling horses. *Journal of Animal Science,* 73, 466-471.

Ott, E.A. & Kivipelto, J. (2002). Growth and development of yearling horses fed either alfalfa or coastal bermudagrass: hay and a concentrate formulated for bermudagrass hay. *Journal of Equine Veterinary Science,* 22, 311-319.

Ott, E., Brown, M., Roberts, G. & Kivipelto, J. (2005). Influence of starch intake on growth and skeletal development of weanling horses. *Journal of Animal Science,* 83, 1033-1043.

Owens, F.N., Dubeski, P. & Hanson, C.F. (1993). Factors that alter the growth and development of ruminants. *Journal of Animal Science,* 71, 3138-3150.

Paço, T.A. & Fradinho, M.J. (2011). The role of extensive grazing systems in Southern Europe horse prodution. In N. T. Prochazka (Ed.), *Pastures: Dynamics, Economics and Management.* (pp. 131-150). New York, NY: Nova Science Publishers.

Pagan, J.D. (2005). Managing growth for different commercial end points. In J.D. Pagan & R.J. Geor (Eds.), *Advances in Equine Nutrition III.* (pp. 319-326). U.K.: Nottingham University Press.

Pagan, J.D. & Jackson, S.G. (1996). The incidence of developmental orthopedic disease on a Kentucky Throughbred farm. *Pferdeheilkunde,* 12, 351–354.

Pagan, J.D., Jackson, S.G. & Caddel, S. (1996). A summary of growth rates of thoroughbreds in Kentucky. *Pferdeheilkunde,* 12, 285–289.

Pagan, J.D., Brown-Douglas, C.G. & Caddel, S. (2006). Body weight and condition of Kentucky Thoroughbred mares and their foals as influenced by month of foaling, season, and gender. In N. Miraglia & W. Martin-Rosset (Eds.), *Nutrition and feeding of the broodmare.* (pp. 245-252). EAAP series Nº 120. Wageningen, the Netherlands: Wageningen Academic Publishers.

Pagan, J.D., Martin, O.A., & Crowley, N.L. (2009). Relationship between body condition and metabolic parameters in Sport Horses, Pony Hunters and Polo Ponies. *Journal of Equine Veterinary Science,* 29, 418-420.

Park, H.-K. & Ahima, R.S. (2015). Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. *Metabolism Clinical and Experimental,* 164, 24-34.

Payne, J.M. & Payne, S. (1987). *The metabolic profile test.* Oxford University Press, UK.

Peacock, M. (2010). Calcium metabolism in health and disease. *Clinical Journal of the American Society of Nephrology,* 5, S23–S30.

Pearce, S.G., Grace, N.D., Firth, E.C., Wichtel, J.J., Holle, S.A. & Fennessy, P.F. (1998a). Effect of copper supplementation on the copper status of pasture-fed young Thoroughbreds. *Equine Veterinary Journal,* 30, 204-210.

Pearce, S.G., Grace, N.D., Firth, E.C., Wichtel, J.J. & Fennessy, P.F. (1998b) Effect of copper supplementation on the copper status of pregnant mares and foals. *Equine Veterinary Journal*, 30, 200-203.

Pearce, S.G., Firth, E.C., Grace, N.D. & Fennessy, P.F. (1998c). Effect of copper supplementation on the evidence of developmental orthopaedic disease in pasture-fed New Zealand Thoroughbreds. *Equine Veterinary Journal*, 30, 211-218.

Pearson, R.A. & Ouassat, M. (1996). Estimation of the liveweight and body condition of working donkeys in Morocco. *Veterinary Records*, 138, 229-233.

Pedrotti, L., Bertani, B., Tuvo, G., Barone, F., Crivellari, I., Lucanto, S. & Redento, M. (2010). Evaluation of bone density in infancy and adolescence. Review of medical literature and personal experience. *Clinical Cases in Mineral and Bone Metabolism*, 7, 102-108.

Perotto, D., Cue, R.I. & Lee, A.J. (1992). Comparison of nonlinear functions for describing the growth curve of three genotypes of dairy cattle. *Canadian Journal of Animal Science*, 72, 773-782.

Pethick. D.W., Lindsay, D.B., Barker, P.J. & Northrop, A.J., 1983. The metabolism of circulating non-esterified fatty acids by the whole animal, hind-limb muscle and uterus of pregnant ewes. *British Journal of Nutrition*, 49, 129-143.

Piccione, G., Bertolucci, C., Foa, A. & Caola, G. (2004). Influence of fasting and exercise on the daily rhythm of serum leptin in the horse. *Chronobiology International*, 21, 405-417.

Piccione, G., Caola, G. & Refinetti, R. (2005). Temporal relationships of 21 physiological variables in horse and sheep. *Comparative Biochemistry and Physiology*, Part A 142, 389 – 396.

Piccione, G., Assenza, A., Borruso, M., Fazio, F. & Caola, G. (2009). Daily pattern of some fatty acids in the athletic horse. *Journal of Animal Physiology and Animal Nutrition*, 93, 7-14.

Platt, H. (1978). Growth and maturity in the equine fetus. *Journal of the Royal Society of Medicine*, 71, 658-661.

Platt, H. (1984). Growth of the equine fetus. *Equine Veterinary Journal*, 16, 247-252.

Porr, C., Kronfeld, D., Lawrence, L., Pleasant, R. & Harris, P. (1998). Deconditioning reduces mineral content of the third metacarpal bone in horses. *Journal of Animal Science*, 76, 1875–1879.

Powell, D.M., Lawrence, L.M., Fitzgerald, B.P., Danielsen, K., Parker, A., Siciliano, P. & Crum, A. (2000). Effect of short-term feed restriction and calorie source on hormonal and metabolic responses in geldings receiving a small meal. *Journal of Animal Science*, 78, 3107-3113.

Pratt-Phillips, S.E., Owens, K.M., Dowler L.E. & Cloninger, M.T. (2010). Assessment of resting insulin and leptin concentrations and their association with managerial and innate factors in horses. *Journal of Equine Veterinary Science*, 30, 127-133.

Prevrhal, S., Fuerst, T., Fan, B., Njeh, C., Hans, D., Uffmann, M., Srivastav, S & Genant, H. K. (2001). Quantitative ultrasound of the tibia depends on both cortical density and thickness. *Osteoporosis International*, 12, 28–34.

Price, J.S. (1998). Biochemical markers of bone metabolism in horses: potentials and limitations? *The Veterinary Journal*, 156, 163-165.

Price, J.S., Jackson, B., Eastell, R., Goodship, A.E., Blumsohn, A., Wright, I., Stoneham, S., Lanyon, L.E. & Russell, R.G.G. (1995a). Age related changes in biochemical markers of bone metabolism in horses. *Equine Veterinary Journal*, 27, 201– 207.

Price, J.S., Jackson, B., Eastell, R., Wilson, A.M., Russell, R.G.G., Lanyon, L.E. & Goodship, A.E. (1995b). The response of skeleton to physical training: a biochemical study in horses. *Bone*, 17, 221– 227.

Price, J., Jackson, B., Gray, J., Harris, P., Wright, I., Pfeiffer, D., Robins, S., Eastell, R. & Ricketts, S. (2001). Biochemical markers of bone metabolism in growing thoroughbreds: a longitudinal study. *Research in Veterinary Science*, 71, 1 – 9.

Prior, B.M., Cureton, K.J., Modlesky, C.M., Evans, E.M., Sloniger, M.A., Saunders, M. & Lewis, R.D. (1997). In vivo validation of whole body composition estimates from dual-energy X-ray absorptiometry. *Journal of Applied Physiology*, 83, 623-630.

Prior, R.L., Hintz, H.F., Lowe, J.E. & Visek, W.J. (1974). Urea recycling and metabolism of ponies. *Journal of Animal Science,* 38, 565-571.

Quiros-Gonzalez, I. & Yadav, V.K. (2014). Central genes, pathways and modules that regulate bone mass. *Archives of Biochemistry and Biophysics*, 561, 130–136.

Ralston, S.L. (1996). Hyperglycemia/hyperinsulinemia after feeding a meal of grain to young horses with osteochondritis dissecans (OCD) lesions. *Pferdeheilkunde*, 12, 320-322.

Ralston, S.L. (2002). Insulin and glucose regulation. *Veterinary Clinics of North America: Equine*, 18, 295-304.

Raub, R.H., Jackson, S.G. & Baker, J.P. (1989). The effect of exercise on bone growth and development in weanling horses. *Journal of Animal Science*, 67, 2508-2514.

Rauch, F. (2005). Bone growth in length and width: the Yin and Yang of bone stability. *Journal of Musculoskeletal and Neuronal Interactions,* 5, 194-201.

Reichmann, P., Moure, A. & Gamba, H.R. (2004). Bone mineral content of the thrird metacarpal bone in Quarter Horse foals from birth to one year of age. *Journal of Equine Veterinary Science*, 24, 391-396.

Reilly, G.C., Currey, J.D. & Goodship, A.E. (1997). Exercise of young Thoroughbred horses increases impact strength of the third metacarpal bone. *Journal of Orthopaedic Researh,* 15, 862-868.

Reller, E., Kivipelto, J. & Ott, E. (2003). Age-related changes for serum bone metabolism markers in Thoroughbred and Quarter Horse foals. *Journal of Equine Veterinary Science,* 23, 117–120.

Rémésy, C., Chilliard, Y., Rayssiguier, Y., Mazur, A. & Demigne, C. (1986). Le métabolisme hépatique des glucides et des lipides chez les ruminants: principales interactions durant la gestation et la lactation. *Reproduction Nutrition Dévelopment,* 26, 205-226.

Rezende, A.S.C., Sampaio, I.B.M., Legorreta, G.L. & Moreira, D.C.A. (2000). Efeito de dois diferentes programas nutricionais sobre o desenvolvimento corporal de potros Mangalarga Marchador. *Revista Brasileira de Zootecnia.* 29, 495-501.

Rho, J.-Y., Kuhn-Spearing, L. & Zioupos, P. (1998). Mechanical properties and the hierarchical structure of bone. *Medical Engineering and Physics*, 20, 92-102.

Richards, F.J. (1959). A flexible growth function for empirical use. *Journal of Experimental Botany*, 10, 290-300.

Riggs, C.M., Vaughan, L.C, Evans, G.P, Lanyon, L.E. & Boyde, A. (1993). Mechanical implications of collagen fibre orientation in cortical bone of the equine radius. *Anatomy and Embryoogy,* 187, 239-248.

Robert, C., Valette, J.-P., Jacquet, S., Lepeule, J. & Denoix, J.-M. (2013). Study design for the investigation of likely aetiological factors of juvenile osteochondral conditions (JOCC) in foals and yearlings. *The Veterinary Journal,* 197, 36–43.

Robling, A.G., Castillo, A.B. & Turner, C.H. (2006). Biomechanical and molecular regulation of bone remodeling. *Annual Review of Biomedical Engineering,* 8, 455–498.

Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J. & Berry, D.P. (2009). Body condition score and its association with dairy cow productivity, health, and welfare. *Journal of Dairy Science,* 92, 5769-5801.

Romagnoli, U., Macchi, E., Romano, G., Motta, M., Accornero, P. & Baratta, M. (2007). Leptin concentration in plasma and in milk during the interpartum period in the mare. *Animal Reproduction Science,* 97, 180-185.

Ropp, J.K., Raub, R.H. & Minton, J.E. (2003). The effect of dietary energy source on serum concentration of insulin-like growth factor-I, growth hormone, insulin, glucose, and fat metabolites in weanling horses. *Journal of Animal Science,* 81, 1581-1589.

Rourke, K.M., Kohn, C.W., Levine, A.L., Rosol, T.J. & Toribio, R.E. (2009). Rapid calcitonin response to experimental hypercalcemia in healthy horses. *Domestic Animal Endocrinology,* 36, 197–201.

Rubin, C.T., Seeherman, H., Qin, Y.-X., Gross, T.S. (2013). The mechanical consequences of load bearing in the equine third metacarpal across speed and gait: the nonuniform distributions of normal strain, shear strain, and strain energy density. *The Journal of the Federation of American Societies for Experimental Biology*, 27, 1887-1894.

Rubio-Martínez, L.M., Cruz, A.M., Gordon, K. & Hurtig, M.B. (2008). Structural characterization of subchondral bone in the distal aspect of third metacarpal bones from Thoroughbred racehorses via micro–computed tomography. *American Journal of Veterinary Research*, 69, 1413–1422.

Rumbaugh, G.E. & Adamson, P.J. (1983). Automated serum chemical analysis in the foal. *Journal of the American Veterinary Medical Association*, 183, 769-772.

Saastamoinen, M. (1990). Factors affecting growth and development of foals and young horses. *Acta Agriculturae Scandinavica,* 40, 387-396.

Salafia, C.M., Minior, V.K., López-Zeno, J.A., Whittington, S.S., Pezzullo, J.C. & Vintzileos, A.M. (1995). Relationship between placental histologic features and umbilical cord blood gases in preterm gestations. *American Journal of Obstetrics and Gynecology,* 173,1058-1064.

Salgueiro, T. (1984). A problemática da produção animal e suas relações com a alimentação. *Série Técnica* Nº 13, Lisboa: Direcção Geral da Agricultura.

Sandgreen, B., Dalin, G., Carlsten, J. & Lundeheim, N. (1993). Osteochondrosis in the tarsocrural joint and osteochondral fragments in the fetlock joints in Standardbred trotters. II. Body measurements and clinical findings. *Equine Veterinary Journal Suppl.*, 48–53.

Santos, R.I.D.G. (2008). *Caracterización genética de la aptitud deportiva del caballo pura sangre Lusitano a partir de variables biocinemáticas al trote.* PhD Tesis. Universidad de Cordoba. Spain.

Santos, A.S. & Silvestre, A.M. (2008). A Study of Lusitano Mare Lactation Curve with Wood's Model. *Journal of Dairy Science*, 91, 760-766.

Santos, A.S., Ferreira, L.M.M., Martin-Rosset, W., Cone, J.W., Bessa, R.J.B. & Rodrigues, M.A.M. (2013). Effect of nitrogen sources on in vitro fermentation profiles and microbial yield using equine caecal contents. *Animal Feed Science and Technology*, 182, 93– 99.

Santos, S.A., Souza, G.S., Oliveira, M.R. & Sereno, J.R. (1999). Using nonlinear models to describe height growth curves in Pantaneiro horses. *Pesquisa Agropecuária Brasileira,* 34, 1133–1138.

Satiel, A.R. & Kahn, C.R. (2001). Insulin signaling and the regulation of glucose and lipid metabolism. *Nature,* 14, 799–806.

Satué, K., Felipe, M., Mota, J. & Muñoz, A. (2011a). Factors influencing gestational length in mares: A review. *Livestock Science,* 136, 287-294.

Satué, K., Felipe, M., Mota, J. & Muñoz, A. (2011b). Gestational length in Carthusian broodmares: effects of breeding season, foal gender, age of mare, year of parturition, parity and sire. *Polish Journal of Veterinary Sciences,* 14, 173-180.

Savage, C.J., McCarthy, R.N. & Jeffcott, L.B. (1993a). Effects of dietary energy and protein on induction of dyschondroplasia in foals. *Equine Veterinary Journal Suppl*., 16, 74-79.

Savage, C.J., McCarthy, R.N. & Jeffcott, L.B. (1993b). Effects of dietary phosphorus and calcium on induction of dyschondroplasia in foals. *Equine Veterinary Journal Suppl*., 16, 80-83.

Seeman, E. (2008). Bone quality: the material and structural basis of bone strength. *Journal of Bone and Mineral Metabolism,* 26, 1-8.

Seeman, E. & Delmas, P.D. (2006). Bone quality - The material and structural basis of bone strength and fragility. *The New England Journal of Medicine,* 354, 2250-2261.

Semevolos, S.A. & Nixon, A.J. (2007). Osteochondrosis: Etiologic factors. *Compendium on Continuing Education: Equine Edition*, 2, 158–164.

Seré, C. & Steinfeld, S. (1996). *World livestock production systems: current status, issues and trends.* FAO Animal Production and Health Paper 127. Food and Agriculture Organization, Rome.

Shahar, R., Zaslansky, P., Barak, M., Friesem, A.A., Currey, J.D. & Weiner, S. (2007). Anisotropic Poisson's ratio and compression modulus of cortical bone determined by speckle interferometry. *Journal of Biomechanics*, 40, 252-264.

Sharir, A., Barak, M.M. & Shahar, R. (2008). Whole bone mechanics and mechanical testing. *The Veterinary Journal*, 177, 8–17.

Sherman, S. & Hadley, E. (1993). Aging and bone quality: an underexplored frontier. *Calcified Tissue International,* 53:S1.

Silva, S.R., Guedes, C.M., Couto, P., Santos, A.S. & Melo-Pinto, P. (2012). Relationship between body condition and neck crest score systems and subcutaneous fat, tissue and muscle ultrasonic measurements in horses. In Saastamoinen, M., Fradinho, M.J., Santos, A.S. & Miraglia, N. (Eds.), *Forage and grazing in horse nutrition*. EAAP series Nº 132, (pp. 381-386). Wageningin, the Netherlands: Wageningin Academic Publishers.

Simmons, H.A. & Ford, E.J.H. (1991). Gluconeogenesis from propionate produced in the colon of the horse. *British Veterinary Journal*, 147, 340-345.

Sims, N.A. & Gooi, J.H. (2008). Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. *Seminars in Cell & Developmental Biology*, 19, 444–451.

Sims, N.A. & Martin, T.J. (2014). Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *BoneKEy Reports,* 3, Article 481.

Sims, N.A. & Vrahnas, C. (2014). Regulation of cortical and trabecular bone mass by communication between osteoblasts, osteocytes and osteoclasts. *Archives of Biochemistry and Biophysics*, 561, 22–28.

Skedros, J.G., Mason, M.W., Nelson, M.C. & Bloebaum, R.D. (1996). Evidence of structural and material adaptation to specific strain features in cortical bone. *The Anatomical Record*, 246, 47-63.

Skedros, J.G., Dayton, M.R., Sybrowsky, C.L., Bloebaum, R.D. & Bachus, K.N. (2003). Are uniform regional safety factors an objective of adaptive modeling/remodelling in cortical bone? *Journal of Experimental Biology*, 206, 2431-2439.

Skedros, J.G., Dayton, M.R., Sybrowsky, C.L., Bloebaum, R.D. & Bachus, K.N. (2006). The influence of collagen fiber orientation and other histocompositional characteristics on the mechanical properties of equine cortical bone. *Journal of Experimental Biology*, 209, 3025-3042.

Skedros, J.G., Mendenhall, S.D., Kiser, C.J. & Winet, H. (2009). Interpreting cortical bone adaptation and load history by quantifying osteon morphotypes in circulary polarized light images. *Bone*, 44, 392-403.

Sloet van Oldruitenborgh-Oosterban, M., Mol, J. & Barneveld, A. (1999). Hormones, growth factors and other plasma variables in relation to osteochondrosis. *Equine Veterinary Journal Suppl*., 31, 45– 54.

Smyth, G.B. Young, D.W. & Duran, S.H. (1993). Maturation of insulin and glucose responses to normal feeding in foals. *Australian Veterinary Journal*, 70, 129-132.

Solé, M., Santos, R., Molina, A., Galisteo, A. & Valera, M. (2014). Genetic analysis of kinematic traits at the trot in Lusitano horse subpopulations with different types of training. *Animal*, 8, 192–199.

Staniar, W.B. (2013). The growing horse. In R.J. Geor, P.A. Harris & M. Coenen (Eds.), *Equine applied and clinical nutrition*. (pp. 243-260). Saunders Elsevier.

Staniar, W.B., Kronfeld, D.S., Treiber, K.H., Splan, R.K. & Harris, P.A. (2004a). Growth rate consists of baseline and systematic deviation components in Thoroughbreds. *Journal of Animal Science*, 82, 1007-1015.

Staniar, W.B., Kronfeld, D.S., Hoffman, R.M., Wilson, J.A. & Harris, P.A. (2004b). Weight prediction from linear measures of growing Thoroughbreds. *Equine Veterinary Journal*, 36, 149-154.

Staniar, W.B., Cubitt, T.A., George, L.A., Harris, P.A. & Geor, R.J. (2007a). Glucose and insulin responses to different dietary energy sources in Thoroughbred broodmares grazing cool season pasture. *Livestock Science*, 111, 164-171.

Staniar, W.B., Kronfeld, D.S., Akers, R.M. & Harris, P.A. (2007b). Insulin-like growth factor I in growing thoroughbreds. *Journal of Animal Physiology and Animal Nutrition*, 91, 390–399.

Statistical Analysis Systems Institute (2002-2010). Version 9.3. SAS Inc., Cary, NC, USA.

Steelman, S., Michael-Eller, E., Gibbs, P. & Potter, G. (2006). Meal size and feeding frequency influence serum leptin concentration in yearling horses. *Journal of Animal Science,* 84, 2391-2398.

Steinfeld, H., Wassenaar, T. & Jutzi, S. (2006). Livestock production systems in developing countries: status, drivers, trends. *Scientific and Technical Review of the Office International des Epizooties (Paris),* 25, 505-516.

Stewart, A.J. (2011). Magnesium disorders in horses. *Veterinary Clinics of North America: Equine,* 27, 149–163.

Sticker, L.S., Thompson, Jr D.L., Bunting, L.D., Fernandez, J.M. & DePew, C.L. (1995a). Dietary protein and (or) energy restriction in mares: plasma glucose, insulin, Nonesterified fatty acid, and urea nitrogen responses to feeding, glucose and epinephrine. *Journal of Animal Science,* 73, 136-144.

Sticker, L.S., Thompson Jr., D.L., Bunting, L.D., Fernandez, J.M., DePew, C.L. & Nadal, M.R. (1995b). Feed deprivation of mares: plasma metabolite and hormonal concentrations and responses to exercise. *Journal of Animal Science,* 73, 3696-3704.

Strand, E., Braathen, L.C., Hellsten, M.C., Huse-Olsen, L. & Bjornsdottir, S. (2007). Radiographic closure time of appendicular growth plates in the Icelandic horse. *Acta Veterinaria Scandinavica,* 49, 19.

Suagee, J.K., Burk, A.O., Quinn, R.W., Petersen, E.D., Hartsock, T.G. & Douglass, L.W. (2008). Effects of diet and weight gain on body condition scoring in Thoroughbred geldings. *Journal of Equine Veterinary Science,* 28, 156-166.

Sykes, A.R. & Field, A.C. (1973). Effects of dietary deficiencies of energy, protein and calcium on the pregnant ewe, IV. Serum total protein, albumin, globulin, tranferrin and plasma urea levels. *Journal of Agricultural Science,* 80, 29-36.

Tabar-Rodriguez, J.J., Cruz, A.M., Monteith, G., Gordon, K., & Hurtig, M.B. (2009). Use of quantitative ultrasonography for noninvasive surveillance of the third metacarpal bone in racing and training Thoroughbreds. *American Journal of Veterinary Reseach,* 70, 1484-1493.

Taichman, R.S. (2005). Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem cell niche. *Blood,* 105, 2631–2639.

Teixeira, J.A.A. (2009). *Avaliação radiográfica de osteocondrose como contributo na selecção de reprodutores equinos da raça Puro Sangue Lusitano*. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária, Lisboa.

Tennant, B.C. (1997) Hepatic function, In J.J. Kaneko, J.W. Harvey & M.L. Bruss (Eds.), *Clinical biochesmestry of domestic animals.* (5th ed.) (pp. 327-352). Academic Press, Inc., London, UK.

Thompson, K.N. (1995). Skeletal Growth Rates of Weanling and Yearling Thoroughbred Horses. *Journal of Animal Science,* 73, 2513-2517.

Thompson, K.N., Jackson, S.G. & Baker, J.P. (1988a). The influence of high planes of nutrition on skeletal growth and development of weanling horses. *Journal of Animal Science,* 66, 2459-2467.

Thompson, K.N., Baker, J.P. & Jackson, S.G. (1988b). The influence of supplemental feed on growth and bone development of nursing foals. *Journal of Animal Science,* 66, 1692-1696.

Tinworth, K.D., Wynn, P.C., Boston, R.C., Harris, P.A., Sillence, M.N., Thevis, M., Thomas, A. & Noble, G.K. (2011). Evaluation of commercial available assays for the measurement of equine insulin. *Domestic Animal Endocrinology,* 41, 81-90.

Tischner, M. (1985). Embryo recovery from Polish-pony mares and preliminary observations on foal size after transfer of embryos to large mares. *Equine Veternary Journal Suppl*., 17 S3, 96–98.

Toribio, R.E., Kohn, C.W., Sams, R.A., Capen, C.C. & Rosol, T.J. (2003). Hysteresis and calcium set-point for the calcium parathyroid hormone relationship in healthy horses. *General and Comparative Endocrinology,* 130, 279–288.

Toribio, R.E. (2011). Disorders of calcium and phosphate metabolism in horses. *Veterinary Clinics of North America: Equine,* 27, 129-147.

Trillaud-Geyl, C., Bigot, G., Jurquet, V., Bayle, M., Arnaud, G., Dubroeucq, H., Jussiaux, M. & Martin-Rosset, W. (1992). Influence du niveau de croissance pondéral sur le développement squelettique du cheval de selle. In *Proceedings of 18ème Journée de la Recherche Equine,* Paris, France, pp. 162-168.

Trillaud-Geyl, C., Fleurance, G., Donabedian, M., Perona, G., Bigot, G., Arnaud, G., Dubroeucq, H. & Martin-Rosset, W. (2004). Effect of body weight gain on the skeleton growth in the sport horses. In *Proceedings of the 55th Annual Meeting of the European Association for Animal Production,* Bled, Slovenia, H2.5.

Trivers, R.L. & Willard, D.E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*, 179, 90-92.

Trumble, T.N., Brown, M.P., Merritt, K.A. & Billinghurst, R.C. (2008). Joint dependent concentrations of bone alkaline phosphatase in serum and synovial fluids of horses with osteochondral injury: an analytical and clinical validation. *Osteoarthritis and Cartilage*, 16, 779-786.

Valberg, S. & Firshman, A. (2009). Insulin resistance – what is it and how do we measure it? In J. Pagan (Ed.), *Advances in Equine Nutrition IV*. (pp. 355-366). Nottingham University Press, UK.

Vale, A.C., Aleixo, I.P., Lúcio, M., Saraiva, A., Caetano-Lopes, J., Rodrigues, A., Amaral, P. M., Rosa, L.G., Monteiro, J., Fonseca, J.E., Vaz, M.F. & Canhão, H. (2013). At the moment of occurrence of a fragility hip fracture, men have higher mechanical properties values in comparison with women. *BMC Musculoskeletal Disorders,* 14, 295.

Valera, M., Blesa, F., Dos Santos, R. & Molina, A. (2006). Genetic study of gestation length in andalusian and arabian mares. *Animal Reproduction Science,* 95, 75-96.

Valette, J.P., Robert, C., Toquet, M.P., Denoix, J.M. & Fortier, G. (2007). Evolution of some biochemical markers of growth in relation to osteoarticular status in young horses: results of a longitudinal study in three breeds. *Equine and Comparative Exercise Physiology,* 4, 23–29.

Valette, J.-P., Robert, C. & Denoix, J.-M. (2008). Use of linear and non-linear functions to describe the growth of young sport- and race-horses born in Normandie. *Animal,* 2, 560-565.

van den Bergh, J.P.W., van Lenthe, G.H., Hermus, A.R.M.M., Corstens, F.H.M., Smals, A.G.H. & Huiskes, R. (2000). Speed of sound reflects Young's modulus as assessed by microstructural finite element analysis. *Bone.* 26, 519–524.

van der Eerden, B.C.J., Karperien, M. & Wit, J.M. (2003). Systemic and local regulation of the growth plate. *Endocrine Reviews,* 24, 782–801.

van der Made, J. (1999). Ungulates from Atapuerca TP6. *Journal of Human Evolution,* 37, 389–413.

van der Made, J. (2013). First description of the large mammals from the locality of Penal, and updated faunal lists for the Atapuerca ungulates e Equus altidens, Bison and human dispersal into Western Europe. *Quaternary International,* 295, 36-47.

Van Doorn, D.A., Everts, H., Wouterse, H., Homan S. & Beynen, A.C. (2011). Influence of high phosphorus intake on salivary and plasma concentrations, and urinary phosphorus excretion in mature ponies. *Journal of Animal Physiology and Animal Nutrition,* 95, 154–160.

van Niekerk, F.E. & van Niekerk, C.H. (1997). The effect of dietary protein on reproduction in the mare. II. Growth of foals, body mass of mares and serum protein concentration of mares during the anovulatory, transitional and pregnant periods. *Journal of South Africa Veterinary Association,* 68, 81-85.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science,* 74, 3583-3597.

van Weeren, P.R. (2005). Equine Osteochondrosis: a challenging enigma. *Pferdeheilkunde,* 21, 285-292.

van Weeren, P.R (2006). Etiology, diagnosis, and treatment of OC(D). *Clinical Techniques in Equine Practice,* 5, 248-258.

van Weeren, P.R. & Barneveld, A. (1999). The effect of exercise on the distribution and manifestation of osteochondrotic lesions in the Warmblood foal. *Equine Veterinay Journal Suppl*., 31, 16–25.

van Weeren, P.R. & Brama, P.A.J. (2001). Physiology and pathology of the equine joint. *Pferdeheilkunde,* 17, 307–318.

van Weeren, P.R. & Brama, P.A.J. (2005). Physiology of growth and development. In V. Juliand & W. Martin-Rosset (Eds.), *The growing horse: nutrition and prevention of growth disorders*. EAAP series Nº114, (pp. 51–60). Wageningen, the Netherlands: Wageningen Academic Publishers.

van Weeren, P.R. & Jeffcott, L.B. (2013). Problems and pointers in osteochondrosis: twenty years on. *The Veterinary Journal*, 197, 96 -102.

van Weeren, P.R., Sloet van Oldruitenborgh-Oosterbaan, M. & Barneveld, A. (1999). The influence of birth weight, rate of weight gain and final achieved height and sex on the development of osteochondrotic lesions in a population of genetically predisposed Warmblood foals. *Equine Veterinary Journal Suppl.,* 26–30.

van Weeren, P.R., Knaap, J. & Firth, E.C. (2003). Influence of liver copper status of mare and newborn foal on the development of osteochondrotic lesions. *Equine Veterinary Journal,* 35, 67-71.

Van Weyenberg, S., Buyse, J. & Janssens, G. (2007). Digestibility of a complete ration in horses fed once or three times a day and correlation with key blood parameters. *The Veterinary Journal*, 173, 311-316.

Van Weyenberg, S., Hesta, M., Buyse, J., Papadopoulos, G.A. & Janssens, G. (2008). Short-term effects of energy changes on plasma leptin concentrations and glucose tolerance in healthy ponies. *The Veterinary Journal*, 178, 233-237.

Vander Heyden, L., Lejeune, J.P., Caudron, I., Detilleux, J., Sandersen, C., Chavatte, P., Paris, J., Deliège, B. & Serteyn, D. (2013). Association of breeding conditions with prevalence of osteochondrosis in foals. *Veterinary Record*, 172, 68.

Vaz, M.F., Canhão, H. & Fonseca, J.E. (2011). Bone: a composite natural material. In: Tesinova P, editor. Advances in composite materials - Analysis of natural and man-made materials, InTech; 2011, p. 195-228. Available from: http://www.intechopen.com/books/advances-in-compositematerials-analysis-of-natural-and-man-made-materials/bone-a-composite-natural-material. Accessed 2014, February 17th.

Vermorel, M. & Martin-Rosset, W. (1997). Concepts scientific bases, structure and validation of the French horse net energy system (UFC). *Livestock Production Science,* 47, 261-275.

Vermorel, M., Martin-Rosset, W. & Vernet J. (1997). Energy utilization of twelve forages or mixed diets for maintenance by sport horses. *Livestock Production Science,* 47, 157-167.

Vervuert, I. & Ellis, A.D. (2013). Developmental orthopedic disease. In R.J. Geor, P.A. Harris & M. Coenen (Eds.), *Equine applied and clinical nutrition*. (pp. 536-548). Saunders Elsevier.

Vervuert, I., Coenen, M., Wedemeyer, U., Chrobok, C., Harmeyer, J. & Sporleder, H. (2002). Calcium homeostasis and intact plasma parathyroid hormone during exercise and training in young Standardbred horses. *Equine Veterinary Journal,* 34, 713–718.

Vervuert, I., Coenen, M. & Zamhofer, J. (2005). Effects of draught load exercise and training on calcium homeostasis in horses. *Journal of Animal Physiology and Nutrition,* 89, 134-139.

Vervuert, I., Winkelsett, S., Christmann, L., Bruns, E., Hoppen, H.-O., Distl, O., Herscht, B. & Coenen, M. (2007a). Evaluation of the influences of exercise, birth date, and osteochondrosis on plasma bone marker concentrations in Hannoverian Warmblood foals. *American Journal of Veterinary Research,* 68, 1319-1323.

Vervuert, I., Bothe, C. & Coenen, M. (2007b). Glycaemic and insulinaemic responses to mechanical or thermal processed barley in horses. *Journal of Animal Physiology and Animal Nutrition,* 91, 263-268.

Verwilghen, D., Busoni, V., Gangl, M., Franck, T., Lejeune, J.-Ph., Vanderheyden, L., Detilleux, J., Grulke, S., Deberg, M., Henrotin, Y. & Serteyn, D. (2009a). Relationship between biochemical markers and radiographic scores in the evaluation of the osteoarticular status of Warmblood stallions. *Research in Veterinary Science*, 87, 319–328.

Verwilghen, D.R., Vanderheyden, L., Franck, T., Busoni, V., Enzerink, E., Gangl, M., Lejeune, J.-P., van Galen, G., Grulke, S. & Serteyn, D. (2009b). Variations of plasmatic concentrations of Insulin-like Growth Factor-I in post-pubescent horses affected with developmental osteochondral lesions. *Veterinary Research Communications,* 33, 701–709.

Vicente, A.A., Carolino, N. & Gama, L.T. (2012). Genetic diversity in the Lusitano horse breed assessed by pedigree analysis. *Livestock Science,* 148, 16–25.

Vicente, A.A., Carolino, N., Ralão-Duarte, J. & Gama, L.T. (2014). Selection for morphology, gaits and functional traits in Lusitano horses: I. Genetic parameters estimate. *Livestock Science,* 164, 1-12.

Vicente, A.P.A. (2015). *Characterization and selection of the Lusitano horse breed*. Dissertação de Doutoramento em Ciências Veterinárias. Faculdade de Medicina Veterinária. Universidade de Lisboa.

Viguet-Carrin, S., Garnero, P. & Delmas, P.D. (2006). The role of collagen in bone strength. *Osteoporosis International,* 17, 319–336.

Virkajärvi, P., Saarijärvi, K., Rinne, M & Saastamoinen, M. (2012). Grass physiology and its relation to nutritive value in feeding horses. In M. Saastamoinen, M.J. Fradinho, A.S. Santos & N. Miraglia (Eds.*), Forages and grazing in horse nutrition*. (pp. 17–43). EAAP series Nº 132, Wageningen, The Netherlands: Wageningen Academic Publishers.

Waller, A. & Lindinguer, M.I. (2006). Hydration of exercise Standardbred racehorses assessed noninvasively using multi-frequency bioelectrical impedance analysis. *Equine Veterinary Journal Suppl.,* 36, 00-00.

Walton, A. & Hammond, J. (1938). The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. *Proceedings of the Royal Society B.*125, 311–335.Watts, N.B. (1999). Clinical utility of biochemical markers of bone remodeling. *Clinical Chemistry,* 45, 1359-1368.

Watts, N.B. (1999). Clinical utility of biochemical markers of bone remodeling. *Clinical Chemistry*. 45, 1359-1368.

Webb, S.P., Potter, G.D., Evans, J.W. & Webb, G.W. (1990). Influence of body fat content on digestible energy requirements of exercising horses in temperate and hot environments. *Journal of Equine Veterinary Science,* 10, 116-120.

Wei, J. & Ducy, P. (2010). Co-dependence of bone and energy metabolisms. *Archives of Biochemistry and Biophysics,* 503, 35–40.

Weiner, S. & Wagner, H.D. (1998). The material bone: structure-mechanical function relations. *Annual Review of Materials Science*. 28, 271–298.

Weisrock, K.U., Winkelsett, S., Martin-Rosset, W., Forssmann, W.-G., Parvizi, N., Coenen, M. & Vervuert, I. (2011). Long-term effects of intermittent equine parathyroid hormone fragment (ePTH-1-37) administration on bone metabolism in healthy horses. *The Veterinary Journal,* 190, e130–e134.

Weller, R., Pfau, T., Babbage, D., Brittin, E., May, S.A. & Wilson, A.M. (2006). Reliability of conformational measurements in the horse using a three-dimensional motion analysis system. *Equine Veterinary Journal,* 38, 610-615.

Westervelt, R.G., Stouffer, J.R., Hintz, H.F. & Schryver, H.F. (1976). Estimating fatness in horses and ponies. *Journal of Animal Science,* 43, 781-785.

Wolter, R. (1994). *Alimentation du cheval*. Paris, France: Éditions France Agricole.

Wright, B., Rietveld, G. & Lawlis, P. (1998). Body condition scoring of horses. Retrieved in Jan. 15th, http://www.omafra.gov.on.ca/english/livestock/horses/facts/98-101.pdf

Yang, P.F., Bruggemann, G.-P. & Rittweger, J. (2011). What do we currently know from *in vivo* bone strain measurements in humans? *Journal of Musculoskeletal and Neuronal Interactions,* 11, 8-20.

Ytrehus, B., Carlson, C.S. & Ekman, S. (2007). Etiology and pathogenesis of osteochondrosis. *Veterinary Pathology,* 44, 429-448.

Zechner, P., Zohman, F., Sölkner, J., Bodo, I., Habe, F., Marti, & Brem, G. (2001). Morphological description of the Lipizzan horse population. *Livestock Production Science*, 69, 163-177.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. & Friedman, J.M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372, 425–432.

Zieba, D.A., Amstalden, M. & Williams, G.L. (2005). Regulatory roles of leptin in reproduction and metabolism: A comparative review. *Domestic Animal Endocrinology*, 29, 166–185.

Zofková, I. (2003). Pathophysiological and clinical importance of insulin-like growth factor-I with respect to bone metabolism. *Physiological Research*, 52, 657–679.

Annex I

Lusitano breed Standard

Adapted from "Lusitano Breed Studbook Regulation" (APSL, 2010) and Lusitano breed characteristics, retrieved from APSL webpage http://www.cavalo-lusitano.com/en/stud-books-lusitano-horse/lusitano-breed, 3rd March, 2015

(100 points attributed to ideal model)

1. **Type:** Middleweight (around 500 kg); medium shaped, sub-convex profile throughout the body (with rounded outlines), which silhouette can be fitted into a square;
2. **Height:** measured at withers using a measuring stick at age of 6:
    - female 1.55 m;
    - male 1.60 m;
3. **Coat:** The most frequently found are all shades of grey and bay.
4. **Temperament:** Noble, generous and ardent but always gentle and able to long suffering;
5. **Movements:** Agile, elevated, forward, smooth and providing a great comfort to the rider;
6. **Aptitude:** A natural ability for concentration, with a great disposition for High School Work and courage and enthusiasm for the "Gineta" exercises (combat, hunting, bullfighting, work with cattle, etc.);
7. **Head:** Well proportioned, of medium length, narrow and dry, with the lower jaw not too pronounced and the cheek tending to be long. Slightly sub-convex profile with the forehead in advance of the bones of the eyebrows: the eyes tend to be elliptical in shape (almond shape), big and alive, expressive and confident. The ears are of medium length, fine, narrow and expressive;
8. **Neck:** Of medium length, arched with a narrow hairline: the junction between head and neck is narrow or fine: the neck is deep in the base and well inserted between the shoulders, rising up from the withers without any marked depression;
9. **Withers:** Well defined and long, with a smooth transition from the back to the neck. Always slightly higher than the croup. In mature males it is covered with fat but is always clearly visible through the shoulders;
10. **Chest:** Of medium size, deep and muscular;
11. **Ribcage:** Well developed, long and deep with the ribs obliquely arched into the joint with the column which promotes a short and full flank;
12. **Shoulders:** Long, oblique and well-muscled;
13. **Back:** Well defined and tending towards the horizontal making a smooth union between the withers and loins;
14. **Loins:** Short, wide, muscular, slightly convex, well connected with the back and croup with which they form a continuous harmonious line;
15. **Croup:** Strong and rounded, well proportioned, slightly oblique, identical in length and width, convex and harmonious profile with the point of hip relatively unobtrusive, giving the croup a transverse section of elliptical shape. The tail emerges from the same line of the croup, being of silky, long and abundant hair;

16. Members: The forelegs are well muscled and harmoniously inclined. The upper arm is straight and muscular. Dry and large knees. The cannons are slightly long, dry and with well-defined tendons. The fetlocks are dry, relatively big and with very little hair. The pasterns are relatively long and oblique. The hooves are of good constitution, well defined and proportioned without being too open; the line of the coronet is not very evident. The buttock is short and convex. The thigh is muscular and tends to be short, and is orientated in such a way that the patella is in the same vertical line of the hip bone, or point of the hip. The leg is slightly long positioning the hock in the same vertical line of the point of the buttock. The hocks are large, strong and dry. The angles of the legs are relatively closed.

Annex II

Body condition score description (Henneke *et al.*, 1983)

1	**Poor** – The horse is emaciated. The spinous processes (backbone), ribs, tailhead and hooks and pins all project prominently. The bone structures of the withers, shoulders and neck are easily noticeable, and no fat can be felt anywhere.
2	**Very thin** – The spinous processes are prominent. The ribs, tailhead and pelvic bones stand out, and bone structures of the withers, neck and shoulders are faintly discernible.
3	**Thin** – The spinous processes stand out, but fat covers them to midpoint. Very slight fat cover can be felt over the ribs, but the spinous processes and ribs are easily discernible. The tailhead is prominent, but individual vertebrae cannot be seen. Hook bones are visible but appear rounded. Pin bones cannot be seen. The withers, shoulders and neck are accentuated.
4	**Moderately thin –** The horse has a negative crease along its back and the outline of the ribs can just be seen. Fat can be felt around the tailhead. The hook bones cannot be seen and the withers, neck and shoulders do not look obviously thin.
5	**Moderate. The threshold level of body condition** – The back is level. Ribs cannot be seen but can easily felt. Fat around the tailhead feels slightly spongy. The withers look rounded and the shoulder and neck blend smoothly into the body.
6	**Moderate to fleshy** – There may be a slight crease down back. Fat around the tailhead feels soft and fat over the ribs feels spongy. There are small deposits along the sides of the withers, behind the shoulders along the sides of the neck.
7	**Fleshy** –There may be a crease down the back. Individual ribs can be felt, but there is noticeable fat between the ribs. Fat around the tailhead is soft. Fat is noticeable in the withers, the neck and behind the shoulders.
8	**Fat** – The horse has a crease down the back. Spaces between ribs are so filled with fat that the ribs are difficult to feel. The area along the withers is filled with fat, and fat around the tailhead feels very soft. The space behind the shoulders is filled in flush and some fat is deposited along the inner buttocks.
9	**Extremely fat** – The crease down the back is very obvious. Fat appears in patches over the ribs and there is bulging fat around the tailhead, withers, shoulders and neck. Fat along the inner buttocks may cause buttocks to rub together, and the flank is filled in flush.

Annexe III

Adaptation of the Henneke *et al.*, 1983 scoring system

Condition	Neck	Withers	Loin	Tailhead	Ribs	Shoulder
1 Poor	Bone structure noticeable	Bone structure easily noticeable	Spinous processes project prominently	Tailhead, pin bones, and hook bones project prominently	Ribs project prominently	Bone structure easily noticeable
Animal extremely emaciated; no fatty tissue can be felt						
2 Very thin	Faintly discernible	Faintly discernible	Slight fat covering over base of spinous processes; transverse processes of lumbar vertebrae feel rounded; spinous processes are prominent	Tailhead prominent	Ribs prominent	Faintly discernible
Animal emaciated						
3 Thin	Neck accentuated	Withers accentuated	Fat build-up halfway on spinous processes but easily discernible; transverse processes cannot be felt	Tailhead prominent but individual vertebrae cannot be visually identified; hook bones appear rounded, but are still easily discernable; pin bones not distinguishable	Slight fat cover over ribs; ribs easily discernible	Shoulder accentuated
4 Moderately thin	Neck not obviously thin	Withers not obviously thin	Negative crease along back	Prominence depends on conformation; fat can be felt; hook bones not discernible	Faint outline discernible	Shoulder not obviously thin
5 Moderate	Neck blends smoothly into body	Withers rounded over spinous processes	Back level	Fat around tailhead beginning to feel spongy	Ribs cannot be visually distinguished, but can be easily felt	Shoulder blends smoothly into body
6 Moderately Fleshy	Fat beginning to be deposited	Fat beginning to be deposited	May have slight positive crease down back	Fat around tailhead feels soft	Fat over ribs feels spongy	Fat beginning to be deposited; point of shoulder not discernible
7 Fleshy	Fat deposited along neck	Fat deposited along withers	May have positive crease down back	Fat around tailhead is soft	Individual ribs can be felt, but noticeable filling between ribs with fat	Fat deposited behind shoulder
8 Fat	Noticeable thickening of neck	Area along wither filled with fat	Positive crease down back	Tailhead fat very soft	Difficult to feel ribs	Area behind shoulder filled in flush with body
Fat deposited along inner buttocks						
9 Extremely fat	Bulging fat	Bulging fat	Obvious positive crease down back	Bulging fat around tailhead	Patchy fat appearing over ribs	Bulging fat
Fat along inner buttocks may rub together, flank filled in flush						

Annex IV

Body condition score description (adapted from INRA-HN-IE, 1997)

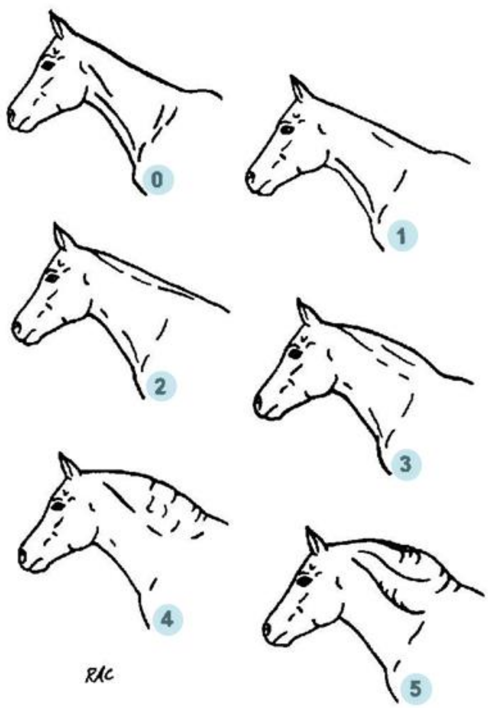
	Region						
	Manual appraisal					Visual appraisal	
Score	**Along the neck**	**Withers**	**Behind the shoulder**	**Ribs**	**Tailhead**	**Back line**	**Croup**
0	Upper edge of the neck emaciated; bone structure quite noticeable; dry muscles.	Quite prominent; skin dried and glued to the spinous processes.	Region highly depressed; shoulder prominent and very dry; the ribs are clearly visible.	Depression among the ribs very pronounced; the connection between the ribs and the transverse processes is visible; dry skin and stuck to the ribs.	Tailhead vertebrae are clearly visible; sacral-tuberal ligament is highly prominent.	Back line quite visible; vertebrae bodies are individualized.	Emaciated; sharp point; ends of the hips and buttocks quite visible.
1	Upper edge of the neck still emaciated, but the bone structure is only slightly perceptible.	Prominent withers; the upper edge of the shoulder is visible.	Empty region, clearly showing the relief of the shoulder.	Ribs and transverse processes still visible; glued skin; no fat deposition	Tail protrudes sharply and inserts in the hip on a hollow surrounding its base; vertebrae and sacral-tuberal ligament are still visible.	Back line accented; spinous processes well marked.	Outline concave; ends of the hips and buttocks clearly visible.
2	Fat accumulation in the upper edge of the neck is already visible; draws up a slight line along the base of the neck.	The withers is marked and dry; the side faces are flat.	Slightly concave region; shoulder relief is well designed; a slight fat deposition is palpable.	The ribs are guessed under the skin; there is a slight fat deposition between the individualized ribs.	The insertion of the tail protrudes from the hip; a slight fat depot is detectable around its base.	Back line marked; processes are a bit covered but still individualized.	The ends of the hips and buttocks still distinguished.
3	Upper edge of the neck slightly curved and well designed; line in the base of the neck almost inexistent.	The withers is slightly prominent.	This region is flatted and the shoulder is identified; a well-defined adipose deposit that slides under the hand is palpable.	The region is plane and uniform; the ribs are not visible and are only detected by palpation; a layer of fat tissue covers the line of the transverse processes.	Tail relatively noticeable despite the fat deposition which involves its base; this fat deposit is firm.	Back line apparent; Non-separable processes covered by a slight fat deposition.	General appearance slightly rounded; the ends of the hips and buttocks guess only.
4	Accumulation of fat in the upper edge of the neck; this presents curved, thick, firm and without line in the base; palpable by a full hand.	The withers is included.	Slightly convex region; a thick and soft fat deposit is palpable.	Fatty depot well-marked over the ribs and sliding under the hand; the rib region is rounded; the ribs are not palpable; heterogeneous fat depots are palpable.	The tail insertion is little detached from the hip; the fat depot that surrounds it is thick and soft.	Back line included; processes covered with a fatty deposit forming a horizontal "bar".	The croup is very rounded and uniform; ends of the hips and buttocks are included and hardly locatable.
5	Upper edge of the neck quite bulged and included in muscle mass; fat accumulation along the neck that is only palpable with a wide open hand.	The withers is included in a fat bulged mass in both faces.	Clearly bulged region; shoulder included; a considerable trembling fat mass is palpable.	Ribs region is bulged; the ribs are covered with a thick layer that slides under the hand; fat deposition is heterogeneous and visible backlit.	The tail is massively included in a "pad" of fat tissue with a spongy consistency.	Back line included; processes immersed in the fat covering the ribs.	Very round; it may appear a ridge between two symmetrical masses "double rump".

Annex V

Cresty Neck Scoring System (Carter *et al*., 2009)

In this system, crest height is measured at half of neck length from the dorsal midline of the neck to estimate differentiation between the crest (tissue apparent above the *ligamentum nuchae*) and neck musculature, identified by palpation and visual assessment. Neck length is measured in a relaxed position, at approximately 45º angle, from the poll to the highest point of the withers.

Score	Description
0	No visual appearance of a crest (tissue apparent above the *ligamentum nuchae).* No palpable crest
1	No visual appearance of a crest, but slight filling felt with palpation
2	Noticeable appearance of a crest, but fat deposited fairly evenly from poll to withers. Crest easily cupped in one hand and bent from side to side.
3	Crest enlarged and thickened, so fat is deposited more heavily in middle of the neck than toward poll and withers, given a mounded appearance. Crest fills cupped hand and begins loosing side to side flexibility.
4	Crest grossly enlarged and thickened, and can no longer be cupped in one hand or easily bent from side to side. Crest may have wrinkles/creases perpendicular to top line.
5	Crest is so large it permanently droops to one side.



Cresty neck scores – adapted from Carter *et al*. (2009)