

PAPER

Nutritional status of *Lusitano* broodmares on extensive feeding systems: body condition, live weight and metabolic indicators

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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, D) were monitored during four years, in a total of 119 gestation/lactation cycles. All mares were kept on pasture, and 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. Glucose, non-esterified fatty acids, 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 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.

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.*, 2006) 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.*, 2006). 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.*, 2006).

The most common method reported for the evaluation of body reserves, mainly regarding body fat tissue, is the body condition scoring (BCS). This method has been designed for horses (Henneke *et al.*, 1983; INRA, 1990; Arnaud *et al.*, 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; Cassil *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

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contents and body reserves (Martin-Rosset *et al.*, 1986).

Changes in broodmare BW and body condition (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.*, 2006; 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 longitudi-

nal 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.

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.

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 \pm 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.

Feeding plans

All mares were maintained on pasture all year and had free access to water. The stud-farms are located between latitude $38^{\circ}88'$ to $39^{\circ}29'$ N and longitude $07^{\circ}67'$ to $08^{\circ}87'W$ 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 $17^{\circ}C$ (B, C and D) and $16^{\circ}C$ (A)

(Instituto de Meteorologia, I.P., Lisboa, Portugal). Temperatures range, in winter, from $-3^{\circ}C$ to $25.2^{\circ}C$ for B, C and D stud-farms and from $-4.5^{\circ}C$ to $21.9^{\circ}C$ for stud-farm A and, in summer, from $11.9^{\circ}C$ to $45.2^{\circ}C$ for B, C and D stud-farms and from $9.4^{\circ}C$ to $41.3^{\circ}C$ for stud-farm A. Pastures are settled mainly on fluvisols and regosols (B and C), cambisols (A) and podzols (D) (FAO, 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 $1.5 \text{ kg head}^{-1} \text{ d}^{-1}$ to $5.5 \text{ kg head}^{-1} \text{ d}^{-1}$), and with grass hay or cereal straw (ranging from 2 to $6 \text{ kg head}^{-1} \text{ d}^{-1}$), according to animals' physiological stage and pasture availability. C and D mares were only supplemented with grass hay (ranging from 5 to $10 \text{ kg head}^{-1} \text{ d}^{-1}$), in periods when pasture was scarce. Dry matter production in similar pastures of the same regions ranged from $40 \text{ kg ha}^{-1} \text{ d}^{-1}$ in February to $90 \text{ kg ha}^{-1} \text{ d}^{-1}$ in April, and decrease to $50 \text{ kg ha}^{-1} \text{ d}^{-1}$ in May (Paço and 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 $104^{\circ}C$. Ground samples were analyzed for ash by burning overnight at $550^{\circ}C$ (Instituto Português da Qualidade, 1983). Crude protein (CP) was measured by Kjeldahl method (ISO, 2005). Crude fibre (CF) analyses were conducted according to the official procedure for feed analysis (ISO, 2000). Neutral detergent fibre (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).

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 Arnaud *et al.* (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 (0.5 kg) was regularly checked.

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, average daily gain (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 was measured with an aluminum standard measuring stick from the ground to the highest point of the withers.

Blood metabolites

On the days of BC and BW assessments, between 8.00 h and 11.00 h and before any compound feed was distributed, blood samples ($\approx 18 \text{ mL}$) 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 $4^{\circ}C$, for 15 min. Plasma and serum samples were

stored at -20°C until analysis. Plasma glucose, urea and albumin concentrations were measured by colorimetric methods in an auto-analyzer (Kone Optima Analyzer, Thermo 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).

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 Inst. 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 \times 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 \times time were studied.

All results are presented as $Lsmeans \pm SEM$, unless stated otherwise (Table 1). When variables did not follow the normal distribution, transformed data (\log_{10} 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.

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.

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 effect of the physiological stage on BW ($P < 0.001$), 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 1 a-d). Besides seasonal factors (*e.g.*, grass abundance in the spring), major BW changes in the broodmare were normally

Table 1. Chemical composition and nutritive value of supplementary feeds (on dry matter basis)^o used in A and B mares.

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

^oValues are presented as means \pm SD. ^fCompound feed composition: oats, 28%; soybean meal 44, 22%; alfalfa dehydrated, 20%; maize, 15%; carob, 8%; sunflower seed meal, 4%; minerals and vitamins, 3%. [§]Number of analysed samples. DE, digestible energy; NE, net energy; MADC, horse digestible crude protein; DP, digestible protein. [†]Estimated according to INRA (2012) system.

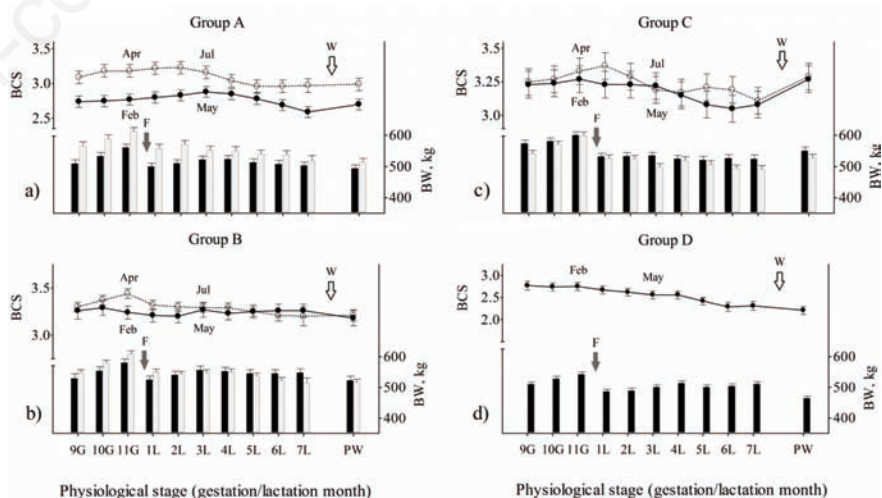


Figure 1. Body condition scoring (BCS) and body weight (BW) according to physiological stage, in mares of groups A, B, C and D (figures a, b, c and d respectively). Solid symbols and continuous lines refers to Feb-Mar foaling mares and open symbols and dotted lines refers to Apr-May foaling mares. In all the figures, foaling is represented by the solid arrow and weaning by the open arrow.

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). Body weight 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 1a). Mares of group D showed, in gen-

eral, 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 1d).

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 1 a-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 1b). 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 1c). 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 1d).

Considering data from all mares, a positive Correlation was found between BC 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; 1993) and, consequently, the growth of nursing foals could be influenced by the amount of body reserves of their dams (Martin-Rosset and Young, 2006). The current study provides an overview of BC 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 BC 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 BC 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

Table 2. Chemical composition and nutritive value of pastures (on dry matter basis) sampled in the spring of 2006 (stud farms A and B) and in the spring of 2008 (stud farms C and D).

	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 [†] , MJ/kg	11.6	11.1	8.9	11.4	10.6	11.5	9.3	
NE [†] , MJ/kg	6.6	6.1	4.3	6.3	5.8	6.3	5.0	
DP [†] , g/kg	136	149	62	16	112	142	70	
MADC [†] , g/kg	122	134	56	152	101	128	63	

DM, dry matter; CP, crude protein; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; DE, digestible energy; NE, net energy; MADC, horse digestible crude protein; DP, digestible protein. [†]Estimated according to INRA (2012) system.

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 5C° to 25C° in mild cli-

mate (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 and Vermorel, 1991). In contrast, the absence of BCS changes in mares of group B during the summer months (Figure 1b) 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 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 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 1d). 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 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.

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

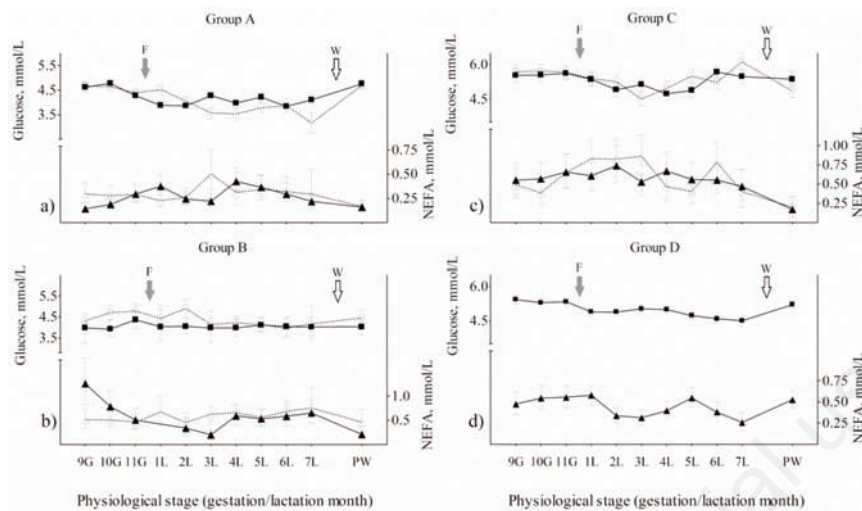


Figure 2. Blood glucose and non-esterified fatty acids (NEFA) concentrations according to physiological stage, in mares of groups A, B, C and D (figures a, b, c and d, respectively). Solid symbols and continuous lines refer to Feb-Mar foaling mares and open symbols and dotted lines refer to Apr-May foaling mares. In all the figures, foaling is represented by the solid arrow and weaning is represented by the open arrow.

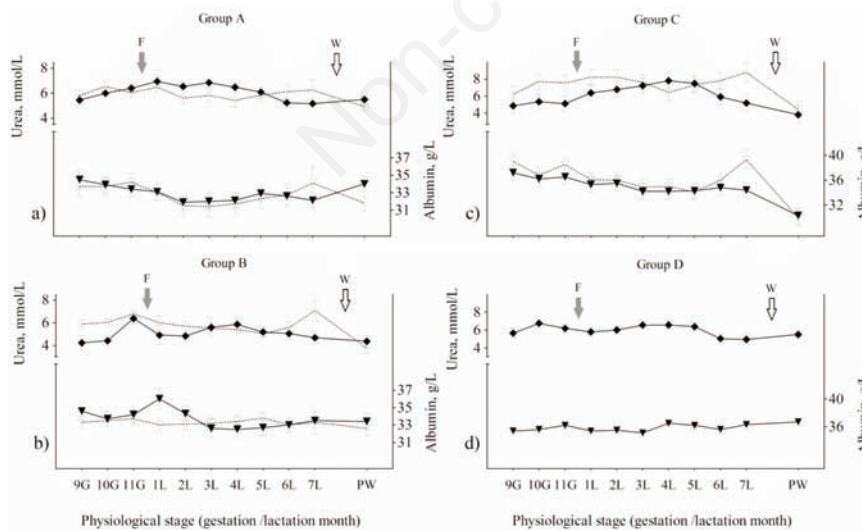


Figure 3. Blood urea and albumin concentrations according to physiological stage, in mares of groups A, B, C and D (figures a, b, c and d, respectively). Solid symbols and continuous lines refer to Feb-Mar foaling mares and open symbols and dotted lines refer to Apr-May foaling mares. In all the figures, foaling is represented by the solid arrow and weaning is represented by the open arrow.

control that keeps it within narrow limits. However, low values may indicate decreased energy 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 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.*, 2003; 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 and 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 concen-

trations 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.*, 1995; Caldeira, 2005; Dugdale *et al.*, 2010). 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. 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). 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.*, 1995). 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.

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). 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). 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

Table 3. Prediction models for body weight and withers height in the four groups of foals, through the first four months of life.

	Group	Foaling season	Model	Foaling season	Fixed effects (Pr>F)		
					Time	Foaling season ×time	Time×time
Body weight, kg							
	A	Feb-Mar Apr-May	BW=57.4+1.418 d-0.00299 d ² BW=57.4+1.347 d-0.00299 d ²	ns	<0.001	0.023	<0.001
	B		BW=51.7+1.191 d-0.00225 d ²	ns	<0.001	ns	<0.001
	C	Feb-Mar Apr-May	BW=53.0+1.338 d-0.00186 d ² BW=53.0+1.249 d-0.00186 d ²	ns	<0.001	0.019	0.001
	D	Feb-Mar	BW=50.7+1.358 d-0.00309 d ²	-	<0.001	-	<0.001
Withers height, cm							
	A		WH=102.0+0.306 d-0.00084 d ²	ns	<0.001	ns	<0.001
	B		WH=100.4+0.294 d-0.00082 d ²	ns	<0.001	ns	<0.001
	C	Feb-Mar Apr-May	WH=98.9+0.373 d-0.00112 d ² WH=98.9+0.343 d-0.00112 d ²	ns	<0.001	0.011	0.001
	D	Feb-Mar	WH=99.1+0.345 d-0.00107 d ²	-	<0.001	-	<0.001

BW, body weight; WH, Withers height; d, days of age; ns, not significant.

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 BC 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.

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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