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Supplement inclusion on post-weaning foals' (Mangalarga Marchador) diet with a protein supplement based on whey protein, glutamine, and lysine

Inclusão de suplemento proteico a base de whey protein, glutamina e lisina na dieta pós-desmame de potros mangalarga marchador

Pierre Barnabé Escodro[1]; Mário Fernando Vilela de Almeida[2]; Tobyas Maia de Albuquerque Mariz[3]; Rebeca Alves Weigel[4]; Yane Fernandes Moreira[5]; Fátima Caroline Soares Borges[5]; Amanda Caroline Gomes Graboschii[5]*; Luan Luthzemberg Ferreira de Andrade[2]; Marcio Calixto Matias[2]; Julimar do Sacramento Ribeiro[3]

Highlights

Concentrate replacement in diet with whey protein, lysin, glutamine supplementation;
Use of protein supplementation increasing body measurements in post-weaning foals;
Post-weaning foal growth (Mangalarga Marchador breed) via protein diet;
Comparison statistics based on usual diet and specific supplementation in foals;

Abstract

The objective of this study was to evaluate the partial replacement effect on body measurements (body mass, muscle thickness, and fat thickness) of the concentrated fraction of foals' diet with a protein supplement containing whey protein, glutamine, and lysine during the post-weaning period. Eighteen Mangalarga Marchador foals were randomly divided into two groups in the post-weaning phase, with nine animals each in the control group (CG) and the group receiving protein supplementation (WG). Based on 4% of their body weight (BW), the CG animals received a diet with roughage to concentrate in a ratio of 50:50, which composed of *Cynodon sp.* hay and a 15% crude protein (CP) commercial horse ration. The

[1] Animal Science Prof., Universidade Federal de Alagoas, UFAL, Viçosa, AL, Brazil. E-mail: pierre.escodro@propep.ufal.br
[2] Masters Students of Animal Science, UFAL, Viçosa, AL, Brazil. E-mail: mfernando11@hotmail.com; luthzemberg_luan@hotmail.com; marciocalixtovet@live.com
[3] Animal Technology Profs., UFAL, Arapiraca, AL, Brazil. E-mail: tobyas.mariz@arapiraca.ufal.br; julimar.ribeiro@arapiraca.ufal.br
[4] Autonomous Veterinary Doctor, Quimtia S.A., São Paulo, SP, Brazil. E-mail: raweigel78@gmail.com
[5] Veterinary Medicine Students, UFAL, Viçosa, AL, Brazil. E-mail: yanemoreira4@gmail.com; fcarolinesborges@gmail.com; amandagraboschii@gmail.com
* Author for correspondence

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WG diet consisted of the same amount of hay used for the CG diet, half the amount of concentrate used in the CG diet, and 102 g of a protein supplement containing whey protein, glutamine, and lysine. During the experiment, five evaluations were performed, starting on the day of weaning (day 0), followed by evaluations conducted at 7, 14, 28, and 42 days after weaning. Body measurements, body mass, and thickness of the fat and muscle layers were assessed at each time point. The substitution of a portion of the commercial diet with protein supplement containing whey protein, glutamine, and lysine did not cause changes in the weight gain or linear measurements of Mangalarga Marchador foals during the post-weaning period; however, the change in diet increased muscle thickness and decreased fat thickness when compared to animals in the control group.
Key words: Amino acids. Foals. Body measurements. Superficial gluteal muscle. Whey protein.

Resumo

Objetivou-se com este estudo, avaliar a substituição de uma parte do concentrado da dieta por suplemento proteico contendo whey protein, glutamina e lisina sobre medidas corporais, massa corporal, espessura muscular e de gordura de potros da raça Mangalarga Marchador durante o pós desmame. Utilizou-se dezoito potros da raça Mangalarga Marchador, divididos aleatoriamente em dois grupos na fase pós-desmame, com 9 animais fazendo parte de Grupo Controle (GC) e outros 9 animais compondo o grupo que recebeu suplementação proteica (GW). Os animais do GC receberam uma dieta de proporção volumoso/concentrado 50:50, considerando-se 4% do PV, composta por ração comercial com 15% PB e feno de Cynodon sp. A dieta do GW foi composta pela mesma quantidade de feno do GC, diferenciando-se na parte concentrada, que foi composta por 50% do concentrado oferecido ao grupo controle acrescido de 102g de suplemento proteico contendo whey protein, glutamina e lisina. Foram realizadas cinco etapas de avaliação de medidas corporais, massa corporal, espessura da camada de gordura e muscular, iniciando no dia do desmame (dia 0) e após 7, 14, 28 e 42 dias. A substituição de parte do concentrado comercial por suplemento proteico contendo whey protein, glutamina e lisina, não acarretou perdas em ganho de peso e medidas lineares de potros da raça Mangalarga Marchador durante o período de 42 dias após o desmame, mas promoveu ganhos em espessura muscular e diminuição de espessura de gordura quando comparados aos animais do grupo controle.
Palavras-chave: Aminoácidos. Cavalos jovens. Medidas corporais. Músculo glúteo superficial. Proteína.

Introduction

Saddle-type animals, a group which the Marchador Mangalarga is part of, reach approximately 80% of their final withers height at six months of age and 90% after completing their first year of life (Spindola et al., 2018). Young and growing horses are exceptionally responsive to protein supplementation, and special attention should be paid to essential amino acids, especially lysine, which is considered the limiting amino acid for these species, that is, the amino acid most likely to limit or increase the use of tissue protein (Silva Inácio et al., 2018).

Several studies have reported positive results with lysine supplementation in foals and relationships between glutamine administration, increased protein synthesis capacity, and muscle mass gains in horses.

Additional benefits to the microbiota, regeneration of the intestinal mucosa, and decreased anisocytosis have also been reported (Carr, 2018). The use of whey proteins extracted from the aqueous portion of milk to feed foals during the weaning phase is still understudied; however, whey proteins are a promising nutritional tool for improving equine morphological and immunological indices during this developmental phase, which is critical for foal and can be subject to marked changes that trigger acute stress conditions (Spindola et al., 2018; Lansade et al., 2018).

The nutritional importance of soluble whey proteins is due to their amino acid profile containing bioactive peptides with high levels of essential amino acids, especially branched-chain amino acids such as leucine, isoleucine, and valine, which are associated with growth factors, reconstruction, and muscle repair (Sousa et al., 2012).

The objective of this study was to evaluate the effect of supplementation with a protein supplement containing whey protein, glutamine, and lysine on various body measurements, namely, body weight, muscle thickness, and fat thickness of Mangalarga Marchador foals during the post-weaning period.

Materials and Methods

The protocol was approved by the Ethics Committee on Animal Use of the Federal University of Alagoas (UFAL) under protocol number 065/2018. The experiment was performed at Haras Formoso 2S (Formoso Stud Farm 2S), located in the city of Cajueiro in Alagoas, north-eastern Brazil, from September to December.

A total of 18 foals in the weaning phase were used, including 12 females and 6 males. The mean age was 158.7 ± 41.3 days, and the mean body score was 3 on a scale of 0 to 5 (Carroll & Huntington, 1988). The experiment was distributed in a completely randomized design with two treatments and nine repetitions. Each diet was considered a treatment. The diets were isonitrogenated and calculated to meet the requirements according to NRC (2007).

The Control Group (CG) consisted of 6 females and 3 males and was subjected to a daily diet of 3 kg of commercial concentrate (Max Equines Potros, Durancho Animal Nutrition, Pesqueira, PE, Brazil), 3 kg of Tifton 85 hay (*Cynodon sp.*), and water "*ad libitum*". This diet is routinely used on farms, with a daily supply of approximately 4% live weight (LW) in a 50:50 roughage/concentrate ratio.

The Protein Supplementation Group (WG) consisted of 6 females and 3 males, subjected to a daily diet of only 1.5 kg of commercial concentrate (Max Equines Potros, Durancho Animal Nutrition), which is equivalent to 50% of that offered to the CG, and 102 g of protein supplement, consisting of 68% crude protein (Equisave Whey, Quimtia, Columbo, PR, Brazil) (Table 1), in addition to 3 kg of Tifton 85 hay (*Cynodon sp.*) and water "*ad libitum*". Table 2 shows the estimation of crude protein supply in the experimental diets.

Table 1
Nutritional composition of commercial concentrate (CC), Tifton hay (TH), and protein concentrate (PC)

Nutrients (%)	CC	TH	PC
Moisture [a]	13.00	17,31	-
CP [a]	18.00	14.53	68.00
ADF [a]	18.00	-	-
P [b]	0.50	-	-
FM [a]	12.00	-	-
MM [a]	10.00	-	-
EE [b]	3.00	1.27	-
Ca [a]	1.50	-	-
Ca [b]	1.00	-	-
DM	87,00	82.37	-
OM	-	92.92	-
TC	-	77.12	-
NDF	-	82.94	-
NDFap	-	81.02	-
TDN	-	58.63	-
GLN [b]	-	-	2.00
LEU [b]	-	-	4.00
LIS [b]		-	20.00

Abbreviations: CP, crude protein; ADF, acid detergent fiber; P, phosphorus; FM, fibrous matter; MM, mineral matter; EE, ether extract; Ca, calcium; DM, dry matter; OM, organic matter; TC, total carbohydrates; NDF, neutral detergent fiber; NDFap, neutral detergent fiber corrected for ash and protein; TDN, total digestible nutrients; GLN, glutamine; LEU, leucine; LIS, lysine
[a] Maximum.
[b] Minimum.

Based on the dry matter offer, Table 3 shows the crude protein consumption where the amount of protein in each diet is equivalent in percentage, but the sort of the amino acid to each diet is distinct, causing divergent final results in the experiment.

Table 2
Estimation of crude protein supply in experimental diets

Ingredients	Experimental Diets	
	CG (g of CP)	WG (g of CP)
Commercial Concentrate	469.8	234.9
Hay	359.1	359.1
Protein Supplement	-	102
Total CP supplied	828.9	696

Abbreviations: CG, control group; WG, protein supplementation group; CP, crude protein; g, gram

Table 3
Protein and energy estimated intake in foals from 0 to 42 days after weaning receiving diets with and without protein supplementation

Ingredients	Experimental Diets	
	CG	WG
DMC (%)	5,25	4,08
DMC CP (g)	910	736
DMC CP (% dmc)	17,1%	17,9%

Abbreviations: DMC, Dry Material Consumption; CP, crude protein; CG, control group; WG, protein supplementation group.

The animals were identified, weighed, dewormed (Equest Pramox: moxidectin 0.4 mg/kg BW + praziquantel 2.5 mg/kg BW) and individually allocated into 20 m² open stalls with permitted communication (Figure 1) from 7:00 to 15:00 and were released collectively at night in an area of 9,000 m². The concentrate was supplied at 7:30 and 15:30, not exceeding 3 kg/animal/day and hay was provided continuously between given times. (Figure 1).

Figure 1. Individual stalls where foals were kept during the day.

During the experiment, five evaluations were performed, starting on the day of weaning (day 0) when the diet adaptation started, followed by evaluations for seven days, when the adaptation period ended, and the diet was served on 14, 28, and 42 days after weaning. Body mass, body measurements, and fat and muscle thickness were estimated. Body mass (BW) was calculated using Crevat and Quetelet's mathematical formula, where BW (kg) = thoracic girth (TG) ³ × 80 (Souza et al., 2017).

Body measurements assessed using a hypometer and a tape measure were as follows: (1) Thoracic girth, drawn along the line of the spinous apophysis of the 7th-8th thoracic vertebra to the corresponding inferior sternal region at the level of the olecranon tuberosity; (2) Thoracic depth (TD), vertical distance between the highest point of the interscapular region and the xiphoid cartilage perpendicular to the ground; (3) Cannon circumference (CaC), measured by the circumference of the middle third of the left metacarpal bone; (4) Body length (BL), tracing between the major tubercle of the humerus and sciatic tuberosity; (5) Rump length (RL), tracing between the ileum wing and the sciatic tuberosity; (6) Withers height (WH) corresponds to the highest point of the interscapular region to the ground; (7) Rump height (RH) corresponds to the highest point of the ileum sacral tuberosity to the ground.

Fat and muscle thickness measurements were performed by ultrasonography using Mindray DP-20 equipment (Mindray Brazil, São Paulo, SP, Brazil) with a 6-8 MHz linear transducer. Fat layer thickness and muscle depth were assessed for both groups at six pre-established points as follows (Gobesso, Martins, Gil, Françoso, & Gonzaga., 2014): (1) Thickness of the fat layer in a cross-sectional cut of the *longissimus dorsi* muscle (FTL), between the 17th and 18th rib bilaterally; (2) Thickness of the fat layer at the tail insertion region (FTT), monitored at 5 cm lateral to the axis of the spine and 7 cm cranial to the base of the tail, on both sides (right and left); (3) Thickness of the fat layer on the superficial gluteus (FTG) at the midpoint of the imaginary line between the tip of the ileum and the ischium bilaterally, approximately 10 cm from the dorsal process of the spine; (4) Thickness of the superficial gluteal muscle (TMG) at the midpoint of the imaginary line between the tip of the ileum and the ischium bilaterally, approximately 10 cm from the dorsal process of the spine.

The values of the studied variables were found to be regular and homogeneous using the Shapiro-Wilk test. Thus, they were subjected to analysis of variance (ANOVA) for repeated measures using R software (Manso et al., 2014). The means were compared using Tukey's test at a 5% significance level ($P < 0.05$). (Figure 2).

Figure 2. Ultrasound evaluation sites: FTL, thickness of the fat layer in a cross-sectional cut of the left *longissimus dorsi* muscle; TMG, left superficial thickness of the gluteal muscle; FTT, thickness of the fat layer in the tail insertion region.

Results and Discussion

The foals were not subjected to concentrate supplementation during lactation, and weaning started at a mean body weight of 160.79 kg in CG and 153.40 kg in WG, which is slightly below the 171.55 kg and 173.5 kg cited by Rezende, Sampaio, Legorreta and Moreira (2000) and Silva Moura et al. (2016), respectively, and above the 127.26 kg cited by Manso et al. (2014) for foals of the Mangalarga Marchador breed.

The body mass and body measurement values for the five assessment points (0, 7, 14, 28, and 42 days) are presented in Table 4.

Table 4
Mass and body measurements obtained from Mangalarga Marchador foals at 0, 7, 14, 28, and 42 days of weaning

Measurements	Group	Time					CV (%)
		0	7	14	28	42	
Mass (TG³ x 80)	CG	160.79	164.34	164.98	167.56	172.61	11.63
	WG	153.40	154.69	159.69	161.42	169.89	13.7
Chest girth (cm)	CG	125.77	126.66	127.11	127.77	129.00	3.95
	WG	124.00	124.33	125.66	126.11	128.33	4.56
Thoracic depth (cm)	CG	59.33[b]	62.55[ab]	63.00[ab]	63.66[a]	64.55[a]	5.14
	WG	61.44	61.55	62.22	62.77	64.44	4.28
Cannon circumference (cm)	CG	15.44[b]	15.88[ab]	15.88[ab]	16.22[a]	16.22[a]	3.63
	WG	15.77	15.77	15.77	15.77	15.88	4.18
Body length (cm)	CG	112.44[c]	116.22[bc]	118.33[abc]	120.11[ab]	122.77[a]	4.87
	WG	113.66[c]	114.11[bc]	116.55[abc]	119.11[ab]	121.00[a]	3.91
Rump length	CG	39.00[c]	39.88[bc]	40.00[bc]	41.229[ab]	42.11[a]	4.13
	WG	38.33[c]	39.22[bc]	40.33[abc]	41.55[ab]	42.00[a]	5.95
Withers height	CG	122.11	123.11	124.88	125.33	126.77	3.3
	WG	123.00	123.22	123.77	125.00	125.66	2.68
Rump height	CG	124.44	126.33	128.55	129.00	129.00	2.93
	WG	126.66	126.88	127.22	128.22	128.55	2.72

Abbreviations: TG, thoracic girth CG; control group, WG; the group with protein supplementation; CV, coefficient of variation
Means followed by different letters in the same row indicate significantly different values (P <0.05) as determined by Tukey's test.

There were no differences in the morphological measurements between treatments, providing the animals with good growing conditions. This result is important, considering that the nutritional deficit during this phase could cause alterations in their growth patterns.

The results obtained from the ultrasound measurements of fat and muscle thickness at the six pre-established points (FTL, TMG, and FTT) are described in Table 5.

With the progression of post-weaning time, the WG treatment resulted in a reduction in the thickness of the fat layer in a cross-sectional cut of the longissimus dorsi muscle (FTL), the thickness of the superficial gluteal muscle (TMG), and the thickness of the fat layer at the tail insertion region (FTT); however, there was an increase in muscle mass and a variation in the thickness of the superficial gluteal muscle, with higher values on day 42. These results could be related to the dietary amino acid profile that causes significant muscular growth in the adipose tissue.

Table 5

Mean values of fat thickness of the longissimus dorsi muscle (FTL), thickness of fat at the tail (FTT), thickness of fat of the superficial gluteus (FTG), and thickness of the superficial gluteal muscle (TMG) obtained by ultrasound examination in Mangalarga Marchador foals at 0, 7, 14, 28, and 42 days of weaning

Ultrasound Measurements	Group	Time					CV (%)
		0	7	14	28	42	
FTL - direct (cm)	CG	0.39±0.11*	0.42±0.12*	0.40±0.10*	0.39±0.09*	0.39±0.08*	24.63
	WG	0.27±0.05[a]	0.27±0.05[a]	0.20±0.02[b]	0.20±0.02[b]	0.17±0.01[b]	23.53
FTL - left (cm)	CG	0.39±0.10*	0.43±0.10*	0.40±0.94*	0.39±0.84*	0.39±0.82*	22.84
	WG	0.27±0.05[a]	0.27±0.04[a]	0.20±0.02[b]	0.20±0.02[b]	0.18±0.01[b]	22.89
FTT - right (cm)	CG	0.30±0.07	0.34±0.06	0.34±0.07*	0.34±0.07*	0.34±0.06*	19.78
	WG	0.32±0.05[a]	0.31±0.04[a]	0.23±0.03[b]	0.23±0.03[b]	0.19±0.02[b]	23.36
FTT - left (cm)	CG	0.31±0.07	0.35±0.06	0.34±0.07*	0.33±0.06*	0.34±0.06*	18.75
	WG	0.31±0.04[a]	0.31±0.04[a]	0.23±0.03[b]	0.23±0.03b	0.19±0.02[b]	22.50
FTG - right (cm)	CG	0.36±0.08*	0.39±0.09*	0.37±0.08*	0.36±0.07*	0.37±0.06*	20.57
	WG	0.29±0.04[a]	0.28±0.03[a]	0.22±0.01[b]	0.22±0.03[b]	0.19±0.01[b]	19.30
FTG - left (cm)	CG	0.35±0.08	0.37±0.06*	0.36±0.07*	0.35±0.07*	0.37±0.05*	19.07
	WG	0.30±0.04[a]	0.28±0.03[a]	0.21±0.02[b]	0.22±0.03[b]	0.19±0.01[b]	20.31
TMG - right (cm)	CG	1.88±0.11	1.93±0.11	1.94±0.10	1.97±0.11	2.03±0.09	5.87
	WG	2.63±0.33*[b]	2.65±0.33*[b]	2.84±0.22*[ab]	2.99±0.21*[ab]	3.07±0.22*[a]	11.00
TMG - left (cm)	CG	1.91±0.14	1.99±0.14	2.00±0.14	2.01±0.14	2.04±0.10	6.76
	WG	2.63±0.34*[c]	2.64±0.34*[bc]	2.84±0.21*[abc]	2.99±0.21*[ab]	3.07±0.21*[a]	11.13

Abbreviations: FTL, fat thickness of the longissimus dorsi muscle; FTT, thickness of fat at the tail; FTG thickness of fat of the superficial; TMG thickness of the superficial gluteal muscle; CG, control group; WG, group with protein supplementation; CV, coefficient of variation

Means followed by different letters in the same row followed by an asterisk in the same column indicate significantly different values ($P < 0.05$) as determined by Tukey's test.

There was no difference between treatments in the thickness of the superficial gluteal muscle and the thickness of the fat layer at the tail insertion region in all evaluated periods.

The weaned animals had a mean age of 158.7 ± 41.3 days, which is within the 4 to 7 months cited in the literature; however, weaning at the stud farm is performed abruptly and in individual stalls. This type of weaning is more stressful than weaning performed in a progressive way, which alleviates the negative effect of definitive weaning on both mother and offspring (Spindola et al., 2018).

Diet adaptation occurred without undesirable episodes for both groups tested, except for insufficient dietary intake of the diet containing protein concentrate (WG) in the first 2 days. There were no episodes of any disease during the 42 days of the experiment.

Rezende et al. (2000) evaluated the weaning phase of foals of this breed from 40 to 365 days of age and showed that this is the phase with the lowest weight gain per day. The authors noted non-significant weight gain between 166 and 202 days (36 days of weaning), and non-supplemented foals weighed 171.5 to 181.59 kg on an average.

In the present study, CG animals obtained similar results to those of Rezende et al. (2000) after 42 days of weaning, with no statistically significant difference between CG and WG animals, but with an increase in weight observed in both groups. Each group showed a difference between body length and rump length; after 42 days, the coefficient of variation (CV) was 11.63% in the CG animals and 13.7% in the WG animals, showing the relative superiority of the WG diet, indicating a better contribution to the animal growth in the post-weaning phase. (tab. 4) There was a difference in the control group (CG) for the cannon circumference and thoracic depth between the time elapsed.

Silva Moura et al. (2016) also found no significant difference in the weight of foals of this breed under the same diet with probiotic or phytase supplementation. The data showed that, even with 234.9 g of concentrate and a daily intake of 132.9 g less crude protein, the foals subjected to the WG diet had similar development compared to those given the CG diet, which minimizes the ingestion of carbohydrates, the risks of gas dilation, and feeding costs. The amino acid profile of the protein fraction of a diet can directly affect the use of this nutrient by animals, especially with regard to essential and limiting amino acids (Staniar et al., 2001). This may explain the performance observed in the animals of the supplemented group.

There were no significant differences in linear body measurements between the CG and WG animals, all of which showed slight growth curves for all measurements during the 42 days of the experiment. In evaluating the measurements within the groups, there were significant differences in the body length and rump length between the diets, and these measurements showed the most changes during the experimental period, as has been cited in other studies related to foals of this breed (Silva Moura et al., 2016). What was unconventional in relation to the other studies was the significant difference between the cannon circumference and the thoracic depth of CG foals; however, when comparing the CVs of the CG and WG diets, there were no significant variations between the groups.

The withers height did not show a significant change during the 42 days of the study. Silva Moura et al. (2016) showed similar results in foals of this breed over a greater time, in which they observed a mean height increase from 121.5 to 123.9 cm after 59 days of weaning, with no significant differences among foals supplemented with or without *Saccharomyces cerevisiae*, probiotics, or phytases. Similarly, Silva Inácio et al. (2018) did not observe any significant growth in foals supplemented with sorghum silage during the Brazilian drought, reporting a CV of 3.65%, which is similar to that obtained in this study. These findings show that, although there was an increase in the withers height at weaning, there were no significant differences in the measurements at 60 days.

One of the greatest concerns of breeders of Mangalarga Marchador is the withers height of adult horses, which must be within the 152 cm recommended by the Associação Brasileira dos Criadores de Cavalos Mangalarga Marchador (Brazilian Association of Mangalarga Marchador

Breeders; ABCCMM). Only a few foals in the traditional management of Brazilian equids are supplemented before one year of age. As mentioned above, the animal can reach 80%-90% of its height by one year of age; however, most of the animals of this breed do not reach the recommended height (Santiago, Rezende, Fonseca, Abrantes, & Lage, 2013; Silva Inácio et al., 2018). We observed that the mean withers height of the animals after 42 days was 83.40% of the withers height recommended by the ABCCMM in the CG animals and 82.65% of the recommended withers height in the WG animals. Further studies are required to compare the correlation between diet and increased withers height up to 12 months of age.

The thickness of the fat layer in the cross-sectional cut of the *longissimus dorsi* muscle (FTL) demonstrated uniformity of measurement between the right and left sides. There were no significant differences in the FTL values between the time elapsed for the CG diet. For the WG diet, a significant difference was observed in the FTL values starting at 14 days, which remained stable for up to 42 days.

The fat thickness at the tail insertion (FTT) and superficial gluteus (FTG) followed the same linearity as the FTL, with a decrease observed in the WG animals starting at 14 days when significant differences between groups also emerged. These results show that protein concentrate supplementation generally decreased fat thickness in the regions measured during the experiment.

There was no difference in the thickness of the TMG in CG animals. However, a significant increase in muscle thickness in the WG animals occurred after 14 days, which was significantly different between the groups. These results demonstrate an increase in muscle thickness in WG animals compared to that in CG animals.

In general, and in the authors' external evaluation, the WG animals had a body score similar to that of the CG animals after 42 d. However, due to the increase in lean mass promoted by the protein concentrate, it was clear that the WG animals were more muscular and morphologically more presentable.

Conclusions

The decrease in the dry-matter-concentrated daily diet followed by supplementation with whey protein, glutamine, and lysine was made with less crude protein but the right type of amino acids, which did not cause changes in weight gain or linear measurements but induced significant improvements related to increased muscle thickness and decreased fat thickness. More studies are needed to evaluate the possible differences in weight and withers height of one-year-old foals; however, protein concentrate may become an alternative to obtain better zoometric indexes.

Animal Welfare Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received. The authors confirm that they have followed the EU standards to protect animals used for scientific purposes.

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