In vivo performance of Italian Heavy Draft Horse weanlings fed two protein levels and slaughtered at two ages

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ABSTRACT: This study aimed at evaluating in vivo performance, growth parameters, intakes, dressing percentage, and blood parameters in Italian Heavy Draft Horse (IHDH) weanlings fed 2 CP levels up to the 2 typical ages of slaughter. Forty-one weanlings were grouped in 8 pens according to sex, age, and BW. After a transition period, animals were randomly assigned to 2 isoenergetic diets containing different CP levels: 10.6 and 11.2% CP in DM for low protein (LP) and 13.2 and 14.7% CP in DM for high protein (HP) diets in the first and second phase, respectively. About half of the animals (n = 22) were slaughtered when aged 13 mo (end of first phase); the remaining animals (n = 19) were slaughtered at 18 mo (end of second phase). Animals were weighed, measured for withers height, and scored in vivo for fleshiness and BCS at 3 wk intervals. Feed intake in each pen was measured weekly, and feed samples were collected every 2 mo. Blood samples from venous jugular were collected in both phases to analyze plasma protein, urea, glucose, bilirubin, hepatic enzymes, and mineral content. Growth parameters were estimated within phase by modeling BW as a function of age using fourth-degree Legendre polynomials. During the first phase, a different linear coefficient (P = 0.051) for the growth curve was observed between females fed a HP or a LP diet, while males showed differences only on quadratic and cubic Legendre coefficients. However, no significant differences were detected in ADG between the CP levels and sexes. In the second phase, Legendre coefficients were not different between treatments for the remaining weanlings, and once again no differences were found on ADG. The DM intake was influenced by diets in both periods, greater in the HP diet as compared with the LP diet (P < 0.001). No differences due to diet were observed for fleshiness or BCS scores at the end of each phase or in the dressing percentage at slaughter. As expected, plasma urea was greater (P < 0.001) in animals fed the HP diet but was within a normal range for healthy horses. In conclusion, a small dietary protein restriction (i.e., on average 3% of DM) did not change the in vivo performance of IHDH weanlings up to 13 or up to 18 mo of age.

Key words: dietary protein level, growth rate, heavy breeds, horses, weanlings

INTRODUCTION

Horse meat is a niche product consistently consumed in some world districts where horses are reared for human consumption (Sarriés et al., 2006). Specific aspects of horse meat are its high bioavailable iron content, the lack of intramuscular fat, and its low cholesterol level (Badiani et al., 1997; Levine, 1998). Young weanlings raised for meat and belonging to heavy breeds are preferred by the meat industry (Tateo et al., 2008), because of their higher growth rate compared with light breeds (Martin-Rosset, 2005). However, few studies on the growing parameters of weanlings have focused on heavy horses (e.g., Austbø, 2005; Delobel et al., 2005), although the noteworthy muscle development of these breeds implies specific feeding needs, such as increased protein requirements (Martin-Rosset et al., 1994). Recent constraints of the European Union Nitrate Directive (European Commission, 1991) have led to a reduction of CP levels in beef animals (Schiavon et al.,...
Growth of weanlings fed two protein levels

This may lead to either positive or negative consequences on animal growth, obviously also in horses, depending on the feed quality and energy requirements (Lewis, 1996; Pagan, 2009). Whereas studies on CP level on the growth of light horses have been already performed (e.g., Graham-Thiers et al., 1999; Stanier et al., 2001), literature on heavy breeds is scarce, although information on other coldblood breeds have been previously reported (Saastamoinen, 1996).

The Italian Heavy Draft Horse (IHDH) is a heavy breed originally developed for draft but nowadays mainly bred for meat production (Mantovani et al., 2005, 2010), with typical slaughter ages at about 12 or 18 mo (Tateo et al., 2005). The present study was aimed at evaluating the effects of CP restriction (i.e., 2 different dietary CP levels) on in vivo performance, growth parameters, selected blood parameters, and dressing percentage of IHDH weanlings reared under standard conditions and isoenergetic diets up to the 2 typical ages of slaughter.

**MATERIALS AND METHODS**

All procedures were performed according to Italian legislation on animal care (Legislative Decree No. 116 of January 27, 1992) and approved by the Ethical Committee for the Care and Use of Experimental Animals of the University of Padova (Padova, Italy).

**Animals and Feeding**

The study was conducted over a period of about 8 mo at the experimental farm of the University of Padova (Legnaro, Italy). Forty-nine IHDH weanlings, 224 ± 42 d old at arrival at farm, were first introduced in the study. The weanlings entered the experimental station in December 2010 and weighed 310 ± 55 kg. All animals were treated against internal parasites (1.29 g/100 kg BW; Equalan duo; Merial Animal Health, Harlow, UK) at arrival and after 6 mo of trial and injected with a broad-spectrum antibiotic as a prophylactic (1 mL/16 kg BW; Norodine 24; Bayer S.p.A., Milano, Italy). Animals were divided into 8 groups (about 6 weanlings per group) on the basis of sex, age, and BW and allocated to 8 pens of about 7 by 5 m bedded with wheat straw that was partially renewed every 3 to 4 d. Health status was monitored daily by a veterinarian during the length of their stay at the farm.

For the first 28 d, all the animals were fed an adaptation diet in which meadow hay was progressively replaced by the experimental diet; indeed, diet varied from the initial 100% of meadow hay to the final 100% of experimental diet, by weekly replacing 25% of hay with experimental diet and maintaining the same proportions in ingredients. The experimental trial started when the 100% of experimental diet was provided to weanlings.

Data from the adaptation period were not included in the study. Thereafter, pens were divided between 2 experimental diets (i.e., low protein [LP] and high protein [HP] diets), randomly assigning 1 pen of females and 3 males to each diet. Diets were given ad libitum in 2 subsequent phases of about 4 mo each (Table 1). The end of each phase corresponded to 1 of the 2 typical ages at slaughtering for the IHDH breed (De Palo et al., 2009).

At the beginning of the trial, some horses (8 males: 6 fed LP and 2 fed HP diets) showed signs of bites situated mainly on the back due to aggressive approaches of other animals in the same pen. The wounded animals were immediately isolated in an infirmary pen and after some days reared in 2 separate pens. They were fed the HP diet for the whole period of the experiment, but they were excluded from the trial.

In vivo data from all the 41 nonwounded horses were retained for the analysis of the first phase, that is, 12 males and 6 females assigned the LP diet and 16 males and 7 females assigned the HP diet. The remaining weanlings were aged 276 ± 35 d at the beginning of the phase and 414 ± 30 d once the phase had ended. The second phase included

<p>| Table 1. Ingredients and composition of the 2 experimental diets (low protein [LP] and high protein [HP] levels), given to animals in the 2 subsequent experimental phases (data are expressed as % on DM basis) |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Item</th>
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<td>2.84</td>
<td>2.92</td>
<td>2.88</td>
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<td>NE,⁴ HFU/kg of DM</td>
<td>0.80</td>
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</table>

¹Vitamin and mineral premix was added per animal (50 g/d) and contained (per kg) 1,000,000 IU of vitamin A, 70,000 IU of vitamin D, 1,200 mg of vitamin E, 5,000 mg of niacin, 1,980 mg of betaine, 800 mg of Fe, 500 mg of Cu, 2,950 mg of Zn, 28 mg of I, 8 mg of Se, and 30 mg of Mo.
²DP = digestible protein (of the INRA French system [Martin-Rosset et al., 2006]).
³DE values were calculated using the NRC equation (NRC, 2007).
⁴NE is expressed as horse feed units (HFU) estimated using the INRA method proposed by Martin-Rosset et al. (2006).
19 weanlings ranging from 424 ± 26 (beginning) to 533 ± 25 d of age (end). In this second phase, a LP diet was given to 5 males distributed in 2 pens and 3 females located in 1 pen, whereas the HP diet was provided for 8 males (in 2 distinct pens) and 3 females in 1 pen. Apart from the wounded animals that were fed but excluded from the trials, all the experimental horses were fed the same HP or LP diet in the 2 phases. During the first phase, the HP diet was formulated (Martin-Rosset et al., 2006; NRC, 2007) to be isoenergetic and achieve 13.2% of CP on a DM basis. The LP diet (10.6% CP) was obtained by reducing the inclusion of soybean meal from 9.5 to 2.4% in DM and increasing corn meal from 13.0 to 17.7% in DM. During the second phase, the diets were formulated to cover the greater nutritional requirements of the animals due to the greater age and the different expected composition of growth (Martin-Rosset et al., 2006; NRC, 2007). Therefore, the protein content reached 14.7 and 11.2% in DM for HP and LP diets, respectively. The inclusion of corn silage was about 20% of DM for both experimental diets and phases (Table 1).

Feed Composition

Samples of the single ingredients of the diets were collected every 2 mo (4 sample collections for a total of 24 samples) to estimate the chemical composition of experimental diets (Table 1). Feeds were analyzed for their composition and NDF and ADF contents (Van Soest et al., 1991; AOAC, 2003). Starch content was determined, following its hydrolysis to glucose (AOAC, 2003), by liquid chromatography (Bouchard et al., 1988). The content of digestible protein for the French system was determined from the chemical composition of feeds using the equations reported by Martin-Rosset et al. (2006). The nutritional values of feeds for the NRC (2007) system (DE; Mcal/kg DM) were calculated from the chemical composition using different equations for forages and for concentrate as suggested by the NRC (2007) system. To calculate the INRA NE, expressed as horse feed units (HIFU/kg DM), the procedure based on digestibility coefficients of OM for feeds of similar chemical composition reported by Martin-Rosset et al. (2006) was applied. Residuals were collected and weighed every 7 d. The DMI was estimated from the average DM daily distributed and the average DM of residuals and computed on a pen basis for intervals of 4 wk during the whole trial. During the feeding experiment, horses had free access to fresh water.

Body Measures

During the experimental phases, BW and withers height were recorded individually at 3-wk intervals, and in vivo fleshiness and body condition were scored by a skilled technician using a 5-point scale both for fleshiness (from 1 = all muscle profiles concave to very concave; poor muscle development; to 5 = all muscle profiles extremely convex; exceptional muscle development considering the profiles of shoulders, loins, rump, thighs, and buttocks; Badiani et al., 1995) and for the BCS (from 1 = very lean to 5 = very fat; Martin-Rosset, 1990).

Slaughter Data

To define the 2 different ages of slaughtering, about half of the animals (n = 22) were slaughtered in May 2011, at the end of the first phase, and the others (n = 19) in November 2011, that is, when the second phase had finished. All carcasses were weighed immediately after slaughter and the dressing percentage was computed as the ratio between the carcass weight and the final BW measured 1 d before slaughter.

Blood Parameters

Blood samples were collected from all animals at Day 29 and Day 176 of the experiment, that is, on the first day of recording belonging to each phase. The samples were obtained from the jugular vein and stored in heparinized tubes under vacuum (Venoject; Terumo, Leuven, Belgium). Plasma was obtained by centrifugation (1,500 × g for 15 min at 4°C) and an indirect Potentiometer Analyzer (Hitachi 911; Roche Boehringer, Mannheim, Germany) was used to estimate total proteins, urea N, glucose, bilirubin, aspartate aminotransferase (AST), γ-glutamyl transferase, creatine kinase (CK), alanine transaminase (ALT), calcium, phosphorus, magnesium, and potassium.

Statistical Analysis

All individual data measured on weanlings (i.e., live BW, withers height, live fleshiness, and BCS scores at the beginning and the end of each phase; blood parameters; carcass weight; and dressing percentage at slaughter) were averaged by phase and pen and analyzed separately by phase using the PROC GLM of SAS (SAS Inst. Inc., Cary, NC) according to the following linear model:

\[ y_{ijk} = \mu + D_i + S_j + e_{ijk}, \]  

[1]

in which \( y_{ijk} = \) average pen value on each variables described above within phase, \( \mu = \) overall mean, \( D_i = \) fixed effect of diet with different protein levels (1 = 2 levels, LP and HP), \( S_j = \) fixed effect of sex (1 = 2 levels, males and females), and \( e_{ijk} = \) random residual error term ~N(0, \( \sigma^2_e \)). The individual ADG obtained by regressing weight on age at different times in each phase (i.e., approximately every 3 wk) was analyzed as pen mean within phase by model [1]. Individual growth parameters were estimated within
phase for D, S, and their interaction by modeling live BW as a function of age using a fourth-degree Legendre polynomial (i.e., intercept, linear, quadratic, and cubic components) as described by Meyer (1998, 2001). In this case the model used was as follows:

\[ y_{ijkl} = \mu + D_i + S_j + (D \times S)_{ij} + \sum_{k=1}^{4} b_k \times L_{jk} + \text{Animal}_{ij} + \epsilon_{ijkl}, \]

in which \( y_{ijkl} \) is individual BW at different ages during the experimental phases, \( \mu \) is overall mean, D and S have the same meaning as above and \( D \times S \) represents their interaction, \( b_k \) is the regression coefficient, \( L_{jk} \) is the value of the \( k \)th Legendre polynomial (i.e., \( k = 1, ..., 4 \)) within \( D \times S \), \( \text{Animal}_{ij} \) is the individual animal effect within \( D \times S \), and \( \epsilon_{ijkl} \) is random residual error term \( \sim N(0, \sigma_e^2) \).

Linear, quadratic, and cubic components of Legendre polynomial within the \( D \times S \) were contrasted to compare the effects of diet within sex.

Feed consumption data (i.e., DM and CP intakes) within each phase, referred to the pen as an experimental unit, were analyzed with the following linear model:

\[ y_{ijkl} = \mu + D_i + S_j + P_k + (D \times P)_{ik} + \epsilon_{ijkl}, \]

in which \( y_{ijkl} \) is average DM or CP intake in the \( l \)th pen (\( l = 8 \) levels), \( \mu \) is overall mean, D and S have the same meaning as above, \( P_k \) is the periodical recording of intakes and residuals (\( k = 5 \) periods in the first experimental phase and \( k = 4 \) in the second experimental phase), \( (D \times P)_{ik} \) is the interaction between D and P, and \( \epsilon_{ijkl} \) is random residual error term \( \sim N(0, \sigma_e^2) \). The degrees of freedom of the \( D \times P \) effect were used to compare diets within each period using the PDIFF (SAS Inst. Inc., Cary, NC) and the Bonferroni correction method for multiple comparisons.

All results are presented as least squares means and relative pooled SE. Significant results were considered at \( P < 0.05 \), and a tendency to significance was assumed at \( P < 0.10 \).

**RESULTS**

**Evaluation of Diets and Intakes**

The LP and HP diets were isoenergetic and, respectively, contained 2.84 and 2.90 Mcal DE/kg DM (NRC, 2007) and 0.80 and 0.81 HFU NE/kg DM (Martin-Rosset et al., 2006) in the first and second phase, respectively (Table 1). In the second phase, a greater inclusion of soybean meal in place of corn meal in the HP diet increased the difference in dietary protein levels between diets as compared with the first phase (the reduction in CP content from the HP to the LP diet was about 20 and 24% in the first and in the second phase, respectively; Table 1). No relevant changes in the other chemical constituents (i.e., NDF, ADF, and lipids contents) were observed between the HP and LP diets (Table 1).

Over the whole experiment, the CP intakes of weanlings fed the LP diet were significantly lower \( (P < 0.01) \) than those of weanlings on the HP diet (Fig. 1). On average, the CP intake in the first phase was 30% lower for the LP diet compared with the HP diet (i.e., 0.80 vs. 1.16 kg/d of CP; Fig. 1A). This difference was due not only to the lower CP level of the LP diet but also to the DM intake, which was lower in weanlings receiving the LP diet than in those receiving the HP diet (7.86 vs. 9.68 kg/d; \( P < 0.001 \); data not shown). During the second phase, the lower CP intake (–20%) of LP diet was confirmed (1.71 vs. 1.36 kg/d; \( P < 0.001 \); data not shown). The average CP intake on a BW basis was 197 and 260 g/100 kg BW for the LP diet and 282 and 312 g/100 kg BW for the HP diet, respectively, in the first and second phase. Dry matter intake was similar for the 2 experimental groups in the second phase (10.92 vs. 11.22 kg/d for the LP and HP diet, respectively; \( P = 0.200 \); data not shown). Both DM and CP intakes were unaffected by the sex (data not shown).
Growth performances of weanlings analyzed in relation to diet and sex and separated by each phase are shown in Table 2. During the first phase, the ADG of weanlings did not differ across the diets and the sex and averaged 1.04 kg/d. At the beginning of the trial, the mean BW of females was 335 kg, that is, similar to that of males, which weighed 339 kg. No differences were observed between the diets and the sex at the beginning of the experiment for withers height, fleshiness, and BCS (Table 2). Similarly, at the end of the first phase, BW, withers height, fleshiness, and BCS were unaffected by the diet and the sex (Table 2).

After the first slaughter date, the number of animals in each experimental group was almost halved (Table 2). During the second phase, growth performances of remaining weanlings were unaffected by the diet and the sex. The average ADG in second phase was 1.24 kg/d whereas in the first phase it averaged 1.04 kg/d. The final BW, withers height, fleshiness, and BCS increased up to 599 kg, 144 cm, 3.03, and 2.71, respectively (Table 2).

Body weight growth parameters of weanlings during the first and second phase described by fourth-degree Legendre polynomials are shown in Fig. 2. During the first phase of the experiment, the BW growth parameters of males on the HP diet were influenced by diet as compared with the growth of males on the LP diet (i.e., quadratic component, \( P < 0.01 \), and cubic component, \( P = 0.033 \); Fig. 2A for males). Therefore, the growth of males fed the LP diet, from 276 ± 35 to 414 ± 30 d of age, was somewhat faster than males fed the HP diet at the beginning, but then it was slightly slower than males fed the HP diet in the middle, and faster again than males fed the HP diet in the second part of the first growing phase (Fig. 2A for males). During the first phase, the females in the LP diet showed different growth parameters than females in the HP diet, with a linear component that was significantly different (\( P = 0.051 \); Fig. 2A for females) and a quadratic

<table>
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<td>–</td>
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<td>0.15</td>
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</tr>
<tr>
<td>Initial BCS,² score</td>
<td>2.47</td>
<td>2.39</td>
<td>0.11</td>
<td>2.42</td>
<td>2.44</td>
<td>0.11</td>
<td>ns</td>
</tr>
<tr>
<td>Final BCS,² score</td>
<td>2.70</td>
<td>2.72</td>
<td>0.08</td>
<td>2.67</td>
<td>2.75</td>
<td>0.08</td>
<td>ns</td>
</tr>
</tbody>
</table>

¹SE is the pooled standard error for the least squares means.
²CP content expressed on DM basis.
³ns = not significant.
⁴Fleshiness was linearly scored in vivo from 1 (all muscle profiles concave to very concave; poor muscle development) to 5 (all muscle profiles extremely convex; exceptional muscle development) considering the profiles of shoulders, loins, rump, thighs, and buttocks.
⁵Scoring system for BCS, from 1, very lean, to 5, very fat; Martin-Rosset, 1990.

**Growth Performance**

Growth performances of weanlings analyzed in relation to diet and sex and separated by each phase are shown in Table 2. During the first phase, the ADG of weanlings did not differ across the diets and the sex and averaged 1.04 kg/d. At the beginning of the trial, the mean BW of females was 335 kg, that is, similar to that of males, which weighed 339 kg. No differences were observed between the diets and the sex at the beginning of the experiment for withers height, fleshiness, and BCS (Table 2). Similarly, at the end of the first phase, BW, withers height, fleshiness, and BCS were unaffected by the diet and the sex (Table 2).

After the first slaughter date, the number of animals in each experimental group was almost halved (Table 2). During the second phase, growth performances of remaining weanlings were unaffected by the diet and the sex. The average ADG in second phase was 1.24 kg/d whereas in the first phase it averaged 1.04 kg/d. The final
component close to significance ($P = 0.061$; Fig. 2A for females). Indeed, females on the LP diet showed a reduced growth rate after the first month of the experiment, thereafter maintaining a growth rate similar to female weanlings in the HP diet (Fig. 2A for females). During the second phase, from 424 ± 26 to 533 ± 25 d of age, the BW growth parameters of males under the 2 dietary treatments were similar (Fig. 2B for males), with no significant differences in the curve parameters, which maintained a parallel shape. Furthermore, the growth curves of females fed LP and HP diets were very similar in the second phase, with none of the 3 components of the Legendre polynomial describing the growth curve affected by the diet (Fig. 2B for females).

**Carcass Traits**

Half of the animals ($n = 22$) were slaughtered at the end of the first phase, at about 417 ± 30 d of age, that is, when they were on average 13 mo old. On the other hand, the remaining animals were harvested at 533 ± 32 d of age (i.e., end of the second phase of the experiment, at about 18 mo of age). The carcass weight was similar for the 2 diets and averaged 306 ± 14 and 375 ± 7 kg for the first and second phase, respectively (data not shown). The dressing percentage was unaffected by the diet and varied from 60.4 ± 0.7 (animals fed the LP diet) to 61.0 ± 0.6% (animals fed the HP diet) for weanlings slaughtered at about 13 mo and from 62.5 ± 0.4 (the LP diet) to 63.1 ± 0.5% (the HP diet) for weanlings slaughtered at about 18 mo of age (data not shown). The value of dressing percentage was not different between sexes in the first phase of the experiment, that is, 61.2 ± 0.69% for females vs. 60.2 ± 0.50% for males ($P > 0.10$; data not shown). However, a tendency of a greater ($P = 0.074$; data not shown) dressing percentage in females than in males was observed at the end of the second phase, that is, 63.9 ± 0.6 vs. 61.7 ± 0.4% for females and males, respectively (data not shown).

**Blood Parameters**

As regards blood parameters, in the first phase, no differences due to the diet or sex were observed in target traits (Table 3), excluding AST, CK, and ALT. These enzymes resulted higher in females than in males ($P < 0.05$). In the second phase, no differences due to the sex were found, whereas the protein level of the diet significantly affected the urea nitrogen (5.42 vs. 6.98 mmol/L for the LP and HP diet, respectively; $P = 0.009$)
Table 3. Least squares means for blood parameters measured on male and female weanlings fed 2 experimental diets (low protein [LP] and high protein [HP] levels), in 2 phases (from 276 ± 35 to 414 ± 30 d of age and from 424 ± 26 to 533 ± 25 d of age)

<table>
<thead>
<tr>
<th>Item</th>
<th>First phase</th>
<th></th>
<th>Second phase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.2% CP</td>
<td>14.7% CP</td>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE¹</td>
<td></td>
<td>SE¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SE¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diet</td>
<td>SE¹</td>
</tr>
<tr>
<td>CP, %</td>
<td>10.6</td>
<td>13.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals, no.</td>
<td>18</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total proteins, g/L</td>
<td>67.7</td>
<td>63.8</td>
<td>1.15</td>
<td>63.7</td>
</tr>
<tr>
<td>Urea N, mmol/L</td>
<td>3.55</td>
<td>4.08</td>
<td>0.36</td>
<td>3.83</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.75</td>
<td>5.45</td>
<td>0.26</td>
<td>5.50</td>
</tr>
<tr>
<td>Bilirubin T, μmol/L</td>
<td>13.1</td>
<td>14.6</td>
<td>0.69</td>
<td>13.8</td>
</tr>
<tr>
<td>Enzymes&lt;d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST, units/L</td>
<td>581</td>
<td>584</td>
<td>33.0</td>
<td>436</td>
</tr>
<tr>
<td>GGT, units/L</td>
<td>13.9</td>
<td>15.2</td>
<td>0.99</td>
<td>15.5</td>
</tr>
<tr>
<td>CK, units/L</td>
<td>518</td>
<td>689</td>
<td>74.7</td>
<td>430</td>
</tr>
<tr>
<td>ALT, units/L</td>
<td>17.6</td>
<td>18.7</td>
<td>1.38</td>
<td>13.0</td>
</tr>
<tr>
<td>Ca, mmol/L</td>
<td>2.92</td>
<td>2.83</td>
<td>0.03</td>
<td>2.86</td>
</tr>
<tr>
<td>P, mmol/L</td>
<td>1.79</td>
<td>1.96</td>
<td>0.07</td>
<td>1.91</td>
</tr>
<tr>
<td>Mg, mmol/L</td>
<td>0.68</td>
<td>0.71</td>
<td>0.01</td>
<td>0.70</td>
</tr>
<tr>
<td>K, mmol/L</td>
<td>4.52</td>
<td>4.50</td>
<td>0.06</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>14.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Total proteins, g/L</td>
<td>64.0</td>
<td>66.4</td>
<td>1.14</td>
<td>65.2</td>
</tr>
<tr>
<td>Urea N, mmol/L</td>
<td>5.42</td>
<td>6.98</td>
<td>0.25</td>
<td>6.12</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.40</td>
<td>4.91</td>
<td>0.13</td>
<td>4.54</td>
</tr>
<tr>
<td>Bilirubin T, μmol/L</td>
<td>10.8</td>
<td>15.8</td>
<td>1.78</td>
<td>11.7</td>
</tr>
<tr>
<td>Enzymes&lt;d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST, units/L</td>
<td>439</td>
<td>424</td>
<td>33.9</td>
<td>415</td>
</tr>
<tr>
<td>GGT, units/L</td>
<td>12.5</td>
<td>15.2</td>
<td>1.59</td>
<td>14.2</td>
</tr>
<tr>
<td>CK, units/L</td>
<td>478</td>
<td>591</td>
<td>110</td>
<td>424</td>
</tr>
<tr>
<td>ALT, units/L</td>
<td>17.2</td>
<td>15.8</td>
<td>1.93</td>
<td>16.2</td>
</tr>
<tr>
<td>Ca, mmol/L</td>
<td>2.88</td>
<td>2.94</td>
<td>0.06</td>
<td>2.89</td>
</tr>
<tr>
<td>P, mmol/L</td>
<td>2.20</td>
<td>2.16</td>
<td>0.04</td>
<td>2.22</td>
</tr>
<tr>
<td>Mg, mmol/L</td>
<td>0.78</td>
<td>0.77</td>
<td>0.02</td>
<td>0.78</td>
</tr>
<tr>
<td>K, mmol/L</td>
<td>4.16</td>
<td>3.98</td>
<td>0.07</td>
<td>4.08</td>
</tr>
</tbody>
</table>

¹SE is the pooled standard error for the least squares means.
²CP content expressed on DM basis.
³ns = not significant.
⁴AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; CK = creatine kinase; ALT = alanine transaminase.

and the plasmatic level of glucose (4.40 vs. 4.91 mmol/L for the LP and HP diet, respectively; P = 0.049).

**DISCUSSION**

This study has indicated that a reduction in protein level in the diet of heavy draft horses reared for meat production does not reflect a lowering of growth and carcass characteristics in the IHDH, both considering an earlier or a later stage of slaughtering typical in the Italian horse meat market (De Palo et al., 2009). Reviewing the different methods for horse feeding standards, Martin-Rosset and Ellis (2005) have shown that protein requirements in growing horses from 6 to 18 mo of age for a given growth rate could be different depending on the system adopted, that is, progressively increasing from 6 to 18 mo of age for the NRC (2007), steady up to 12 mo, and then decreasing from 12 to 18 mo for the INRA (Martin-Rosset, 1990). Other methods proposed in Germany, The Netherlands, or Scandinavia depict different dynamics of protein requirement from 6 to 18 mo of age, although in the range of 6 to 12 mo of age, the German system follows a pattern similar to the NRC system and the other 2 systems are closer to the INRA requirements (Martin-Rosset and Ellis, 2005). However, in spite of these different dynamics in CP requirement suggested by different feeding standards, it has been reported that optimal growth can be obtained with a CP concentration in the diet ranging from 12 to
17% (Saastamoinen, 1996; Staniar et al., 2001). However, most of studies have been performed considering light breeds and standard requirements for the heavy draft breeds have been reported only by the French system (Martin-Rosset and Ellis, 2005). Nonetheless, the INRA standards for growing and fattening heavy draft breeds suggest a range of corrected digestible protein from 9 to 12% on DM basis, which is equivalent to a 13 to 17% of CP considering an average digestibility of 0.7, as in our study (Martin-Rosset, 1990; Martin-Rosset et al., 1994; Martin-Rosset and Ellis, 2005). Therefore, the INRA CP derived values for heavy breeds equal those suggested for lighter breeds by the NRC. On the other hand, it should be taken into account that feeding standards are not always well validated on horse. For example, in a recent study of Mantovani and Bailoni (2011), it has been demonstrated that both commonly used feeding standards systems, that is, the NRC and INRA systems, fail to predict accurately the exact needs of either energy or protein in stallions during the reproductive season. Differences between feeding systems are also dependent on the breed and/or the type of horse used to develop requirements for a given horse category. For coldblood horse breeds, for example, Saastamoinen (1993) found a greater energy expenditure in Finnhorses than in warmblood horses or in Standardbred. In addition, differences between coldblood Icelandic horses and Standardbred were reported also by Sverrisdóttir et al. (1994) and more recently by Ragnarsson and Jansson (2011).

The relationship between growth performance and CP level have been reported as depending more on the quality of the protein (i.e., AA composition) than on the amount of protein given to the animal, especially during the first year of growth (Ott et al., 1981; Graham et al., 1994). For example, Staniar et al. (2001) demonstrated that thoroughbred foals supplemented on pasture with 14 or 9% of CP, that is, respectively, with a normal or lower than suggested requirements (Schryver et al., 1987), showed equal growth and condition score by fortifying the low protein diet with 0.6% lysine and 0.4% threonine. In addition, Saastamoinen and Koskinen (1993) have reported that foals fed diets including a high protein supplementation (0.32% lysine and 0.18% methionine) showed faster growth from weaning to 12 mo than foals supplied with a low quality protein. Graham et al. (1994) suggested that threonine is the second-limiting AA for yearling horses fed a concentrate containing corn, oats, and soybean meal with medium quality coastal bermudagrass hay. More recently, Winsco et al. (2011) attempted to demonstrate that methionine could also be a limiting factor for the growth of the weanling Quarter Horse, but due to a common intake among experimental groups of lysine and threonine, different methionine inclusion levels were correlated to neither different growth parameters nor N balance.

In our experiment, the levels of lysine and threonine in LP diets, estimated by considering the average AA content of feeds reported by the NRC (2007), were lower than those suggested by the NRC (2007) or reported by Ott (2001). In particular, the lysine allowances of LP diets were 57 and 76% of the NRC requirements and 82 and 79% of Ott’s requirements (Ott, 2001) during the first and second phase of the experiment, respectively. However, the content of lysine in the HP diets was close to or greater than requirements reported by both Ott (2001) and the NRC (2007). In addition, the levels of threonine estimated in all diets and phases of the experiment were lower than those suggested by Ott (2001), varying from 79 and 61% in LP diets (in the first and second phase, respectively) and from 77 and 91% in HP diets (in the same order). On this basis, the results of similar growth performances of weanlings belonging to the 2 experimental groups (LP and HP diets) during the whole experiment were unexpected. However, few experimental data about the exact requirements of limiting AA in growing horses of heavy breeds are available in the literature. Moreover, for the IHDH weanlings, no information on the chemical and tissue composition of the body gain (bone, muscle, and fat) is available considering a BW range from 300 to 480 kg. In addition, the contents of lysine and threonine should be directly analyzed in single feeds and/or in mixed diets because of the high variability of these AA in forages and concentrates (NRC, 2007). However, information about AA content in common feedstuffs and about AA requirements and intake in horses are available in literature (e.g., Hintz and Cymbaluk, 1994; Martin-Rosset and Tisserand, 2004). The attempt at reducing the protein dietary level by increasing the availability of limiting AA is widespread in other animal species, particularly in great growth performance animals such as heavy pigs (i.e., pigs slaughtered at about 160 kg for dry-cured ham production; Gallo et al., 2014).

The average growth rate observed in our study was not in agreement with data reported for other heavy breeds. For example, the ADG of horses in this study resulted lower than the ADG indicated by Martin-Rosset (2005) for French heavy breeds but greater than observations of Delobel et al. (2005) in Ardennes draft horses between 8 and 18 mo of age. However, these differences in growth rate could be attributable to the effects of selection practices on this trait in different heavy breeds.

The dynamic of growth measure in this study indicated a curvilinear pattern in IHDH horses in both periods of the study, that is, between 9 and 18 mo of age. Many other authors have reported that the growth curve in horses can be predicted better from nonlinear functions (Austbo, 2005; Delobel et al., 2005; Greene et al., 2005; Martin Rosset, 2005; Valette et al., 2008). However, to our knowledge, this is the first time that Legendre polynomials have been used to describe the growth of young...
horses. Growth parameters may be expressed by different
equations, such as linear functions, logarithmic curves,
or sigmoid or polynomial curves (Valette et al., 2008).
Legendre polynomials make variation in traits over time
easy to model (Meyer, 2001) and have the advantage of
being orthogonal and linearly independent (Shaeffer and
Dekkers, 1994; Meyer, 1998). Moreover, Legendre poly-
nomials have been widely applied in modeling the genetic
aspects of growth curves in some livestock species such
as pig as well as both dairy and beef cattle (Veerkamp
and Thompson 1999; Huisman et al., 2002; Arango et al.,
2004). In our study, Legendre polynomials allowed a di-
rect comparison of IHDH growth performance depending
on protein levels in the diet, the sex, and their interaction,
and also allowed statistical inference statistical inference
among the parameter of the curves. As regards the latter,
this study demonstrated that a reduction in protein levels
in the diet can reduce the growth rate but only moderately
and in a not definitive manner, allowing a recovery of
growth performance later during the experiment. These
kinetics were evident particularly in males and during
the first experimental phase, that is, from 9 to 13 mo of
age. The occurrence of compensatory growth under envi-
ronmental and dietary limiting conditions, that is, by re-
ducing CP supply, has been commonly reported in horse
literature (e.g., Martin-Rosset, 2005; Martin-Rosset and
Ellis, 2005). Moreover, compensatory growth similar to
the findings of the present study has also been reported
by Schiavon et al. (2010) in feeding Piemontese young
bulls 2 protein levels. In addition, Schiavon et al. (2013)
reported slight consequences on overall ADG by reducing
the dietary CP density from 139 to 102 g CP/kg DM in
finishing bulls and heifer crosses of dairy or dual purpose
cows sired by Belgian Blue males.

Furthermore, carcass traits of animals fed different di-
et did not differ in the present study, and carcass weight
and dressing percentage of LP and HP groups were simi-
lar at the end of both phases of fattening. The average
dressing percentages obtained in the present study are
in agreement with data reported for heavy draft breed at
about 12 mo of age in France but lower than those indicat-
ed for older animals by the same authors (Trillaud-Geyl
et al., 1984). However, the French indication for heavy
horses at 18 mo of age or later encompasses a longer pe-
riod, including finishing at pasture, with the aim of reduc-
ing feeding costs. Otherwise, the Italian market situation,
despite its higher feeding costs, is usually based on indoor
intensive fattening up to typical slaughter ages (12 or 18
mo) after weaning that can include or not (depending on
availability) a postweaning period on pasture (De Palo et
al., 2009). Comparing other species, particularly cattle,
in other experiments in which a reduction of CP was ad-
ministered during fattening, no differences were found
between feeding treatments at slaughter. Indeed, neither
in indoor fattening (Schiavon et al., 2010) nor in feed-
lots (Vasconcelos et al., 2006) did differences emerge in
dressing percentage between animals fed a lower dietary
protein than standard requirements.

Among metabolic parameters measured during the
study, the LP diet was associated with a lower level of
plasma urea nitrogen, which is in agreement with the
presence of a lower CP intake and with observations also
reported by Vasconcelos et al. (2006) in feedlot steers
fed different protein levels. However, the values of urea
nitrogen observed in this study for animals fed a HP diet
were comparable to those reported by Calamari et al.
(2010) for adult saddle horses receiving a diet integrated
with different sources and doses of selenium. In spite of
a great amount of literature on horse blood chemistry,
only few studies have investigated blood parameters on
young heavy horses. However, recently, Aoki and Ishii
(2012) have reported results on blood metabolic changes
in the first 4 wk of life in foals of different heavy draft
breeds. Among the metabolites analyzed in the latter
study, the amounts of plasma urea nitrogen are in agree-
ment with our findings in animals fed a LP diet during
the first phase (i.e., 3.55 mmol/L of urea). However, a
recent study on Spanish horses has indicated that urea
nitrogen levels at 12 mo of age should be within the
range of 6.1 to 10.0 mmol/L (Muñoz et al., 2012), that
is, greater than values observed in the present research
except for animals fed the HP diet in the second phase of
the study (i.e., 6.98 mmol/L). The glucose level detected
in our study for horses in the HP diet was slightly greater
than in animals fed the LP diet. These results are lower
than those reported for heavy foals by Aoki and Ishii
(2012) or for 1-yr-old Spanish horses by Muñoz et al.
(2012) but in agreement with values underlined by Stull
and Rodiek (1988), Calamari et al. (2010), and Aoki and
Ishii (2012) for the adult horses. All measured enzymes,
although not different among experimental groups,
were greater than the values observed by Calamari et al.
(2010). Particularly, the values of AST, alkaline phos-
phatase, and CK were, respectively, 1.5, 2, and 3 times
greater than values reported by Calamari et al. (2010).
Moreover, data of Muñoz et al. (2012) in 12-mo-old
Spanish horses were also different from results reported
in this study. However, weanlings on a fattening regi-
men could be characterized by more liver enzyme ac-
tivities than young or adult horses in normal or restricted
feeding regimens (Gossett and French, 1984).

All hematological measures for minerals were in a
physiological range in our study and in agreement with
values of Calamari et al. (2010) but lower than values of
Nielsen et al. (1998a,b) for calcium and phosphoros.
Comparing the 2 phases of our experiment, the phosphou-
rus level in the second phase, that is when growth rate of
animal was faster, resulted greater than in the first phase.
Therefore, a greater mobilization of this mineral could be hypothesized during faster growth, in accordance with observation of Nielsen et al. (1998a), although performed during training on young Quarter Horse.

In conclusion, this study demonstrates for the first time in growing heavy horses that a dietary protein restriction (i.e., on average 3% of DM) did not affect growth performance or the carcass traits of animals up to 13 or 18 mo of age. The lowering of dietary protein level can enable a reduction in feeding costs of horse meat production and a positive effect on N pollution, in accordance with environmental regulations. Further investigations on the role of different limiting AA could better explain the efficiency of protein utilization in growing horses.

LITERATURE CITED


