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Effects of low-protein diets and rumen-protected conjugated linoleic acid on production and carcass traits of growing double-muscled Piemontese bulls[1]

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ABSTRACT: The effects of low-protein (LP) diets and rumen-protected CLA on DMI, ADG, carcass traits, and health status of double-muscled Piemontese young bulls were investigated. Forty-eight bull calves (BW = 237 ± 24 kg) were divided in 4 groups and housed in 12 fully slatted pens. Bulls were fed 2 diets differing in CP density [high-protein (HP) diet: CP = 145 g/kg of DM; LP diet: CP = 108 g/kg of DM] and top-dressed with 80 g/d of rumen-protected CLA or 65 g/d of hydrogenated soybean oil. Orts were collected weekly and feed intake was estimated on a pen basis, with 3 replicated pens for each treatment. Each bull was weighed monthly and examined for alterations of the locomotion system by using the locomotion score as an index of lameness and by counting the number of swollen joints. Carcass quality traits were measured at slaughter, after a feeding period of 332 d. Compared with HP, LP reduced ADG only during the first 4 mo of the trial (1.30 vs. 1.53 kg/d, $P = 0.003$). However, because of compensatory growth, over the whole trial, no significant effects attributable to CP or to additive were found on final BW (668 kg), ADG (1.19 kg/d), DMI (8.50 or 86 g/d per kg of $BW^{0.75}$), dressing percentage (67.3%), carcass conformation (5.2 points), and carcass fat covering (1.87 points). Feed efficiency was affected by a CP × additive interaction ($P = 0.030$), with CLA improving feed efficiency when added to the LP diets, whereas feed efficiency was reduced with the HP diets. The addition of both LP and CLA reduced the number of bulls presenting swollen joints ($P = 0.001$), and LP improved the locomotion score ($P = 0.021$) compared with HP. It was concluded that 10.8 g/kg of CP density in the diet is sufficient for double-muscled Piemontese bulls. The reduction in CP density from 145 to 108 g/kg of DM, in addition to reducing the feeding cost, allows a strong reduction in N consumption without negative consequences on growth performance and carcass traits.

Key words: conjugated linoleic acid, double-muscling, lameness, locomotion score, Piedmontese breed, protein requirement

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INTRODUCTION

Protein requirements of double-muscled (**DBM**) breeds of cattle may be underestimated as compared with more traditional breeds. Double-muscled sires are increasingly used in Europe in cross-breeding programs (Dal Zotto et al., 2007, 2009; Penasa et al., 2009). Compared with other cattle, DBM animals have less bone, less fat, more muscle, a greater dressing percentage (Shahin and Berg, 1985), and likely a reduced intake (Trillat, 1967; Vissac, 1968; Geay et al., 1982). The reduced intake and the greater potential for lean growth suggest that DBM bulls require diets with increased energy and protein density (Fiems et al., 1990). The Piemontese (also known as Piedmontese) breed exhibits DBM in almost all offspring (Albera et al., 2004) but information on the intake and production traits of this breed is limited (Albera et al., 2001). Contextually, in the intensive farms of Italy, cereals and corn silage-based diets with CP at 140 to 150 g/kg of DM are commonly fed to conventional breeds of cattle (Cozzi,

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2007). Constraints introduced by the European Union Nitrate Directive (European Commission, 1991) and the cost of soybeans are forcing farmers to adopt reduced-protein diets for cattle (Yan et al., 2007). Some farmers are also using CLA in combination with low-protein (**LP**) diets. The use of CLA is thought to be beneficial because it decreases fat deposition and slightly increases lean tissue growth and feed efficiency in nonruminant animals (Park et al. 1997). Because CLA has also been proposed to have protein-sparing effects (Park et al., 1997; Pariza et al., 2001), the effect on cattle of CLA in combination with LP diets is of interest. In cattle, to prevent rumen hydrogenation of these biologically active molecules, CLA must be supplied in rumen-protected forms (Perfield et al., 2004). This work was aimed at exploring the effects of conventional and LP corn grain- and corn silage-based diets with or without the addition of rumen-protected CLA on DMI, growth performance, and carcass traits of DBM Piemontese bulls. The main hypotheses tested in this work were that on DBM Piemontese bulls, a strong reduction of the dietary CP density from approximately 145 to 108 g/kg of DM would exert a relevant reduction in ADG (>0.100 kg/d), at least in the first phase of growing, and that the addition of rumen-protected CLA would improve the growth performance of bulls fed LP-density diets.

MATERIALS AND METHODS

This project followed the Guideline for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Consortium, 1988) and was approved by the Ethical Committee for the Care and Use of Experimental Animals of the University of Padova (Legnaro, Italy).

Animals and Diets

The trial was carried out at the experimental farm of the University of Padova (Legnaro, Italy). Forty-eight DBM Piemontese bulls, 202 ± 18 d old, were used. The number of experimental pens (4 bulls per pen) was quantified, as described by Lerman (1996), as the minimal number to detect significant differences ($P = 0.05$; power = 85%) in ADG among treatments of 0.100 kg/d, with an anticipated within-group SD for ADG of 0.150 kg/d. The bulls arrived at the experimental station in January 2008 and were weighed and blocked by BW and age and assigned to 4 experimental groups of 12 animals each. The bulls in the 4 experimental groups were housed in 12 fully slatted-floor pens with 4 animals, each with similar BW. Thus, for each treatment, 3 pens with 4 animals each were used. Slatted flooring was chosen because it is the predominant floor type in Italian intensive beef cattle farms, because it does not require bedding material and has a low labor cost to remove slurry. In addition, slatted floors are preferable to the deep litter system because they ensure better hygienic conditions and fewer gastrointestinal diseases, particularly in cases of scarce availability of bedding materials (Cozzi et al., 2009). After arrival, all bulls were vaccinated against bovine rhinotracheitis virus, parainfluenza$_3$ virus, and modified live bovine respiratory syncytial virus (Cattlemaster 4, Pfizer Italia Srl, Roma, Italy) and injected with 2.5 mg/kg of BW of Tulathromycin, a broad-spectrum antibiotic as a prophylactic (Draxxin, Pfizer Italia Srl).

For the first 28 d, all the bulls were fed an adaptation diet in which meadow hay was progressively replaced by the experimental control diet; data from this period were excluded from the analysis. Thereafter, animals in each group were fed 1 of 4 experimental diets for 332 d. The 4 diets were the result of a combination of 2 concentrations of soybean meal with the addition of hydrogenated soybean oil (**HSO**) or rumen-protected CLA (Table 1). The high-protein (**HP**) ration was formulated to achieve 145 g/kg of DM of CP density. The LP ration was obtained by reducing the soybean meal from 126 to 33 g/kg of DM and increasing all the other ingredients accordingly. To minimize differences in composition among treatments, a common basal diet, corresponding to the composition of the LP diet, was prepared daily using a mixer wagon equipped with a computer-assisted weighing scale, calibrated monthly. Net energy content and fermentable OM content of the diets, based on actual feed composition, were computed according to the French energy system (Sauvant et al., 2004) and the Dutch protein system (Tamminga et al., 1994), respectively. In the French energy system, NE content is computed from the ME by considering a single efficiency coefficient (k_{mg}) resulting from a weighted mean of the efficiencies for maintenance (k_m) and BW gain (k_g) for a given intake.

The CLA supplement (provided by Sila, Noale, Italy) consisted of methyl esters of CLA bound to a silica matrix and coated with HSO contained fatty acids in the triglyceride form. The lipid-coated CLA was composed of 800 g/kg of lipid, 178 g/kg of ash, and 22 g/kg of moisture. The lipid portion contained 456 g/kg of palmitic and stearic acids, 79.2 and 76.8 g/kg of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA isomers, respectively, and 91 g/kg of other fatty acids. In this trial, the dose of CLA (80 g/d) was established to supply 6.3 and 6.1 g/d of *cis*-9, *trans*-11 C18:2 and *trans*-10, *cis*-12 C18:2 isomers, respectively. This dosage is close to that used by others (Gillis et al., 2004) in beef cattle. Because the CLA product was manufactured using HSO as the coating material, the animals not treated with CLA received a daily amount of HSO (65 g/d) to supply the same amounts of lipid as the CLA treatment. The HSO was composed of 990 g/kg of lipid and 878 g/kg of fatty acids, almost exclusively represented by palmitic and stearic acids (866 g/kg). Both the additives (HSO and CLA) were distributed daily by top dressing and mixing.

Table 1. Ingredient composition of diets

Item	Experimental diet[1]			
	HP_{HSO}	HP_{CLA}	LP_{HSO}	LP_{CLA}
Total mixed ration ingredient, g/kg of DM				
Corn grain, ground	360	360	400	400
Corn silage	250	250	276	276
Dried sugar beet pulp	102	102	113	113
Soybean meal	126	126	33	33
Wheat bran	63	63	70	70
Wheat straw	60	60	66	66
Vitamin and mineral mix[2]	24	24	26	26
Calcium soap	8	8	9	9
Hydrogenated soybean oil	7	7	7	7
Top dressing, g/d				
Hydrogenated soybean oil	65	—	65	—
Rumen-protected CLA	—	80	—	80

[1]The high-protein (HP) and low-protein (LP) diets contained 145 and 108 g of CP/kg, respectively; HSO = 65 g/d of top-dressed hydrogenated soybean oil; CLA = 80 g/d of top-dressed commercial CLA (product coated with HSO; SILA, Noale, Italy).

[2]Contained per kilogram: 120 g of calcium, 56 g of sodium, 17 g of magnesium, 16 g of phosphorous, 240,000 IU of vitamin A, and 15,000 IU of vitamin D_3.

Measurements, Controls, and Analysis

The amount of each feed ingredient loaded into the mixer wagon and the weight of the mix uploaded in the manger of each pen were recorded daily. The orts that remained in the mangers were weighed and sampled by pen weekly. Samples of each feed ingredient of the diets and orts were analyzed for their proximate composition (AOAC, 2000) and their NDF, ADF, ADL, and AIA contents (Van Soest et al., 1991). Starch content was determined, after its hydrolysis to glucose (AOAC, 2000), by liquid chromatography (Bouchard et al., 1988). All the bulls were individually weighed monthly on the same day. Because animals in pens were not fed individually, DMI and feed efficiency were computed on a pen basis.

An operator, licensed for carcass evaluation according to the SEUROP grading system (European Commission, 1991), evaluated the body condition of each bull monthly. Body condition was expressed in terms of expected carcass conformation and fat covering according to the SEUROP grid (European Commission, 1991). Thus, conformation was linearly scored from S+ (all muscle profiles extremely convex; superior muscle development) to P− (all muscle profiles concave to very concave; very poor muscle development) by considering the profiles of shoulders, loins, rump, thighs, and buttocks. Conformation was transformed into numerical terms: S+ = 6.33; S = 6; S− = 5.66; …, P+ = 1.33; P = 1.00; P− = 0.66. Ultrasound measurement is not applicable on these animals because their fat covering is not thick enough to ensure reliable estimates of fat covering (Greiner et al., 2003). Thus, fat covering was scored linearly by a combined visual and palpation approach, considering the presence and the thickness of subcutaneous fat depots at specific points of the body, from 1 (very lean: no palpable fat is detectable; the ribs, bone structure, and head of the tail are very prominent) to 5 (very fat: thick fat depots are present over the shoulders, the ribs, and around the head of the tail; bone structure is no longer visible, the animal is fleshy). Later, these evaluations were correlated with the corresponding values of SEUROP conformation and fat covering evaluated on the carcasses after slaughter.

Health status was monitored daily by a veterinarian following the experimental protocol for animal care. Toward the middle of the trial (195 d after the beginning), 1 bull in the HP diet with added HSO died. After macroscopic postmortem examination, the veterinarian determined that the unexpected death was likely due to heart failure. During the second part of the trial, some bulls exhibited swollen joints and mild symptoms of lameness. From each bull, rumen fluid was sampled and analyzed for pH (rumenocentesis) as described by Nordlund et al. (2004) to evaluate possible conditions of acidosis. After the appearance of these signs, each bull was examined monthly and classified for lameness by locomotion scoring. Locomotion scoring has been found useful in assessing the severity, duration, and prevalence of subclinical and clinical lameness, and it is also used to compute foot and leg score indexes for genetic evaluation of dairy cows (McDaniel, 1997). Hence, lameness was assessed using the method proposed by Sprecher et al. (1997), which observes deviations from normal posture and walking by using a 1 to 5 rating system (1 = sound; 2 = mildly lame, the animal develops an arched-back posture only while walking and the gait is normal; 3 = moderate, not clinically lame, an arched-back posture is evident while both standing and walking and a short stride with 1 or more limbs is observed; 4 = clinically lame, an arched-back posture is always evident and the gait is described as one deliberate step at a time, the animal favors one or more limbs or feet; 5 = severely lame, the animal demonstrates reluctance to bear weight on 1 or more limbs or feet). Lameness observations were recorded each time the animal was weighed as the animal walked from its pen to the weighing station for a distance of approximately 10 to 40 m. At the weighing station, the feet and legs of each animal were carefully examined by counting the number of swollen joints, as evidence of synovitis. During the entire trial, only 2 animals presented clinical lameness (locomotion scores of 4 and 5). The veterinarian indicated that the health status of 1 bull was compromised (locomotion score = 5). Therefore, this bull was moved to an infirmary pen and given an intramuscular injection (0.08 mg/kg) of dexamethasone sodium phosphate. All the data from this animal were excluded from the statistical analysis. The same injection treatment was applied to another animal with traumatic lameness and a locomotion score of 4. After treatment, the health status of this bull quickly improved such that after 10 d, its locomotion score decreased to 2 and the bull was not removed from the trial.

Ambient temperature outside the stable was measured continuously and averaged on a daily basis over the whole trial with a digital thermometer (Opticom MCC-10, Opticom, Venlo, the Netherlands).

Carcass Traits

When the bulls reached approximately 660 kg of BW, the prevalent slaughter weight in the Italian market, the animals were fasted for 1 d and slaughtered (age at slaughter: 562 ± 18 d). Carcasses were individually weighed and scored for conformation and fat covering according to the SEUROP system (European Commission, 1991). Dressing percentage was computed as the ratio between the carcass weight 24 h after slaughter and BW.

Statistical Analysis

Data recorded from each bull (BW, ADG, body condition, locomotion data, and carcass traits) were statistically analyzed with the following model using the GLM procedure (SAS Inst. Inc., Cary, NC):

$$y_{ijk} = \mu + B_i + T_j + BT_{ij} + e_{ijk},$$

where y is the experimental observation; μ is the overall mean; B is the effect of the block of pens with lighter, medium, or heavier initial BW (i = 1, . . ., 3); T is the effect of treatment (j = 1, . . ., 4) as a combination of the 2 amounts of CP and the 2 additives; and e is residual error (k = 1, ..., 12). The BT interaction corresponds to the pen effect. The B effect based on initial BW was introduced in the model as a block factor. Data on initial BW and SEUROP score served as covariates with the corresponding values during the trial, to correct for initial individual variability. For BW and ADG, the pen was considered the experimental unit, so 3 observations for each treatment (the mean of 4 bulls) were used. For body condition, locomotion data, and carcass traits, the bull was considered the experimental unit. Orthogonal contrasts, using the proper lines of errors, were used to evaluate the effects of CP, additive, and the interaction of CP × additive.

The same model without the BT interaction was used to analyze the DMI data and feed efficiency; there were 3 observations for each treatment because these data were recorded on a pen basis. The effect of treatment was tested using the pen as an error line. Orthogonal contrasts were used to evaluate the effects of CP, additive, and the interaction of CP × additive. The in vivo conformation and fat covering scores evaluated the day before slaughter were correlated with the corresponding values assessed on the carcasses of the slaughtered bulls.

RESULTS

Evaluation of the Diets

The LP diets contained less CP and greater concentrations of lipid, ash, starch, NDF, ADL, and AIA compared with the HP diets because the only difference between the 2 diets was the amount of soybean meal included (Table 2). The 4 diets were isoenergetic and contained 7.8 MJ/kg of DM of NE_m and NE_g and 580 g/kg of DM of fermentable OM. The ratios between the RDP and fermentable OM were 154 and 118 g/kg for the HP and LP diets, respectively. Consequently, the RDP balance, calculated as described by Tamminga et al. (1994), was +2.6 and RDP was −18.6 g/kg of DM for the HP and LP diets, respectively. The value of an RDP balance close to zero indicates that HP was well balanced in terms of availability of energy and N for rumen microbes, whereas a negative value of LP indicates a marked shortage of RDP.

ADG and BW

Over the whole trial, ADG did not differ across treatments and averaged 1.17 kg/d (Table 3). The reduction in dietary CP density reduced ADG only during the initial 120 d of the trial (1.53 vs. 1.31 kg/d for HP and LP, respectively, $P = 0.003$). The compensatory growth exhibited by LP during the middle part of the trial was not significant (1.11 vs. 1.21 kg/d for HP and LP, respectively), but the BW of the young bulls differed ($P = 0.002$) only at the end of the first period. No significant effect resulting from the addition of CLA was observed on BW ($P = 0.43$) and ADG ($P = 0.42$).

DMI and Feed Efficiency

Over the course of the experiment, DMI increased from 8.02 in the initial period to 10.21 kg/d in the final period (Table 4). When expressed per unit of $BW^{0.75}$, DMI ranged between 90 and 100 g/kg of $BW^{0.75}$ per day during the initial period of the trial, and averaged 86

Table 2. Chemical composition and nutritional value of diets

Item	Experimental diet[1]			
	HP_{HSO}	HP_{CLA}	LP_{HSO}	LP_{CLA}
Chemical composition,[2] g/kg of DM				
Starch	357	357	393	393
NDF	287	287	303	303
ADF	137	137	145	145
CP	145	145	108	108
Ash	49	49	48	48
Lipids	41	41	42	42
Nutritional value				
ME,[3] MJ/kg of DM	11.9	11.9	11.7	11.7
NE,[3] MJ/kg of DM	7.8	7.8	7.8	7.8
Fermentable OM,[4] g/kg of DM	588	588	577	577
RDP,[4] g/kg of DM	90.8	90.8	68.0	68.0
RUP,[4] g/kg of DM	54.3	54.3	40.2	40.2
RDP balance,[4] g/kg of DM	2.6	2.6	−18.6	−18.6

[1]The high-protein (HP) and low-protein (LP) diets contained 145 and 108 g of CP/kg, respectively; HSO = 65 g/d of top-dressed hydrogenated soybean oil; CLA = 80 g/d of top-dressed commercial CLA (product coated with HSO, SILA, Noale, Italy).

[2]Values computed from the chemical analysis of each feed ingredient in triplicate and from the mean of the actual daily loads of each feed ingredient recorded by mixer wagon computer.

[3]Values computed according to the actual chemical composition and the French energy system (Sauvant et al., 2004).

[4]Values computed according to the actual chemical composition and the Dutch protein system (Tamminga et al., 1994).

g/kg of $BW^{0.75}$ per day over the whole trial. The effect of treatments on voluntary DMI was very limited and was never significant.

From the initial to the final period, the G:F ratio decreased from 0.176 to 0.085 kg/kg of DM. During the initial period of the trial, reflecting the effects on growth rate, the reduction in CP decreased ($P = 0.001$) feed efficiency from 0.189 to 0.163. No significant effects of CP were found in later periods ($P = 0.47$ and 0.36 for the middle and the final period, respectively), and the overall feed efficiency was on average 0.132.

During the initial period, CLA decreased feed efficiency when used on HP diets (−0.014) and increased feed efficiency (+0.010) when added to LP diets, and the corresponding interaction (CP × additive) was significant ($P = 0.023$). The same effects were observed for the data regarding feed efficiency measured over the whole trial. In this case, the P-value of the CP × additive interaction was 0.030.

Status of the Locomotion System

The monthly mean for daily ambient temperature was always below 24°C, even though between June and September there were 24 d with an average temperature greater than 25°C; however, the temperature was greater than 27°C for only for 7 d.

During August, when the highest ambient temperatures were reached (daily mean of 24°C), approximately one-half of the bulls presented some mild signs of lame-

Table 3. Body weight and daily BW gain

Time, d	Treatment[1,2]					*P*-value		
	HP_{HSO}	HP_{CLA}	LP_{HSO}	LP_{CLA}	RMSE[3]	CP	Additive	CP × additive
BW, kg								
At d 0	281	280	276	277	15	0.23	0.88	0.42
After 120 d	470	453	432	437	23	0.002	0.28	0.09
After 233 d	594	577	569	576	42	0.28	0.63	0.32
Before slaughter, 332 d	686	655	659	672	52	0.64	0.43	0.09
ADG, kg/d								
Initial period, d 0 to 120	1.60	1.45	1.28	1.32	0.19	0.003	0.28	0.09
Middle period, d 121 to 233	1.11	1.11	1.19	1.22	0.31	0.25	0.93	0.83
Final period, d 234 to 332	0.91	0.77	0.88	0.95	0.27	0.24	0.54	0.12
Entire trial, d 0 to 332	1.23	1.13	1.13	1.17	0.15	0.48	0.42	0.08

[1]The high-protein (HP) and low-protein (LP) diets contained 145 and 108 g of CP/kg, respectively; HSO = 65 g/d of top-dressed hydrogenated soybean oil; CLA = 80 g/d of top-dressed commercial CLA (product coated with HSO, SILA, Noale, Italy).

[2]Data are the means of 3 pen observations (with 4 animals for pen).

[3]Root mean square error.

Table 4. Feed efficiency and DMI

	Treatment[1,2]					*P*-value		
Time, d	HP_{HSO}	HP_{CLA}	LP_{HSO}	LP_{CLA}	RMSE[3]	CP	Additive	CP × additive
DMI, kg/d								
Initial period, d 0 to 120	8.16	7.99	8.09	7.87	0.21	0.48	0.17	0.84
Middle period, d 121 to 233	8.21	8.16	8.66	8.30	0.31	0.15	0.28	0.42
Final period, d 234 to 332	10.29	10.00	10.26	10.31	0.50	0.63	0.69	0.58
Entire trial, d 0 to 332	8.82	8.66	8.94	8.76	0.27	0.52	0.30	0.95
Feed efficiency,[4] kg/kg of DM								
Initial period, d 0 to 120	0.196	0.182	0.158	0.168	0.007	0.001	0.57	0.023
Middle period, d 121 to 233	0.136	0.136	0.137	0.147	0.014	0.47	0.59	0.59
Final period, d 234 to 332	0.088	0.077	0.085	0.091	0.011	0.36	0.67	0.23
Entire trial, d 0 to 332	0.139	0.130	0.127	0.134	0.005	0.19	0.85	0.030

[1]The high-protein (HP) and low-protein (LP) diets contained 145 and 108 g of CP/kg, respectively; HSO = 65 g/d of top-dressed hydrogenated soybean oil; CLA = 80 g/d of top-dressed commercial CLA (product coated with HSO, SILA, Noale, Italy).

[2]Data are the means of 3 pen observations (with 4 animals for pen).

[3]Root mean square error.

[4]Feed efficiency was computed as ADG/DMI.

ness (locomotion score = 2 or 3). One animal with a locomotion score of 4 recovered quickly, whereas another one with an impaired walk (locomotion score = 5) was removed from the HP diet with HSO. No other cases of clinical lameness were detected.

Data regarding the effects of treatments on the percentage of bulls presenting alterations of the locomotion system are shown in Table 5. Compared with the HP diet, the LP diet reduced (improved) the locomotion score after 273 ($P = 0.034$) and 301 ($P = 0.021$) d of trial, reduced the percentage of bulls presenting swollen joints in 1 or more legs after 273 ($P = 0.001$), 301 ($P = 0.001$), and 332 ($P = 0.025$) d of trial, and reduced the average number of altered legs per bull after 233 ($P = 0.016$) and 273 ($P = 0.033$) d of trial. Compared with HSO, CLA supplementation reduced the number of bulls presenting joint alterations at 273 ($P = 0.019$) and 332 ($P = 0.001$) d of trial.

Body Condition

After 120 d of trial, as observed for BW and ADG, body condition, expressed in units of SEUROP conformation score (Table 6), was greater for HP compared with LP (4.07 vs. 3.72 points, $P = 0.005$). However, during the middle part of the trial, the conformation score increased less with HP than with LP (+0.48 vs. +0.74 points, respectively; $P = 0.010$) such that at the

Table 5. Status of the locomotion system

	Treatment[1,2]					*P*-value		
Time, d	HP_{HSO}	HP_{CLA}	LP_{HSO}	LP_{CLA}	RMSE[3]	CP	Additive	CP × additive
Locomotion score[4]								
After 233 d	1.5	1.5	1.3	1.0	0.7	0.10	0.40	0.48
After 273 d	1.8	1.6	1.4	1.0	0.7	0.034	0.15	0.65
After 301 d	1.5	1.5	1.1	1.0	0.7	0.021	0.76	0.91
Before slaughter, 332 d	1.6	1.3	1.2	1.1	0.7	0.14	0.28	0.49
Bulls with altered legs,[5] %								
After 233 d	54.5	58.3	41.7	50.0	32.0	0.45	0.67	0.87
After 273 d	63.6	50.0	33.3	16.7	20.9	0.001	0.019	0.91
After 301 d	36.4	41.7	25.0	16.7	15.0	0.001	0.89	0.46
Before slaughter, 332 d	36.4	25.0	33.3	8.3	15.0	0.021	0.001	0.42
Altered legs,[5] No./bull								
After 233 d	1.0	1.3	0.4	0.6	0.9	0.016	0.41	0.91
After 273 d	1.0	0.8	0.4	0.3	0.9	0.033	0.51	0.96
After 301 d	0.6	0.7	0.3	0.3	0.8	0.11	0.96	0.96
Before slaughter, 332 d	0.5	0.4	0.3	0.2	0.7	0.26	0.47	0.94

[1]The high-protein (HP) and low-protein (LP) diets contained 145 and 108 g of CP/kg, respectively; HSO = 65 g/d of top-dressed hydrogenated soybean oil; CLA = 80 g/d of top-dressed commercial CLA (product coated with HSO, SILA, Noale, Italy).

[2]Data are the means of 12 observations, except for HP_{CLA}, in which 2 bulls were excluded because of health problems.

[3]Root mean square error.

[4]Locomotion score, using a 1 to 5 numerical rating system (1 = sound; 5 = severely impaired walking), was used as tool to evaluate lameness (Sprecher et al., 1997).

[5]Alterations were assessed by counting the number of swollen carpal or tarsal joints; 1 case of traumatic lameness was also observed.

Table 6. Average value and increase in BCS expressed in terms of conformation and fatness as suggested by the SEUROP grid for carcass classification

	Treatment[1,2]					*P*-value		
Item	HP_{HSO}	HP_{CLA}	LP_{HSO}	LP_{CLA}	RMSE[3]	CP	Additive	CP × additive
Conformation[4]								
At beginning, 0 d	3.14	3.33	3.22	3.22	0.41	0.80	0.80	0.31
After 120 d	4.06	4.08	3.80	3.65	0.32	0.005	0.64	0.20
After 233 d	4.49	4.61	4.53	4.40	0.40	0.53	0.98	0.19
Before slaughter, 332 d	5.20	5.32	5.10	5.07	0.46	0.34	0.66	0.34
Change in conformation[4]								
Initial period, d 0 to 120	0.80	0.82	0.54	0.39	0.31	0.006	0.49	0.98
Middle period, d 121 to 233	0.43	0.53	0.73	0.75	0.31	0.010	0.55	0.78
Final period, d 234 to 332	0.71	0.71	0.57	0.67	0.25	0.34	0.40	0.80
Entire trial, d 0 to 332	1.94	2.06	1.84	1.81	0.46	0.21	0.83	0.98
Fat covering[5]								
After 120 d	1.0	1.0	1.0	1.0	—	—	—	—
After 233 d	1.6	1.3	1.9	1.3	0.4	0.15	0.001	0.44
Before slaughter, 332 d	1.7	1.3	1.7	1.5	0.5	0.66	0.10	0.54
Change in fat covering[5]								
Initial period, d 0 to 120	0.0	0.0	0.0	0.0	—	—	—	—
Middle period, d 121 to 233	0.6	0.3	0.9	0.3	0.4	0.16	0.001	0.44
Final period, d 234 to 332	0.1	0.0	−0.2	0.2	0.6	0.52	0.20	0.26
Entire trial, d 0 to 332	0.7	0.3	0.7	0.5	0.5	0.66	0.10	0.54

[1]The high-protein (HP) and low-protein (LP) diets contained 145 and 108 g of CP/kg, respectively; HSO = 65 g/d of top-dressed hydrogenated soybean oil; CLA = 80 g/d of top-dressed commercial CLA (product coated with HSO, SILA, Noale, Italy).

[2]Data are the means of 12 observations, except for HP_{CLA}, in which 2 bulls were excluded because of health problems.

[3]Root mean square error.

[4]Conformation was linearly scored in vivo from S+ (all muscle profiles extremely convex; exceptional muscle development) to P− (all muscle profiles concave to very concave; poor muscle development) considering the profiles of shoulders, loins, rump, thighs, and buttocks (S+ = 6.33; P− = 0.66).

[5]Fat covering was linearly scored in vivo by a combined visual and palpation approach considering the presence and thickness of subcutaneous fat depots at the base of the tail, ribs, and shoulders (1 = very lean; 5 = very fat).

end of the middle period ($P = 0.53$) and at the end of the trial ($P = 0.34$), no significant difference in conformation attributable to CP was detected. The final conformation score averaged 5.2, corresponding to the letter E+ of the SEUROP classification system (where E = excellent).

As expected for a DBM breed, the fatness score was very low during the whole trial, and at the end, it averaged 1.5 points on a numerical rating system from 1 (very lean) to 5 (very fat). Compared with HSO, the addition of CLA reduced ($P = 0.001$) the value of fat covering only during the middle period of the trial.

Carcass Traits

No significant effects on carcass traits attributable to CP, the additive, or their interaction were observed (Table 7). Carcass weight averaged 450 kg and dressing percentage averaged 67.3%. The SEUROP classifications performed on the carcasses for both conformation and fat covering were in agreement with the evaluations obtained in vivo. In fact, the correlations between the estimates of conformation (18 classes) and fat covering (5 classes) evaluated in vivo at the end of the trial with the corresponding values assessed on the carcasses were 0.79 and 0.60, respectively.

DISCUSSION

DMI and Dietary CP

Arthur (1995) indicated that DBM bulls have reduced DMI compared with other cattle because of the small size of their gastrointestinal tracts. The low DMI combined with the increased muscle mass of DBM may lead to greater CP requirements (Fiems et al., 1990; Arthur, 1995). The current work is one of the few reporting DMI data of DBM Piemontese bulls fed corn grain and corn silage diets. In this experiment, the average DMI was more than 90 g/d per kg of $BW^{0.75}$ from 280 to 450 kg of BW and approximately 80 g/d per kg of $BW^{0.75}$ for the rest of the trial through to slaughter. These figures are slightly greater than those found for DBM Belgian Blue bulls fed corn silage diets (De Campeneere et al., 1999).

In ruminants, voluntary DMI could be affected by the amount of RDP, and a DMI reduction with decreasing proportions of RDP was expected, based on the results of Valkeners et al. (2008) with DBM Belgian Blue bulls. In this trial, the reduction in dietary CP density exclusively caused by a reduction in the proportion of soybean meal did not affect DMI. The substantial lack of effects in this experiment suggested that, in the LP diet, the low supply of RDP could have been partially

Table 7. Carcass traits

Item	Treatment[1,2]				RMSE[3]	*P*-value		
	HP_{HSO}	HP_{CLA}	LP_{HSO}	LP_{CLA}		CP	Additive	CP × additive
Cold carcass weight, kg	468	444	438	450	38	0.44	0.56	0.11
Dressing percentage	67.7	67.6	66.8	67.2	1.3	0.10	0.80	0.50
SEUROP classification								
Conformation[4]	5.17	5.30	5.27	5.11	0.58	0.97	0.92	0.34
Fat covering[5]	1.93	1.86	1.86	1.83	0.17	0.45	0.31	0.72

[1]The high-protein (HP) and low-protein (LP) diets contained 145 and 108 g of CP/kg, respectively; HSO = 65 g/d of top-dressed hydrogenated soybean oil; CLA = 80 g/d of top-dressed commercial CLA (product coated with HSO, SILA, Noale, Italy).

[2]Each data are the mean of 12 observations, except for HP_{CLA}, in which 2 bulls were excluded because of health problems.

[3]Root mean square error.

[4]SEUROP scoring system for carcass conformation from S+ (all muscle profiles extremely convex; exceptional muscle development) to P− (all muscle profiles concave to very concave; poor muscle development) considering the profiles of shoulders, loins, rump, thighs, and buttocks (S+ = 6.33; P− = 0.66).

[5]SEUROP scoring system for carcass fat covering (1 = very lean; 5 = very fat).

compensated for by N recycling (Valkeners et al., 2008). Considering the large proportion of concentrates in the 2 diets and the reduced development of the forestomachs in DBM cattle (Arthur, 1995), it is also possible that the shortage of RDP in the LP could have been partially compensated for by a shorter residence time of the feed in the forestomachs and by a partial shift in the site of digestion of feeds from the rumen to the intestine.

Growth Performance and Dietary CP

The average age of the bulls at slaughter was slightly greater (562 vs. 523 d) than the average age at slaughter found in a survey conducted on 804 young bulls from 109 sires fattened in 124 farms of the Piemonte region (Boukha et al., 2007). In the current study, carcass weight was also slightly greater (450 vs. 417 kg) but the carcass weight:age ratio was very similar (0.801 vs. 0.797 kg/d, respectively) to that found in the survey by Boukha et al. (2007). In addition, the growth rate achieved to approximately 1 yr of age in this trial was very similar (1.43 vs. 1.40 kg/d, respectively) to that shown, at the same age, by 988 young DBM Piemontese bulls under performance testing (Albera et al., 2001). The average BW at 11.5 mo of age was 448 vs. 434 kg for the bulls in this trial and the performance-tested bulls, respectively. In addition, the decrease in growth rate of Piemontese bulls after 1 yr of age was expected because this is a medium-framed DBM breed. Because of the slow increase in fat covering, Piemontese bulls are often slaughtered at different ages, depending on the cost of the feed ingredients, trends of carcass prices, and farm conditions. The reason most breeders prolong the fattening periods until 18 to 20 mo of age is, in many cases, due to the need to dilute the very high cost of the stock calf on a heavier carcass. Such comparisons indicated that the growth performance obtained in this trial quite accurately represented those commonly achieved on commercial farms of Piemontese bulls, where bulls are commonly fed rations based on hay and concentrates.

Over the whole trial, ADG averaged 1.16 kg/d, dressing percentage averaged 67.3%, and carcass fat covering score was below 2 points. These results confirmed that the in vivo performance and carcass traits of DBM Piemontese subjects are in the same order of magnitude as those observed on DBM Belgian Blue bulls fed corn silage and cereal-based diets (Arthur, 1995; De Campeneere et al., 1999; Biagini and Lazzaroni, 2005a,b). The strong similarity in carcass traits obtained in this trial with those obtained on DBM Belgian Blue bulls is remarkable. De Campeneere et al. (1999) and Fiems et al. (2002, 2003) indicated dressing percentages ranging from 66.6 to 68.0%, a SEUROP conformation index ranging from 3.7 to 5.7 points, and a fat covering index ranging from 1.86 to 2.20 points. A direct comparison of Piemontese and Belgian Blue DBM bulls confirmed that the carcasses of the 2 breeds are also very similar in terms of commercial cuts (Biagini and Lazzaroni 2005a) and percentages of separable meat (80.0%), fat (6.9%), and bone (13.1%).

The correlations between the in vivo estimates of carcass conformation and fat covering with the corresponding values assessed on the carcasses were good (0.79 and 0.60, respectively), notwithstanding the subjective nature of these evaluations and the limited number of scores used for fat covering. Similar results were reported by Bittante et al. (1992) and Jansen et al. (1985), who found correlations between in vivo estimates and actual carcass conformation of 0.77 and 0.70, respectively. Andersen et al. (1981), in the final report of the working group of the European Commission on performance testing of bulls, stated that predictive parameters should be used for the estimation of muscle mass or muscle content and that they may be assessed visually, and also that morphological characteristics (conformation, fleshiness, and fatness) are inexpensive but very rough predictors of composition; however, they may also be of commercial value in their own right. Jansen et al. (1985) found that body measurements of an objective nature were poorer predictors of carcass conformation than in vivo classification. For fat covering as well, the correlations between the in vivo and postmortem evaluations found in the current experiment were good, particularly considering that ultrasound measurement of fat covering is not applicable in DBM bulls because of the very scarce thickness of fat covering. Thus, the results of this experiment confirm, as proposed by Bittante et al. (1992), the possibility of using in vivo carcass evaluation systems for developing selection criteria of young bulls performance tested for beef production.

The data regarding the in vivo fatness score during the trial highlighted the fact that the reduction in feed efficiency during the trial in DBM Piemontese bulls was not due to an increase in fat deposition and needs to be investigated further. Moreover, the same data confirm the known ability of Piemontese young bulls to maintain an almost constant degree of carcass fatness for a long period, which allows the animals to be slaughtered early or late without greatly affecting carcass composition while carcass conformation is improved.

In the intensive farms in Italy, cereals and corn silage-based diets with 140 to 150 g/kg of DM of protein density are commonly applied (single-phase feeding) to cattle of conventional breeds (Cozzi, 2007); similarly, in the United States, Galyean (1996) found that CP densities ranging from 125 to 144 g/kg of DM are commonly used in the feedlot cattle industry for finishing beef cattle. The increased potential for lean growth and the moderate feed intake capability have suggested that diets with energy and protein densities greater than those applied on conventional cattle should be used for DBM bulls (Fiems et al., 1990). For DBM Belgian Blue bulls, Boucqué et al. (1984) indicated that ration CP density should exceed 140 g/kg of DM, whereas a content of 120 g/kg was found to be sufficient for non-DBM Belgian Blue bulls. Later, De Campeneere et al. (1999) suggested CP densities of 160, 143, and 120 g/kg of DM as adequate for DBM Belgian Blue bulls at 350 to 460, 460 to 570, and 570 to 680 kg of BW, respectively. In this trial, no benefits were achieved by using 145 (as opposed to 108) g/kg of DM of CP density. Even though during the initial period bulls fed the LP ration grew slower and were less efficient than those receiving the HP ration, at the conclusion of the study, because of compensatory growth shown in the middle period, they did not show relevant differences in total DMI, ADG, feed efficiency, or carcass traits. Our results showed a compensation rate of 109% for ADG (LP:HP) and of 154% for the in vivo SEUROP conformation score in the middle period. The significant difference in ADG observed in the initial period between HP and LP indicated that when these animals were less than approximately 450 kg of BW, their rate of growth was reduced when fed LP diets. Considering that De Campeneere et al. (1999) suggested 160 g of CP/kg of DM for DBM Belgian Blue bulls with BW from 350 to 460 kg, it cannot be ruled out that the greatest CP content used in this trial was not sufficient for ensuring the maximum growth during the initial period. Phase feeding with decreasing dietary CP can also be considered in those situations in which the farm size is adequate, but more information is required to assess the relevance of phase feeding.

Conditions of the Locomotion System and Dietary CP

The greater incidence of abnormalities to the locomotion system in this trial during the summer was not surprising. Compared with conventional cattle, DBM young bulls have thinner bones and are more susceptible to stress, caused by greater ambient temperatures and housing conditions (Arthur, 1995). De Campeneere et al. (2002) reported that 60% of all DBM Belgian Blue bulls showed lesions in at least 1 of their legs. Ruis-Heutinck et al. (2000) reported that 78% of DBM Belgian Blue bulls had lesions in the carpal joint of their forelegs. In these last experiments, housing conditions (closed barn vs. open-front stable) were far more important for health status than the feeding system, because no evidence of severe and persistent acidosis was found (De Campeneere et al., 2002). Despite the risk of increasing the rate of culling (Cerchiaro et al. 2005), fully slatted flooring is predominant in many European countries because it does not require bedding material, reduces the labor cost, and keeps the animals cleaner and less exposed to gastrointestinal disorders (Cozzi et al., 2009). Regarding the effect of the ambient temperatures, EU-SCAHAW (2001) indicated that above 27°C, a negative effect on cattle welfare could occur, and temperatures greater than 25°C were found to significantly increase the rectal temperature in DBM animals compared with non-DBM animals (Halipré, 1973). The ambient temperatures recorded during the current experiment indicated that the animals could have been exposed to moderate heat stress for a short time. During the period of moderate heat stress in August, more than one-half of the bulls presented some leg alteration. On dairy cows, increased lameness was associated with increased standing time under conditions of heat stress (Sanders et al., 2009). The pH of the rumen fluid averaging 6.7 ± 0.3 suggested the absence of acidosis. The incidence of problems decreased during the later month, despite the increase in BW.

Although the incidence of leg alterations was great, the condition of the locomotion system was not seriously compromised because the deviations from normal posture and walking were small. No adverse effect on growth performance of the bulls was observed because the decrease in growth rate shown in the last part of the trial occurred when the incidence of leg alterations was decreasing. Moreover, for the LP diet, the locomotion score remained within the range of normality (maximum value of 1.4 on a 1 to 5 scale) during the entire trial, despite the slatted flooring of the pens.

The role that protein may have in the development of leg, foot, and hoof pathologies is an open question. Manson and Leaver (1988), who compared 161 vs. 198 g/kg CP diets in dairy cattle between 3 and 26 wk postpartum, found that the HP diet significantly increased (worsened) locomotion scores and the incidence and duration of clinical lameness in dairy cows. Increased rumen-degradable N was indicated as a possible factor in increased lameness and laminitis (Logue et al., 1989; Bargai et al., 1992). Some other hypotheses have indicated allergic histaminic reactions to certain types of proteins (Nilsson, 1963) or a link between greater protein supplementation and protein degradation end products (Bazeley and Pinset, 1984). Notwithstanding the small number of subjects involved in this trial, the results support the hypothesis that a reduced CP density could exert positive effects on the health status of the locomotion system of growing bulls. The greater incidence of swollen joints observed in August was likely due to the combined effects of high ambient temperature, greater dietary CP, and the type of flooring.

Effects of CLA

Research performed on laboratory animals, pigs, and dairy cattle has shown that CLA supplementation improves animal performance, increases feed efficiency, alters lipid metabolism, reduces animal adiposity, increases lean body mass, and alters the immune response (Pariza et al., 2001). The multiple physiological effects reported for CLA appear to be the result of multiple interactions of the biologically active CLA isomers with numerous metabolic pathways (Pariza, 2004). Isomers of CLA are formed during ruminal biohydrogenation of linoleic and linolenic acids and in tissues of animals from *trans*-11 C18:1, another intermediate in the biohydrogenation of unsaturated fatty acids (Pariza et al., 2001). In this experiment, indirect effects on CLA supply attributable to administration of HSO can be ruled out because of the almost complete saturation of the lipids supplied with HSO.

Among the various CLA isomers, 2 in particular have been implicated as biologically active. The *cis*-9, *trans*-11 isomer has anticarcinogenic effects and enhances growth and feed efficiency, whereas the *trans*-10, *cis*-12 CLA isomer has strong repartitioning properties that result in reduced body fatness (Pariza, 2004). The reduction in body fat appears to be due mostly to a reduction in body fat accretion and not to a mobilization of the body fat that had already accumulated before the experiment (Pariza, 2004). Park et al. (1997) and Pariza et al. (2001) also suggested that muscle mass may be preserved or enhanced as a result of CLA-induced changes in the regulation of some cytokines that profoundly affect skeletal muscle catabolism and immune function.

Little information is available on the effects of rumen-protected CLA on the growth performance of growing cattle. Gillis et al. (2004) documented that feedlot Angus × Hereford heifers receiving a CLA supplementation for 30 or 60 d before slaughter, at a dosage similar to that used in this trial, increased ADG and feed efficiency only during the first 30 d of treatment with respect to controls, but that no differences were observed over the whole trial. In this trial, the addition of CLA exerted a significant reduction in the in vivo fatness score, but only during the middle part of the growth period and not during the finishing period. The effectiveness of the *trans*-10, *cis*-12 CLA isomer in reducing body fat was likely not fully explicated in this trial because of the very poor propensity of the DBM subjects to deposit fat in their body, so the observed effects of a CP-CLA interaction on feed efficiency were more likely due to the action of the *cis*-9, *trans*-11 CLA isomer. In this regard, it can be observed that CLA addition tended to interact with CP on growth rate and, in agreement with the report by Gillis et al. (2004), CLA addition did not have any influence on DMI. Both during the initial period and over the entire trial, feed efficiency was affected by the interaction of CP × additive: it was observed that CLA administration decreased feed efficiency on the HP diets and increased feed efficiency on the LP diets. These results support the hypothesis proposed by Park et al. (1997) and Pariza et al. (2001) that muscle mass could be preserved or enhanced by CLA, but in the current trial, this was observed only under conditions of a shortage of dietary protein. The results of the current experiment also indicated that LP rations and CLA addition may help in reducing the lameness score and incidence of swollen joints.

In conclusion, this experiment partially covered the lack of knowledge on the production and carcass traits of DBM Piemontese bulls fed cereals and corn silage-based diets. Many similarities have been found in production and carcass traits between DBM Piemontese and DBM Belgian Blue young bulls. It was shown that greater dietary CP density is not required for DBM Piemontese bulls, even if their voluntary feed intake is somewhat less and their protein deposition is comparable with or greater than that of other conventional continental beef cattle. The reduction in CP density from 145 to 108 g/kg of DM, besides reducing the feeding cost, induced a strong reduction in N consumption without negative consequences on growth performance and carcass traits. Nevertheless, ADG was increased by the high dietary protein diet during the initial period; therefore, phase feeding may be considered an alternative if the farm size is adequate.

It was also shown that both the LP diet and CLA supply may have a positive effect on leg and hoof health conditions. The combination of the 2 factors allowed an improvement in feed efficiency, supporting the hypothesis that CLA could exert some saving effects on protein catabolism, but only under conditions of dietary CP shortage. Considering the cost of CLA, further investigations with smaller doses, shorter periods of administration, or both are needed.

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