



Universitat Autònoma de Barcelona

Universitat Autònoma de Barcelona

Facultat de Veterinària

Departament de Ciència Animal i dels Aliments.

Official Master's Degree in Quality of Food of Animal Origin

Thesis presented to overcome the 15 credits of module Master's Thesis of Master in Quality
Food of Animal Origin

**Effect of increasing forage in feedlot diets on feed intake, time spent ruminating and
carcass quality**

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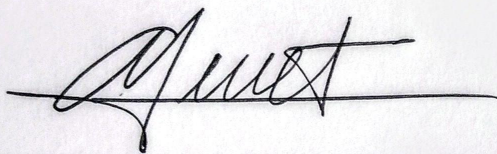
Bellaterra, 3rd July, 2018

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INFORMA

Que el trabajo titulado: "Effect of increasing forage in feedlot diets on feed intake, time spent ruminating and carcass quality" ha sido realizado bajo mi supervisión o tutela por el Sr. Ricardo Sebastián Abril Mejía dentro del módulo Trabajo Fin de Máster del Máster Oficial de Calidad de Alimentos de Origen Animal de la Universitat Autònoma de Barcelona.

Bellaterra, 3 de Julio de 2018

A handwritten signature in black ink, appearing to read 'Alfred', is written over a horizontal line. Below the line is a dotted line.

Dr. Alfred Ferret

Abbreviations

ADF: Acid Detergent Fiber

ANOVA: Analysis of Variance

BW: Body Weight

CP: Crude Protein

DM: Dry matter

DMI: Dry matter intake

NDF: Neutral Detergent Fiber

TMR: Total mixed ration

VFA: Volatile Fatty Acids

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Abstract

An experiment was conducted to ascertain the effects of increasing forage in feedlot diets on feed intake, time spent ruminating and carcass quality. Twenty-four Simmental heifers were blocked in four body weight groups (260, 241, 230, and 209 kg). Each group had 6 heifers, which were randomly assigned to 1 of 2 experimental treatments. There were in total 12 heifers per treatment. The experiment was performed in 4 periods, with 28 days per period. During the last week of each experimental period, measurements and samplings were made for further analysis. Treatment diets consisted of total mixed rations with two main ingredients, a concentrate and a forage source: a) barley straw at 10% of inclusion (BS 10%); b) alfalfa hay at 19% of inclusion (AH 19%). Diets were offered daily at 0900 on an ad libitum basis. Eating and ruminating behavior was video recorded for 24 h over three non-consecutive days in each experimental period and feed intake was controlled by means of feed bunks mounted on digital platform scales. In total 2,112 hours of video were analyzed. Results show that there were no significant differences in eating, ruminating and total chewing activities. The intakes of DM, DM from concentrate, NDF, and NDF from forage did not show differences among treatments, but DM from the forage was higher in AH 19% ($P < 0.001$). These results are in agreement with the similar carcass quality recorded among animals, with a back fat score that tended to be greater in animals fed BS 10% in correspondence with the lower forage consumed by them.

1.Introduction

Around the world, beef is an important source of protein, minerals and vitamins in human diet. Europe has historically been a beef exporter but at the beginning of the 21st century the trade balance shifted, making imports bigger than exports due basically to economic and health issues (Zjalić et al., 2006). At present, bovine production represents, in terms of value, 8.1 % of total agricultural output and 18.8 % of animal output, excluding animal products, such as milk. Between 2007 and 2014, the number of non-dairy cows decreased by 4 %, from 12.5 to 12 million heads, and between 2009 and 2014 beef production from heifers and bulls fell in both the EU-28 (- 7 %) and the EU-15 (- 8 %). However, there might now be an opportunity to recover bovine production in response to an increase in demand for meat from calves aged under 8 months and from young cattle aged between 8 and 12 months (Marquer et al., 2015).

Worldwide, there is now a variety of more efficient beef production systems. In Europe, two main types can be distinguished: in western Europe, pasture based systems and in the central-eastern parts of Europe and the Mediterranean, cereal-based systems (Zjalić et al., 2006). Combinations of these systems are also commonly found in the Pyrenees, where farmers are obliged to give shelter and feed to their animals in winter, and in spring move them to mountainous areas where pasture is rich in nutrients (Casasús et al., 2002). Intensive beef production systems are the most common method of fattening cattle in dry lands in Europe where pasture is insufficient.

1.1 Performance in beef production

Beef cattle, in this intensive production system, are fed a high-energy diet that is formulated to optimize growth rate, feed efficiency, animal health and well-being, and carcass quality at the lowest possible cost (NRC, 2000). As growth rate increased production systems had to become more efficient and reliable to provide more animal protein. With the green revolution, new methods of raising animals were proposed and the most adequate for the new population needs was the intensive production model. In general, these production systems typically involve small to large herd sizes, with animals confined in limited spaces enhancing their ability to produce either beef or milk. Animals under these systems are fed on different ingredients as they become available throughout the year, and which are more suitable to their nutritional needs. These systems are more professionalized than extensive methods, using high levels of resources but also yielding high volumes of meat and milk (FAO, 2012).

In beef production, to achieve better daily gain weights, ruminant feeds must be restructured so they can provide animals with all the nutritional requirements, offering them the possibility to show their true genetic potential. In this case, animals are raised on concentrates and grains providing them several advantages such as faster growth, less land use and time consumption. For consumers, beef has more acceptable qualities such as flavor, appearance, tenderness and lower cost (Severe and Zobell, 2011).

Several studies dealing with beef production, especially in the fattening period, show that animals allowed to graze had a lower daily gain and reached a commercial maturity later than animals confined indoors with modified diets due to the amount of energy expenditure for grazing (Cozzi et al., 2009). Studies with bulls and steers also show that even though their physiological characteristics are relevant, the addition of different levels of concentrate in their diet increases the daily weight gain (Molleta et al., 2014). Studies with young Holstein calves (dairy breed) demonstrate that in intensive systems animals reach higher slaughter weights in less time as a result of greater digestibility of concentrate (Dias et al., 2017).

1.2 Ruminal Acidosis

High concentrate diets used for achieving better results in beef production are typically high in non-fiber carbohydrates to promote high daily weight gains. The primary non-fiber carbohydrate is starch, which is much more rapidly fermented in the rumen than structural carbohydrates such as cellulose and hemicelluloses (Sniffen et al., 1992). Rapid fermentation of carbohydrates leads to rapid production of volatile fatty acids (VFA), which are readily dissociated, causing a decrease in ruminal pH (Aschenbach et al., 2011). When the dissociation of VFA is greater than the removal of protons from the rumen, ruminal pH decreases (Penner et al., 2007).

Ruminal pH is a critical factor to maintain the normal and stable function of the rumen because of its key role in physiological functions, mainly motility and absorption. In addition, it controls microbial populations and allows fermentation of the diet. Ecological conditions within the rumen must be kept within limits to maintain normal microbial growth and metabolism, and thus the well-being of the host ruminant. Cellulolytic organisms grow optimally at pH 6.7 (Van Soest, 1994). Acidosis, characterized by low ruminal pH (Nagaraja and Titgemeyer, 2007), is thought to be a prevalent digestive disorder in feedlot cattle fed high-concentrate diets. Acidosis can be categorized in two different forms: acute and sub-acute, often called clinical and subclinical acidosis. The economic impact of subacute ruminal

acidosis is great because, although animals may not appear to be sick, it will affect feed intake and general performance (González et al., 2012).

The physiological mechanism regulating ruminal pH primarily involves the neutralization of hydrogen ions with bicarbonate supplied in saliva (Bailey and Balch, 1961) and from ruminal bicarbonate secretion by the ruminal epithelium (Penner et al., 2009). Protons are primarily removed from the rumen in a condensation reaction with ruminal bicarbonate, the majority of which is supplied through salivary secretion (Bailey and Balch, 1961), and the absorption of VFA across the ruminal epithelium (Allen, 1997).

Saliva is added to feed during chewing. It lubricates feed, which allows cattle to swallow particles, provides a means of recycling nitrogen from plasma into the rumen, buffers the VFA produced during microbial digestion of feeds, adds fluid to the ruminal environment for fermentation, provides nutrients for the ruminal microorganisms, inhibits foam formation and prevents bloat, and facilitates the passage of digesta through the gastrointestinal tract (Beauchemin, 2001). Parotid saliva has a pH of about 8.2, and is strongly buffered between 6 and 7 because it contains a high bicarbonate and moderate phosphate content (McDougall, 1948). Thus, saliva plays an important role in buffering pH of the rumen contents and preventing ruminal acidosis. Considerably less saliva is produced when cattle are fed grain diets compared with forage-based diets.

Increasing the time spent eating and ruminating in beef cattle is expected to increase the volume of saliva secreted each day. Total rumination time is highly variable, ranging from 2 to 6 h/d for feedlot cattle fed high-grain diets (Beauchemin, 2001). Assuming a resting salivation rate of 30 mL/min and a salivation rate during chewing (sum of eating and ruminating) which is three times higher, total saliva production in most beef cattle would rarely be expected to exceed 100 L/d.

The relationship between feeding management, feed intake, animal performance, and the incidence of metabolic disorders such as ruminal acidosis remains unclear (Schwartzkopf-Genswein et al., 2003). Nutritionists and feedlot managers attribute subclinical acidosis and reduced performance to erratic feeding behavior and intake by cattle, which is believed to result in losses of as much as \$15 to 20 per animal. Although several studies have concluded that large variations in intake by cattle fed high-concentrate diets may cause digestive disturbances (Fulton et al., 1979; Britton and Stock, 1987), few studies have confirmed that variability in ad libitum feed intake reduces growth performance of cattle. However, considering that the

relationships between ruminal acidosis and performance variability could exist, we hypothesized that these digestive disorders caused by low forage (fiber) intake and less time spent chewing could also affect carcass quality. Therefore, the main objective of this experiment was to ascertain the effect of increasing forage in feedlot diets on feed intake, time spent ruminating and carcass quality. This study is part of a project with the aim to assess the effects of forage inclusion in beef cattle diets on performance, carcass and meat quality.

2. Materials and Methods

Animal procedures were approved by the Institutional Animal Care and Use Committee of the Universitat Autònoma de Barcelona in accordance with the European directive 2010/63/EU.

2.1 Animals and Housing

Twenty-four Simmental heifers were used in this experiment. When they arrived at the experimental farm, the heifers were 188.9 ± 2.06 d old and had an average initial body weight (BW) of 235.6 ± 4.19 kg. They were allotted in groups of 3 in roofed pens. Each pen had a concrete floor and was 5 m long and 2.5 m wide ($12.5 \text{ m}^2/\text{pen}$). The space of each pen was divided in 2 different areas. The feeding area was equipped with a feed bunk and a water trough, and the resting area bedded with wood shavings. The continuous pens were separated by a metal fence with a bar design that allowed animals contact between pens.

2.2 Experimental Design

Heifers were blocked in four BW groups (260, 241, 230, and 209 kg). Each group had 6 heifers that were randomly assigned to 1 of 2 experimental treatments. There were in total 12 heifers per treatment. The experiment was performed in 4 periods, with 28 days per period. During the last week of each experimental period, measurements and samplings were made for further analysis.

2.3 Experimental Diets

Diets, offered on ad libitum basis as total mixed ration (TMR), were formulated to be isoenergetic and isonitrogenous (NRC, 2000). Treatment diets were (Table 1): a) TMR with 10% barley straw (BS10%), and b) TMR with 19% alfalfa hay (AH19%). The diet was offered once a day at 0900 throughout the experiment.

Table 1. *Ingredients and chemical composition of the diets*

Item	Diets ¹	
	BS 10%	AH 19%
Ingredient composition, % of DM		
Barley Straw	10	-
Alfalfa hay	-	19
Corn, ground	35	41.5
Barley, ground	43	31.5
Soybean meal, 44%CP	9	5
Salt	0.7	0.7
Sodium bicarbonate	1	1
Calcium carbonate	0.5	0.5
Dicalcium phosphate	0.4	0.4
Vitamin-mineral premix ²	0.4	0.4
Chemical composition, % DM		
CP	11.9	13.1
NDF	23.8	21.2
ADF	7.7	8.8
Ether extract	2	2
Ash	4.8	7.5
NFC ³	57.5	56.2
ME ⁴ , Mcal/kg of DM	2.83	2.81

¹BS 10% = TMR with 10% of barley straw; AH 19% = TMR with 19% of alfalfa hay

²Nutral Terneros® (NUTRAL, S.A., Colmenar Viejo, Madrid, Spain): vitamin and mineral premix contained per kg premix (as fed): 1.500 kIU vitamin A, 500 kIU vitamin D₃, 3.75 g vitamin E, 0.5 g vitamin B1, 0.5 g vitamin B2, 0.25 g vitamin B6, 1.25 mg vitamin B12, 15.0 g Zn, 2.5 g Fe, 83.3 g S, 55.0 mg Co, 2.5 g Cu, 7.5 g Mn, 100.0 mg I, 100.0 mg Se

³NFC: nonfiber carbohydrates calculated as 100 – (CP + ash + NDF + EE)

⁴According to NRC (2000)

To better characterize the diets, two cannulated heifers per treatment were used to evaluate the effects of diets on ruminal fermentation (Table 2). Samples were taken after 2 weeks of diet adaptation on 3 non-consecutive days. Ruminal samples were taken with an electric vacuum pump connected to a 1-m iron tube that was introduced through the cannula to reach different locations within the rumen and obtain a 300-mL sample. Sampling times were as follows: immediately before feeding, and at 4, 8, 12, 16 and 24 h after feeding. The ruminal fluid was squeezed through four layers of cheesecloth and pH was measured immediately with a glass electrode pH meter (model 507; Crisson Instruments SA, Barcelona, Spain).

Table 2. *Characterization of the diets based on their effects on ruminal pH*

	Time postfeeding					
	Hour 0	Hour 4	Hour 8	Hour 12	Hour 16	Hour 24
Diets ¹						
BS 10%	7.30	6.25	6.01	5.53	6.41	7.51
AH 19%	7.54	6.05	6.34	6.57	6.87	7.43

¹ BS 10%= TMR with 10% of barley straw; AH 19% = TMR with 19% of alfalfa hay

2.4 Data collection

To record feed intake, an automated system was used. Feed bunks (120 L capacity) were mounted on waterproof digital platform scales in each stall (model DI-160, DIGI I's Ltd, Maesawa-cho, Isawa-gun, Iwake, Japan). Individual feed intake was monitored with an electronic ear tag on each heifer (Allflex HDX ULTRA HP ISO 982, Azasa, Madrid, Spain), which was detected by an antenna (Allflex panel reader, Azasa, Madrid, Spain) placed next to each feed bunk. Each scale was programmed to transmit the feed weight at intervals of 5 s. The information was downloaded onto a computer with appropriate data capture software (LabView, National Instruments Corporation, Austin, TX, USA).

2.5 Chemical analysis

Feed samples were dried in a forced air oven at 60°C for 48 h for later chemical analysis. Samples were ground in a hammer mill through a 1-mm screen (P. PRAT SA, Sabadell, Spain) and retained for analysis. Dry matter content was determined by drying samples for 24 h at 103°C in a forced-air oven, and ash content according to AOAC (1990; ID 950.05). Nitrogen content was determined by the Kjeldahl procedure (AOAC, 1990; ID 976.05). Ether extract was performed according to AOAC (1990; ID 920.39). The NDF and ADF contents were

determined sequentially by the procedure of Van Soest et al. (1994) using a thermostable α -amylase and sodium sulfite, and expressed on an ash-free basis.

2.6. Feeding behavior

2.6.1 Eating, ruminating and total chewing activities

To analyze eating and ruminating activities, animal behavior was recorded for 24 hours on three nonconsecutive days in the last week of each experimental period. In total, 2,112 hours of video were recorded and analyzed. For this purpose, a video recording system was installed (model VS-101P VioStor NVR, QNAP Systems Inc., XizhiCity, Taipei County, Taiwan). A digital color camera (model VIVOTEK IP7142, VIVOTEK INC., ChungHO, Taipei County, Taiwan) was located at a height of 3 meters in front of the feeding area. For night recording, an infrared light equipped with photoelectric cells ($\lambda = 830$ nm and 500 W; Dennard 2020, Hants, UK) was also installed. A heifer was considered to be eating when it had its head in the feed or the water bunk, or was chewing or swallowing food with its head over it. Ruminating activity was defined as the time when heifers were regurgitating, masticating, and swallowing the bolus, either lying or standing in the bed area. Total chewing was obtained after the sum of eating and ruminating times. These activities were expressed as minutes per day.

2.6.2 Sampling Method

To analyze behavior, the unit of study was one hour (60 minutes), divided in intervals of 5 minutes. These activities were assumed to span 60 s every 5 minute-period (Madruga et al., 2017).

2.7 Carcass quality measurements

Heifers were allotted in the farm and fed the corresponding diet until each BW block reached the target weight of 400 kg. Heifers were then transported in block to a commercial slaughterhouse (Sabadell, Spain) 5.8 km from the UAB experimental farm. Heifers were slaughtered using standard procedures in an EU-licensed abattoir. The animals' BW was registered immediately before transfer to the abattoir. After slaughter, hot carcass weight was recorded, and carcass back fat and conformation scores were classified according to the EU classification system into 1, 2, 3, 4 and 5 and S, E, U, R, O, P categories, respectively (EU Regulation No 1234/2007 and No 1249/2008).

2.8 Statistical Analysis

For eating and ruminating activities data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model contained the fixed effects of treatment, period and treatment x period interaction, and the random effects of block and animal nested within block. The day was considered a repeated measure. For intake variables, the same model was used but without considering the day as a repeated measure. For categorical variables not normally distributed (carcass conformation and back fat score), rank transformation was used. Rank-transformed data were analyzed by the Tukey Multiple Comparisons test of the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

3. Results

3.1 Intake

Dry matter intake was not different between diets, being on average 7.24 and 7.35 for BS 10% and AH 19%, respectively (Table 3). Considering the concentrate to forage proportion of the diets, and assuming that this proportion did not change during TMR consumption, DM intake from concentrate did not vary between diets. In contrast, DM intake from the forage source was greater in AH 19% than in BS 10% (1.40 vs 0.72 kg/d, respectively; Table 3, $P < 0.001$). Neutral detergent fiber and NDF intake from the forage was not different between diets.

Table 3. Dry matter and NDF intake of heifers fed TMR diets with barley straw and alfalfa hay

Item	Diets ¹		SEM	P-value
	BS 10%	AH 19%		
Dry matter intake, kg/d	7.24	7.35	0.460	0.812
Dry matter intake from concentrate, kg/d	6.53	5.96	0.280	0.165
Dry matter intake from forage, kg/d	0.72	1.40	0.053	<0.001
NDF intake, kg/d	1.72	1.56	0.104	0.125
NDF intake from forage, kg/d	1.24	1.12	0.053	0.122

¹ BS 10%= TMR with 10% of barley straw; AH 19% = TMR with 19% of alfalfa hay

3.2 Eating, ruminating and total chewing activities

Time spent for eating, ruminating and total chewing was not different between diets (Table 4). On average, heifers spent 171 minutes eating, 387 minutes ruminating, and total chewing time was 558 minutes.

Table 4. Feeding behavior of heifers fed TMR diets

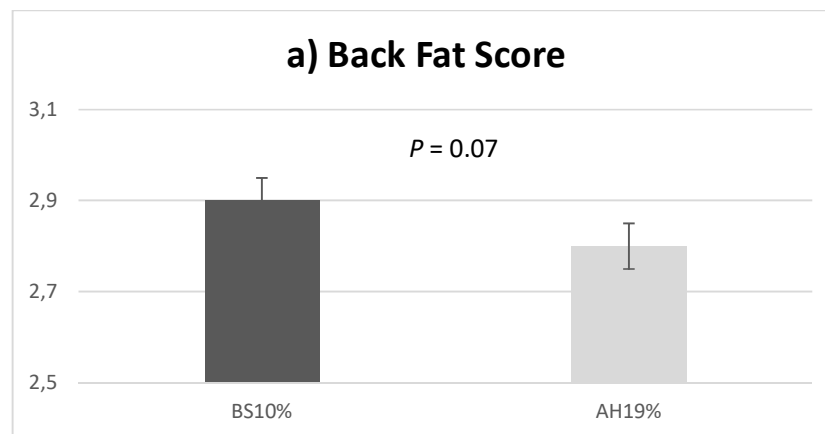
Item	Diets ¹		SEM	P-value
	BS 10%	AH 19%		
Eating, min/d	167.2	174.8	10.01	0.451
Ruminating, min/d	395.9	378.0	23.37	0.455
Total chewing, min/d	566.9	550.2	19.13	0.384

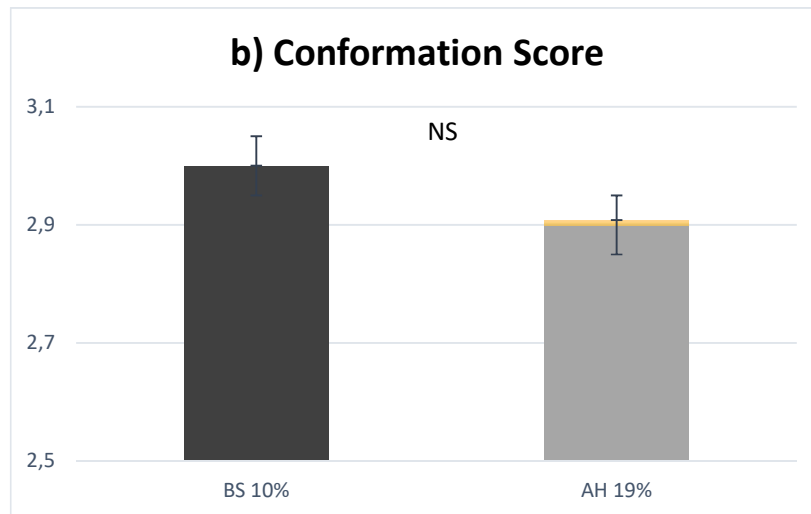
¹BS 10%= TMR with 10% of barley straw; AH 19% = TMR with 19% of alfalfa hay

3.3 Carcass Quality

Carcass back fat from heifers fed BS 10% tended to be fattier than in those fed AH 19% (Figure 1; $P = 0.07$). Conformation score was not different between carcasses in both diets (Madruga, personal communication, 2018).

Figure 1. Carcass back fat (a) and conformation score (b) of heifers fed with TMR diets





4. Discussion

In the present study, two experimental TMR diets, with barley straw (BS 10%) and alfalfa hay (AH 19%), were formulated according to the NRC (2000) to be isoenergetic and isonitrogenous, to achieve similar animal performance. After the evaluation of total dry matter intake, dry matter intake from concentrate, total NDF intake and NDF from forage, results did not differ between treatments. In the case of dry matter intake from forage, significant differences were observed in response to the increased amount of forage in AH 19% treatment. No differences in DM and NDF intakes resulted in no differences in time spent eating, ruminating and total chewing. These results disagree with those obtained by Madruga et al. (2018), who used similar diets to ours. Animals fed AH 19% ate more DM and NDF than those fed BS 10%, and consequently spent more time ruminating. This could be explained by the fact that in our experiment we had 3 animals per pen, so animals were competing for feed, while in the other experiment there was only one animal per pen. According to González et al. (2012), environmental and social factors can affect animal feeding behaviors. In particular, social interactions among animals allotted in groups and more limited feed bunk space when animals are housed in a group pen, can limit normal eating behaviors reducing directly eating, ruminating and total chewing activities.

Carcass back fat and conformation scores were classified according to the EU classification system into 1, 2, 3, 4 and 5 and S, E, U, R, O, P categories, respectively (EU Regulation No 1234/2007 and No 1249/2008). In both cases, means did not differ significantly

between treatments, but carcasses of heifers fed BS 10% tended to have a greater back fat score than in AH 19%. This last result could be due to the lesser amount of forage consumed by these animals. Carcass of animals fed with higher concentrate diets usually have better conformation and back fat scores. Realini et al. (2004) working with animals fed with a high concentrate diet had better carcass scores than animals fed pasture. This difference can be explained by the ability of these diets to maintain higher grow rates with high fat back deposition in less time than diets with a lesser amount of concentrate (Muir et al. 1998).

5. Conclusion

In this experiment, the inclusion of alfalfa hay at 19% did not affect intake and feeding behaviors in comparison with a diet with a greater proportion of concentrate. Moreover, carcasses were very similar in quality. Thus, the original hypothesis that an increased fiber intake could help to reduce digestive disorders, improving animal performance and carcass quality, could not be verified.

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