Feedlot performance of bulls and steers fed on three levels of concentrate in the diets

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ABSTRACT. Current study evaluated the effect of different sexual groups and different concentrate levels in diets on animal performance, feed efficiency and carcass characteristics of bovines finished in feedlot. Ninety four bulls and 75 steers Purunã with average age 19 months at the beginning of the feedlot period were used. Animals were maintained in individual pens during 116 days and fed with a diet of corn silage and three concentrate levels (0.8, 1.1, and 1.4% of body weight). The concentrate was formulated with 25% soybean meal, 73% corn grain, 1.0% of a mineral mix and 1.0% limestone and adjusted at every 28 days. The interaction between sexual groups and concentrate levels was not significant for any of the studied variables. Bulls were more efficient than steers ones, with higher average daily gain (1.33 vs. 1.12 kg day⁻¹), better feed conversion ratio (6.6 vs. 7.6 kg DM intake kg⁻¹ gain), and higher carcass gain (86.6 vs. 69.2 kg). Increase in concentrate level in diet did not influence animal performance even though a higher DM was observed for animals fed on 1.4% (8.8 kg) and 1.1% (8.4 kg) levels of concentrate when compared with those fed on 0.8% level (7.7 kg).

Keywords: efficiency, feed intake, sexual group, weight gain.

Desempenho de bovinos não castrados e castrados alimentados com três níveis de concentrado na dieta

RESUMO. Este trabalho avaliou o efeito de diferentes classes sexuais e níveis de concentrado na dieta sobre o desempenho animal, eficiência alimentar e características de carcaça de bovinos terminados em confinamento. Noventa e quatro machos não castrados e 75 castrados da raça Purunã com 19 meses de idade no início do período experimental foram usados. Os bovinos foram mantidos em baias individuais durante 116 dias e alimentados com uma dieta a base de silagem de milho e três níveis de concentrado (0,8; 1,1 e 1,4% do peso corporal). Os concentrados foram formulados com 25% de farelo de soja, 73% de milho, 1% de sal mineral e 1% de calcário; ajustados a cada 28 dias. A interação grupo sexual e níveis de concentrado não foi significativa para nenhuma das variáveis estudadas. Os machos não castrados foram mais eficientes do que os machos castrados, com maior ganho em peso (1,33 vs. 1,12 kg dia⁻¹), melhor conversão alimentar (6,6 vs. 7,6 kg de ingestão de MS kg⁻¹ de ganho em peso) e maior ganho em peso de carcaça (86,6 vs. 69,2 kg). O aumento dos níveis de concentrado na dieta não influenciou o desempenho animal, embora maior ingestão em MS tenha sido observada para os animais alimentados com 1,4% (8,8 kg) e 1,1% (8,4 kg) níveis de concentrado quando comparado com os animais alimentados com níveis de 0,8% de concentrado (7,7 kg).

Palavras-chave: eficiência, ingestão de alimentos, grupo sexual, ganho em peso.

Introduction

Brazil has the largest commercial cattle herd in the world, with approximately 185 million animals and a production of approximately 8.5 million tons of carcass each year (FAPRI, 2013). Approximately 16% (1.4 million tons) of the total is exported to several countries worldwide (FAPRI, 2013). In fact, Brazil is a principal meat exporter in the world due to herd size, pasture area, high grain production, co-products utilization in animal foods and a 2.5% increase in profitability during the last decade (ANUALPEC, 2013). Cattle in Brazil are finished on a pasture system (approximately 90% of slaughtered animals), according to ANUALPEC (2013) and only 3.5 million animals are slaughtered from feedlot system. So that beef production could be improved, mainly aiming at reducing slaughter age and improving meat quality (ROTTA et al., 2009; FUGITA et al., 2012; ITO et al., 2012), the feedlot system, associated with high levels of concentrate in the diet, is being increasingly used in Brazil (PRADO et al., 2000; DUCATTI et al., 2009; FUGITA et al., 2012). Increasing energy
density in cattle diet increases and improves animal performance and carcass characteristics. Further, it has a direct influence on quantitative and qualitative meat aspects (DUCATTI et al., 2009; ROTTTA et al., 2009; DIAN et al., 2010; PRADO et al., 2011).

On the other hand, meat bulls' production is low in Brazil. According to Bretschneider (2005), slaughterhouses discriminate bulls because most have dark meat and because of the great development of forelegs to the detriment of hindquarters, where the best carcass cuts are found (PEROTTO et al., 2009). These authors observed that the decrease in slaughter age of bulls to two years or less opens up new perspective to produce bulls' meat, already on the market in several European countries. Sex has an important influence on growth pattern, body development and carcass composition of beef cattle (PRADO et al., 2009; ROTTTA et al., 2009). Aggressive behavior, low tenderness rates and dark color of bull meat (FIELD, 1971) have been some of the reasons bulls were and still are castrated. Differences in productivity between bulls and steers are mainly manifested after puberty (LUNSTRA et al., 1978) attained at an average age of 14 months. During puberty the testes primarily produce androgens, among which testosterone is the most potent (LEE et al., 1990). Androgens are responsible for the development of male accessory organs, external secondary sexual characteristics and male behavior. The anabolic property of androgens, especially testosterone, influences the average daily gain of bulls in up to 15% than steers, with only average 3% higher daily feed intake (ROTTTA et al., 2009).

On the other hand, increase in energy density in diets by providing larger quantities of concentrates may improve feed efficiency and animal performance (MISSIO et al., 2010) and alter carcass characteristics and meat quality (ROTTTA et al., 2009). Current research was performed to study animal performance and feed efficiency of bulls and steers finished in feedlot and fed on three concentrate level in the diets.

Material and methods

Local, animals, housing and diets

Current experiment, approved by the Department of Animal Production of the State University of Maringá (CIOMS, 1985), was conducted at the Experimental Station of the Model Farm of the Agronomic Institute of Paraná (IAPAR) in Ponta Grossa, Paraná State, South Brazil.

One hundred sixty-nine Purunã animals (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim) were used in a factorial arrangement. The animals were weighed and distributed into two sexual groups (bulls = 94; steers = 75) and three diet groups (0.8, 1.1, and 1.4% of concentrate). The animals were selected at random and castrated at 210 days of age by standard surgical method, as described by Bretschneider (2005). All animals were injected of local anesthetic into each testis and into the scrotum on the day of castration. They also received a 20 mL of antibiotic injected in the neck. After the animals were de-wormed and vaccinated, they were allocated into individual pens (8.0 m² for each animal) in a feedlot system. After a 14 days diet adaptation period, the animals were weighed. Experiment started when animals had an average initial body weight (BW) of 324 ± 33.3 kg and an average age of 12 ± 0.9 months. The animals were kept in feedlot during 116 days.

During the feedlot period the animals were fed twice a day on a diet of corn silage and concentrate (soybean meal, corn grain, mineral salt and limestone) (Table 1). Concentrate volume was adjusted every 28 days when the animals were weighed after a 16 hours rest. The animals' BW and concentrate and corn silage intake were recorded monthly until day 116 of the period experimental when the bulls reached a final BW of 465 ± 57.3 kg.

Table 1. Chemical composition of ingredients in diets (% on DM).

<table>
<thead>
<tr>
<th>Item</th>
<th>Soybean meal</th>
<th>Corn grain</th>
<th>Corn silage</th>
<th>Concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>49.1</td>
<td>8.93</td>
<td>5.66</td>
<td>16.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>1.30</td>
<td>3.36</td>
<td>2.13</td>
<td>4.55</td>
</tr>
<tr>
<td>Mineral salt</td>
<td>6.63</td>
<td>0.89</td>
<td>3.06</td>
<td>2.38</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>43.0</td>
<td>86.8</td>
<td>89.1</td>
<td>77.1</td>
</tr>
<tr>
<td>Non fibrous carbohydrates</td>
<td>37.0</td>
<td>68.9</td>
<td>45.6</td>
<td>58.9</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>6.00</td>
<td>17.9</td>
<td>43.5</td>
<td>18.2</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>13.1</td>
<td>4.40</td>
<td>26.8</td>
<td>5.92</td>
</tr>
<tr>
<td>Total digestible nutrients’</td>
<td>82.2</td>
<td>80.8</td>
<td>60.5</td>
<td>78.8</td>
</tr>
</tbody>
</table>

Data obtained from the Laboratory of Food Analysis - IAPAR, "Data from the NRC - 2000).

Animal performance and feed intake

Animals were weighed once at the beginning of the experiment and then once every 28 days (after a fasting from solid food for a period of 16h) for the duration of the experiment (116 days) so that animal performance could be determined. Daily feed intake was estimated as the difference between supplied feed and refusals in the trough. During the collection period, samples of the supplied feed and refusals were collected and a representative composite sample was drafted per animal in each treatment for analyses.

Carcass characteristics

After reaching the final BW the animals were transported to the slaughterhouse. The truck's stocking density was 1 animal m² and the transport distance was...
10 km. At the slaughterhouse, the animals were housed in collective pens for approximately 12h (overnight) before slaughter. The animals were slaughtered at a commercial slaughterhouse according to industrial practices in Brazil. Carcasses were identified, weighed (hot carcass weight–HCW) and chilled for 24h at 4°C. After chilling, the right half of the carcass was used to determine the carcass quantitative characteristics. The right half of the carcass was used to determine physical characteristics. Hot carcass dressing (HCD) is the percentage of the individual animal’s dressing defined by the ratio HCW:BW and multiplied by 100.

**Chemical analyses**

Dry matter content of the ingredients (silage and concentrate mix) was determined by oven-drying at 65°C for 24h. Analytical DM content of the oven-dried samples was determined by drying at 105°C for 5 hours, following (AOAC, 1998 - method 930.15). OM content was calculated as the difference between DM and ash contents, with ash determined by combustion at 550°C for 5 hours. The heat stable alpha-amylase was utilized to determined NDF and ADF (MERTENS, 2002). Nitrogen (N) content was determined by the Kjeldahl method (AOAC, 1998 - Method 976.05) and total carbohydrates (TC) were obtained by the following equation: TC = 100 – (%CP + %EE + %Ash) (SNIFFEN et al., 1992). Non-fiber carbohydrates (NFC) were determined as the difference between TC and NDF. Total digestible nutrient (TDN) content of the diets was obtained by methodology described by Kearl (1982). The samples were analyzed at the Laboratory of Food Analysis – IAPAR.

**Statistical analysis**

All variables were previously submitted to normality test by Univariate proceeding (SAS, 2004). The variables that had normal 5% level of probability distributions using the Shapiro-Wilk test distributions were analyzed by the method of least squares (GLM procedure – SAS, 2004) by the linear model:

\[ Y_{ijk} = \mu + SG_i + CL_j + SG \times CL_j + \varepsilon_{ijk} \]

where:
- \( Y_{ijk} \) = observation value in the animal k-th, j-th level of concentrate and the i-th sexual groups;
- \( \mu \) = overall mean of the characteristic Y;
- \( SG_i \) = fixed effect of the i-th sexual groups, where \( i = 1 \) (steers) and \( i = 2 \) (bulls);
- \( CL_j \) = effect of the j-th level of concentrate in the diet, where \( j = 1 \) (0.8%), \( j = 2 \) (1.2%) and \( j = 3 \) (1.4%); \( \varepsilon_{ijk} \) = random effect peculiar to each observation.

The characteristics for which normal distribution was not accepted were then transformed by the square root method, according to the following formula:

\[ Y' = \sqrt{Y} \]

where:
- \( Y' \) = the value of the variable on the scale transformed by the square root.

Then the transformed variables (\( Y' \)) were also subjected to the normal Univariate test procedure at 5% probability (SAS, 2004). The variables in the transformed scales that had normal 5% level of probability distributions using the Shapiro-Wilk test distributions were then analyzed by the method of least squares (GLM procedure – SAS, 2004) by the linear model:

\[ Y'_{ijk} = \mu + SG_i + CL_j + SG \times CL_j + \varepsilon_{ijk} \]

where:
- \( Y'_{ijk} \) = square root of the observation in the animal k-th, j-th level of concentrate and the i-th sexual groups;
- \( \mu \) = overall mean of the characteristic Y;
- \( SG_i \) = fixed effect of the i-th sexual groups, where \( i = 1 \) (steers) and \( i = 2 \) (bulls);
- \( CL_j \) = effect of the j-th level of concentrate in the diet, where \( j = 1 \) (0.8%), \( j = 2 \) (1, 2%) and \( j = 3 \) (1.4%); \( CL_j \times SG_i \) = fixed effect of the interaction between sexual groups and the concentrate level; \( \varepsilon_{ijk} \) = random effect peculiar to each observation.

The characteristics whose normal distribution was not restored by square root transformation were then analyzed by the method of generalized linear models (NELDER; WEDDERBURN, 1972) using the GENMOD procedure at 5% probability (SAS, 2004). In the case of the continuous variable, a model was used that included the fixed effects of sexual groups, concentrate level and the interaction between these main effects, recommending gamma distribution as a systematic part of the model and logarithmic function as link function.

So that results could be presented and discussed, average transformed variables analyzed by the method of generalized linear models or square root were converted to the original scale, respectively rising squared values or its antilogarithm.

**Results and discussion**

Since interaction between sexual groups vs. concentrate levels was not significant for all variables
studied, the effects of sexual groups and concentrate levels were presented and discussed separately (Table 2).

Dry matter intake (kg day\(^{-1}\) or kg 100 kg\(^{-1}\) of BW) of silage and concentrate and total were similar for bulls and steers (Table 2). Mean dry matter intake was 8.28 kg or 2.14% BW. Likewise, feed intake of other nutrients was similar for bulls and steers. Overall, the dry matter intake varied from 2.0 to 2.5% of BW in cattle finished in feedlot and fed on a diet with high energy density (MERTENS, 1994; NRC, 2000; MAGGIONI et al., 2009). Thus, feed intake was normal for the animals.

Crude protein and TDN intake by bulls and steers were 0.83 and 5.64 kg day\(^{-1}\); or rather, below the recommended values for bulls with two years in finishing period and weight gain between 1.3 and 1.5 kg day\(^{-1}\) (NRC, 2000). Mean NDF intake was 2.75 kg day\(^{-1}\), although the low NDF intake (0.5% of BW) did not influence the feed intake of all nutrients (MERTENS, 1994; MAGGIONI et al., 2009).

Dry matter conversion was better for bulls than for steers (Table 2). In general, bulls have better dry matter conversion (FIELD, 1971; BRETSCHNEIDER, 2005; PRADO et al., 2009) when compared to that of steers, perhaps due to high production of anabolic hormones by the testicles (LUNSTRA et al., 1978; LEE et al., 1990).

Table 2. Feed intake of nutrients by cattle of different sexual groups and fed on three concentrate levels in the diets.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Sexual groups</th>
<th>Concentrate levels</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bulls</td>
<td>Steers</td>
<td>S(^{-1}) CL Sse. CL</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Silage intake(^{**})</td>
<td>4.79</td>
<td>4.73</td>
<td></td>
</tr>
<tr>
<td>Concentrate intake(^{**})</td>
<td>3.57</td>
<td>3.30</td>
<td></td>
</tr>
<tr>
<td>Total dry matter intake(^{**})</td>
<td>8.44</td>
<td>8.12</td>
<td></td>
</tr>
<tr>
<td>Silage:concentrate ratio(^{**})</td>
<td>57.2</td>
<td>58.6</td>
<td></td>
</tr>
<tr>
<td>Dry matter intake, kg kg BW(^{-1})</td>
<td>2.07</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>Organic matter(^{**})</td>
<td>8.20</td>
<td>7.89</td>
<td></td>
</tr>
<tr>
<td>Crude protein(^{**})</td>
<td>0.85</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Neutral detergent fiber(^{**})</td>
<td>2.78</td>
<td>2.72</td>
<td></td>
</tr>
<tr>
<td>Acid detergent fiber(^{**})</td>
<td>1.52</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>Ether extract(^{**})</td>
<td>1.20</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>Total carbohydrates(^{**})</td>
<td>7.08</td>
<td>6.83</td>
<td></td>
</tr>
<tr>
<td>Non fibrous carbohydrates(^{**})</td>
<td>4.33</td>
<td>4.15</td>
<td></td>
</tr>
<tr>
<td>Total digestible nutrients(^{**})</td>
<td>5.76</td>
<td>5.52</td>
<td></td>
</tr>
<tr>
<td>Dry matter conversion(^{**})</td>
<td>6.61</td>
<td>7.56</td>
<td></td>
</tr>
</tbody>
</table>

\(^{**}\)Body weights; \(^{**}\)Average daily gain (ADG) kg; \(^{**}\)Total daily intake (kg); \(^{**}\)Concentrate levels (kg); \(^{**}\)Values with normal distribution (GLM, SAS 2004); \(^{**}\)Values which normal distribution was determined after transformation by \(\sqrt{Y}\); \(^{**}\)Variables with normal distribution did not determine after transformation by \(\sqrt{Y}\) (Gemmound proceedings, SAS, 2004); All = (CS): a, b, c (NC); Means followed by different letters in the same row are different (p < 0.05).

Corn silage intake was lower for animals fed on higher concentrate levels in the diet (Table 2). There was thus a substituting effect of silage by concentrate intake. In general, when there is an increase concentrate a decrease in intake roughage occurs (MISSIO et al., 2009). Contrastingly the concentrate intake was higher for animals fed on high concentrate levels in the diets. These results showed that a higher increase of concentrate levels in the diet also increases feed intake, as observed by other researchers (MISSIO et al., 2009).

High concentrate intake was responsible for higher dry matter, crude protein, total carbohydrates, non fibrous carbohydrates and total nutrients digestible in the diets (Table 2). However, NDF, ADF and ether extract rates were similar among the diets.

Feed intake, controlled by physiological, physic and psychogenic factors (MERTENS, 1994; MAGGIONI et al., 2009), is the result of several interrelated mechanisms which determine the final response of animals (SILVA et al., 2012). López-Caballero et al. (2002) observed that the dominance of a control mechanism over the other mechanism may be associated with dietary quality in energy density and diet digestibility, but its correlation on feed intake may be positive or negative.

Dry matter conversion was similar among the three diets (Table 2). According to Euclides Filho et al. (1997), an increased level of concentrate improved feed conversion since an increase in energy density resulted in greater energy intake and therefore less feed was needed for weight gain, and thus better feed conversion. However, no change in feed conversion occurred in current experiment due to the increased level of concentrate in the diet. Similar results were reported by Calder (2004) but were not reported in the study by Missio et al. (2009). These authors found that an increase in the level of concentrate in the diet improved feed conversion ratio as a result of higher energy density diets with higher concentrate proportions.

Initial weight was higher for bulls than for steers because the latter were castrated at 210 days. In fact, animals’ castration stress reduces their performance (BRETSCHNEIDER, 2005). As has been noted in the case for initial weight, the final weight and average daily gain (ADG) were higher for bulls (491 and 1.33 kg) than for steers (439 and 1.12 kg) (Table 3). In general, on similar handling and feeding bulls has higher weight gain than steers (RICETTI et al., 2008; PRADO et al., 2009; ROTTA et al., 2009).

High growth of bulls when compared to that of steers...
or even female cattle seems to be due to high production of anabolic hormones by the testicles (LUNSTRA et al., 1978; LEE et al., 1990).

Carcass gain, carcass efficiency deposition and muscle efficiency deposition were better for bulls than for steers (Table 3). Carcass efficiency and muscle efficiency deposition are the ratio of kg of carcass and muscle deposited and carcass gain (kg). However, fat efficiency deposition was similar for bulls and steers.

Concentrate levels had no effect on animal performance (Table 3). Low ADG (1.25 kg) may be explained by low protein (0.83 kg) and total nutrients digestible (5.74 kg) intake per day by animals. Altogether, the ADG for crossbred cattle is above 1.5 kg (ROTTA et al., 2009; DIAN et al., 2010; MAGGIONI et al., 2010).

Similar results were observed by López-Campos et al. (2012) who reported no difference in ADG of animals fed on 1.0 or 2.0% concentrate in the diet, although it was expected by the authors that the ADG could be higher due to the high concentrate level in the diet. However, the increase in ADG of animals finished in feedlot with increasing level of concentrate in the diet has been reported by other authors (BRONDANI et al., 2004).

Table 3. Animal performance and feed efficiency from bulls and steers fed on three concentrates levels in the diets.

<table>
<thead>
<tr>
<th>Item</th>
<th>Sexual groups</th>
<th>Concentrate levels</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bulls</td>
<td>Steers</td>
<td></td>
</tr>
<tr>
<td>Initial weight, kg</td>
<td>330</td>
<td>319</td>
<td>300</td>
</tr>
<tr>
<td>Final weight, kg</td>
<td>491</td>
<td>439</td>
<td>468</td>
</tr>
<tr>
<td>Average daily gain, kg day-1</td>
<td>1.30</td>
<td>1.12</td>
<td>1.10</td>
</tr>
<tr>
<td>Carcass gain, kg</td>
<td>86.7</td>
<td>86.1</td>
<td>74.5</td>
</tr>
<tr>
<td>Carcass efficiency deposition</td>
<td>11.7</td>
<td>14.2</td>
<td>12.8</td>
</tr>
<tr>
<td>Muscle efficiency deposition</td>
<td>17.7</td>
<td>22.8</td>
<td>19.8</td>
</tr>
<tr>
<td>Fat efficiency deposition</td>
<td>64.9</td>
<td>67.2</td>
<td>68.4</td>
</tr>
</tbody>
</table>

Abbreviations: *Sexual groups*, †Concentrate levels, ‡Dry matter intake (kg) carcass gain (kg), ‡Dry matter intake (kg) muscle gain (kg), ‡Dry matter intake (kg) fat gain (kg). Variables with normal distribution (GLM proceeding, SAS (2004)).

Conclusion

Finishing bulls in feedlot system is an important alternative to the beef cattle industry since there is approximately a 19% weight gain and 25% carcass gain. Moreover, there is a 14% improvement on the conversion of dry matter when compared to that of steers.

On the other hand, the concentrate level in the diets had no effect on animal performance, feed efficiency and carcass characteristics. Thus, the bulls and steers could have low concentrate (0.8%) supplementation in the diet.

Acknowledgements

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References


FUGITA, C. A.; PRADO, I. N.; JOBIH, C. C.; ZAWADZKI, F.; VALERO, M. V.; PIRES, M. C. O.; PRADO, R. M.; FRANÇOZO, M. C. Corn silage with and without enzyme-


KEARL, L. C. Nutrient requirements of ruminants in developing countries. 1st ed. Utah: International Feedstuffs Institute; Utah Agricultural Experiment Station; Utah State University, 1982.


PRADO, I. N.; ABRAHÃO, J. J. S.; ZAWADZKI, F.; VALERO, M. V.; MARQUES, J. A.; ITO, R. H.; PEROTTO, D. Composição química e perfil de ácidos graxos do músculo Longissimus de bovinos de diferentes grupos genéticos alimentados com silagem de sorgo ou cana-de-açúcar e terminados com 3, 4 ou 4, 8 mm de espessura de gordura de cobertura. Semina: Ciências Agrárias, v. 32, n. 4, p. 1461-1476, 2011.


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