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Ammonium dipropionate in the total mixed ration does not change the ingestive behavior but improves the productive performance of feedlot bulls

A adição do dipropionato de amônio na ração total misturada não altera o comportamento ingestivo, mas melhora o desempenho produtivo de novilhos confinados

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Abstract
The objective of the present study was to evaluate the productive performance, the ingestive behavior, the apparent digestibility of the diet, and the carcass characteristics of beef steers finished in confinement under the effect of ammonium dipropionate in the diet, and the fractionation or not in the supply of the diet. The experimental design was completely randomized, in a 2 x 2 factorial scheme, totaling four treatments, as follows: Diet without ammonium dipropionate provided twice a day; Diet without ammonium dipropionate given once daily; Diet with ammonium dipropionate provided twice daily; Ammonium dipropionate diet provided once daily. Thirty-two ½ Angus ½ Nellore bulls were used, with an average age of 11 months. The use of ammonium dipropionate in the overall average increased average daily gain, dry matter intake, and carcass gain. The diet provided twice a day provided, on average, greater weight gain, greater carcass gain, and better feed conversion. When evaluating the association between treatments, the use of dipropionate plus the diet supplied twice a day showed greater daily carcass gain during the experimental period and higher hot carcass weight (1.251 kg, 111.4 kg, and 308.6 kg respectively), as well as ensuring better apparent digestibility of dry matter (74.57%). With the data obtained in the present study, it is possible to state that it is advisable to use ammonium dipropionate while maintaining the fractionation of the diet for beef steers in the finishing phase.
Keywords: animal performance; carcass characteristics; chemical stabilizer; diet digestibility; feed management.

Resumo
O objetivo do presente estudo foi avaliar o desempenho produtivo, o comportamento ingestivo, a digestibilidade aparente da dieta e as características de carcaça de novilhos de corte terminados em confinamento sob efeito do dipropionato de amônio na dieta, e do fracionamento ou não no fornecimento da dieta. O delineamento experimental foi inteiramente casualizado, em um esquema fatorial 2 x 2, totalizando quatro tratamentos, sendo: Dieta sem dipropionato de amônio fornecida duas vezes ao dia; Dieta sem dipropionato de amônio fornecida uma vez ao dia; Dieta com dipropionato de amônio fornecida duas vezes ao dia; Dieta com dipropionato de amônio fornecida uma vez ao dia. Utilizou-se 32 novilhos ½ sangue Angus ½ sangue Nelore, com idade média de 11 meses. O uso do dipropionato de amônio na média geral aumentou o ganho médio diário, a ingestão de matéria seca, e o ganho de carcaça. A dieta fornecida duas vezes ao dia proporcionou na média geral, maior ganho de peso, maior ganho de carcaça e melhor conversão alimentar. Ao avaliar a associação entre os tratamentos, o uso de dipropionato mais a dieta fornecida duas vezes ao dia mostrou maior ganho de carcaça diário, durante o período experimental e maior peso de carcaça quente (1,251 kg, 111,4 kg e 308,6 kg respectivamente), assim como garantiu melhor digestibilidade aparente da matéria seca (74,57%). Com os dados obtidos no presente estudo é possível afirmar que é recomendável utilizar o dipropionato de amônio mantendo o fracionamento da dieta para novilhos de corte em fase de terminação.
Palavras-Chave: características de carcaça; desempenho animal; digestibilidade da dieta; estabilizante químico; manejo alimentar.

1. Introduction

Adequate feeding management is essential for a successful production system, and it must guarantee the supply of nutrients compatible with animal requirements. The fractional supply of the total mixed ration (TMR) to the animals is a common practice in many farms, however, the choice of which management to adopt must be thoroughly evaluated. Supplying TMR to cattle in a feedlot system only once a day can be a good strategy in terms of reducing labor costs [(1)], however, when the diet

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contains silage in its composition, the longer exposure time to oxygen favors the activity of spoilage microorganisms, causing its deterioration that can reach the TMR, deteriorating the other ingredients [2].

When adopting this food management, the inclusion of propionic acid to the TMR at the time of its supply reduces losses due to its potential for microbiological control, and thus, ensures the maintenance of TMR quality, its greater use, improved productive performance, allowing to reduce the number of times the diet will be provided throughout the day [3,4]. Providing the diet twice a day, even if it is more expensive, makes the TMR stay fresh in the trough for longer, reducing the activity of spoilage microorganisms and can stimulate the intake of dry matter by the animals [5,1]. Adding propionic acid to the TMR in management with supply twice a day can further enhance the productive performance and intake, due to its preservative role, as mentioned above, and also enhance flavor [6].

Some studies using organic acids such as propionic acid have been developed, and these aimed to evaluate their effectiveness in preserving dietary nutrients throughout the day, that is, their potential to reduce losses and deterioration of nutrients from food [7,4]. Organic acids are used in the food industry to prevent deterioration and prolong the shelf life due to their antimicrobial activity and are characterized as food additives and preservatives [8, 9, 10], which ensures the maintenance of the quality of the TMR for longer periods [11].

Seeking to elucidate gaps on these topics, especially in beef cattle, the present study evaluated the effect of ammonium dipropionate (antimicrobial stabilizer based on propionic acid) on TMR and also evaluated the fractional and non-fractional supply of the diet on the performance, carcass traits, and ingestive behavior of feedlot bulls in the finishing phase. The hypothesis was that the use of the stabilizer reduces the frequency of daily TMR supply, without impairing animal performance.

2. Material and methods

The experiment was carried out at the Laboratory of Food Analysis and Ruminant Nutrition, and at the Didactic, Research, and Extension Unit in Beef Cattle - Feedlot of the Animal Production Center (NUPRAN) along with the Master's Program in Veterinary Sciences, Agricultural Sciences Sector and Environmental Studies, State University of the Midwest (CEDETEG/ UNICENTRO), located in the municipality of Guarapuava, state of Paraná, Brazil (25º23'02" S and 51º29'43" W). All experimental procedures were previously submitted to the Animal Research Ethics Committee (CEUA/UNICENTRO), having been approved for execution (Official Letter 056/2019).

Experimental animals were 32 bulls, ½ Angus and ½ Nellore, from the same herd, with an average age of 11 ± 2 months, an initial average body weight of 350 ± 5 kg, previously dewormed. This was a 2 x 2 factorial completely randomized experimental design, aiming to evaluate the inclusion of ammonium dipropionate in the diet combined with fractional and non-fractional supply, constituting four treatments: Diet without ammonium dipropionate supplied twice a day; Diet without ammonium dipropionate once a day; Diet with ammonium dipropionate twice a day; Diet with ammonium dipropionate diet once a day.

The facilities consisted of 16 feedlot pens, with 15 m^2 each (2.5 m x 6.0 m). Each pen had a concrete trough measuring 2.30 m long, 0.60 m wide, and 0.35 m high, and a metal trough equipped with a float for automatic water replenishment. The experimental period lasted 105 days, with 16 days for adaptation to diets and facilities, plus 89 days for data collection, divided into 3 periods, with two evaluation periods of 28 days each and a third period of 33 days.

The ammonium dipropionate used was the commercial product Mold-Zap® 55, registered with the Ministry of Agriculture, Livestock and Food Supply (MAPA) by the company Alltech, under number PR-05224 00049, REV.03.11 TB/REV1013PF, and is classified as a preservative additive based on propionic acid at a dose of 550 g kg^{-1}, ammonium hydroxide at a dose of 120 g kg^{-1} and water. Animals assigned to treatments that received the fractional diet were fed at 06h00 and 17h00, and the animals that did not receive the fractional diet were fed only at 17h00. All diets were provided "ad libitum". At the time of feeding, ammonium dipropionate was applied and homogenized. The dose used was 1.25 mL kg^{-1} TMR, which is recommended by the manufacturer.

Voluntary intake was recorded daily, by weighing the amount offered and the leftovers from the previous day, considering the adjustment of daily intake, to allow leftovers at 5% of the total supplied. Diets consisted of 40% corn silage and 60% concentrate. The concentrate was prepared in the commercial feed factory of Cooperativa Agrária (Guarapuava, Paraná, Brazil), based on soybean meal, wheat bran, corn grain, malt radicle, calcitic limestone, dicalcium phosphate, livestock urea, common salt, mineral vitamin premix, as pellets. Samples of corn silage and concentrate were dried in a forced air oven at 50°C for 72 hours, to determine the partially dry matter. The pre-dried samples were ground in a Wiley mill with a 1mm diameter sieve and subsequently sent for chemical analysis.

The pre-dried samples of corn silage and concentrate were analyzed for contents of dry matter (DM), mineral matter (MM), ether extract (EE), and crude protein (CP), according to techniques described in AOAC

[12]. The content of neutral detergent fiber (NDF) was obtained according to Van Soest et al.[13], using thermostable α-amylase and acid detergent fiber (ADF), according to Goering and Van Soest[14]. Total digestible nutrient (TDN) contents were calculated according to equations proposed by Weiss et al.[15]. To determine the total dry matter, samples were oven-dried at 105ºC [16], and to determine the P and Ca contents, analyses were carried out according to Tedesco et al.[17].

Table 1. Chemical composition of silages and experimental diets

Composition	Corn silage	Concentrate	TMR
Dry matter, %	28.99	90.40	59.70
Mineral matter, % DM	2.85	8.43	5.64
Crude protein, % DM	7.54	21.35	14.45
Ether extract, % DM	2.99	2.92	2.96
Neutral detergent fiber, % DM	47.05	24.72	35.89
Acid detergent fiber, % DM	26.61	8.65	17.63
Lignin, % DM	3.68	1.40	2.54
Ca, %	0.15	1.49	0.82
P, %	0.23	0.51	0.37
Total digestible nutrients, %	69.21	80.90	75.06

Premix guaranteed level per kg concentrate:16,000 IU vit. A; 2,000 IU vit. D3; 25 IU vit. E, 0.36 g S; 0.74 g Mg; 3.6 g Na; 0.52 mg Co; 22.01 mg Cu; 1.07 mg I; 72.80 mg Mn; 0.64 mg Se; 95.20 mg Zn and 40 mg sodium monensin.

Bulls were individually weighed at the end of each experimental period, after solid fasting for 10 hours. For animal performance, body weight (BW), dry matter intake, expressed in kg animal day^{-1} (DMI), dry matter intake, expressed as a percentage of body weight (DMI, % BW), average daily weight gain (ADG, kg day^{-1}) and feed conversion (FC, kg kg^{-1}). DMI was measured through the difference between the daily amount of food supplied and the amount of leftovers from the previous day. DMI, % BW was obtained by the ratio of DMI to the mean BW for the period, multiplied by 100 (DMI, % BW= DMI/BW*100). ADG was considered as the difference between the final (BWf) and initial BW (BWi) of the experimental period divided by the evaluated days (ADG = BWf - BWi/28 and/or 33). FC was obtained by the ratio of DMI to ADG (FC= DMI/ADG).

During the experiment, feces of each pen were daily scored, by visual inspection. This evaluation followed the methodology adapted from Looper et al.[18] and Ferreira et al.[19]. The ingestive behavior was analyzed during the second evaluation period (day 42 of the experimental period), in a continuous time of 48 hours, starting at noon on the first day and ending at noon on the third day of evaluation. Observations were taken by seven observers per shift, for 48 hours, in a rotation system every 6 hours, with readings taken at regular intervals of 3 minutes.

Animal behavior data, represented by idleness, rumination, and liquid and solid intake, were expressed in hours day^{-1}. On the same occasion, following the same methodology, the frequency of occurrence of feeding, watering, urination, and defecation activities, expressed in number of times per day, was observed. For nocturnal observations, the environment was maintained with artificial lighting, a condition kept since the beginning of the experimental period.

The apparent digestibility of the diet was evaluated in two moments, also in a continuous time of 48 hours, one at the end of the first (initial phase) and another at the end of the third (final phase) experimental period; and from these two evaluations, the mean values were obtained. For this analysis, a homogeneous sample of the produced feces was taken and stored under refrigeration at intervals of six hours. After two consecutive days of collection, these were homogenized to form a composite sample for laboratory analysis. At the end of each shift, the total volume produced was estimated and the fecal pH was determined.

Concomitantly, food was collected once a day during the apparent digestibility test, which was freezer-stored. After the end of the evaluation, samples were thawed, and homogenized to form a composite sample, per pen and treatment. The daily intake of food and leftovers was determined. Samples of diets and feces were dried in a forced air oven at 55°C to constant weight, and corrected for the total dry matter at 105°C, for determination of dry matter content. DM of leftovers and feces of each experimental unit was determined by the same procedures adopted in diet analysis.

The DM digestibility coefficient (DC) of the experimental diets was calculated using the equation: DC (%) = [(g ingested nutrient – g excreted nutrient) / g ingested nutrient] x 100. At the end of the experimental period, animals were fasted for solids for 10 hours for weighing relative to the last evaluation period, and after that, animals were fed and weighed before transportation to the slaughterhouse, obtaining the farm weight.

Carcass gain in the feedlot period (CG) expressed in kg was obtained by the difference between hot carcass weight at slaughter and the initial body weight (BW) of the animals under a theoretical carcass yield of 50%. Taking the feedlot period of 89 days as a basis, the average carcass gain (ACG) was also calculated, expressed in kg day^{-1}, which is obtained by the ratio of CG to BW, as well as the efficiency of transformation of the dry matter ingested into carcass (ETC), expressed in kg DM kg carcass and the efficiency of transformation of weight gain into carcass, obtained by the ratio of ACG to ADG (ACG / ADG), expressed in %. For the calculations, hot carcass weights were used.

Five development measures were taken in the carcasses: carcass length, leg length, arm length, arm perimeter, and round thickness according to the methodologies suggested by Muller [20]. At the time of slaughter, non-carcass components were weighed (head, tongue, tail, leather, paws, and testicle, which are called external components; and empty heart, kidneys, liver, lungs, spleen, full and empty rumen-reticulum, full and empty abomasum, full and empty omasum, full and empty intestines, which are called vital organs).

Data collected for each variable were tested by analysis of variance with comparison of means at 5% significance, using the SAS statistical software[21]. The analysis of each variable followed the statistical model: Yij = μ + DFi + ADj + (DFi*ADj)ijk + Eijkl; where: Yij = dependent variables; μ = Overall mean of all observations; DFi = diet fractionation effect of order "i"; ADj = Effect of ammonium dipropionate of order "j"; DFi*ADj = effect of the interaction between diet fractionation of order "i" and the use of ammonium dipropionate of order "j"; and Eijkl = Residual random effect.

3. Results and discussion

There was no interaction (P>0.05) between the inclusion of ammonium dipropionate and diet fractionation, on the performance of feedlot finished bulls (Table 2). For ADG kg day^{-1}, DMI kg day^{-1}, and DMI % BW, there was a

Table 2. Performance of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet.

Ammonium dipropionate	Frequency of supply	Feedlot period 0 to 28 days	Feedlot period 0 to 56 days	Feedlot period 0 to 89 days
		Average daily weight gain, kg day^{-1}		
Without	One	1.393	1.509	1.458
Without	Two	1.598	1.665	1.670
With	One	1.589	1.641	1.535
With	Two	1.857	1.864	1.833
Mean without ammonium dipropionate	1.496 b	1.587 b	1.564 b	
Mean with ammonium dipropionate	1.723 a	1.752 a	1.684 a	
Mean with one meal	1.491 B	1.575 B	1.496 B	
Mean with two meals	1.728 A	1.765 A	1.751 A	
		Dry matter intake, kg day^{-1}		
Without	One	9.29	9.94	10.06
Without	Two	9.16	9.42	9.51
With	One	9.54	9.76	9.77
With	Two	10.44	10.78	10.88
Mean without ammonium dipropionate	9.22 b	9.68 b	9.78 b	
Mean with ammonium dipropionate	9.99 a	10.27 a	10.33 a	
Mean with one meal	9.41 A	9.85 A	9.91 A	
Mean with two meals	9.80 A	10.10 A	10.20 A	
		Dry matter intake, % BW		
Without	One	2.16	2.20	2.12
Without	Two	2.20	2.14	2.04
With	One	2.30	2.23	2.13
With	Two	2.34	2.28	2.18
Mean without ammonium dipropionate	2.18 b	2.17 b	2.08 b	
Mean with ammonium dipropionate	2.32 a	2.26 a	2.16 a	
Mean with one meal	2.23 A	2.21 A	2.13 A	
Mean with two meals	2.27 A	2.21 A	2.11 A	
		Feed conversion		
Without	One	6.80	6.75	7.08
Without	Two	5.76	5.67	5.70
With	One	6.08	5.99	6.41
With	Two	5.63	5.81	5.94
Mean without ammonium dipropionate	6.28 a	6.21 a	6.39 a	
Mean with ammonium dipropionate	5.86 b	5.90 a	6.17 a	
Mean with one meal	6.44 A	6.37 A	6.74 A	
Mean with two meals	5.70 B	5.74 B	5.82 B	

Mean values, followed by different lowercase letters in the same column, differ from each other by F-test at 5%, in the comparison between the presence and absence of ammonium dipropionate in the feed. Mean values, followed by different uppercase letters in the same column, differ from each other by F-test at 5%, in the comparison between fractional and non-fractional diet supply.

significant difference (P<0.05) between the inclusion or not of ammonium dipropionate in the diet in both evaluation periods, whereas the FC showed a difference only in the first period (0 to 28 days). For both parameters, the use of ammonium dipropionate was better than not using it, that is, it increased daily intake and weight gain, and improved FC in the first period. As for fractionating or not the diet supply, there was a significant difference (P<0.05) for ADG kg day^{-1} and FC. Animals that received the diet twice a day showed greater weight gain and better FC compared to the animals that received the diet once a day in both evaluation periods.

According to Kung et al.[11], the addition of propionic acid to TMR at the time of supply delayed food spoilage in the trough, reducing pH and temperature after 24 hours of exposure to O_2. In addition, these authors state that one of the main objectives in using a stabilizer like ammonium dipropionate is to guarantee the potential of animal performance due to the provision of a stable and quality diet. Given this, although TMR stability was not evaluated in the present study, a better performance was observed, in the average daily gain of animals receiving food with better aerobic stability. This was achieved by the presence of ammonium dipropionate that preserved the TMR, or the fractional supply of the diet, which shortened the time the TMR remained in the trough, and animals had access to smaller portions with higher quality twice a day.

Preserved foods exposed to O_2 for longer tend to show the proliferation of microorganisms that cause heating and loss of nutritional value, generating a food of dubious quality, and reduced consumption by the animals[22]. Propionic acid guarantees greater stability to the TMR and enhances the flavor [6], thus these may have been the factors that influenced the increase in dry matter intake, both expressed in % body weight and in kg day^{-1}, which ensured greater average daily weight gain, kg day^{-1} when ammonium dipropionate was used.

The use of additives to inhibit undesirable fermentation favors the aerobic stability of preserved foods and results in food with reduced losses caused by exposure to O_2, maintaining its nutritional value [23]. Propionic acid-based products can improve the aerobic stability of preserved foods after exposure to O_2 [11, 24], thus the inclusion of ammonium dipropionate at the time of feeding the animals reduced losses in nutritional quality due to the good stability of the TMR, explaining the better FC compared to the group that did not receive ammonium dipropionate. The same may have occurred concerning diet fractionation, where the animals that were given two meals during the day, reached a better FC due to the time in which the diet remained exposed to O_2.

Table 3 lists data on carcass gain and efficiency of the transformation of the dry matter ingested into carcass. For CG, kg, and ACG, kg day^{-1}, there was an interaction between the inclusion of ammonium dipropionate and diet fractionation. Animals that received two meals with the inclusion of ammonium dipropionate showed the highest mean values for CG, kg and ACG, kg day^{-1} (111.4 kg and 1.251 kg, respectively), but did not differ from animals fed once or twice a day without the inclusion of ammonium dipropionate in the diet, whereas the animals that were fed once a day with the inclusion of ammonium dipropionate had the lowest mean values for CG, kg and ACG, kg day^{-1} (91.7 kg and 1.030 kg, respectively). Regarding the parameters of ETC and ACG ADG^{-1}, there was no interaction between the use of ammonium dipropionate and fractionation or not of the feeding. Only the ETC showed a statistical difference (P<0.05), where animals fed twice a day, on average, were more efficient.

Table 3. Gain and efficiency of carcass gain of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet.

Ammonium dipropionate	Frequency of supply		Mean
	One	Two	
GC, kg			
Without	96.4 ab*	98.6 ab*	97.5
With	91.7 b*	111.4 a*	101.5
Mean	94.0	105.0	
ACG, kg day^{-1}			
Without	1.083 ab*	1.108 ab*	1.096
With	1.030 b*	1.251 a*	1.141
Mean	1.057	1.180	
ETC, kg DM kg gain^{-1}			
Without	9.33	8.61	8.97
With	9.52	8.70	9.11
Mean	9.42 A	8.66 B	
ACG ADG^{-1}, %			
Without	68.5	66.4	67.5
With	67.5	68.3	67.9
Mean	68.0	67.4	

Mean values, followed by different uppercase letters in the same column or different lowercase letters in the same row differ from each other by F-test at 5%. *Breakdown of the interaction between the use or not of ammonium dipropionate and feeding frequency.

The best ETC, as well as the highest mean value of GC and ACG of animals fed twice a day, is positive since the greater weight gain of these animals (Table 2) was converted into carcass and not into viscera, which in some cases may occur. The reduction in the frequency of supplying the diet to the animals may imply variations in ruminal functions (uniformity of rumen fermentation, reduced fiber digestion, and pH instability) throughout the day, and to obtain the best animal performance, such variations need to be avoided [25,26,27,28]. Therefore, these variations probably did not occur in the animals fed twice a day, ensuring better performance. In addition, the inclusion of ammonium dipropionate in the TMR is advantageous, when combined with the fractional supply of the feed, since it resulted in an increase of 17.68% in carcass gain, compared to the group that received ammonium dipropionate along with the non-fractional supply.

Table 4 lists results on farm body weight and the quantitative characterization of carcasses at slaughter. There was an interaction (P<0.05) between the inclusion or not of ammonium dipropionate and the fractional or non-fractional supply of the diet, for farm body weight, hot carcass weight, and carcass length, where the animals fed a diet with ammonium dipropionate twice a day were superior to the others (557.5 kg, 308.6 kg and 133.25 cm, respectively). The other parameters in Table 4 showed no interaction, however when analyzing the individual effect of the treatments, a significant difference (P<0.05) was detected for fat thickness, where the animals that received ammonium dipropionate in the diet were superior to those that did not receive it (5.50 mm versus 4.94 mm, respectively). The other evaluated parameters showed no statistical difference (P>0.05).

The greatest thickness of subcutaneous fat for animals receiving ammonium dipropionate in the diet may be related to greater preservation of soluble carbohydrates in the diet. A higher concentration of these carbohydrates and a possible association with ammonium dipropionate may favor the production of propionic acid in the rumen, and according to Kozloski [29] and Gonçalves et al. [30], when propionic acid enters the gluconeogenesis cycle, it becomes a glucose precursor, stimulates insulin production, and when the animal is in a positive energy balance, this hormone is responsible for activating lipogenesis

Values of fat thickness found in the present study are satisfactory, and important, because when there is little fat covering the carcass, qualitative losses may occur during carcass cooling, darkening its external face and shortening muscle fibers, which impair the visual pattern and the tenderness of the meat, leading to a commercial devaluation [20, 31]. In this context, it is worth emphasizing the importance of adding the antimicrobial in the TMR, as it improved performance (Table 3 and Table 4), and resulted in better carcass finishing. Even with no significant effect, the carcass yield deserves attention, because regardless of the fractional supply of the diet or the inclusion of the antimicrobial agent, carcass yield was greater than 54%, which is considered satisfactory. Carcass yields of this magnitude become attractive for meatpacking industries as they generate greater profitability [32].

Table 4. Farm body weight and carcass traits of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet.

Ammonium dipropionate	Frequency of supply		Mean
	One	Two	
	Farm body weight, kg		
Without	519.6 b*	520.9 b*	520.3
With	506.5 b*	557.5 a*	532.0
Mean	513.1	539.2	
	Hot carcass weight, kg		
Without	284.8 b*	284.8 b*	284.8
With	276.7 b*	308.6 a*	292.6
Mean	280.7	296.7	
	Carcass length, cm		
Without	129.38 bc*	129.88 b*	129.63
With	127.69 c*	133.25 a*	133.25
Mean	128.53	131.56	
	Carcass yield, %		
Without	54.8	54.7	54.7
With	54.9	55.3	55.1
Mean	54.8	55.0	
	Fat thickness, mm		
Without	4.88	5.00	4.94 b
With	5.63	5.38	5.50 a
Mean	5.25	5.19	
	Round thickness, cm		
Without	18.19	18.81	18.50
With	18.63	18.69	18.66
Mean	18.41	18.75	
	Arm length, cm		
Without	38.19	39.88	39.03
With	38.06	38.75	38.41
Mean	38.13	39.31	
	Arm perimeter, cm		
Without	43.44	41.81	42.63
With	40.94	43.56	42.25
Mean	42.19	42.69	

Mean values, followed by different uppercase letters in the same column or different lowercase letters in the same row differ from each other by F-test at 5%. *Breakdown of the interaction between the use or not of ammonium dipropionate and feeding frequency.

Non-carcass components represented by internal organs showed no interaction between the use of ammonium dipropionate and diet fractionation (Table 5). When evaluating the non-carcass components considering the inclusion or not of ammonium dipropionate, only the liver weight showed a difference (P<0.05), where the animals that received ammonium dipropionate in the diet had greater liver weights compared to those that did not receive it (1.07 kg against 0.93 kg, respectively). Therefore, when evaluating the non-carcass components considering the fractional or non-fractional supply of the diet, there was no significant difference (P>0.05).

Table 5. Mean proportion of non-carcass components of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet.

Ammonium dipropionate	Frequency of supply		Mean
	One	Two	
Hearth weight, % BW			
With	0.31	0.30	0.30
Without	0.32	0.32	0.32
Mean	0.31	0.31	
Lung weight, % BW			
With	0.93	0.88	0.90
Without	0.92	0.96	0.94
Mean	0.93	0.92	
Spleen weight, % BW			
With	0.32	0.33	0.32
Without	0.33	0.37	0.35
Mean	0.33	0.35	
Kidney weight, % BW			
With	0.19	0.22	0.20
Without	0.20	0.21	0.21
Mean	0.20	0.22	
Liver weight, % BW			
With	0.92	0.94	0.93 b
Without	1.10	1.04	1.07 a
Mean	1.01	0.99	
Full rumen-reticulum weight, % BW			
With	7.61	7.70	7.66
Without	7.73	7.61	7.67
Mean	7.67	7.65	
Empty rumen-reticulum weight, % BW			
With	2.69	2.75	2.72
Without	2.83	2.72	2.78
Mean	7.76	2.73	
Full abomasum weight, % BW			
With	0.46	0.41	0.43
Without	0.41	0.41	0.41
Mean	0.43	0.41	
Empty abomasum weight, % BW			
With	0.35	0.36	0.36
Without	0.39	0.35	0.37
Mean	0.37	0.36	
Full intestine weight, % BW			
With	4.12	4.22	4.17
Without	4.22	4.26	4.24
Mean	4.17	4.24	

Mean values, followed by different uppercase letters in the same column or different lowercase letters in the same row differ from each other by F-test at 5%.

The liver plays an essential role in metabolic pathways. Food intake, energy requirements for maintenance, and weight gain directly influence the development of this organ [33]. Therefore, the heaviest livers were those of the animals that consumed the most DM (Table 2). The external components of the carcass, represented by the head, tongue, paws, tail, leather, and testicles (Table 6), showed no interaction (P>0.05) between the presence or absence of ammonium dipropionate in the feed and fractional or non-fractional supply of the diet, and likewise, when analyzing these components separately, there was no significant difference (P>0.05).

Table 6. Mean proportion of external components of the carcass of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet.

Ammonium dipropionate	Frequency of supply		Mean
	One	Two	
Head weight, % BW			
Without	2.23	2.22	2.23
With	2.18	2.12	2.15
Mean	2.21	2.17	
Tongue weight, % BW			
Without	0.17	016	0.17
With	0.18	0.17	0.18
Mean	0.18	0.17	
Paws weight, % BW			
Without	2.27	2.36	2.31
With	1.96	1.90	1.93
Mean	2.11	2.13	
Tail weight, % BW			
Without	0.25	0.25	0.25
With	0.24	0.26	0.25
Mean	0.25	0.25	
Leather weight, % BW			
Without	9.76	9.82	9.79
With	9.09	8.68	8.89
Mean	9.42	9.25	
Testicle weight, % BW			
Without	0.32	0.32	0.32
With	0.27	0.29	0.28
Mean	0.30	0.30	

Mean values, followed by different uppercase letters, in the same column, or different lowercase letters, in the same row, differ from each other by F-test at 5%.

Studies of non-carcass components represented by the internal (Table 5) and external (Table 6) organs of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet are scarce in the literature. However, the lack of differences for these components is important from the producers' point of view, since they are not remunerated for non-carcass components.

The fecal score (Table 7) showed no differences (P>0.05) between treatments or periods evaluated. This lack of difference is a good result and demonstrates that regardless of the fractional supply of the diet or the inclusion of ammonium dipropionate, animals maintained an ideal score according to the classification by Kononoff et al. [34], where the feces had a pasty consistency, producing piles from 2.54 to 5.08 cm in height, which according to these authors is normal and demonstrates adequate health of the animals.

Table 7. Fecal score of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet.

Ammonium dipropionate	Frequency of supply	Feedlopt period		
		0 to 28 days	0 to 56 days	0 to 89 days
		Fecal score		
Without	One	3.08	3.04	3.04
Without	Two	3.11	3.07	3.05
With	One	3.08	3.04	3.04
With	Two	3.07	3.05	3.04
Mean without ammonium dipropionate	3.09	3.06	3.05	
Mean with ammonium dipropionate	3.08	3.05	3.04	
Mean with one meal	3.08	3.04	3.04	
Mean with two meals	3.09	3.06	3.05	

Mean values followed by different lowercase letters, in the same column, differ from each other by F-test at 5%, in the comparison between the presence and absence of ammonium dipropionate in the feed. Mean values followed by different uppercase letters, in the same column, differ from each other by F-test at 5%, in the comparison between fractional and non-fractional diet supply.

Table 8 lists the data on fecal output, fecal dry matter content, and fecal pH, in addition to the apparent digestibility of dry matter. Of these, there was interaction (P<0.05) between treatments only for fecal output and apparent dry matter digestibility. Fecal output in both natural and dry matter was higher for animals fed twice a day that received ammonium dipropionate in the diet (16.68 kg and 2.84 kg, respectively). The higher fecal output is justified due to higher DM intake (Table 2). The apparent digestibility of DM was higher in animals fed twice a day that received ammonium dipropionate in the diet, followed by animals fed once a day that received ammonium dipropionate in the diet (74.57% and 73.66%, respectively).

The greater apparent digestibility for the diets with ammonium dipropionate may be a consequence of lower deterioration that occurs in the silage after exposure to O_2. According to Horst et al. [35], when there is food degradation after its exposure to O_2, molds emerge, DM reduces and digestibility decreases. When evaluating the effect of including or not a propionic acid-based additive in the TMR combined with the number of meals (1 or 2 times) to dairy cows, Dias et al. [36] observed no significant differences (P<0.05) in fecal analysis, citing only a trend towards higher NDF digestibility.

Table 8. Fecal output, dry matter content, apparent digestibility of dry matter, and fecal pH of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet.

Ammonium dipropionate	Frequency of supply		Mean
	One	Two	
	Fecal output, kg NM day^{-1}		
Without	16.44 b*	15.50 b*	15.97
With	14.98 b*	16.68 a*	15.83
Mean	15.71	16.09	
	Fecal output, kg DM day^{-1}		
Without	2.78 b*	2.65 b*	2.72
With	2.58 b*	2.84 a*	2.71
Mean	2.68	2.74	
	Fecal dry matter content, %		
Without	17.06	17.13	17.09
With	17.26	17.04	17.15
Mean	17.16	17.08	
	DM digestibility, %		
Without	72.29 c*	72.32 c*	72.31
With	73.66 b*	74.57 a*	74.11
Mean	72.98	73.45	
	Fecal pH		
Without	7.47	7.55	7.51
With	7.42	7.51	7.46
Mean	7.45	7.53	

Mean values followed by different lowercase letters, in the same column, differ from each other by F-test at 5%, in the comparison between the presence and absence of ammonium dipropionate in the feed. Mean values followed by different uppercase letters, in the same column, differ from each other by F-test at 5%, in the comparison between fractional and non-fractional diet supply. *Breakdown of the interaction between the use or not of ammonium dipropionate and feeding frequency.

Table 9 lists the parameters of ingestive behavior represented by idleness, rumination, water, and food consumption expressed in hours day^{-1}, under the effect of the presence or absence of ammonium dipropionate in the diet combined with the fractional or non-fractional supply of the diet, according to the feedlot phase, and there was no statistical difference (P>0.05) for the parameters.

Table 9. Ingestive behavior of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply of the diet.

Ammonium dipropionate	Frequency of supply		Mean
	One	Two	
Idleness, hours day^{-1}			
Without	14.14	15.48	14.81
With	14.07	13.93	14.00
Mean	14.11	14.70	
Rumination, hours day^{-1}			
Without	6.80	5.44	6.12
With	6.82	6.90	6.86
Mean	6.81	6.17	
Water consumption, hours day^{-1}			
Without	0.30	0.27	0.28
With	0.27	0.27	0.27
Mean	0.28	0.27	
Feed intake, hours day^{-1}			
Without	2.77	2.83	2.80
With	2.84	2.91	2.88
Mean	2.81	2.87	

Mean values, followed by different uppercase letters, in the same column, or different lowercase letters, in the same row, differ from each other by F-test at 5%.

According to Bürger et al. [37] and Pinto et al. [38], ingestive behavior is mainly influenced by the type and concentration of energy in the diet offered to the animals. Therefore, the lack of differences in behavioral parameters is important, as it shows that there were no variations between the diets tested in the present study. Table 10 presents the ingestive behavior parameters represented by the frequency of occurrence of feeding, drinking, urination, and defecation, expressed in times per day, under the effect of the presence or absence of ammonium dipropionate in the diet combined with the fractional or non-fractional supply of the diet, according to the feedlot period, and there was also no statistical difference (P>0.05) for the parameters.

The results in Tables 9 and 10 indicated that adding or not adding ammonium dipropionate and/or fractioning or not the supply of the diet does not interfere with the ingestive behavior, but reinforces the idea that the main factor of change in the ingestive behavior of cattle is the composition of the diet these animals were given, that is, the forage: concentrate ratio, digestibility of dietary constituents, energy level and particle size [39, 40, 41].

Table 10. Ingestive behavior of feedlot finished bulls under the effect of the presence or absence of ammonium dipropionate in the feed combined with the fractional or non-fractional supply

Ammonium dipropionate	Frequency of supply		Mean
	One	Two	
Feeding, times day^{-1}			
Without	20.3	20.1	20.2
With	21.7	20.0	20.8
Mean	21.0	20.1	
Drinking, times day^{-1}			
Without	8.3	8.8	8.5
With	8.3	8.8	8.5
Mean	8.3	8.8	
Urinating, times day^{-1}			
Without	9.4	6.4	7.9
With	6.5	9.1	7.8
Mean	8.0	7.8	
Defecating, times day^{-1}			
Without	10.7	7.9	9.3
With	9.8	11.8	10.8
Mean	10.2	9.8	

Mean values, followed by different uppercase letters, in the same column, or different lowercase letters, in the same row, differ from each other by F-test at 5%.

4. Conclusions

The inclusion of ammonium dipropionate caused an overall average increase in weight gain, dry matter intake, and carcass gains. The supply of the diet twice a day also promoted, in the overall average, an increase in the average weight gain and carcass gains, as well as improved the feed conversion of the animals. The combination of ammonium dipropionate with the supply of the diet twice a day improved the carcass parameters and resulted in better apparent digestibility of dry matter.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M. Neumann and T. Durman. *Data curation*: M. Neumann. *Formal Analysis*: M. Neumann. *Funding acquisition*: M. Neumann. *Project administration*: E. R. Almeida. *Methodology*: M. Neumann and T. Durman. *Supervision:* E. R. Almeida. *Investigation:* A. M. Souza, F. B. Cristo, E. Baldissera and E. E. Bremm. *Visualization*: E. R. Almeida and A. M. Souza. *Writing (original draft):* E. R. Almeida. *Writing (review & editing)*: M. Neumann and A. M. Souza.

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