DOI: 10.5433/1679-0359.2016v37n6p4221

Production performance and safety of meat from beef cattle finished in feedlots using salinomycin in the diet

Desempenho produtivo e inocuidade da carne de bovinos terminados em confinamento com salinomicina na dieta

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Abstract

This study assessed production performance, carcass characteristics, serum parameters, residue depletion of meat, and economic performance during the finishing period of steers in feedlots using salinomycin in their diet. A total of 32 steers were finished in a feedlot on a diet comprising corn silage and concentrate (50:50), with or without added salinomycin (120 mg per animal day^{-1}). Study design was completely randomized, with eight repetitions. Results indicated that the use of salinomycin increased both weight gain (1.582 vs. 1.304 kg) and feed conversion (6.16 vs. 7.25 kg kg^{-1}). No significant alterations were observed in feed intake (9.52 vs. 9.25 kg animal day^{-1}), serum parameters, or apparent diet digestibility. The withdrawal period of 16 h prior to slaughter promoted a lower salinomycin concentration (0.25 μg kg^{-1}) in organs and edible tissues, which is below the levels allowed by legislation. Animals finished with salinomycin also exhibited greater warm carcass weight (287.76 vs. 275.81 kg) and better economic performance (profit margin increments of R$ 84.20 per animal). Salinomycin use in feedlot-finished steers promoted both production and economic performance improvements without added implications to either animal or consumer health since there were no significant residues found in any edible tissues.
Key words: Growth promotion additives**.** Feed conversion. Ionophores. Withdrawal period.

Resumo

Objetivou-se avaliar o desempenho produtivo, as características de carcaça, os parâmetros séricos, a depleção residual na carne e a economicidade da terminação de novilhos em confinamento com salinomicina na dieta. Foram confinados 32 animais com dieta constituída de silagem de milho e concentrado (50:50), adicionada ou não de salinomicina (120 mg por animal dia^{-1}). O delineamento foi inteiramente casualizado com 8 repetições. O uso de salinomicina melhorou o ganho de peso (1,582 vs 1,304 kg) e a conversão alimentar (6,16 vs 7,25 kg kg^{-1}). Não houve alterações significativas no consumo

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Received: Nov. 26, 2015 – Approved: Aug. 10, 2016

de alimento (9,52 vs 9,25 kg animal dia^{-1}), nos parâmetros séricos e na digestibilidade aparente da dieta. O período de carência de 16 h antes do abate promoveu concentrações de salinomicina inferiores a 0,25 µg kg^{-1} nos órgãos e tecidos comestíveis, cujos valores estão abaixo dos permitidos na legislação. Animais terminados com salinomicina apresentaram maior peso de carcaça quente (287,76 vs 275,81 kg) e melhor resultado econômico, com incremento de R$ 84,20 por animal na margem de lucro. O uso de salinomicina para novilhos em confinamento promoveu melhorias no desempenho produtivo e econômico, sem prejudicar a saúde dos animais e dos consumidores, pois não houve resíduos significantes nos tecidos comestíveis.
Palavras-chave: Aditivo promotor de crescimento**.** Conversão alimentar. Ionóforo. Período de carência.

Introduction

Finishing cattle in feedlot in Brazil is an activity that is currently growing at around 6% per year. On average, 8 to 10% of all the bovines slaughtered each year are produced using cattle fattening operations performed through the feedlot system (ANUALPEC, 2014). Additionally, because feed efficiency is the indicator that best correlates with profitability in the feedlot system, the use of feed additives thus becomes an opportunity to increase bovine production and economic performance.

Ionophores are among the many additives available in the Brazilian industry, and these act by manipulating rumen fermentation through the selection of microbiota, facilitating better efficiency of energy and protein metabolism, and reducing the incidence of digestive disorders, all of which are factors that may increase overall productivity (BERGEN; BATES, 1984).

Ionophores are produced through the fermentation of different strains of *Streptomyces sp*., and act by causing ion transport alterations in bacterial cell membranes. These substances form a liposoluble complex with cations and mediate their transport through lipid membranes, resulting in osmotic imbalances within cells, losses of function, and bacterial death (RANGEL et al., 2008).

There are more than 120 ionophores described in literature (NAGARAJA et al., 1997). In Brazil, only six are approved for use in animal feed: lasalocid (cattle, poultry, and rabbits), monensin (cattle and poultry), salinomycin (pigs, cattle, and poultry), maduramicin (poultry), narasin (poultry), and semduramicin (poultry) (BRASIL, 2008).

According to Kobayashi (2010), the ionophores used in cattle production promote the overall reduction of gram-positive bacteria populations and methanogenic ciliate protozoa, which possess methanogenic bacteria both internally and superficially. Therefore, ionophores are capable of promoting a reduction in rumen methane and an increase in digestive efficiency.

Improvements in feed efficiency through energy metabolism results from increases in propionic acid production by gram-negative, ionophores-resistant bacteria, which consume free H^+ ions in the rumen in order to form this acid. Thus, methane production is reduced due to lower levels of free H^+ ions necessary for its formation, and because of reductions in the populations of gram-positive methanogenic bacteria present (OLIVEIRA et al., 2006; ZEOULA et al., 2008). This is a positive outcome, as the production of methane is responsible for the loss of 2 to 12% of food energy, and its release into the atmosphere contributes to environmental pollution (MITSUMORI; SUN, 2008).

Furthermore, ionophores may improve the efficiency of protein use in the diet, since they inhibit the growth of proteolytic bacteria in the rumen; this occurs through decreased loss of nitrogen by accumulation of ammonia in the rumen and via increased amino acid absorption in the intestine (YANG; RUSSELL, 1992). In general, feedlot finishing diets have a high concentration of non-fibrous carbohydrates, which may pose a risk to animal health due to digestive disorders. Use of ionophores, however, can reduce the number of incidences and the severity of some of these

disorders (NAGARAJA; LECHTENDBERG, 2007).

Grain overload speeds up rumen fermentation and the production of short chain fatty acids, which are associated with decreased saliva production, resulting in a pH reduction and favoring the proliferation of gram-positive bacteria such as *Streptococcus bovis*. These bacteria produce lactic acid, which is a strong acid that exacerbates conditions such as ruminal acidosis. Another effect of intense proliferation of *Streptococcus* and protozoa in the rumen is the production of mucopolysaccharides, which increases ruminal fluid viscosity and may cause bloat (BERGEN; BATES, 1984). Ionophores acts reducing the population of these bacteria; however, they do not have an effect over the types of bacteria that use lactic acid produced through rumen fermentation, such as *Selenomonas ruminantium*, thus controlling the occurrence and severity of conditions such as acidosis and bloat (CHENG et al., 1998).

In Brazil, three ionophores are available for use in bovine production, with sodium monensin being the most used. The majority of studies aimed at determining optimal doses and clarifying the effects of using salinomycin in the diets of beef cattle were performed in the 1980s. Since then, however, reports on the use of this additive in literature are scarce, although some recent studies related to the development of commercial products containing this additive have encouraged research focused on clarifying its efficacy.

The objective of this study was to assess production performance, carcass characteristics, serum parameters, residue depletion and withdrawal period in meat, and economic performance of finishing steers in feedlot utilizing a diet containing salinomycin.

Materials and Methods

The work was performed at the Animal Production Centre (NUPRAN) of Midwest State University (UNICENTRO), in Guarapuava, Paraná, Brazil, after the project was approved by the ethics committee for the use of animals in research (CEUA/UNICENTRO) under protocol number 027/2014 on 07 June 2014.

According to Köppen classification, the climate in this region is of type Cfb (humid mesothermal subtropical), with an annual average precipitation rate of 1,944 mm, an average annual minimum temperature of 12.7°C and maximum of 23.5°C, and relative air humidity levels of 77.9% at an altitude of 1,026 m.

A total of 32 steers mixed Angus × Charolais × Nelore, all from the same herd, were used in this study, with an initial average weight of 374 kg and average age of 12 months. These bovines were previously dewormed and divided into two groups, and were then balanced by weight and body condition in order to form the experimental units. The treatments comprised providing the animals with a diet of corn silage and concentrate (50:50), either without (control) or with ionophore (salinomycin).

The tested ionophore used was salinomycin (Saligran® G120, provided by the company Impextraco Latin America Ltda), which has still not been registered as a bovine feed additive with the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA). Obtaining this registry was also one of the primary goals of this study. This product was given at a dose of 1 g animal day^{-1}, which was sufficient given the available amounts of salinomycin (120 mg). The salinomycin used in this experiment is one of the additives currently authorized by MAPA to be used in beef cattle feed as a performance enhancer at doses of 100 to 120 mg animal day^{-1} (BRASIL, 2008).

The bovines w*/ere housed in 16 pens of feedlot, semi-covered, with an area of 15 m^2, that included a cement feeder and water dispenser controlled with a float. Each pen contained two bovines and was considered an experimental unit. The experimental

design was completely randomized, consisting of two treatments with eight repetitions, utilizing a scheme of plots subdivided over time for variables related to animal performance.

Feedlot had a duration of 112 days, with the initial 28 days focused on adaptation. Subsequently, three periods of 28 assessment days were performed. The animals were weighed at the beginning and end of each period, after fasting from solids for 12 hours (h). Feeding management was performed twice a day, at 6:00 and 17:00 h, and daily intake was calculated based on the difference in weight between the amount of food offered and the remaining leftovers from the previous day. Adjustments in diet provision were performed daily, with the goal of food being offered "ad libitum" while simultaneously considering average leftover rates of 5% of dry matter (DM).

The diet was formulated to promote the gain of 1.5 kg of body weight per animal per day (NRC, 2000) (Table 1). The diets were provided in the form of total mixed ration (TMR), with the group of animals treated with salinomycin receiving the product over the TMR in order to guarantee ingestion. The concentrate was made from a mix of soybean meal, soybean hulls, barley roots, ground corn kernels, calcitic lime, dicalcium phosphate, common salt, livestock urea, and mineral premix.

Table 1. Bromatological analysis of corn silage, concentrate, and experimental diets.

Parameters	Corn silage	Concentrate	Diet[1]
Dry matter content (%)	35.04	89.59	65.23
Crude protein (% of DM)	5.66	21.33	13.50
Mineral matter (% of DM)	2.24	9.68	5.96
Ethereal extract (% of DM)	3.03	3.95	3.49
Neutral detergent fiber (% of DM)	43.86	29.31	36.59
Acid detergent fiber (% of DM)	23.48	13.41	18.45
TDN, (% of DM)	71.40	75.60	73.50
Ca (% of DM)	0.14	1.67	0.91
P (% da DM)	0.22	0.58	0.40

[1]Composition: 50% of corn silage and 50% of concentrate;
TDN = total digestible nutrients; Ca= calcium; P= phosphorus.

Feed samples were collected from both rations and leftovers throughout the experiment. From these samples, content of crude protein (CP), mineral matter (MM), ethereal extract (EE), neutral detergent fiber (NDF) with thermostable α-amylase, and acid detergent fiber (FDA), were analyzed according to the methods described by Silva and Queiroz (2006). The total digestible nutrients (TDN) content was calculated according to equations proposed by Weiss et al. (1992).

In the evaluation of animal performance during feedlot, daily dry matter intake (DDMI, kg d^{-1}), intake expressed as a percentage of body weight (DMI, % BW), average daily weight gain (ADG, kg d^{-1}), and feed conversion (FC) were measured.

To assess the apparent digestibility of DM in the diets, total cattle fecal samples from bovines in each experimental unit were collected for three consecutive days during an intermediate phase of the feedlot period. Immediately following excretion, the feces were weighted and sampled in each 6 h period. After 72 h of collection, a representative feces sample was formed through dehydration. In subsequent laboratory analyses, the apparent digestibility of DM in the diet (AD) was determined using the expression: AD (%) = [(DM intake in g -DM excreted in g) ÷ DM intake in g] × 100.

On days 0, 28, 56, and 84, blood samples were collected to evaluate the serum biochemical profiles of liver and kidney function indicators. A 20 mL sample of blood from one animal per experimental unit was collected from the coccygeal vein, with 10 mL stored in a tube containing EDTA for fibrinogen quantification, and the remaining 10 mL stored in a tube also containing EDTA for quantification of gamma glutamyl transferase (GGT) and aspartate aminotransferase (AST), total serum protein, albumin, urea, and creatinine.

At the end of the feedlot period, after fasting from solids for 12 h, the bovines were weighed as they were loaded into the refrigerator, located at a distance of 5 km. The slaughtering process followed the standard flow of a commercial abattoir, according to applied legislation for bovine slaughter. The weight of the warm carcass was registered to calculate yield, carcass length, arm length, arm perimeter, thigh thickness, and subcutaneous fat thickness at the 12th rib level, according to the methodologies outlined by Muller (1987). The non-integrant components of the carcass were also weighed.

To evaluate residue depletion of salinomycin in edible tissues, the 16 bovines that originally received salinomycin were given food without salinomycin at intervals of 56 h (4 bovines), 32 h (6 bovines), and 16 h (6 bovines) before slaughter. Fat, muscle, liver, and kidney tissue samples from the bovines were collected during slaughter. Samples from one bovine from the control group (without salinomycin) were also collected, and were used to calibrate residue detection. These procedures were performed according to recommendations contained in guidelines VICH GL48 (2011).

Tissue samples were frozen at -20°C and shipped to the Quimiplan Analysis and Consulting Ltda. laboratory for quantification of salinomycin levels through high performance liquid chromatography, coupled with sequential mass spectrometry. Expected quantification limits (QLIs) were 10 µg kg^{-1} for muscle and fat, 175 µg kg^{-1} for liver, and 250 µg kg^{-1} for kidney, with respect to levels prescribed in current Brazilian legislation (BRASIL, 2004), according to the guidelines for residue maximum limits (RML) of salinomycin, as observed in the Japanese Positive List (JAPAN, 2014) and in Health Canada (CANADA, 2014).

In the performance of carcass weight gain evaluation, variables were calculated using an initial carcass theoretical yield of 50%, which is a value used conventionally when there is a lack of resources to perform bovine slaughtering at the beginning of the evaluation. Carcass gain in the feedlot period (CGF) was calculated, obtained by the difference between warm carcass weight at slaughter and initial theoretical weight (IBW = initial bodyweight × 0.5). The average daily gain of carcass (ADGC) was calculated based on an 84-day period of feedlot (ADGC = CGF ÷ 84). It was considered that carcass gain yield (CGY) demonstrated the percentage that the carcass gain represents with regard to average daily weight gain (ADG) of the live animal (CGY = ADGC ÷ ADG), and that the feed conversion in carcass (FCC) represents the relation between DDMI and ADGC.

In terms of economic analysis, the average of regional prices that occurred in 2014 were considered, as follows: finished cattle at R$ 120.29 per kg, unfinished cattle at R$ 4.61 per kg live, corn silage at R$ 0.25 per kg of DM, concentrate formulated with 19% of CP at R$ 712.43 per ton of DM, salinomycin at R$ 8.08 per kg of product Saligran® G120.

In the statistical analyses of data regarding performance in feedlot and under serum biochemical profiles, the experimental design was completely randomized, comprising two treatments, with eight repetitions, in a scheme of plots subdivided over time, during three evaluation periods. In terms of animal performance, each repetition comprised one pen with two bovines. In contrast, for the serum biochemical profile, samples were collected from one bovine per pen. Data collected for each variable were subjected to analysis of variance, with

comparison of averages at 5% of significance by Tukey's test for the variable's performance and the t test for the serum biochemical profile, using the statistical program SAS (1993).

For parameters related to the apparent digestibility of DM in diets, carcass characteristics and non-integrant components of the carcass, and carcass gain performance, the experimental design was completely randomized comprising two treatments with eight repetitions for apparent digestibility of DM (one pen containing two bovines) and 16 repetitions for the carcass data (two bovines from each pen). The data collected for each variable was then submitted for analysis of variance at 5% of significance, using the statistical program SAS (1993).

Statistical analysis of residue depletion of salinomycin in bovine tissues was performed according to the model established in the guidelines of EMEA (1996), which recommended that the withdrawal period and depletion of products should be determined from linear regression analysis through the "software Withdrawal-time Calculation Program WT1.4", using a confidence interval of 95%. In order for it to be possible to outline the depletion curve for determining the withdrawal period, at least three groups of data (three times the restriction of the active ingredient before slaughter) are required, with at least one group possessing values above the RML and another group having values bellow the RML. In addition, to fulfill the requirements, linearity analysis was performed (F test), and homogeneity of variance (Bartlett test) and normality (Shapiro-Wilk) were calculated.

Results and Discussion

There was no significant ($P > 0.05$) interaction between treatments and periods of evaluation for ADG, DDMI, DMI, and FC, as well as no significant ($P > 0.05$) difference noted among feedlot periods for ADG and DMI (Table 2). DDMI and FC, however, did show significant differences ($P < 0.05$) between periods, with an increase in DDMI and decrease in FC over time in the feedlot, regardless of the use of salinomycin. These responses are normal in the finishing phase of bovines, since DDMI increases due to increase in live weight, and FC decreases because the greatest proportion of fat gain occurs in the most advanced stages of the finishing process. The stability of ADG demonstrated that bovine management in the feedlot was efficient, allowing for high rates of gain during the period.

In this study, the inclusion of salinomycin did not promote alterations ($P > 0.05$) in the DDMI and DMI (Table 2). However, in terms of general averages, bovines treated with salinomycin showed increases ($P < 0.05$) of 21% in ADG (1.582 vs. 1.304 kg per animal day^{-1}) and increase of 15% in FC (6.16 vs. 7.25 kg kg^{-1}), as compared to that found in the control diet. The results of improved ADG and FC corroborate findings presented by other authors, including Ferrell et al. (1983), Merchen and Berger (1985), and Zinn (1986a, b), with the values found here being above those found in those particular studies.

Conversely, Reffett-Stabel et al. (1989) used doses of 0, 50 and 100 mg per animal day^{-1} of salinomycin in the feed of steers during feedlot. These animals also received 0.81 kg per animal day^{-1} of a supplement with 32.7% of CP and corn silage "ad libitum", in all treatments, with no observed effect in ionophore over ADG (average of 0.7 kg) and FC (average of 6.95 kg kg^{-1}). Nevertheless, these authors did observe a reduction of 4.76 and 15.5% in DDMI when using doses of 50 and 100 mg of salinomycin, respectively.

Table 2. Effects of salinomycin on production performance of steers in feedlot.

Treatments	Periods of zero-grazing			Average	*SD
	1st (0-28 days)	2nd (29-56 days)	3rd (57-84 days)		
	Average daily weight gain (kg per animal)				
Control	1.127	1.491	1.292	1.304 B	0.316
Salinomycin	1.571	1.554	1.621	1.582 A	
Average	1.349 a	1.522 a	1.456 a		
	Daily dry matter intake (kg per animal)				
Control	8.20	9.26	10.28	9.25 A	1.074
Salinomycin	8.50	9.60	10.47	9.52 A	
Average	8.35 b	9.43 ab	10.37 a		
	Intake in relation to body weight (%)				
Control	2.09	2.16	2.19	2.15 A	0.203
Salinomycin	2.16	2.19	2.17	2.17 A	
Average	2.12 a	2.17 a	2.18 a		
	Feed conversion (kg kg^{-1})				
Control	6.95	6.33	8.48	7.25 A	1.683
Salinomycin	5.64	6.30	6.53	6.16 B	
Average	6.29 b	6.31 b	7.50 a		

Averages, followed by different uppercase letters, in the column, differ among them by the F test at 5%.
Averages, followed by different lowercase letters, in the row, differ among them by Tukey's test at 5%.
*SD: standard deviation.

In Brazil, Nuñez (2008) obtained values of DDMI of 7.79 kg, DMI of 1.83%, ADG of 1.43 kg, and FC of 5.38 kg kg^{-1}, in the finishing of Nelore steers subjected to a diet containing 23% of corn silage and 13 ppm of salinomycin. Sitta (2011) assessed the effect of ionophores (20 and 30 ppm of monensin or 13 ppm of salinomycin) associated with a non-ionophore additive (15 ppm of virginiamycin) for Nelore steers in feedlot. This study noted that the association of salinomycin and virginiamycin promoted DDMI similar to that of the control (without additive) (9.85 vs. 9.89 kg); however, the association with monensin (20 or 30 ppm) promoted a decrease in DDMI (average of 9.14 kg).

In bovines fed highly concentrated diets, ionophores may suppress food intake. According to Rogers and Davis (1982), a decrease in feed intake occurred, likely due to factors such as increased time of feed retention in the rumen and increased diet energy density, as a result of lower methane production and higher propionic acid (the main gluconeogenic agent responsible for feed intake control) production during digestion.

In a literature review, Sitta (2011) also demonstrated that the use of monensin for bovines in feedlot reduced on average 6.33% of DDMI and 1.8% of ADG, and improved FC by 5.64%. Conversely, salinomycin use reduced DDMI by 3.15%, increased ADG by 1.19%, and increased FC by 4.44%. Apparently, sodium monensin caused a more significant reduction in DDMI than salinomycin, a fact that may be related to palatability, as previously suggested by Baile et al. (1979).

Merchen and Berger (1985) assessed doses of salinomycin (0, 5.5, 11, 16.5 or 22 ppm) against the use of monensin (22 ppm) in rations used to feed steers during feedlot using 90% of concentrate. They also obtained increased weight gain by using additives, but without affecting intake and feed efficiency when compared to the control, for both ionophores.

Despite the high number of studies and time spent in research on this particular topic,

responses regarding the use of ionophores on bovine performance in feedlot are inconsistent in the literature, mainly concerning DDMI, which is possibly influenced by interactions between ionophore doses and feed characteristics.

No significant effect (P > 0.05) of salinomycin was noted with regard to the daily production of excrement (Table 3). The AD was higher (P < 0.05) in the bovines that received the ration with salinomycin (70.96% against 69.29%).

Table 3. Effect of salinomycin on excrement production, in natural (NM) or dry (DM) matter, along with the apparent diet digestibility of steers in feedlot.

Parameters	Treatments		Average	Prob.	*SD
	Salinomycin	Control			
Excrement production (kg d^{-1} of NM)	16.27	16.72	16.50	0.473	1.193
Dry matter of excrement (%)	17.63	17.08	17.36	0.099	0.582
Excrement production (kg d^{-1} of DM)	2.86	2.85	2.85	0.894	0.162
Apparent digestibility of DM (%)	70.96	69.29	70.13	0.044	1.655

*SD: standard deviation.

Nuñez (2013) observed higher AD of the rations (p=0.12) in Nelore bovines consuming 13 ppm of salinomycin in diets with 80% concentrate and sugar cane silage, as compared to the control (without ionophores) (68.56% vs. 66.49%).

According to Van Soest (1994), ionophores have the ability to reduce ruminal degradability of protein, which may promote alterations in the quality of the protein that reaches the intestine. Furthermore, the higher efficiency of energy metabolism promoted using rations containing ionophores, such as the increase in propionic acid proportion and reduction in methane production, may also influence AD.

It was possible that the supply of salinomycin promoted an increase (P < 0.05) of 11.95 kg in the warm carcass weight. There was no effect (P > 0.05) of salinomycin over other carcass characteristics (Table 4).

Table 4. Effect of salinomycin on carcass characteristics and performance of carcass gain of steers finished in feedlot.

Parameters	Treatments		Average	Prob.	*SD
	Salinomycin	Control			
Warm carcass weight (kg)	287.76	275.81	281.78	0.004	59.089
Carcass yield (%)	56.77	57.04	56.90	0.960	1.047
Fat thickness (mm):	4.39	4.58	4.48	0.767	1.255
Carcass length (cm)	131.63	131.31	131.47	0.867	3.602
Thigh thickness (cm)	20.75	19.47	20.11	0.076	1.231
Arm length (cm)	39.63	38.56	39.09	0.213	1.552
Arm perimeter (cm)	44.06	42.06	43.06	0.060	1.477
Average daily gain of carcass (kg d^{-1})	1.195	1.048	1.122	0.083	0.199
Carcass weight gain in feedlot (kg)	100.4	88.1	94.2	0.085	16.673
Carcass gain yield (%)	76.12	80.73	78.42	0.072	3.725
Feed conversion in carcass (kg kg^{-1})	8.02	9.06	8.54	0.048	1.358

*SD: standard deviation.

The use of salinomycin promoted greater (P < 0.10) ADGC and CGF, and improved (P < 0.05) FCC. However, CGY was higher (P=0.07) in the control group.

Non-integrant components of the carcass were not altered (P > 0.05) (Table 5) by supplementing rations with salinomycin.

Table 5. Effect of salinomycin on non-integrant components of the carcasses of steers finished in feedlot.

Parameters (% of live weight)	Treatments		Average	Prob.	*SD
	Salinomycin	Control			
Heart	0.39	0.38	0.38	0.835	0.038
Liver	1.26	1.33	1.29	0.085	0.091
Lungs	0.78	0.85	0.81	0.411	0.125
Kidneys	0.23	0.22	0.22	0.789	0.020
Spleen	0.43	0.48	0.45	0.463	0.121
Rumen/reticulum filled	9.27	8.58	8.93	0.185	0.749
Rumen/reticulum empty	1.67	1.65	1.66	0.983	0.122
Abomasum filled	0.65	0.63	0.64	0.942	0.183
Abomasum empty	0.54	0.49	0.51	0.559	0.140
Intestines filled	5.88	5.92	5.90	0.393	1.006
Head	2.79	2.78	2.78	0.781	0.172
Tongue	0.18	0.20	0.19	0.084	0.013
Leather	10.39	10.33	10.36	0.841	0.707
Tail	0.31	0.29	0.30	0.225	0.023
Legs	2.31	2.27	2.29	0.918	0.131

*SD: standard deviation.

Similarly, Merchen and Berger (1985), Gibb et al. (2001), and Sitta (2011) did not observe differences in yield and carcass characteristics of steers on diets using salinomycin.

Ferrell et al. (1983) observed lower carcass yields from bovines with diets containing 15 ppm of salinomycin when compared to the group fed without additives. The authors concluded that this effect was due to the greater carcass weight of bovines grown with salinomycin. It is suggested that lower CGY (P=0.07) in bovines fed with salinomycin (Table 4) is caused by greater final live and warm carcass weight.

The use of salinomycin did not significantly alter the results of serum biochemical markers related to the liver, inflammatory, and kidney functions, suggesting an absence in the effect of salinomycin over the physiological functions of bovines. Regardless of use of salinomycin, biochemical markers of liver, inflammatory, and kidney functions showed results within reference values for the species, with the exception of GGT and fibrinogen on day 84 (Table 6).

According to Kaneko (2008), GGT presents high variations at the serum level in bovines. Thus, the sole use of this test to evaluate liver lesions is not indicated. Therefore, despite the fact that the level of GGT was above the reference level on day 84, it is not possible to indicate the occurrence of liver lesions, since the remaining parameters did not show any alteration for bovines from both treatments. According to Silva et al. (2009), high doses of ionophores increase the serum level of AST. Despite this, the salinomycin dose used in this study did not change the AST level. However, as an exception, alteration in the liver was not observed during the slaughtering process.

Table 6. Effect of salinomycin on serum biochemical markers related to liver, inflammatory, and kidney function of steers finished in feedlot over different evaluation days.

Treatments	Evaluation times				*Reference values
	Day 0	Day 28	Day 56	Day 84	
			Plasmatic protein (g dL^{-1})		
Control	6.44 ± 1.20	7.18 ± 0.66	6.86 ± 0.33	6.73 ± 0.64	
Salinomycin	6.60 ± 1.02	7.25 ± 0.47	6.47 ± 0.42	6.45 ± 0.52	6.97 - 8.85
**P	0.775	0.798	0.072	0.363	
Effect of additive (S)			***Prob>F = 0.6770		
Effect of Time (T)			***Prob>F = 0.0573		
Interaction S*T			***Prob>F = 0.5296		
			Albumin (mg dL^{-1})		
Control	2.06 ± 0.44	2.23 ± 0.24	2.59 ± 0.18	2.56 ±0.32	
Salinomycin	2.19 ± 0.25	2.19 ± 0.16	2.56 ± 0.22	2.72 ± 0.38	2.82 - 3.55
**P	0.490	0.734	0.814	0.402	
Effect of additive (S)			***Prob>F = 0.9726		
Effect of Time (T)			***Prob>F = 0.0251		
Interaction S*T			***Prob>F = 0.3989		
			Plasma fibrinogen (g dL^{-1})		
Control	700.0 ± 400	325.0 ± 148.8	375.0 ± 198.2	712.5 ± 216.7	
Salinomycin	500.0 ± 239.1	387.5 ± 229.5	485.7 ± 157.4	625.0 ± 166.9	300 - 700
**P	0.249	0.530	0.250	0.382	
Effect of additive (S)			***Prob>F = 0.2991		
Effect of Time (T)			***Prob>F = 0.0046		
Interaction S*T			***Prob>F = 0.2344		
			Aspartate aminotransferase (AST. UI L^{-1})		
Control	62.50 ± 30.63	63.21 ± 27.72	77.50 ± 9.74	63.75 ± 7.27	
Salinomycin	51.88 ± 8.29	74.75 ± 16.37	69.86 ± 12.88	65.25 ± 10.12	47.94 - 89.38
**P	0.371	0.332	0.226	0.739	
Effect of additive (S)			***Prob>F = 0.8864		
Effect of Time (T)			***Prob>F = 0.0433		
Interaction S*T			***Prob>F = 0.1893		
			Gamma glutamyl transferase (GGT. UI L^{-1})		
Control	22.3 ± 5.7	26.0 ± 6.1	27.0 ± 9.0	35.9 ± 13.2	
Salinomycin	18.6 ± 5.6	24.0 ± 3.7	23.3 ± 5.2	31.0 ± 6.7	9.2 - 24.3
**P	0.219	0.441	0.343	0.374	
Effect of additive (S)			***Prob>F = 0.1292		
Effect of Time (T)			***Prob>F = 0.0034		
Interaction S*T			***Prob>F = 0.9475		
			Urea (mg dL^{-1})		
Control	27.75 ± 4.27	32.88 ± 5.30	31,63 ± 6,14	28,13 ± 4,70	
Salinomycin	27.63 ± 6.67	33.88 ± 10.74	30,43 ± 8,96	29,00 ± 6,85	28,70 - 48,84
**P	0.965	0.818	0,772	0,771	
Effect of additive (S)			***Prob>F = 0.9608		
Effect of Time (T)			***Prob>F = 0.4909		
Interaction S*T			***Prob>F = 0.5339		

continue

continuation

			Creatinine (mg dL^{-1})		
Control	1.71 ± 0.25	1.73 ± 0.31	1.61 ± 0.32	1.70 ± 0.33	
Salinomycin	1.53± 0.29	1.64 ± 0.31	1.66 ± 0.20	1.65 ± 0.12	1.08 - 1.88
**P	0.184	0.577	0.747	0.693	
Effect of additive (S)		***Prob>F = 0.5058			
Effect of Time (T)		***Prob>F = 0.8750			
Interaction S*T		***Prob>F = 0.2975			

*Adapted from Kaneko (2008); Lopes et al. (2007); and Cardoso et al. (2011).
**P Value related to T test (P<0.05).
***Prob Values >F related to the Multivariate Analysis of Variance (MANOVA; P<0.05).

In the assessment, at the 84-day mark, the fibrinogen value was above reference in the bovines from the control group; however, this occurred with no statistical significance. Fibrinogen is considered the main indicator of acute inflammation in cattle (KANEKO, 2008).

Levels of plasma protein, albumin, fibrinogen, AST, and GGT presented significant alterations (P < 0.10) throughout the evaluation period, regardless of treatment. These variables were shown to be dependent on age and animal development; therefore, serum content of blood metabolites changes over time from birth until adulthood (DOORNENBAL et al., 1988). These alterations are related to changes in metabolism and body composition due to alterations in the deposition rate of bone, muscular, and fat tissue, until body maturity (OWENS et al., 1995).

Some researchers in previous studies also observed that serum content of creatinine had a positive correlation with muscle mass in bovines (WUTHIER; STRATTON, 1957; SILVA et al., 2013). In this study, supplementation of salinomycin in rations resulted in higher ADG; however, significant differences were not detected in serum creatinine, which is in contrast with the findings of the aforementioned researchers.

Residues of salinomycin in the samples of tissues from bovines were not found, regardless of the withdrawal period performed (Table 7).

Table 7. Salinomycin content in bovine tissues following different periods of intake suspension and slaughter (withdrawal periods).

Tissues	Recommended residue maximum limits (RML)		[3]Quantification limit (QLI) 56 h 32 h	Salinomycin content found during the withdrawal periods		
	[1]Japanese Positive List	[2]Health Canada		16 h		
			($\mu g\ kg^{-1}$)			
Muscle	20	-	10	<0.25	<0.25	<0.25
Fat	20	-	10	<0.25	<0.25	<0.25
Liver	400	350	175	<0.25	<0.25	<0.25
Kidney	500	-	250	<0.25	<0.25	<0.25

Source: [1]Japan (2014).
[2]Canada (2014).
[3]The QLI value should correspond up to half the minimum value of RML established for each tissue.

It was not possible to determine the residue depletion curve of salinomycin in bovine tissues according to withdrawal periods through linear regression as recommended by the EMEA (1996), since significant concentrations of the substance were not detected in any of the analyses performed. According to EMEA (1996), in cases in which all concentrations are below RML from first time of collection, the data does not comply with the mathematical model proposed by the method, and therefore, it is not possible to determine the withdrawal period through linear regression.

In these cases, when not enough data is available to outline the depletion curve, it is safe to consider the period of time in which the concentrations are below RML, with a 10% increase, in order to compensate for biological variations. Thus, considering the time required for transport of the bovines from the farm, plus 12 h for resting and fasting before slaughter, salinomycin may be indicated as fully exhausted in the finishing period of bovines, and thus it is not required to further withdraw the product prior to slaughter. Nevertheless, as a precaution due to biological variations, and with respect to the timeframe of 16 h for withdrawal before slaughtering, it is recommended that the use of salinomycin is suspended 24 h prior to slaughter.

In a study performed by EFSA (2008), edible tissues of bovines treated with 0.9 mg kg^{-1} live weight of labeled sodium salinomycin (C^{14}) were tested, and results below QLI (59 µg kg^{-1}) in the kidney, muscle, and fat were observed. Conversely, high concentrations were detected in the liver, which corresponded to 2.263 µg kg^{-1} and 1.548 µg kg^{-1} after 12 and 36 hours of withdrawal period, respectively. In the present experiment, the dose used corresponded approximately to 1/3 of the dose used in the above-mentioned study.

Doses of 1.5 mg kg^{-1} of body weight in calves, and 8 $mg.kg^{-1}$ in steers, were considered toxic, causing cardiovascular disorders, tremors, and inappetence. Doses of 10 mg kg^{-1} of body weight in steers were considered lethal, causing lung emphysema, necrosis of the cardiac muscle, and focal liver necrosis (EFSA, 2008).

In this experiment, we observed that the use of salinomycin in bovines in the finishing period promoted an overall increase of R$ 84,20 in the profit margin per animal (Table 8).

Table 8. Economic analysis of salinomycin supplementation in the finishing of steers in feedlot.

Parameters (R$ per animal)	Treatments	
	Salinomycin	Control
Daily cost of diet	4.60	4.47
Daily cost with salinomycin	0.008	0.00
Total cost of diet during evaluation period	386.80	375.17
Cost of unfinished cattle	1.724.56	1.724.56
Gross income	2.307.64	2.211.81
Profit margin	196.28	112.08

Conclusions

The use of salinomycin in the finishing of steers in feedlot promoted increases in weight gain and improved feed conversion, without corresponding changes in food intake. The improvements in production performance promoted by salinomycin increased profit margins per animal by 75%.

The use of salinomycin in the diet of bovines in the finishing process does not affect either animal or consumer health, since neither residues in edible tissues nor alterations in bovine serum biochemical parameters were detected. Sustaining the use of salinomycin 24 hours prior to slaughtering is recommended.

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