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Homeopathic additives and virginiamycin® in grazing beef cattle[1]

Aditivos homeopáticos associados à virginiamicina® para bovinos de corte em pastejo

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ABSTRACT - The aim of this study was to evaluate the effects of homeopathic additives and the non-ionophore antibiotic virginiamicin® on intake, digestibility, metabolic characteristics and carcase characteristics in Nellore bulls under supplemented grazing. Twelve uncastrated Nellore bulls were used, with an initial mean weight of 346.04 ± 13.33 kg and initial age of 20.00 ± 2.00 months. The animals were kept in individual paddocks of *Urochloa decumbens* Stapf. (syn. *Brachiaria)* and divided into two treatments: Lipomax® - supplement concentrate with homeopathic additives (Convert H®, Sodo 100®, Figotonus®) and a non-ionophore antibiotic (Virginiamycin®); Control - supplement concentrate containing the same protein content, but with no additives. The experiment was carried out in a completely randomised design. Intake, the apparent digestibility of the dietary constituents and the metabolic characteristics of the bulls were not affected by supplementation with additives (P>0.05). Lipomax® improved weight gain in the animals (P<0.05) and the hot and cold carcase weight was also higher in animals supplemented with Lipomax® (P<0.05). The proportion of muscle and fatty tissue in the carcase was not affected by supplementation with additives (P>0.05). Supplementation with homeopathic additives and virginiamicina® had a positive effect on performance and on hot and cold carcase weight in grazing Nellore bulls.

Key words: Antibiotic. Nellore. Pasture. Supplement.

RESUMO - O objetivo com este trabalho foi avaliar os efeitos de aditivos homeopáticos associados ao antibiótico não ionóforo virginiamicina® sobre o consumo, a digestibilidade, as características metabólicas e as características de carcaça de tourinhos Nelore suplementados em pastejo. Foram utilizados 12 tourinhos Nelore, não castrados, com peso inicial médio de 346,04 ± 13,33 kg e idade inicial de 20,00 ± 2,00 meses. Os animais foram alojados em piquetes individuais de *Urochloa decumbens* Stapf. (syn. *Brachiaria)*, e divididos em dois tratamentos: Lipomax® - suplemento concentrado com aditivos homeopáticos (Convert H®, Sodo 100®, Figotonus®) e antibiótico não ionóforo (Virginiamicina®); e Controle - suplemento concentrado com mesmo teor de proteína, sem aditivos. O experimento foi conduzido em delineamento inteiramente casualizado. O consumo, a digestibilidade aparente dos constituintes da dieta e as características metabólicas dos tourinhos não foram afetados pela suplementação com aditivos (P>0,05). O Lipomax® melhorou o ganho de peso dos animais (P<0,05). O peso de carcaça quente e fria também foram maiores nos animais suplementados com Lipomax® (P<0,05). As proporções de músculo e tecido adiposo na carcaça não foram afetadas pela suplementação com aditivos (P>0,05). A suplementação com aditivos homeopáticos associados a virginiamicina® afetaram de maneira positiva o desempenho e o peso de carcaça quente e fria de tourinhos Nelore em pastejo.

Palavras-chave: Antibiótico. Nelore. Pastagem. Suplemento.

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INTRODUCTION

In the search for tools that help maximise results and improve financial return from livestock activity, additives are used to manipulate rumen fermentation and increase the efficiency of nutrient digestion and absorption (BENATTI *et al*., 2017; SOLOMON; TULLETT, 1988). Hao *et al*. (2014) pointed out that the use of additives is an increasingly common tool in production systems as a way of reducing costs, improving feed conversion and weight gain, and/or benefitting the health and metabolism of the animal, thereby contributing to better performance, especially during the growth phase and termination.

Virginiamycin® is a non-ionophore antibiotic produced from the fermentation of *Streptomyces virginae*. It shows great potential for stabilising rumen fermentation by changes in the population of rumen bacteria, besides having greater control over the production of lactate and methane, as it acts directly on the species that produce these compounds (LEMOS *et al*., 2016; NAGARAJA; TAYLOR, 1987; OLIVEIRA *et al*., 2017). Due to these characteristics, virginiamycin® has been used to adapt animals to high-concentrate diets (LEMOS *et al*., 2016; MONTANO *et al*., 2015), reducing the risk of metabolic disorders. On the other hand, the results of using this compound in roughage-based diets have not yet been consolidated (ALVES NETO *et al*., 2018; FERREIRA *et al*., 2015).

By contrast, homeopathy is a therapeutic technique whose basic principle is 'like curing like' and the use of dynamised medicines, i.e. medicines prepared from plant, animal or mineral substances (GEMELLI; PEREIRA, 2018). Its mechanism of action involves highly complex physical manifestations, and it is characterised by the use of medicines at minimal doses (EBERT *et al*., 2017). In animal nutrition, homeopathy has been used to promote animal performance, substituting the use of chemotherapeutics (CHABEL *et al*., 2009; ÍTAVO *et al*., 2010; MARAFON *et al*., 2014).

However, results evaluating the use of homeopathy together with non-ionophore antibiotics are still relatively scarce and contradictory. As such, the aim of this study was to evaluate the effects of Lipomax® commercial supplement concentrate, which includes in its composition, homeopathic additives (Convert H®, Sodo 100®, Figotonus®) and virginiamycin® non-ionophore antibiotic, on intake, the digestibility of dietary constituents, and the metabolic and carcase characteristics of Nellore bulls under supplemented grazing.

MATERIAL AND METHODS

This study complies with the rules and standards of the Ethics Committee on the Use of Animals of the State University of Mato Grosso do Sul (No 01/2016).

The experiment was conducted on the Aquidauana Campus of the State University of Mato Grosso do Sul. A 12-hectare area of *Urochloa decumbens* Stapf. (syn. *Brachiaria*) was used, which was divided into 12 paddocks, each of 1.00 hectare, where 12 individual uncastrated Nellore bulls were kept, with an initial mean weight of 346.04 ± 13.33 kg and initial age of 20.00 ± 2.00 months.

The experiment was conducted in a completely randomised design with six experimental units (animals) per treatment. After initial weighing, the animals were randomly divided into two treatments: six animals received Lipomax® commercial supplement concentrate with homeopathic additives (Convert H®, Sodo 100®, Figotonus®) and virginiamycin® non-ionophore antibiotic throughout the experimental period (Lipomax®). The remaining animals received supplement concentrate containing the same protein content (Table 1), but with no additives (Control). The supplement concentrate used in the Control treatment was formulated with maize, soya meal, cottonseed, vegetable oil and urea.

The supplements were offered daily at 10:00, in an amount equal to 0.5% of body weight. To adjust the amount of supplement offered, the animals were weighed every 28 days without fasting. The animals were also weighed after a liquid fast of 16 hours at the beginning and end of the experiment to evaluate the average daily gain (ADG). The experiment lasted for 100 days.

Forty-five days after the beginning of the experiment, a digestibility trial was carried out to evaluate intake and nutritional characteristics. LIPE®, supplied to the animals via oesophageal probe as one 500-mg capsule per day, was used to estimate faecal production (SILVA *et al*., 2010). The intake of supplement concentrate was determined from the difference in weight of the offered supplements and the daily quantity of leftovers. Indigestible neutral detergent fibre (iNDF) was used as an internal indicator to estimate forage intake (DETMANN *et al*., 2001).

The digestibility trial lasted for seven days. The LIPE® was applied between day one and day five. On day four of the trial, forage was collected in each paddock, in a simulation of manual grazing, to estimate the intake and apparent digestibility coefficients of the forage. Faeces were collected in the stable directly from the rectum of the animals, between day three and day six of the trial. The faeces were collected once a day for four days at different times (06:00, 10:00, 14:00 and 17:00) to obtain a composite sample.

Table 1 - Percentage composition of the supplements and forage used in the experiment

Component (%)	Control	Lipomax®*
Chemical Composition of the Supplements		
Dry matter	91.50	94.40
Mineral matter	12.50	17.20
Ether extract	9.70	5.20
Crude protein	18.20	18.50
NDFap[1]	13.50	7.50
NFC[1]	46.10	51.60
Additives		
Virginiamycin®	0.000	0.013
Convert H®	0.000	0.200
Sodo 100®	0.000	0.200
Figotonus®	0.000	0.200
Chemical Composition of the Forage		
Dry matter	31.76	32.90
Mineral matter	8.10	7.79
Ether extract	2.53	2.16
Crude protein	5.36	5.16
NDFap[1]	55.95	56.43
NFC[1]	20.23	20.88

*Guaranteed levels: calcium 52g/kg; phosphorus 4000 mg/kg; sodium 7500 mg kg^{-1}; sulphur 1300 mg kg^{-1}; cobalt 5 mg kg^{-1}; copper 75 mg kg^{-1}; iodine 6.20 mg kg^{-1}; manganese 87 mg kg^{-1}; selenium 0.9 mg kg^{-1}; zinc 200 mg kg^{-1}; lysine 50 mg kg^{-1}; methionine 99 mg kg^{-1}; vitamin A 50000 U.I. kg^{-1}; vitamin D3 15000 U.I. kg^{-1}; vitamin E 50 U.I. kg^{-1}. [1]Neutral detergent fibre corrected for ash and protein. Non-fibrous carbohydrates

On the seventh and last day of the trial to evaluate the nutritional characteristics, spot urine samples were collected from spontaneous urination, and blood samples by jugular venepuncture, four hours after offering the supplement. The urine samples were diluted in H_2SO_4 (0.036 N) and frozen at -20 °C (VALADARES *et al*., 1999), for later analysis to determine the creatinine, urea and total nitrogen. The blood samples were collected immediately after collecting the urine and sent to the laboratory for serum urea analysis. Creatinine was quantified using the kinetic colorimetric method; the levels of urinary and serum urea were determined using the fixed-time kinetic method.

The samples of forage and faeces were dried in a forced ventilation oven (55 °C/72 hours) and processed in a knife mill (1 mm). The samples of forage, supplement and faeces were evaluated for dry matter (DM, method INCT-CA G-003/1), mineral matter (MM, method INCT-CA M-001/1), crude protein (CP, method INCT-CA N-001/1), ether extract (EE, method INCT-CA G-005/1), neutral detergent fibre (NDF, method INCT-CA F-002/1) corrected for ash (NDIA, method INCT-CA M-002/1) and protein (NDIP, method INCT-CA N-004/1), and indigestible neutral detergent fibre (NDFi, method INCT-CA F-009/1) as per the techniques described by Detmann *et al*. (2012). The non-fibrous carbohydrate (NFC) content was estimated as per Detmann and Valadares Filho (2010):

$$NFC = 100 - [(\%CP - \%CPu + \%U) + \%NDFap + \%EE + \%MM)$$

where: *CPu* = urea; *CP* content; *U* = urea content.

The forage dry matter intake (FDMI) was estimated by using NDFi as an internal marker, adjusting the equation proposed by Detmann *et al*. (2001):

$$FDMI\ (kg\ day^{-1}) = ((DMF \times NDFi_{faeces}) - (DMi_{supl} \times NDFi_{supl}))/NDFi_{forage}$$

where: *DMF* = faecal dry matter (kg day^{-1}); $NDFi_{faeces}$ = indicator concentration in the faeces; DMi_{supl} = supplement dry matter intake (kg); $NDFi_{supl}$ = indicator concentration in the supplement; $NDFi_{forage}$ = indicator concentration in the forage.

When the animals reached a mean weight of 450 kg, they were transported to an industrial slaughterhouse 10 km

from the experimental site. During pre-slaughter management at the slaughterhouse, the animals were fasted for 24 hours. The animals were stunned by brain concussion, followed by sectioning of the jugular vein, removal of the hide, and evisceration. After evisceration, the liver was collected and washed prior to weighing.

The carcases were identified, divided into two halves with the help of an electric saw and weighed to obtain the hot carcase weight, after which the hot carcase yield was determined. After the slaughter process, the carcases were taken to the cold room where they remained for 24 hours at a temperature of -5 °C; the cold carcase weight and cold carcase yield were then taken.

Carcase length was measured on the left half-carcase of each animal, from the anterior edge of the pubis bone to the medial cranial edge of the first rib. A perpendicular cut was also made in the *Longissimus dorsi* muscle on the left half-carcase, between the 12th and 13th ribs, where the subcutaneous fat thickness was measured with the aid of a digital caliper.

To evaluate the colour of the meat and fat, a portable colorimeter was used as per the *HunterLab* colour system, whose coordinates *L*, *a* and *b* indicate respectively: the luminosity, which is influenced by the amount of water on the surface of the meat, a result of the water retention capacity and the amount of fat; the red content, which reflects the amount of red pigment present in the myoglobin and cytochrome C; and the yellow content, which is associated with the presence of carotenoids. Three colour readings were taken on the *Longissimus dorsi* muscle in the region of the 13th rib of the left half-carcase of each animal.

The proportion of muscle, fatty tissue and bone on the carcass was estimated by physically separating the section corresponding to the 9th, 10th and 11th ribs from the left half-carcase, known as the HxH section, following the procedure described by Hankins and Howe (1946). The proportions in the carcase were estimated as per the equations developed by the above authors:

$$Proportion\ of\ muscle\ Y = 16.08 + 0.80\ X \quad (1)$$

$$Proportion\ of\ fatty\ tissue\ Y = 3.54 + 0.80\ X \quad (2)$$

$$Proportion\ of\ bone\ Y = 5.52 + 0.57\ X \quad (3)$$

where: *X* is the percentage of the corresponding component in the HxH section.

To evaluate the weight and feed conversion data, the PROC MIXED procedure of the SAS University software (SAS Institute Inc., Cary, CA, USA) was used. The PROC GLM procedure was used to evaluate the other performance data, nutritional characteristics and carcass data. The mean values of the treatments were compared by t-test at a significance level of 5%.

RESULTS AND DISCUSSION

It is important to point out that the animals did not suffer any environmental or health challenge during the experimental period which could have intensified the performance of the homeopathic products used, since one of the functions of these products is to control the adverse effects of stress.

The total consumption of forage dry matter, concentrate dry matter and total dry matter did not vary ($P>0.05$) between treatments (Table 2). According to Alves Neto *et al*. (2018), virginiamycin® is an additive with little or no effect on intake, having a greater effect on rumen fermentation. There was no significant difference between the treatments under evaluation ($P>0.05$) for the roughage to concentrate ratio. In the Control treatment, supplement intake corresponded to 9% of the total DM intake; in the Lipomax® treatment this was equal to 14%.

There was a significant difference between treatments ($P<0.05$) for the consumption of ether extract (EE). This seems to have been caused by the higher concentration of this component in the supplement of the Control treatment, as it included cottonseed and vegetable oil among its ingredients.

The consumption of crude protein (CP), neutral detergent fibre corrected for ash and protein (NDFap), non-fibrous carbohydrates (NFC) and total digestible nutrients was similar between treatments ($P>0.05$), due to the similarity of the composition and consumption of the supplement concentrates.

EE digestibility was higher in the diet of animals fed the Control treatment ($P<0.05$; Table 3), which can be explained by the greater consumption of this component in this treatment. However, the digestibility coefficients for CP, NDFap and NFC were similar between diets ($P>0.05$).

The similarity between the concentration of blood urea nitrogen, urine urea nitrogen and total urinary nitrogen in both treatments ($P>0.05$), shows that the usage efficiency of the metabolisable protein intake was similar between treatments (Table 4).

Although there was no difference in intake, digestibility or nitrogen use in the animals of either treatment, those that received Lipomax® showed a higher ($P<0.05$) average daily gain and, consequently, greater ($P<0.05$) final weight. As a result, the animals that received Lipomax® weighed around 30 kg more by the end of the experiment than those in the Control treatment (Table 5).

Increased weight gain is an expected result of the use of additives. According to Ferreira *et al*. (2015), among the positive effects of virginiamycin®, increased weight gain in the animals stands out.

The use of homeopathic additives to promote animal growth still gives contradictory results, however their use is intended to reduce the stress in animals caused by improper handling or even by nutritional

Table 2 - Ingestive characteristics of bulls receiving concentrate supplement with or without Lipomax® additives

Parameter	Treatment		C.V., %	P-value
	Control	Lipomax®		
	kg d^{-1}			
Forage dry matter[1]	8.42	7.99	15.10	0.561
Concentrate dry matter[1]	0.84	1.27	51.07	0.205
Total dry matter[1]	9.26	9.26	12.68	0.996
Roughage:Concentrate ratio[2]	91:09	86:14	6.66	0.169
Ether extract[1]	0.34	0.23	19.46	0.006
Crude protein[1]	0.86	0.83	22.05	0.797
NDFap[1]	5.92	5.67	15.43	0.634
Non-fibrous carbohydrates[1]	1.37	1.72	19.81	0.072
Total digestible nutrients[1]	6.38	6.18	16.36	0.746
	g kg^{-1} BW			
Total dry matter[1]	21.34	22.27	12.90	0.578
Forfage dry matter[1]	18.47	20.30	17.57	0.374
NDFap[1]	14.28	13.12	17.99	0.433

NDFap = neutral detergent fibre corrected for ash and protein; [1]Estimated values for intake; [2]in % pasture

Table 3 - Apparent digestibility coefficient (%) of the dietary components of bulls receiving supplement concentrate with or without Lipomax® additives

Parameter	Treatment		C.V., %	P-value
	Control	Lipomax®		
Ether Extract	79.00	67.90	7.19	0.004
Crude protein	78.10	74.90	7.46	0.359
NDFap[1]	68.40	67.60	7.88	0.817
Non-fibrous carbohydrates	73.90	74.20	11.41	0.949

[1]Neutral detergent fibre corrected for ash and protein

Table 4 - Blood urea nitrogen, urine urea nitrogen and total urinary nitrogen in bulls receiving supplement concentrate with or without Lipomax® additives

Parameter	Treatment		C.V., %	P-value
	Control	Lipomax®		
Blood urea nitrogen[1]	15.12	14.75	10.38	0.690
Urine urea nitrogen[2]	35.89	33.01	28.62	0.622
Total urinary nitrogen[2]	45.88	55.12	24.85	0.231

[1]in mg dl^{-1}; [2]in g d^{-1}

Table 5 - Performance of bulls receiving concentrate supplement with or without Lipomax® additives

Parameter	Treatment		C.V., %	P-value
	Control	Lipomax®		
Initial weight[1]	343.70	348.40	3.97	0.563
Final weight[1]	428.80	459.10	4.71	0.033
Average daily gain[2]	0.85	1.11	19.50	0.047
Feed conversion[3]	11.30	8.60	23.70	0.069

[1]in kg; [2]in kg d^{-1}; [3]in kg kg^{-1}

imbalance (GEMELLI; PEREIRA, 2018), and has a positive impact on animal performance (RIBEIRO *et al*., 2011; SILVA *et al*., 2011).

Little is known about the action of homeopathic products on animal physiology. However, according to Gemelli and Pereira (2018), homeopathic products can affect the hypothalamic-pituitary-adrenal axis, where the energy information of these products is sensed or captured by nerve endings in the mucosa of the mouth and the digestive tract. Once captured, this energy information reaches the central nervous system, triggering corrective or stimulatory actions that result in improved productivity.

Among the additives included in the Lipomax® treatment, the aim of Convert H® is to make the animal less reactive to situations that cause stress, and improve feed conversion (MARAFON *et al*., 2014) by altering the metabolism of the absorbed nutrients. The greater weight gain for a similar intake seen here, seems to confirm these assumptions, although the presence of additives in the supplement has shown only a tendency ($0.05 < P < 0.10$) to significantly affect feed conversion.

With the carcase characteristics, there was a significant increase in hot and cold carcase weight for the Lipomax® treatment compared to the Control ($P<0.05$; Table 6). As animals that received the Lipomax® treatment had a greater final weight, their carcases were expected to be heavier.

However, the difference in slaughter weight seen between animals that received the Lipomax® treatment and those that received the Control, was not enough to significantly increase ($P>0.05$) carcase yield. The greater carcase yield that is expected as the animals become larger (BERG; BUTTERFIELD, 1976), is only seen when there is a greater difference in slaughter weight among the animals. There was no significant difference between treatments in terms of subcutaneous fat thickness, liver weight or carcase length ($P>0.05$).

The colour of the meat, although not influencing sensory or palatability characteristics, is an important factor in the purchase decision of the consumer. Carotenoids, a group of pigments present in nature, in addition to contributing to the colour and nutritional quality of food, have also been used to indicate animals finished on pasture, since the presence of these compounds in the cattle carcase is directly linked to their intake (ÁLVAREZ *et al*., 2015).

The meat colour in the animals that received Lipomax® had a lower ($P<0.05$) value for *b*. The *b* parameter indicates the yellow intensity, which is usually set by the number of carotenoids in the pasture. As pasture intake was similar in both treatments, this may indicate less absorption and deposition of these compounds in the meat due to the use of the additives under evaluation. However, no account was found in the literature that pointed out this effect when using any of the additives evaluated here. The treatments evaluated in the present experiment were unable to effectively alter ($P> 0.05$) the other parameters of meat or fat colour.

The estimated amounts of muscle and fatty tissue in the carcase did not differ ($P> 0.05$) with the use of additives in the concentrate (Table 7).

The greater ($P<0.05$) amount of bone in the animals that received the additives (Lipomax®), together with the similarity in carcase size, points to a physiological effect that seems to increase bone density, probably due to a greater deposition of minerals in the bones.

In studies with non-ruminant animals, it was found that virginiamycin® can have a positive effect on the growth of intestinal microvilosities, increasing the sites of nutrient absorption (LINDEMANN *et al*., 2010; McCORMICK *et al*., 2016; STEWART *et al*., 2010). In high-fibre diets (RAVINDRAN *et al*., 1984), virginiamycin® also acts on the retention time of nutrients in the gastrointestinal tract, which may have increased the absorption of some minerals, such as calcium and phosphorus, thereby increasing bone density, as seen in this study.

Table 6 - Carcass characteristics of bulls receiving concentrate supplement with or without Lipomax® additives

Parameter	Treatment		C.V., %	P-value
	Control	Lipomax®		
Hot carcase weight[1]	242.00	255.00	3.74	0.039
Hot carcase yield[2]	56.40	55.60	3.31	0.471
Cold carcase weight[1]	236.00	249.00	3.88	0.040
Cold carcase yield[2]	55.10	54.30	2.77	0.391
Carcase length[3]	125.00	125.00	2.27	0.492
Fat thickness[4]	3.56	3.00	31.50	0.371
Liver weight[1]	4.75	5.03	8.74	0.283
Colour of the meat				
L	36.90	61.10	3.55	0.813
a	19.30	17.30	9.11	0.068
b	16.00	14.00	7.11	0.009
Colour of the fat				
L	61.00	61.10	7.51	0.980
a	19.60	17.40	28.00	0.478
b	26.50	24.00	12.30	0.183

[1]in kg; [2]in % fasted body weight; [3]in cm; [4]in mm

Table 7 - Tissue estimate in the carcase of bulls receiving concentrate supplement with or without Lipomax® additives

Parameter	Treatment		C.V., %	P-value
	Control	Lipomax®		
Muscle[1]	161.00	168.00	6.83	0.264
Bone[1]	34.50	38.70	7.65	0.027
Fatty tissue[1]	50.70	50.80	16.40	0.983

[1]in kg

CONCLUSION

Supplementation using homeopathic additives and non-ionophore antibiotics improves the performance and hot and cold carcase weight of grazing Nellore bulls.

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