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Research Article

Low doses of monensin for lambs fed diets containing high level of ground flint corn

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ABSTRACT: Two experiments were proposed to evaluate the addition of monensin for lambs fed diets containing a high level of mature ground flint corn. The experimental diets were as follows: no inclusion of monensin (M0) and inclusion of 8 (M8), 16 (M16) and 24 mg kg^{-1} of monensin (M24). In experiment 1, eight cannulated wethers were divided into a double 4 × 4 Latin square experimental design to evaluate nutrient digestibility, plasma parameters and rumen fermentation. The experiment lasted 112 days, divided into four periods of 28 days each. In experiment 2, ninety-two lambs were used in a randomized block design to evaluate the performance over 56 days. In experiment 1, doses of monensin had no effect on nutrient intake ($p \geq 0.07$) and digestibility ($p \geq 0.09$). There was a quadratic effect for acetate molar proportion ($p = 0.01$), acetate to propionate ratio ($p = 0.04$) and rumen pH ($p < 0.01$). However, there was no effect on the molar proportion of propionate and butyrate. The monensin decreased linearly the total SCFA concentration ($p < 0.01$). The inclusion of monensin increased glucose ($p < 0.01$) and decreased lactate concentration in plasma ($p = 0.05$). In experiment 2, monensin decreased dry matter intake ($p = 0.04$). However, there was a quadratic effect for average daily gain ($p = 0.03$) and feed efficiency ($p < 0.01$), with the greatest values observed for the M8 diet. Thus, the inclusion of 8 mg kg^{-1} of dry matter diet (DM) improves ruminal fermentation and plasma parameters, resulting in greater growth performance in lambs.
Keywords: feed efficiency, propionate, sheep

Introduction

In ruminant nutrition, monensin is the main additive used in high concentrate diets (Oliveira and Millen, 2014), selecting for specific bacteria groups and promoting changes fermentation, ruminal pH and, consequently, improving animal health and performance (Schelling, 1984). Supplying optimum levels of feed additives in ruminant diets translate to financial savings and increases in production efficiency.

Despite the considerable importance of providing optimum levels of monensin, there is no definitive recommendation for the inclusion of this additive in lamb diets. The initial studies evaluating doses of monensin for lambs demonstrated that the use of lower doses, between 5 and 11 mg kg^{-1} in a DM diet, were able to improve rumen fermentation (Baran et al., 1986), decrease the coccidian oocyst (Calhoun et al., 1979) and increase energy retention and growth performance of lambs (Nockels et al., 1978; Joyner et al., 1979). In the 1990s most of the studies used doses above 25 mg of monensin kg^{-1} in the DM diet and this dosage showed inefficient action in a number of experiments (Gonzalez-Momita et al., 2009; Ítavo et al., 2011).

Martins et al. (2018) observed that the use of 25 mg of monensin kg^{-1} of DM in diets containing a high level of flint corn ground for lambs did not affect the ruminal concentration of short chain fatty acids (SCFA) and pH. Considering that the action of feed additives can be influenced by diet composition and ingredient processing (Meyer et al., 2013), the present study tested the hypothesis that lower doses of monensin (8 or 16 mg kg^{-1} of diet DM) increases the propionate in the rumen and controls ruminal pH. Consequently, lower doses of monensin increases the nutrient digestibility and alters the metabolic profile of lambs compared with control and high doses. Furthermore, monensin would increase the performance of lambs fed diets containing a high level of mature ground flint corn. The objective of this trial was to evaluate the effects of decreasing inclusion concentrations of monensin (24, 16, 8, and 0 mg kg^{-1} diet DM) in diets containing a high level (72.0 % of DM) of mature ground flint corn in ruminal fermentation, nutrient digestibility, plasma concentrations of metabolites, and growth performance of feedlot lambs.

Materials and Methods

Two experiments were conducted at Piracicaba, in the state of São Paulo, Brazil (22°43′30″ S, 47°38′51″ W, altitude of 528 m). Both experiments were reviewed and approved by the Animal Care and Use Committee (number 9433031014).

Experiment 1

Eight ruminally fistulated Dorper × Santa Inês wethers, with an initial body weight (BW) of 68.72 ± 4.22 and approximately 10 months of age, were used to evaluate their ruminal characteristics, nutrient digestibility, nitrogen balance and plasma parameters. The wethers were dewormed with subcutaneous moxidectin at a dosage of 1 mL 50 kg BW^{-1} and received 2 mL of subcutaneous ADE vitamin supplements on the right side of the neck, 5 days before the beginning

of the experiment. They were placed in metabolism crates (1.30 × 0.55 m) provided with a feeding trough, drinking trough, and a system for collecting feces and urine. The metabolism crates were kept in an indoor environment and protected from direct sunlight and rain.

The wethers were divided into a double 4 × 4 Latin square experimental design with four treatments and eight replications. The experiment lasted for 112 days, divided into four periods of 28 days each. The first seven days were used as a wash-out, to avoid the residual effect related to the treatment received in the previous period. From the eighth day of each period, the wethers received the experimental diets.

The experimental diets were defined by the addition of increased levels of monensin (Rumensin 200, Elanco Animal Health, São Paulo, SP, Brazil). The levels used were: no inclusion of monensin (M0); 8 mg of monensin kg^{-1} of DM (M8); 16 mg of monensin kg^{-1} of DM (M16); and 24 mg of monensin kg^{-1} of DM (M24). Diets were formulated according to NRC (2007) recommendations for growing lambs. The proportion of the ingredients and the chemical composition of the diets are presented in Table 1.

Corn and coastcross hay were coarsely ground using a grinder with a screen comprising a 10 mm sieve, and mixed with soybean, urea, limestone, mineral mix and ammonium chloride using a horizontal mixer. In diets containing monensin, the ionophore was added into a mixer for subsequent homogenization. The experimental diets were weighed on an electronic scale with an accuracy of 1 g and offered *ad libitum* at 08h00.

The orts were recorded daily to determine the DMI. A sample was taken daily from offered feed and orts of each experimental unit and kept at –18 °C for analysis. To determine the nutrient digestibility in the total digestive tract and nitrogen balance, on days 21 to 25 of the experiment the total fecal and urine production were individually collected every day. Urine was collected in containers with a sufficient amount of 6 N HCl to prevent ammonia volatilization, maintaining pH below 3.0. Feces and urine were quantified using an electronic scale (Marte AC-10K) at 08h00, and a sample (10 %) of daily production from each wether was collected and stored at –18 °C.

Rumen fluid was collected on days 26, 27 and 28 of the experiment. Samples were collected every 3 h related to feeding for 24 h, totaling eight collections per day which were carried out for three consecutive days to minimize the effect of collection day, to avoid favoring or prejudicing any treatment. At each interval, a representative sample of ruminal content was collected via cannula, and filtered to obtain 200 mL of ruminal fluid, which was used to measure the pH in a digital potentiometer. The solid part of the ruminal content was returned to the rumen. The analyses of

Table 1 – Ingredients and chemical composition of the experimental diets (g kg^{-1} of DM).

Item	Diets[1]			
	M0	M8	M16	M24
Ingredient proportion				
Coastcross hay	100	100	100	100
Ground corn	720	720	720	720
Soybean meal	140	140	140	140
Urea	5.0	5.0	5.0	5.0
Mineral mix[2]	15.0	15.0	15.0	15.0
Ammonium chloride	5.0	5.0	5.0	5.0
Limestone	15.0	15.0	15.0	15.0
Monensin[3], mg kg^{-1} of DM	0.00	8.00	16.0	24.0
Chemical composition				
Experiment 1				
DM[4], as fed basis	894.0 ± 2.52	892.3 ± 1.03	892.3 ± 1.02	895.4 ± 1.63
OM[5]	949.2 ± 0.81	949.0 ± 0.92	949.9 ± 0.92	950.6 ± 1.02
CP[6]	171.8 ± 1.03	169.1 ± 2.44	174.5 ± 2.81	169.6 ± 4.12
NDF[7]	154.5 ± 4.83	152.8 ± 6.04	150.4 ± 2.13	158.3 ± 2.82
ADF[8]	58.1 ± 2.74	56.7 ± 1.12	52.9 ± 2.14	56.3 ± 3.04
Experiment 2				
DM, as fed basis	879.7 ± 1.52	880.3 ± 2.03	887.1 ± 3.51	885.7 ± 1.51
OM	942.8 ± 1.61	941.0 ± 2.61	942.9 ± 1.63	944.4 ± 4.03
CP	178.0 ± 1.02	176.8 ± 3.01	175.6 ± 4.01	176.3 ± 1.51
NDF	178.2 ± 1.12	178.6 ± 2.92	179.2 ± 1.02	182.6 ± 3.51
ADF	65.0 ± 1.13	62.7 ± 1.34	63.8 ± 2.24	65.0 ± 3.94

[1]M0 = diet without added fed additives; M8 = 8 mg kg^{-1} of monensin; M16 = 16 mg kg^{-1} of monensin; M24 = 24 mg kg^{-1} of monensin (DM basis). [2]Composition: 13 % Ca, 8 % P, 1 % Mg, 7 % S, 22 % Cl, 15 % Na, 1,100 mg kg^{-1} Mn, 500 mg kg^{-1} Fe, 4,600 mg kg^{-1} Zn, 300 mg kg^{-1} Cu, 40 mg kg^{-1} Co, 55 mg kg^{-1} I, and 30 mg kg^{-1} Se. [3]Rumensin 200 (Elanco Brazil, Sao Paulo, SP, Brazil).[4]Dry matter. [5]Organic matter. [6]Crude protein. [7]Neutral detergent fiber. [8]Acid detergent fiber.

SCFA and ammonia nitrogen were carried out in terms of per animal per hour so that an aliquot of 50 mL of each harvest schedule would be stored so as to produce a sample with 150 mL per animal h^{-1} of collecting.

Plasma samples were collected every 3 h related to feeding, from day 26 to 28 by venipuncture of the jugular vein into a vacuolated tube containing sodium fluoride as antiglycolytic and potassium EDTA as anticoagulant and a vacuolated tube containing clot activator and gel.

Samples of feed, orts and feces were dried in a forced-air oven at 60 °C (AOAC, 1990; #930.15). Subsequently, samples were ground through a 1-mm Wiley Mill screen. The final DM concentration was determined after oven-drying the samples at 105 °C (AOAC, 1990; #934.01) and ash concentration was obtained by incineration (AOAC, 1990; method #942.05). Sequential detergent fiber analyses were used to determine neutral detergent fiber (NDF; Van Soest et al., 1991) and acid detergent fiber (ADF; Goering and Van Soest, 1970) with an Ankom 2000 fiber analyzer. Sodium sulfite and heat-stable α-amylase were added in the NDF analysis. Total N was determined according to AOAC (1990; method #968.0) and the crude protein (CP) was obtained by multiplying the total N content by 6.25.

The SCFA concentration in rumen fluid was determined according to Ferreira et al. (2016). The quantification of SCFA was performed using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (7683B) and a fused-silica capillary column, 25 m in length with an internal diameter of 320 µm, containing 0.20 µm cyanopropyl polysiloxane. Ammonia nitrogen was determined with a colorimetric method that was described by Chaney and Marbach (1962) and adapted for a microplate reader with a 550 nm absorbance filter.

The plasma parameters were determined in the Automatic System for Biochemistry - Model SBA-200. Commercial kits were used to determine plasma glucose (Ref. 133-1: 500), urea (Ref. 104) and lactate (Ref.: 116). Insulin and insulin-like growth factor concentration (IGF-I) were determined by a chemiluminescence immunoassay using the Immulite 1000 commercial kit.

Statistical procedures were conducted using PROC MIXED in SAS (Statistical Analysis System, version 9.0). All data were submitted to the Shapiro-Wilk test to verify the normality of the residuals, the removal of outliers, and homogeneity of variances using the Levene test.

The data for SCFA, rumen pH, ruminal ammonia concentration and plasma parameters were analyzed as repeated measures over time. Both diet and time were considered a fixed effect. Animal, period, and square were considered as a random effect. The data were put into covariance matrices and defined according to the lowest value obtained for Akaike's information criterion corrected (AICC). The means from variables were obtained by the LSMEANS command. The effects of diet, hour and diet × hour interaction were defined by the ANOVA *F*-test. Orthogonal polynomials for diet responses were determined by linear, quadratic and cubic effects.

The data for nutrient intake, digestibility and nitrogen balance, and the diet were considered a fixed effect and animal, period and square as a random effect. The means were obtained by the LSMEANS command. All analyzed variables were considered significant where $p \leq 0.05$.

Experiment 2

Ninety-two Dorper × Santa Inês cross lambs were used, 40 males and 52 females, with an initial BW of 21.23 ± 1.22 kg and 61.53 ± 3.13 days of age, to evaluate the average daily gain (ADG), dry matter intake (DMI) and feed efficiency (FE). All lambs were dewormed with subcutaneous moxidectin and received subcutaneous ADE vitamin supplements, five days before the beginning of the experiment. The lambs were kept in an individual tie-stall system, with a slatted floor, individual feeder, and water bunk.

The experiment lasted 56 days, divided into four 14-days periods, with the objective of evaluating the monensin effect over time. The experimental design used was a randomized complete block, with four treatments and twenty-three replications. The lambs were blocked according to sex, age and initial BW.

The experimental diets were the same as described in Experiment 1. The feed amount offered was calculated according to previous intake, adjusted when needed, so that refused feed would not exceed 5 % of daily intake. Orts were recorded at the end of each period to determine the DMI. The lambs were weighed after 14-h-fast on days 0, 14, 28, 42 and 56. In each interval, the ADG and feed efficiency were calculated.

Statistical procedures were conducted using PROC MIXED in SAS (Statistical Analysis System, version 9.0). All data were submitted to the Shapiro-Wilk test to verify the normality of the residuals, the removal of outliers, and homogeneity of variances using the Levene test.

The data for ADG, DMI and FE were analyzed as repeated measures over time. The diet and time were considered a fixed effect. Block was considered as a random effect. The data were put on covariance matrices and defined according to the lowest value obtained for Akaike's information criterion corrected (AICC). The means from variables were obtained by the LSMEANS command. The effects of diet, period and diet × period interaction were defined by the *F*-test of ANOVA. Orthogonal polynomials for diet responses were determined by linear, quadratic and cubic effects.

The data for lamb's BW the diet was considered a fixed effect and block as a random effect. The means were obtained by the LSMEANS command. All analyzed variables were considered significant where $p \leq 0.05$.

Results

Exp. 1. The doses of monensin did not affect the nutrient intake ($p \geq 0.07$) and digestibility ($p \geq 0.09$; Table 2). In addition, the experimental diets did not affect the nitrogen balance in feedlot lambs ($p \geq 0.07$; Table 3).

There were no diet and time interactions for rumen parameters ($p \geq 0.06$; Table 4). There was a quadratic effect for the molar proportion of acetate ($p = 0.01$) and acetate to propionate ratio ($p = 0.04$), with the lowest values observed for the M16 diet. The experimental diets did not affect the molar proportions of propionate, butyrate, isobutyrate, valerate and isovalerate. There was a quadratic effect for rumen pH ($p < 0.01$), the highest values were observed for M16 and the lowest values for M0. Linear effects were observed for total SCFA concentration ($p < 0.01$) and nitrogen ammonia ($p = 0.05$) in ruminal fluid. There was a time effect for acetate ($p < 0.01$), propionate ($p < 0.01$), isobutyrate ($p < 0.01$), isovalerate ($p < 0.01$), acetate to propionate ratio ($p < 0.01$), total SCFA concentration ($p < 0.01$) and rumen pH ($p < 0.01$). The highest molar proportion of propionate was observed 18 h after feeding. Furthemore, the lowest acetate concentration and, consequently, lowest acetate to propionate ratio was observed 18 h after the feed offer. The molar proportion of isobutyrate and isovalerate decreased until 12 h with a subsequent increase in the concentration. The total SCFA increased over time, with the highest concentration observed 18 h after feeding. The highest rumen pH was observed before any feed offer and the lowest value 12 h after feeding.

Effects of diet × hour were not detected ($p \geq 0.07$) for plasma parameters (Table 5). There was a quadratic effect ($p < 0.01$) for plasma glucose concentration in which wethers fed diets containing 16 mg of monensin kg of DM^{-1} had a greater plasma glucose concentration compared to M0. In addition, monensin decreased ($p = 0.05$) the plasma lactate concentration. Doses of monensin did not affect the plasma concentration of insulin and the IGF-I. There was an hour effect for all plasma parameters, and the highest glucose concentration was observed 9 h after feeding. Peak concentrations of plasma lactate, insulin, and IGF-1 occurred 15 h after feeding.

Exp. 2. Effects of diet × period were not detected ($p \geq 0.23$) for DMI, ADG and FE (Table 6). A linear effect was detected for feed DMI ($p = 0.04$). However, there was a quadratic effect for ADG ($p = 0.03$). Inclusion of 8 mg of monensin increased ADG compared to control, in contrast, inclusion of 16 or 24 mg of monensin reduced

Table 2 – Nutrient intake and digestibility in wethers fed diets containing levels of monensin.

Item	Diets[1]				SEM[2]	*p*-value[3]		
	M0	M8	M16	M24		L	Q	C
Intake, g d^{-1}								
DM[4]	1802	1862	1832	1730	101.32	0.20	0.07	0.92
OM[5]	1699	1753	1728	1634	95.58	0.23	0.08	0.95
NDF[6]	318.3	331.7	326.4	315.7	18.45	0.72	0.15	0.71
ADF[7]	115.7	115.8	115.5	112.2	6.79	0.42	0.57	0.85
CP[8]	332.3	340.5	333.8	318.2	15.60	0.11	0.08	0.84
Digestibility, g kg^{-1}								
DM	832.7	833.7	833.9	832.1	7.01	0.96	0.82	0.97
OM	851.3	855.3	856.9	853.0	6.74	0.79	0.49	0.90
NDF	657.3	651.5	659.1	664.8	15.6	0.87	0.75	0.46
ADF	610.9	624.0	591.7	607.5	17.8	0.56	0.94	0.21
CP	808.4	807.3	827.0	821.3	9.04	0.09	0.76	0.17

[1]M0 = diet without added fed additives; M8 = 8 mg kg^{-1} of monensin; M16 = 16 mg kg^{-1} of monensin; M24 = 24 mg kg^{-1} of monensin (DM basis). [2]SEM = standard error of the mean. [3]L = linear effect; Q = quadratic effect; C = cubic effect. [4]Dry matter. [5]Organic matter. [6]Neutral detergent fiber. [7]Acid detergent fiber. [8]Crude protein.

Table 3 – Nitrogen balance in wethers fed diets containing levels of monensin.

Item	Diets[1]				SEM[2]	*p*-value[3]		
	M0	M8	M16	M24		L	Q	C
N intake, g d^{-1}	53.16	54.48	53.41	50.91	2.50	0.11	0.08	0.84
Fecal N, g d^{-1}	10.12	10.46	9.32	9.44	0.58	0.07	0.77	0.11
Urinary N, g d^{-1}	18.80	21.76	20.36	19.13	1.88	0.96	0.26	0.58
N absorbed, g d^{-1}	43.05	44.02	44.08	41.46	2.17	0.33	0.11	0.72
N retention								
g d^{-1}	24.25	22.26	23.72	22.71	1.99	0.73	0.81	0.51
g kg^{-1} of N intake	456.4	402.5	446.1	445.2	33.0	0.95	0.43	0.34
g kg^{-1} of N absorbed	565.3	497.1	540.7	541.6	40.3	0.88	0.40	0.40

[1]M0 = diet without added fed additives; M8 = 8 mg kg^{-1} of monensin; M16 = 16 mg kg^{-1} of monensin; M24 = 24 mg kg^{-1} of monensin (DM basis). [2]SEM = standard error of the mean. [3]L = linear effect; Q = quadratic effect; C = cubic effect.

Table 4 – Rumen short chain fatty acids (SCFA), ammonia and rumen pH in wethers fed diets containing levels of monensin.

Item	Diets[1]				SEM[2]	*p*-value[3]				
	M0	M8	M16	M24		L	Q	C	H	D × H
SCFA, mM 100 mM^{-1}										
Acetate	56.91	49.50	49.38	53.11	2.83	0.16	0.01	0.67	< 0.01	0.48
Propionate	29.45	34.26	34.09	31.52	3.95	0.59	0.15	0.82	< 0.01	0.51
Butyrate	9.47	11.97	12.65	11.30	1.54	0.17	0.06	0.96	0.61	0.29
Isobutyrate	0.71	0.77	0.73	0.67	0.06	0.57	0.32	0.79	< 0.01	0.27
Valerate	1.51	1.68	1.39	1.41	0.24	0.17	0.45	0.07	0.73	0.42
Isovalerate	1.92	1.88	1.76	1.72	0.26	0.33	0.99	0.84	< 0.01	0.06
Acetate to propionate	2.17	1.63	1.59	2.02	0.36	0.64	0.04	0.98	< 0.01	0.42
Total, mM	100.95	96.11	93.47	88.37	5.26	< 0.01	0.96	0.69	< 0.01	0.18
pH	5.43	5.76	5.77	5.68	0.08	< 0.01	< 0.01	0.16	< 0.01	0.22
Ammonia, mg dL^{-1}	17.93	15.69	15.38	14.87	1.23	0.05	0.42	0.65	0.09	0.70

[1]M0 = diet without added fed additives; M8 = 8 mg kg^{-1} of monensin; M16 = 16 mg kg^{-1} of monensin; M24 = 24 mg kg^{-1} of monensin (DM basis). [2]SEM = standard error of the mean. [3]L = linear effect; Q = quadratic effect; C = cubic effect; H = hour effect; D × H = diet and hour interaction.

Table 5 – Plasma parameters in wethers fed diets containing levels of monensin.

Item	Diets[1]				SEM[2]	*p*-value[3]				
	M0	M8	M16	M24		L	Q	C	H	D × H
Glucose, mg dL^{-1}	69.59	74.12	75.07	73.33	2.63	< 0.01	< 0.01	0.77	< 0.01	0.23
Lactate, mg dL^{-1}	9.16	7.38	7.66	7.20	0.55	< 0.01	0.05	0.07	0.01	0.23
Insulin, ulU mL^{-1}	12.76	13.38	12.31	11.88	2.52	0.65	0.77	0.78	< 0.01	0.43
IGF-I, ng mL^{-1}	257.55	264.74	272.27	276.09	36.22	0.48	0.93	0.96	< 0.01	0.07

[1]M0 = diet without added fed additives; M8 = 8 mg kg^{-1} of monensin; M16 = 16 mg kg^{-1} of monensin; M24 = 24 mg kg^{-1} of monensin (DM basis). [2]SEM = standard error of the mean. [3]L = linear effect; Q = quadratic effect; C = cubic effect; H = hour effect; D × H = diet and hour interaction.

Table 6 – Body weight (BW), average daily gain (ADG), dry matter intake (DMI) and feed efficiency (FE) of lambs fed diets containing levels of monensin.

Item	Diets[1]				SEM[2]	*p*-value[3]				
	M0	M8	M16	M24		L	Q	C	P	D × P
Age, d	66.5	67.6	66.5	67.3	2.13	0.81	0.91	0.46	-	-
DMI, g d^{-1}	857.9	898.3	841.5	804.5	35.07	0.04	0.09	0.26	< 0.01	0.23
ADG, g	242.2	266.3	248.4	225.8	8.25	0.76	0.03	0.06	< 0.01	0.86
FE, gain feed^{-1}	0.28	0.30	0.30	0.28	0.01	0.65	< 0.01	0.81	< 0.01	0.95
Initial BW, kg	21.22	21.25	21.26	21.18	1.12	0.88	0.62	0.89	-	-
Final BW, kg										
Period 1	23.42	23.90	23.51	22.96	1.00	0.06	0.02	0.44	-	-
Period 2	26.99	27.32	27.03	26.12	1.08	0.03	0.04	0.99	-	-
Period 3	30.52	31.04	30.45	29.53	1.23	0.03	0.04	0.60	-	-
Period 4	33.50	34.72	34.18	33.09	1.37	0.38	0.02	0.56	-	-

[1]M0 = diet without added fed additives; M8 = 8 mg kg^{-1} of monensin; M16 = 16 mg kg^{-1} of monensin; M24 = 24 mg kg^{-1} of monensin (DM basis). [2]SEM = standard error of the mean. [3]L = linear effect; Q = quadratic effect; C = cubic effect; P = period effect; D × P = diet and period interaction.

ADG compared to 8 mg of monensin. Consequently, a quadratic effect was observed for FE ($p < 0.01$), and the highest values were observed for M8 and M16. In addition, there was a quadratic effect for final BW at P1 ($p = 0.02$), P2 ($p = 0.04$), P3 ($p = 0.04$) and P4 ($p = 0.02$), and the highest BW was observed for lambs fed 8 mg of monensin kg of DM^{-1}.

Effects of period were detected for DMI ($p < 0.01$), ADG ($p < 0.01$) and FE ($p < 0.01$). The DMI increased during the experimental periods while the ADG and FE decreased over time.

Discussion

The main action of monensin in rumen parameters is the alteration of the molar proportion of SCFA, which increases the molar proportion of propionate and reduces the molar proportions of butyrate and acetate (Prange et al., 1978). The effects of monensin on SCFA molar proportions are a consequence of the effect on ruminal bacteria, which favors more efficient fermentation pathways (Capelari and Powers, 2017). The greater availability of propionate may result in

increased glucose supply (Duffield et al., 2012) via the hepatic gluconeogenic flux (Baird et al., 1980), which may increase plasma glucose concentration, as observed in the present study.

The effects of monensin on rumen fermentation is dose-dependent. Ellis et al. (2012) related differences in propionate, butyrate and acetate proportions when the doses of monensin were different. In meta-analysis conducted by Martins et al. (2018), the author reported that the inclusion of 25 mg kg^{-1} of monensin in diets containing a high level of flint corn ground for wethers did not affect the molar proportion of SCFA, rumen pH and ammonia nitrogen concentration, questioning the dosage to be used for feedlot lambs. In the present study, the inclusion of monensin (0, 8, 16 and 24 mg kg^{-1}) altered the molar proportions, decreased the acetate and, consequently, the acetate-to-propionate ratio. The quadratic response showed that the dose of monensin used directly affects the ruminal metabolic processes, resulting in different concentrations of SCFA, and the use of 16 mg kg^{-1} of DM was the most effective dose in promoting the reduction of the acetate-to-propionate ratio. The ratio of lipogenic (acetate and butyrate) and glucogenic (propionate) SCFA is a determinant of methane synthesis in the rumen and hindgut, indicating representative carbon loss and an unproductive use of diet energy (Kebreab et al., 2009).

The data obtained by the present study suggest that the low doses of monensin in diets containing a high level of mature ground flint corn for lambs did not affect the butyrate concentrate in the rumen fluid. However, a number of authors have related a decrease in butyrate concentration with the inclusion of monensin in diets (Ellis et al., 2012), and this reduction may be associated with the inhibition of *Butyrivibrio fibrisolvens* (Zotti et al., 2017).

The inclusion of monensin modulates DMI feed by progressively increasing the duration and the frequency of DMI (Zotti et al., 2017), reducing the daily variation of feed intake, especially when the inclusion of concentrate is greater than 85 % in diets (Stock et al., 1995). In experiment 1, DMI was not affected by the inclusion of monensin in the diets, and thus the input of OM into the rumen was the same among treatments. Therefore, the hypothesis is that monensin modulated the frequency of intake and affected the ruminal fermentation process, reducing the total SCFA concentration, and decreasing the lactate production, maintaining a higher ruminal pH, associated with lower plasma lactate concentration.

The reduction in ammonia concentration in ruminal fluid by the inclusion of monensin observed in the present study is an event commonly reported in the literature. Monensin has the ability to decrease protein degradation in the rumen with a consequent reduction in ammonia concentration (Whetstone et al., 1981). Chen and Russell (1991) observed that monensin presented minor effects in the ruminal proteolysis process; however, it was effective in reducing amino acid deamination.

Increasing the retention of nitrogen by ruminants leads to decreases in N excretion, optimizing the use of protein (Castillo et al., 2001). Kebreab et al. (2001) proposed a predictive model in which cattle with an intake of 500 g of N d^{-1} would have approximately 80 % of total dietary nitrogen excreted in urine. Certain factors can affect the utilization of N by ruminants, such as dietary protein concentration, nitrogen composition of the protein, their interaction with the nutrients (Firkins and Reynolds, 2005), degradability and energy status of the animals (Kebreab et al., 2002). The inclusion of monensin may change the metabolic path in ruminant digestion, improve the protein absorption in the small intestine (Russell and Strobel, 1989) and increase nitrogen retention in lambs (Joyner et al., 1979). However, in the present study increasing the doses of monensin did not affect nitrogen absorption and retention.

It is expected that, since it causes changes in rumen fermentation (Ellis et al., 2012) and decreases DMI (Rogers and Davis, 1982), monensin would improve nutrient digestibility. In the present study, despite the effects on ruminal fermentation, monensin did not affect nutrient intake and digestibility. Many studies have evaluated the effects of the supply of monensin to ruminants on nutrient digestibility parameters, but the results found in the literature are controversial. These effects may be influenced by the conditions of the particular experiment, concentration of monensin and diet characteristics (Galloway et al., 1993). McCann et al. (1990) related that the inclusion of monensin increases the digestibility of fiber and protein when the forage content in the diet decreases. Rodrigues et al. (2001) observed a low effect of the inclusion of monensin on the nutrient digestibility and the results are dependent on the fiber content of the diets. Furthermore, the authors concluded that the effects on digestibility are not due entirely to the decrease in dry matter intake caused by monensin.

Metabolic profile is a tool that assists in the diagnosis of metabolic disorders and nutritional deficiencies. The effects of monensin on the metabolic profile are not consistent in the literature. Mears et al. (1987) performed three experiments on growing lambs and observed no effect of the inclusion of monensin on plasma insulin in any of the trials. In a meta-analysis performed by Duffield et al. (2008), the inclusion of monensin in the diet of dairy cows had no effect on plasma insulin concentration. However, it increased plasma glucose concentration by up to 3 %. The increase in glucose concentration was dependent on the dose used in the experimental diets, and would most likely be a function of monensin in the production of proponiate (Duffield et al., 2008). In the present study, the doses of monensin increased plasma glucose concentration in lambs fed high concentrate diets, although there was no increase in circulating insulin.

The use of monensin is commonly reported to decrease DMI (Joyner et al., 1979; Duffield et al., 2012).

However, the effect on the DMI response is dependent on the concentration of monensin used and diet composition (Hemphill et al., 2018). Enhanced propionate production by low doses of monensin may improve DMI by promoting insulin secretion and suppressing lipolysis, but may provide enough oxidative substrate to directly increase hepatic energy concentration and suppress feed intake with higher concentration (Allen et al., 2009). The decrease in DMI is normally related in studies using high-concentrate diets, especially higher amounts of readily fermentable carbohydrates (Bergen and Bates, 1984). During experiment 2, the increase in doses of monensin concentration decreased the DMI in lambs fed diets containing a high level of mature ground flint corn.

Duffield et al. (2012) related that certain factors are able to influence the estimation of the effect of monensin on ADG, such as study location, type of animal feed and doses, where higher doses of monensin were associated with a smaller effect on ADG. For feedlot lambs there is no consensus regarding the levels of monensin used in diets. Nockels et al. (1978) reported that the 5.5 and 11 mg of monensin kg^{-1} increased the BW gain, and lambs receiving 22 or 33 mg of monensin kg^{-1} showed similar performance when compared with control. Joyner et al. (1979) observed an improvement in feed conversion in lambs fed diets containing 5 mg of monensin kg^{-1}. Similarly, Horton and Stockdale (1981) observed increases in ADG and FE when 11 mg of monensin kg^{-1} was used whereas the use of 33 mg reduced the DMI without affecting ADG or FE. In addition, the inclusion of 5.5 mg of monensin kg^{-1} increased the FE of lambs fed high energy diet intake (3.18 Mcal NE) when compared to higher doses (Calhoun et al., 1979). Our study offered reassurance that for lambs fed a diet containing a high level of mature ground flint corn, 8 mg kg^{-1} of DM of monensin is the best concentration to be used, as it allows for greater body weight gain and feed efficiency.

Conclusion

The concentration of monensin evaluated in the current experiment demonstrated that the inclusion of 8 mg kg^{-1} of DM improves ruminal fermentation and the plasma parameters, indicating higher efficiency of energy retention These results are supported by the increase in growth performance of lambs fed 8 *vs*. 0, 16 and 24 mg of monensin kg^{-1} of DM. In addition, the data support the effect of monensin on DMI as being dose-dependent, as a high concentration of monensin (24 mg kg^{-1}) results in lower intake, with changes in fermentation and plasma parameters similar to those observed when lower concentrations were included. Thus, our conclusion was that the inclusion of 8 mg of monensin kg^{-1} of DM has the potential to be used for feedlot lambs fed diets that include a high level of mature ground flint corn.

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Authors' Contributions

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