LAIRANA ALINE SARDINHA

Use of narasin for lactating ewes and feedlot lambs

Pirassununga

2020

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Dissertação/Tese apresentada ao Programa de Pós-Graduação em Nutrição e Produção animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para a obtenção do título de Mestre/Doutor em Ciências.

Departamento:

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Área de concentração:

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Orientador:

Prof. Dr. Daniel Montanher Polizel

De acordo:_____

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CERTIFICADO

Certificamos que a proposta intitulada "Desempenho, perfil metabólico e composição do leite de ovelhas alimentadas com dieta contendo narasina e o desempenho de suas crias", protocolada sob o CEUA nº 5020291118 (ID 005926), sob a responsabilidade de **Alexandre Vaz Pires** *e equipe; LAIRANA ALINE SARDINHA* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 12/12/2018.

We certify that the proposal "Performance, metabolic profile and milk composition of ewes fed on a diet containing narasine and the performance of their offspring", utilizing 78 Ovines (males and females), protocol number CEUA 5020291118 (ID 005926), under the responsibility of **Alexandre Vaz Pires** *and team; LAIRANA ALINE SARDINHA* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 12/12/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 12/2018 a 02/2018 Área: Nutrição E Produção Animal

Origem:	Animais provenientes de outros projetos						
Espécie:	Ovinos	sexo:	Machos e Fêmeas	idade:	1 a 7 anos	N:	78
Linhagem:	Santa Inês/Dorper			Peso:	40 a 60 kg		

Local do experimento: Departamento de Zootecnia da Escola Superior de Agricultura "Luiz e Queiroz" Universidade de São Paulo

São Paulo, 13 de dezembro de 2018

Profa. Dra. Anneliese de Souza Traldi
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretária
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

UNIVERSIDADE DE SÃO PAULO
ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ"

Comissão de Ética no Uso de Animais – CEUA
www.esalq.usp.br – fone (19)34294400

CERTIFICADO

Certificamos que a proposta intitulada: "Efeito da utilização de dieta com diferentes teores de feno contendo narasina sobre o desempenho, carcaça e morfometria ruminal de cordeiros confinados" registrada com o número de protocolo, n° 2019-21, sob a responsabilidade do Prof. Alexandre Vaz Pires, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal-CONCEA, e foi aprovada pela Comissão de Ética no Uso de Animais (CEUA) da Escola de Agricultura Luiz de Queiroz-ESALQ/USP, em reunião ordinária no dia 14 de novembro de 2019.

Finalidade	**( ) Ensino (x) Pesquisa Científica**
Vigência da autorização	25/11/2019 a 25/03/2020
Espécie/Linhagem/raça	Ovino Dorper x Santa Inês
Nº de animais	44
Idade/Peso	120 dias /20 a 30 kg
Sexo	Macho
Origem	Sistema Intensivo de Produção de Ovinos e Caprinos (SIPOC) - Escola Superior De Agricultura "Luiz De Queiroz"

CERTIFICATE

This is to certify that study: "Effect of diet with different levels of narasin-containing hay on performance, carcass and ruminal morphometry of confined lambs", protocol number nº 2019-21 under the responsibility of Alexasdre vaz Pires, has been approved by the Institutional Animal Care and Use Committee, College of Agriculture "Luiz de Queiroz", Piracicaba, SP, Brazil, University of São Paulo.

Piracicaba, 14 de novembro de 2019

Prof. Dr. Gerson Barreto Mourão
Coordenador da CEUA/ESALQ/USP

EVALUATION FORM

Author: SARDINHA, Lairana Aline

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Dissertation submitted to the Postgraduate Program in Nutrition and Production Animal of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Master's degree in Sciences.

Date: _____/_____/_____

Committee Members

Prof._____

Institution:_____ Decision:_____

Prof..._____

Institution:_____ Decision:_____

Prof._____

Institution:_____ Decision:_____

To my parents Cátia and Luiz who are my inspiration for studies.

My brothers, Lorena and Gustavo, for teaching me to love without limits.

To my husband, Lucas for being by my side at all times and making my world a better place.

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“ Nunca deixem que lhe digam que não vale a pena acreditar no sonho que se tem, ou que seus planos nunca vão dar certo, ou que você nunca vai ser alguém”

- Renato Russo.

RESUMO

SARDINHA, L.A. **Uso de narasina para ovelhas lactantes e cordeiros confinados**. 2020. 100 p. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

Experimento I: O objetivo foi avaliar a inclusão de narasina sobre o desempenho de ovelhas e suas crias durante a fase de lactação. Foram utilizadas 32 ovelhas com dez dias de lactação, juntamente com suas crias. O delineamento experimental foi em blocos completos casualizados. As dietas consistiram em 50% de feno e 50% de concentrado. Os tratamentos foram definidos pela ausência (**N0**) ou inclusão de 13 mg/kg de MS de narasina (**N13**). O experimento teve duração de 70 dias. A inclusão de narasina não afetou o CMS das ovelhas; no entanto, aumentou a produção de leite (P <0,01) e a eficiência alimentar (EA; P = 0,02). As ovelhas alimentadas com N13 apresentaram maior produção de gordura (P <0,01), proteína (P <0,01), lactose (P = 0,04) e sólidos totais no leite (P <0,01). A inclusão de narasina na dieta das ovelhas aumentou a concentração de glicose no sangue (P = 0,05) nas semanas 8, 9 e 10. Os cordeiros de N0 tiveram maior CMS inicial (P <0,01) nas semanas 7, 8, 9 e 10; no entanto, o GMD e o PC no desmame e após o desmame foram semelhantes entre os tratamentos. **Experimento II:** o objetivo do estudo foi avaliar a inclusão de teores de FDN e inclusao de narasina sobre o desempenho, parametros de carcaça, fermentação ruminal e histologia das papilas em cordeiros confinados. Quarenta e quatro cordeiros foram confinados em um sistema tie-stall. O delineamento experimental utilizado foi o de blocos completos cazualizados, em esquema fatorial 2 x 2, sendo o fator I o uso de 20 ou 25% de FDN (na MS) e o fator II a inclusão de narasina (N0) ou inclusão de 13 mg/kg de MS de narasina (N13). O experimento durou 112 dias. Os níveis de FDN nas dietas não afetaram o GMD. No entanto, a dieta contendo 25% de FDN resultou em aumento do CMS dos cordeiros (P = 0,04), consequentemente, reduziu o EA (P <0,01). A inclusão de 13 mg/kg de MS de narasina aumentou a EGS (P <0,01), o EPC (P <0,01) e o CMS (P = 0,04). A inclusão de 20% de FDN resultou em aumento do EPC (P = 0,03) e peso de gordura perirrenal (P = 0,05). A narasina reduziu o teor de proteínas (P = 0,02) e aumentou o extrato etéreo (EE; P <0,01) da carne. Houve interação entre os teores de FDN e a inclusão de narasina na proporção molar de propionato (P = 0,02) e a relação acetato:propionato (P <0,01). A inclusão de narasina reduziu a concentração molar de acetato (P <0,01), aumentou o pH ruminal (P = 0,05) e a concentração total de AGCC (P <0,01), a proporção molar de propionato (P = 0,02) e a relação acetato-propionato (P = 0,03) na fermentação cecal. Dietas contendo 25% de FDN e as dietas com inclusão de narasina resultaram em alterações histológicas das papilas.

Palavras-chaves: Ionóforos. Ovelhas. Cordeiros. Confinamento. Leite.

ABSTRACT

SARDINHA, L.A. **Use of narasin for lactating ewes and feedlot lambs**. 2020. 100 p. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

The objective of this project was to evaluate the supply of narasin on the performance of ewes and their lambs in the lactation phase (Exp. I) and feedlot lambs (Exp. II). **Experiment I**: Thirty-two ewes with ten days of lactation were used, together with their lambs (s). The experimental design was randomized complete blocks. The diets consisted of 50% hay and 50% concentrate. The treatments were defined by the no narasin inclusion (N0) or inclusion of 13 mg/kg DM of narasin (N13). The experiment lasted 70 days. The inclusion of narasin did not affect the ewe's DMI, however, milk production ($P < 0.01$) and feed efficiency (FE; $P = 0.02$) increased. Ewes fed N13 had a greater fat ($P < 0.01$), protein ($P < 0.01$), lactose ($P = 0.04$) and total solids in milk ($P < 0.01$). Narasin inclusion in ewe's diet increased blood glucose concentration ($P = 0.05$) at wk 8, 9 and 10. The lambs of N0 ewes had a greater starter DMI ($P < 0.01$) at wk 7, 8, 9 and 10; however, the ADG and BW at weaning and after weaning were similar between treatments ($P > 0.05$). **Experiment II**: The objective of the study was to evaluate in inclusion of NDF contents and the inclusion of narasin on performance, carcass parameters, rumen fermentation and histology of the papillae in feedlot lambs. Forty-four lambs were confined in a tie-stall system. The experimental design used was a randomized blocks in a 2 x 2 factorial arrangement, with factor I being the use of 20 or 25% NDF (in DM) and factor II no narasin inclusion (N0) or inclusion of 13 mg / kg DM of narasin (N13). The experiment lasted 112 days. NDF levels in the diets did not affect the ADG. However, diet containing 25% NDF resulted in increase in lambs' DMI ($P = 0.04$) consequently, reduced the FE ($P < 0.01$). Inclusion of 13 mg / kg DM of narasin increased SFT ($P < 0.01$), BWT ($P < 0.01$) and DMI ($P = 0.04$). The inclusion of 20% NDF resulted in increased BWT ($P = 0.03$) and perirenal fat weight ($P = 0.05$). The narasin reduced protein content ($P = 0.02$) and increased ether extract (EE) ($P < 0.01$) of the meat. There was interaction between the NDF contents and the inclusion of narasin on the molar proportion of propionate ($P = 0.02$), and the acetate-to-propionate ratio ($P < 0.01$). Inclusion of narasin reduced the molar ratio of acetate ($P < 0.01$), increased ruminal pH ($P = 0.05$) and total SCFA concentration ($P < 0.01$), propionate molar ratio ($P = 0.02$), the acetate-to-propionate ratio ($P = 0.03$) on the cecal fermentation. Diets containing 25% NDF and the diets containing narasin resulted in histological changes in the papillae.

Keywords: Ionophores. Ewes. Lambs. Feedlot. Milk.

LIST OF FIGURES

LIST OF TABLES

SUMMARY

1. INTRODUCTION

Since the beginning of civilization, man and ruminants have shared a long history of production and are now based on the use of new technologies, especially in terms of nutritional aspects. The nutritional management adopted today, using diets with greater inclusion of concentrate or roughage, changes in the physiology and ruminal fermentation process, since the type of food eaten by the animal will change the microbial population, the motility of the gastrointestinal tract and nutrient absorption. In addition, rumen fermentation is closely linked to production efficiency, and is affected by the quality of the ingredients used in the diet, grain processing and microbiota manipulation, which can result in positive effects on yield.

One of the most studied and used ways to manipulate the rumen fermentation process is by the use of feed additives. Increased energy retention, reduced metabolic disorders, increased weight gain, reduced DM intake, increased feed efficiency (FE) and reduced methane production are some of the consequences of using ruminant feed additives (TEDESCHI, 2003; DUFFIELD et al., 2012; GONZÁLEZ et al., 2012).

Narasin is an ionophore produced by the bacteria *Streptomyces aurofaciens* and has been used in industry for several years as a coccidiostatic agent for birds (BERG; HAMILL, 1978). Due to the greater efficiency in inhibiting lactic acid production and increasing propionate production at lower dosages than other ionophores, interest in the use of this molecule in recent years has increased. However, the literature has little information on the use of narasin in ruminant nutrition, especially for sheep.

This research project aims to evaluate the effects of the use of narasin on sheep performance in different phases, as well as in different nutritional management.

2. LITERATURE REVIEW

2.1. Narasin

Narasin is a non-human-class ionophore antibiotic produced by *Streptomyces aureofaciens* bacteria, with molecular form $C_{43}H_{72}O_{11}$, molecular weight 765 Daltons and soluble in alcohol, acetone, chloroform and ethyl acetate (BERG; HAMILL, 1978). Like other ionophores, narasin has selective affinity, with affinity for Na +> K + = Rb +> Cs +> Li + (WONG et al., 1977). With the ability to change ionic gradients and electrical potentials in cell membranes, it ultimately impairs the functions and metabolism of microbial cells. The greater applicability of the molecule is for coccidiostatic control in domestic poultry due to its coccidiostatic characteristics (JEFFERS et al., 1988), but its ability to carry ions across the cell membrane has made it passive for use in ruminant nutrition.

The use of narasin has shown promising results in *in vitro* tests. Berg and Hamill (1978) observed the action of narasin on Gram-positive bacteria and fungi, which could result in increased energy efficiency during the rumen fermentation process. In addition, Nagaraja et al. (1987) observed that narasin increased the molar concentration of propionate at lower doses compared to monensin and lasalocid, and was more efficient in reducing lactic acid production than the other additives evaluated.

The intrinsic characteristics of the molecule increased the interest in the study and use of this ionophore in ruminant nutrition, aiming at coccidiostatic control, alter the rumen microbial population, changes in fermentation parameters and consequent productive response. However, research using narasin as a feed additive for ruminants is still restricted, and the first reports found in the literature were cited by Strasia et al. (1987), in which the authors evaluated the inclusion of different additives in feedlot diets.

2.1.1. Metabolism

The first reported study using narasin in the ruminant diet was conducted by Nagaraja et al. (1987), in which the authors tested ionophores and non-ionophores additives and found that narasin was more effective in reducing lactic acid production compared to the other additives evaluated.

Polizel et al. (2017a), evaluated the effects of the inclusion of sodium monensin (25 mg/kg DM) and narasin (10 or 20 mg/kg DM) on ruminal fermentation parameters of cattle fed 90% diets. concentrated during the adaptation period. The authors found that there was no change in the molar ratio of acetate, propionate and butyrate during the first seven days of feeding with the experimental diets.

Polizel (2017b) evaluated the effects of monensin and narasin on ruminal fermentation parameters, nutrient digestibility and nitrogen balance of lambs. Experimental diets were composed of 10% roughage (coastcross hay) and 90% concentrate. The control treatments were 25 ppm monensin or 5 ppm, 10 ppm or 15 ppm narasin. The authors reported that the lambs fed with the ionophores presented lower DM and nutrient intake compared to the control diet. Regarding nutrient digestibility, lambs that were fed with ionophores presented greater DM, OM and NFC digestibility compared to the control group. In addition, an increasing linear effect on digestibility was observed with increasing doses of narasin added to the diet. For diets containing high concentrate (90% DM) for feedlot lambs, the inclusion of narasin increased the molar ratio of propionate and reduced the acetate:propionate ratio.

Some studies were carried out with the objective of evaluating the effects of narasin in diets containing high forage content.

Polizel et al. (2019) evaluating the ruminal fermentation parameters of cattle fed high roughage diets receiving control (no narasin), 13 mg/kg DM and 20 mg/kg DM of narasin, observed that narasin doses increased the molar proportion of propionate, decreased acetate, and consequently altered the acetate:propionate ratio. These changes in rumen fermentation parameters were obtained when narasin was supplied once a day (every 24 hours).

Miszura et al (2018) evaluated the effect of three additives (narasin, lasalocid and virginiamycin) on the ruminal fermentation characteristics of steers fed diets containing 99% of forage and 1% of concentrate. The experimental treatments were: control, 13 ppm narasin, 20 ppm lasalocide and 20 ppm virginiamycin, and the additives were supplied once a day. The

results showed that narasin increased propionate, total SCFA concentration and decreased acetate-to-propionate ratio compared to the other diets used.

Limede et al. (2019) evaluated the effects of three additives (narasin, salinomycin and flavomycin) on ruminal fermentation parameters of steers fed diets containing 99% of forage and 1% of concentrate. The treatments used were control, 13 ppm narasin, 20 ppm salinomycin and 3 ppm flavomycin. Narasin once again increased the proportion of propionate, and decreased butyrate, acetate and acetate: propionate ratio when compared to the other additives and to the control treatment.

Regarding the effects of narasin on the metabolism of sheep fed high forage diets, Polizel et al. (2016a) evaluated the effects of increasing doses of narasin (0, 8, 16, 24 and 32 mg / kg DM) for lambs fed diets containing high roughage. The hay used was bermuda grass, containing 91% DM, 67.2% NDF and 32.1% ADF. The total concentration of SCFA increased linearly and reduced the ammoniacal nitrogen concentration in the rumen fluid with the narasin inclusion. In addition, narasin did not affect DMI and linearly increased NDF digestibility.

Oliveira (2018) evaluated the frequency of narasin supply on ruminal fermentation parameters and nutrient digestibility in sheep fed diets containing high quality forage (16% CP), being 95% coastcross hay and 5% ground corn (ionophore supply vehicle). The treatments used were: Control (C): daily corn supply without ionophore, N24: daily narasin supply at 13 ppm, N48: narasin supply every 48 hours, on the first day 26 ppm of on the second day corn without the additive (average 13 ppm narasin / day), and N72: supply of narasin every 72 hours, being offered on the first day 39 ppm narasin while on the second and third day corn without ionophore (average 13 ppm narasin/day). The supply of narasin daily (N24) and every 48 hours (N48) increased molar concentration of propionate and total concentration of SCFA, and decreased acetate: propionate ratio.

Pasqualino et al. (2018) evaluated the residual effect of narasin during the wash-out period (after dietary additive removal) in lambs fed diets containing coastcross hay (95% in DM) and concentrate (5% DM). The study showed that there was no effect on the molar ratio of acetate, butyrate, branched chain fatty acid and ruminal pH. However, there was interaction between diet × day after narasin withdrawal for propionate, acetate-to-propionate ratio and ammonia nitrogen concentration. The authors verified that the increase in the propionate molar proportion by the inclusion of narasin remained persistent until the 4th day after the additive withdrawal from the diets. In addition, the decrease in ammonia nitrogen concentration and acetate: propionate ratio lasted for up to 3 days after dietary withdrawal of narasin, concluding

that from the 5th day after dietary withdrawal of narasine, there is no residual effect of the additive on ruminal parameters of sheep.

2.1.2. Performance

In ruminant nutrition, one of the first reports of narasin use in vivo was performed by Strasia et al. (1987), studying different types of additives for cattle (monensin, monensin + tylosin, narasin, narasin + tylosin, salinomycin) and concluded that narasin-fed animals tended to increase weight gain compared to monensin-fed animals.

Recently, Gobato et al. (2017) evaluated the inclusion of 0 or 1300 mg narasin / kg mineral mixture for heifers fed high concentrate diets. The authors reported that the average narasin intake was 10.6 mg narasin / kg of total DM, and in this study it was also reported that the inclusion of narasin in the mineral mixture did not affect the intake of the supplement, as well as the consumption of DM. Experimental treatments did not affect the ADG, however, heifers that received the mineral mixture containing narasin showed an 8.5% increase in feed efficiency.

Regarding the use of narasin in diets containing high concentrate, only one experiment was found to evaluate the effect on lamb performance. Polizel et al. (2016b) observed that the inclusion of 5, 10 and 15 mg narasin / kg DM in diets containing 90% concentrate resulted in linear increase in ADG, FE and final body weight. Another important point noted by the authors was that the inclusion of narasin did not affect DMI.

It is noteworthy that in the study conducted by the authors no measurements were made to determine if there was any effect of the molecule on carcass and meat quality of lambs, and no studies reporting the effect of narasin on these variables were found in the literature. It is not known whether animals exposed to a longer feedlot time, is longer than 56 days, would have altered performance, or even if narasin would be beneficial if ingested over the long term.

Studies with narasin for cattle fed diets containing high roughage began when Silva et al. (2015) evaluated the inclusion of 0, 650 and 1300 mg of narasin per kg of mineral mixture offered to heifers fed with *tifton 85*. The authors observed that the inclusion of the additive in the mineral mixture did not affect the supplement and total DM intake.

In a study by Polizel et al. (2017a) the authors evaluated the supply of 0, 71.5 and 110 mg of narasin/day through the mineral mixture to grazing Nellore steers. The experiment lasted 84 days, and the animals remained rotated in paddocks of 1 hectare each, formed with *Brachiaria brizantha*. The authors reported that the inclusion of narasin did not affect the mineral mix intake. In addition, calves fed 71.5 and 110 mg narasin / day showed greater weight gain compared to control treatment during the first 56 days of the study. However, there was no effect in the last 28 days of the study. Considering the study as a whole (84 days of experiment), the animals fed with 71.5 and 110 mg narasin / day presented an increase in the ADG of 16.63% and 18.66%, respectively, compared to the control treatments.

Polizel et al. (2018) evaluated the effect of inclusion of narasin in mineral salt on the performance of steers grazing on 1 ha each *Brachiaria brizantha* paddocks, while each paddock was continuously grazed for 28 days, followed by a 28-day rest period. The experiment lasted 112 days. The experimental treatments were the control treatment (no additives), and the inclusion of 13 ppm narasin, added to the mineral salt. There was no interaction between treatment and period for ADG and DMI, however, the inclusion of narasin in mineral salt did not affect DMI and increased ADG and final body weight.

Polizel et al. (2019) evaluated the effect of narasin on protein supplementation on the performance of *Brachiaria brizantha* grazing bulls. The experimental treatments were control (no additive inclusion) and the inclusion of 13 ppm narasin. There was no interaction between treatment and period for GMD and supplement intake. Inclusion of narasin in the protein supplement did not affect supplement intake, increased ADG and final body weight, concluding the inclusion of narasin in the protein supplement increases the performance of cattle.

Miszura et al. (2019) evaluated the effect of three additives (narasin, lasalocide and virginiamycin) on the performance of beef cattle. The experimental diets consisted of 96% coastcross and 4% of concentrate (additive delivery vehicle, supplied once a day). The treatments were control, 13 ppm narasin, 20 ppm lasalocid and 20 ppm virginiamycin. There was an effect on DMI, ADG, FE and BW, showing that the inclusion of 13 ppm narasin increased the performance of cattle when compared to the other additives.

Limede et al. (2019) evaluated the effect of different feed additives on the performance of cattle fed high roughage diets. The diets were composed of 96% coastcross and 4% ground corn (additive delivery vehicle supplied once a day). The experimental treatments were: control (without additive), 3 ppm flavomycin, 13 ppm narasin and 20 ppm salinomycin. There was an effect on DMI, ADG and final BW, but there was no effect on FE. The authors found that the

inclusion of narasin increased the DMI, ADG and final BW compared to the other additives studied.

Regarding the supply of narasin to sheep fed diets with high forage content, only one experiment was found in the literature consulted. Oliveira et al. (2018) evaluated the inclusion of narasin (13 mg / kg DM) and the frequency of delivery (daily, every two days or every three days) on the performance of lambs fed 95% fourage diets (11.2% CP). The authors reported that daily supply of narasin or every two days did not affect the DMI, however, increased the lamb's ADG and FE.

2.1.3. Lactation

Ewes and lamb nutrition during the lactation period is of great importance for the success of sheep farming, since sheep milk production is directly related to lamb performance, and the milk is the main feed for lambs during the early stages of development

Changes promoted by ionophores in rumen fermentation, such as the increase in propionate, result in improvements in the animal's energy balance, which is of great importance to animals during the lactation phase. The increase in propionate molar concentration increases hepatic gluconeogenesis, thus contributing to the increase in milk production (OBA; ALLEN, 2003).

Three studies evaluated the effects of the inclusion of narasin in ewes diets during the lactation period. Assis et al. (2018) evaluated the inclusion of narasin doses on DMI, ewes milk production and composition and the performance of their lambs, with a diet containing 50% concentrate and 50% roughage. The experimental diet consisted of *Brachiaria brizantha* hay, corn, soybean meal and minerals. Treatments consisted of a control diet without the addition of narasin or the addition of 13, 20, or 27 ppm narasin. The authors observed a quadratic response for DMI, in which the highest intake was observed in the diet containing 13 ppm narasin. There was a quadratic response for milk yield, with the greater production observed for ewes fed 13 ppm of narasin. In addition, there was a linear decrease in milk protein and somatic cell count in response to the inclusion of narasin in the diets.

The authors concluded that the use of 13 ppm narasin in the diet of lactating sheep proved to be effective for increasing milk production, and that diets containing 27 ppm narasin in DM is not recommended.

Martins et al. (2018), observed that the narasin inclusion (13 mg/kg DM) in the diet containing high levels of soybean hulls inclusion for ewes did not affect the DMI, but provided

a 22.2% increase in milk yield. In addition, narasin promoted a reduction in protein concentration, but had no effect on the concentration of fat, lactose and total milk solids.

2.2. Feedlot lambs

The production of sheep meat in Brazil has a high expansion capacity, due to high market demand and limited supply in the international market. Technologies that reduce costs in animal production and improve production rates are becoming increasingly indispensable for successful sheep farming.

Thus, studies are conducted in search of new strategies for animal feed, highlighting the introduction of high concentrate diets for the lambs in intensive production system and short cycle under feedlot condition (ARRIGONI et al., 2013).

Due to the growth in areas intended for agriculture and also the seasonality of forage production, associated with the growing demand for animal products (CIRNE et al., 2014), it is necessary to intensify areas of animal production, where feedlot has become increasingly adopted by producers in Brazil.

Although the production cost is higher when compared to a pasture rearing, this system has greater economic advantages (BERNARDES et al., 2015). The feedlot is able to provide an increase in the supply of sheep meat, with better quality standardized carcasses (ORTIZ, 2011), carcasses from early slaughtered animals, which guarantee the producer differentiated prices in the sale of this product, faster return of the meat capital and the ability to manipulate the slaughter weight of lambs and the degree of finishing desired by the consumer market (OLIVEIRA et al., 2015), enables large-scale production in small areas (MEDEIROS et al., 2009), improves The herd's dietary conditions (FRESCURA et al., 2005) provide lower parasitic load and enable sheep production during the off-season and lack of food (LAGE et al., 2011).

However, analyzing the costs of feedlot, Pacheco et al. (2014) found that dietary expenses, especially those with concentrated feed, account for about 80% of the fraction of the costs. In order to be viable of feedlot lambs should be taken into account the time of stay of the animals in the feedlot, price of cereals grains, compatibility of the nutritional level with the genetic potential of the animal, weight gain and consumer market (SOUZA et al., 2014).

Aiming at higher feed efficiency of feedlot lambs, the use of diets containing large amount of grains that are quickly fermentable in the rumen optimizes energy intake per kilo of dry matter (STEELE et al., 2009). However, feeds with high proportion of fermentable carbohydrates results in an increased acid production, which causes a decrease in rumen pH.

The rumen pH is an important factor on microbial populations, fermentation products and rumen physiological functions, such as motility and absorption (NAGARAJA; TITGEMEYER, 2007).

Thus, it is necessary to improve feed management with diets containing a high proportion of energy, protein and low fiber to optimize animal performance. However, a minimum amount of NDF in needed to maintain ruminal health, adequate fermentation pattern without excessive gas accumulation and ruminal pH drop, providing adequate chewing, salivation and rumination to reduce incidence of metabolic disorders.

2.2.1. Fiber content in lamb diets

Diets formulated for feedlot lambs demand a high energy, because the performance of the lambs is related to the genetic potential, and to the quality of the diet offered (SANTANA, 2015).

Studies on fiber content and composition in diets for small ruminants are gradually intensifying as new feeding strategies replace fibrous feed with concentrated feed to increase production and reduce costs without harming animal performance.

Diets with excess fiber have low energy density and promote reduced intake leading to low productivity. The NDF content and digestibility may affect the DMI and affect the lamb's performance (TURINO et al., 2007). However, high concentrate diets have higher concentrations of metabolizable energy and result in higher conversion of net energy to gain due to dilution of maintenance energy requirement (NRC, 2007).

Efforts to quantify the fibrous fraction and its importance in the ruminant diet go back to the nineteenth century with the publication of Weende's proximate composition method. Weende's method proposed the determination of "raw fiber" by acid and basic intake, which is still used today to characterize diets, especially for non-ruminant animals. However, the method has some criticism, due to the fact that part of lignin is solubilized and accounted for as non-nitrogenous extract, and also because the crude fiber in some cases has higher digestibility than non-nitrogenous extract (VAN SOEST. 1963).

In the 60's there were great advances to improve the chemical characterization of fibers, allowing the understanding of their correlations with animal responses. The new techniques consisted of methods for quantifying components such as cellulose, hemicellulose and lignin (VAN SOEST, 1963).

The technique called Neutral Detergent Insoluble Fiber (NDF) was developed to account for the main components of the cell wall of plant cells, cellulose, hemicellulose and lignin (MERTENS, 2001).

By incubating the sample in a specific detergent, the cellular content, composed mainly of protein, minerals and non-structural carbohydrates is solubilized and separated from the cell wall by filtration. This technique has been modified over the years, and new steps have been added to minimize cell wall protein and mineral requirements (VAN SOEST; ROBERTSON; LEWIS, 1991).

The NDF is an important chemical fraction in the diet of small ruminants, because besides influencing performance, it stimulates chewing activity and saliva secretion. According to Mertens (2001), a reduction in dietary fiber level can result in a series of cascading events such as lower chewing, decreased rumen pH, changes in microbial populations and reduced acetate-to-propionate ratio.

According to Mertens (2001) with the creation of minimum cost diet formulation programs, there was encouragement in the development of a quantitative method to ensure that the minimum forage requirement is established to ensure that the animal remains a healthy ruminant. However, for lambs intended for meat production, longevity is not the main objective, thus allowing the use of a low NDF fiber content in diets.

Nutritional systems such as Cornell Net Carbohydrate and Protein System (CNPS) for cattle (FOX et al., 2004) and sheep (CANNAS et al., 2004) set minimum requirements for NDF levels in diets, which are between 20 and 24.5. %, where below these values, rumen fermentation and microbial protein synthesis would be negatively altered.

Cardoso et al. (2006) evaluated different levels of NDF in the diet of feedlot lambs (25, 31, 37 and 43% of NDF), did not observe significant difference in the time spent with feeding and rumination. However, the authors concluded that the addition of increasing NDF content to the diet caused a linear decrease in DMI and a linear reduction in average daily gain and a worsening in feed conversion with increasing fiber content.

Ferreira et al. (2011) evaluating the effect of soybean hull inclusion rate in high concentrate lamb diets on DMI effects, digestibility and nitrogen balance. The experimental diets consisted of a control diet with 70% corn and 0% soybean hulls, and the other diets where

corn was replaced by the inclusion of soybean hulls at rates of 15, 30 or 45% of the original corn concentration. resulting in 24.5, 27.3, 35 and 39.5 of NDF. The was authors observed a linear increase in DMI with the increase of soybean hull inclusion in the diet. The acetate concentration increased linearly, while quadratic effects were observed on propionate concentration and acetate-to-propionate ratio with increasing soybean hull in the diet. Ruminal pH and nitrogen concentration increased linearly when corn was replaced by soybean hulls.

The authors concluded that replacing corn grain with up to 45% soybean hulls resulted in a tendency to decrease the apparent digestibility of DM and increase the digestibility of NDF, presenting the fermentation pattern that would reduce the risk of acidosis.

Gallo et al. (2019) conducted a study to evaluate the effects of different NDF levels in diets containing high concentrate on performance, ruminal, blood and carcass aspects of finishing lambs. Experimental treatments consisted of 15, 20 and 25% NDF levels based on DM. The diets were composed of corn, soybean meal, minerals and millet silage being affected to the animals twice a day. The results obtained were that the lambs fed with the lowest fiber content presented the best carcass yield. Blood parameters, rumen SCFA production and protozoan population were not affected by different NDF levels. High concentrate diets with 15% NDF resulted in better feedlot weight gain, producing a good carcass and without lamb health problems and without affecting ruminal parameters.

The same authors evaluated the influence of lamb finishing systems, performance and carcass and meat quality. The treatments consisted of grazing lambs, semi-confinement or confinement. The lambs were slaughtered with average live weight of 35 kg. The authors observed that pasture-finished animals had lower weight gain, were slaughtered at older age, lower carcass weight, lower loin eye area, lower stem compaction index, and lighter shoulder and shoulder weights when compared to other production systems. There was no difference between lambs finished in semi-confinement and confinement for the evaluated characteristics.

The authors concluded that different production systems affect animal performance as well as carcass and meat quality.

3. MILK YIELD AND COMPOSITION FROM EWES FED DIETS CONTAINING NARASIN AND THEIR LAMBS' PERFORMANCE

3.1. ABSTRACT

The changes promoted by feed additives in ruminal fermentation, especially increasing the availability of propionate, can improve the energy balance of an animal, which is of great importance in the lactation period. This trial aimed to evaluate the inclusion of narasin in the diet of lactating ewes on milk yield, composition, dry matter intake (**DMI**) and plasma metabolites of the ewes and growth rate of lambs. Thirty-two lactating ewes (59.0 ± 2.42 kg) were assigned to a randomized complete block design. The experimental diets contained 500 g/kg of dry matter (**DM**) of *coast cross* (Cynodon dactylon (L.) Pers) hay and 500 g/kg DM of concentrate, and the treatments were: **N0 -** no narasin inclusion; **N13 -** inclusion of 13 mg of narasin/kg DM. Once a week, from wk 2 to 10 of lactation, ewes were separated from their lambs, injected with oxytocin and milked mechanically to empty the udder. After 3 h, the milk production was recorded, using the same procedure, and sampled to evaluate the composition. The blood samples were taken weekly, 4 hours after feeding. The average daily gain (**ADG**) and starter DMI of the lambs were evaluated weekly from wk 2 to 12 of age. The inclusion of narasin did not affect ($P = 0.93$) DMI of ewes; however, it increased milk production ($P < 0.01$) and feed efficiency ($P = 0.02$; **FE**). Ewes fed N13 had a greater milk fat ($P < 0.01$), protein ($P < 0.01$), lactose ($P = 0.04$) and total solids production ($P < 0.01$). Narasin inclusion in ewe's diet increased plasma glucose concentration ($P = 0.05$) at wk 8, 9 and 10, however, there was no effect on plasma urea concentration ($P = 0.96$). The lambs of N0 ewes had a greater starter DMI ($P < 0.01$) at wk 7, 8, 9 and 10; however, the ADG and body weight (**BW**) at weaning and after weaning were similar between treatments ($P > 0.05$). The results showed that the inclusion of 13 mg of narasin/kg DM improved the milk production and FE of the ewes without altering the composition of the milk. The lower initial consumption of concentrate by N13 lambs before weaning was caused by the higher production of milk. The results obtained in the present study demonstrate the possible productive gain with the inclusion of narasin in diets for lactating ewes.

Key words: additives, feed efficiency, glucose, milk production, sheep

List of abbreviations: DMI, dry matter intake; DM, dry matter; N0, no narasin inclusion; N13, inclusion of 13 mg/kg of narasin in dry matter; ADG, average daily gain; FE, feed efficiency; BW, body weight; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; EE, ether extract; NFC, non-fiber carbohydrates; BCS, body condition score; AOAC, association of official analytical chemists; FCM, fat corrected milk; FPCM, fat and protein corrected milk.

3.2. INTRODUCTION

The survival and development of lambs are directly linked to the production of milk, since this is the first food ingested by the newborn. It is common to use feed management strategies to improve milk composition and production in an effort to optimize lamb productivity and carcass quality. To that end, the feeding with additives is of interest for increasing milk production of ewes.

The changes promoted by ionophores in ruminal fermentation, further increasing the availability of propionate, result in improvements in energy balance, because the increase of the molar concentration of propionate increases hepatic gluconeogenesis, contributing to the increase of milk production (Oba and Allen, 2003). Monensin and lasalocid are the most studied ionophores, and their use in dairy cows increased energy status during the transition and early lactation periods (McGuffey et al., 2001).

Narasin is an ionophore produced by *Streptomyces aureofaciens* bacteria, with a molecular formula of $C_{43}H_{72}O_{11}$ and is soluble in alcohol, acetone, chloroform and ethyl acetate (Berg and Hamill, 1978). The ability of narasin to carry ions through cell membranes made it possible for tests of population control of bacteria. In an in vitro study, Nagaraja et al. (1987) observed that narasin increased the molar concentration of propionate with lower doses in relation to monensin and lasalocid and was more effective in the decrease in the production of lactic acid than the other additives evaluated. Narasin inclusion in diets for feedlot lambs increased the average daily gain (**ADG**), feed efficiency (**FE**) and final body weight (**BW**; Polizel et al., 2016). However, the effects of narasin on milk production, milk composition and plasma parameters in lactating ewes have not been described.

In this context, we hypothesized that the inclusion of narasin in the diet of ewes post-partum will increase milk production and lamb performance, without changing milk composition and dry matter intake (**DMI**). Hence, the objective of the present study was to evaluate the inclusion of narasin in diets for lactating ewes on DMI, milk production and composition, plasma metabolites of the ewes, and growth of suckling lambs.

3.3. MATERIALS AND METHODS

This study was carried out at sheep facilities of the Department of Animal Science, "Luiz de Queiroz" College of Agriculture (**ESALQ**), University of São Paulo (**USP**), in Piracicaba, State of São Paulo, Brazil. All procedures using animals followed the guidelines recommended by the Animal Care and Use Committee of the USP, protocol number **5020291118**.

3.3.1. Animals and experimental design.

Thirty-two Dorper x Santa Inês ewes with initial BW of 59.0 ± 2.42 kg were housed indoor and individually allotted with their lambs in pens (1.5 × 3.5 m) with a concrete floor, feed bunk, mineral box, waterer and a creep feeding system (0.80×1.0m). Eighteen ewes had single births (nine/treatments) and fourteen had twin births (seven/treatment), with eighteen females (nine/treatment) and twenty-eight male lambs (fourteen/treatment). At lambing, all ewes were dewormed with moxidectin 1% (Cydectin, Fort Dodge Saúde Animal Ltda, Campinas, SP, Brazil) according to label directions.

After 7 ± 1.2 d in milk, ewes were divided into a randomized complete block design, with two diets and 16 blocks. The blocks were defined according to date of lambing, type of birth, sex of the offspring and initial BW of the ewes. Four blocks included ewes nursing single female lambs; five blocks included ewes nursing single male lambs; and seven nursing twins (four male-male; two female-female, and 1 male-female). The experimental period was from 2 to 10 wk post-partum for ewes, and their lambs were evaluated until 12 wk of age.

3.3.2. Feed management, collection of samples and methodologies.

After forming the blocks, ewes were fed the control diets containing 500 g/kg of dry matter (**DM**) of *coast cross* (*Cynodon dactylon* (L.) Pers) hay, and 500 g/kg DM of concentrate, to

adapt them to the experimental facilities and feeding management. The treatments were: **N0** - control diet with no fed additives; and **N13 -** inclusion of 13 mg of narasin/kg DM (ZIMPROVA, Elanco Animal Health, Indianapolis, IN). The dose used was determined according to previous studies (Assis et al., 2019). The diets (Table 1) were isonitrogenous and balanced according to the recommendations of the National Research Council (NRC, 2007).

The concentrate ingredients were weighed using an electronic scale with a 10 g accuracy (Marte, LC 100 São Paulo, Brazil) and mixed using a horizontal mixer with a capacity of 500 kg (Lucato, Limeira, Brazil). The hay was ground using a shredder (Nogueira ® DPM – 4, Itapira, Sao Paulo, Brazil) with a 10 mm screen. Narasin was previously mixed with the concentrate (Lucato, Limeira, Brazil) and supplied to the sheep as a total mixed ration. The animals had *ad libitum* access to the feed and fresh water. The diet was offered daily and orts were collected and weighed weekly in order to calculate DMI. Amounts of feed offered to ewes were calculated according to previous DMI, and adjustments were done when needed so that refused feed did not exceed 0.1 kg of daily intake. Feeds and orts were sampled weekly, and frozen at -20°C for later analysis.

Ewes were weighed without fasting for 3 consecutive days at the beginning and at the end of the experimental period. The initial BW and final BW were determined as the average of the 3-weight data. On the days of weighing, the body condition score (**BCS**) was also assessed by classifying the ewes with grades from 1 (thin) to 5 (fat), with an increment of 0.25 (Russel et al., 1969).

To measure the milk production, once a week (wk 2 to 10), the ewes were separated from their lambs, and mechanically milked (Camp Agri, model GL300, São Paulo, Brazil) twice a day after an intravenous injection of 10 IU of synthetic oxytocin (Univet, São Paulo, Brazil) at 1000 and 1300 h (Susin et al., 1995). The first milking was performed to empty the mammary gland and the milk was discarded. The second one was used to measure milk yield in 3h. The total milk produced per ewe in this interval was weighed on an electronic scale accurate to 0.1 g (Marte AC-10K). After weighing, milk was homogenized and 20 mL was collected and preserved in bromopol Broad Spectrum Microtabs ® II (2-bromo-2-nitropropane-1.3-diol, D & F Control Systems, Inc., Dublin, CA, USA).

From 2 to 10 wk of lactation, blood samples were collected weekly at 4 h after offering the diet from the ewe's jugular vein into Vacutainer tubes containing sodium fluoride as a glycolytic inhibitor and the anticoagulant EDTA (Greinr Bio-One Brazil, Americana, SP,

Brazil). Immediately after drawing, blood samples were centrifuged at 3,000 x g at 4°C for 15 min. After centrifugation, 2 aliquots were obtained from the plasma and were stored separately at -18°C, which were then used to determine the concentration of glucose and urea.

The determinations of plasma glucose and urea were performed using specific commercial enzymatic kits from LABTEST diagnostic S.A. (Lagoa Santa, MG, Brazil; Ref.: 85), in as automatic biochemistry system (SBA – 200, CELM, Barueri, SP, Brazil).

3.3.3. Lamb performance.

Lambs had *ad libitum* access to feeding starter from wk 2 to 12 of age. The starter was composed by 645 g/kg DM ground corn, 190 g/kg DM soybean meal, 5 g/kg DM ammonia chloride, 15 g/kg DM mineral mix, 5 g/kg DM limestone, 40 g/kg DM molasses and 100 g/kg DM milk replacer. The starter was available in a creep feeding system (0.80 × 1.0 m). To avoid access to the ewes' feed bunk, lambs were kept in a leash system, allowing them to nurse and to reach the creep feeder and water (Polizel et al., 2017; Parente et al., 2018). The orts of creep feeding were weighted weekly in an electronic scale with a 1 g accuracy (Marte ®, LC 100 São Paulo, Brazil) for DMI calculations. Lamb BW was measured weekly after a 3 h fast to calculate the ADG. Lamb DMI was obtained during the preweaning (14 to 70 d of age) and postweaning (70 to 84 d of age) periods.

3.3.4. Chemical analysis

The samples of feed and orts were dried (MA035 - Marconi, Piracicaba, São Paulo, Brazil) at 55° for 72 h and ground through a 1-mm in Wiley mill (Marconi, Piracicaba, Brazil). The DM was determined by drying the samples in an oven at 105 ° C for 24 hours (Association of Official Agricultural Chemists; **AOAC**, 1990; #934.01), and ash was determined by incinerating the samples in muffle at 550 ° C for 4 hours (AOAC, 1990; #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) concentrations in an

Ankom 2000 fiber analyzer (Ankom Tech. Corp., Fairport, NY). Heat-stable α-amylase and sodium sulfite were included in the NDF analysis. Total nitrogen concentration was determined using the Leco TruMac® N apparatus (Leco Corporation, St. Joseph, MI, USA; AOAC, 1990; #968.06). The crude protein (**CP**) was calculated by multiplying the total nitrogen by 6.25. The ether extract (**EE**) concentration was determined according to AOAC (1990; #920.29). Non-fiber carbohydrates (**NFC**) of the diets were estimated according to the following equation: NFC = 100 - [(%Total CP - %CP urea + % urea) + %FDN + %EE + %ash] (Hall, 2000).

Milk samples were analyzed for protein, fat, lactose, and total solids. Fat, protein, and lactose concentrations were determined by infrared spectrometry (Bently 2000; Bently Instruments Inc., Chasca, MN, AOAC, 1990). Milk correction calculations for fat (6.5%; **FCM**) and protein (5.8%; **FPCM**) were performed as described by Pulina and Nudda (2002). The FE was calculated for milk production, FCM and FPCM considering the DMI in each week.

3.3.5. Statistical analysis

Pen was the experimental unit for all statistical analyses. Statistical analyses were performed using the MIXED procedure of SAS (SAS version 9.0; SAS Inst. Inc., Cary, NC). All data were submitted to the Shapiro-Wilk test to verify the normality of the residuals, the removal of "outliers" using the Studentized residuals, and homogeneity of variances using the Levene test.

The data for intake, milk production, milk composition, performance of the lambs and plasma metabolites were analyzed as repeated measures over time. The statistical model used was: $y_{ijk} = \mu + D_i + b_j + e_{ij} + T_k + D_iT_k + b_jT_k + e_{ijk}$, in which μ = overall mean, D_i = fixed effect of diet, b_j = random block effect, e_{ij} = random error A, T_k = fixed effect of time, D_iT_k = fixed effect of diet × time interaction, b_jT_k = random effect of block × time interaction, and e_{ijk} = random error B. All analyzed data as repeated measures were put on covariance matrices and tested for "compound symmetry, heterogeneous compound symmetry, autoregressive, autoregressive heterogeneous, unstructured, banded, variance components, toeplitz, and heterogeneous toeplitz" and defined according to the lowest value obtained for Akaike's information criterion. The treatment means were obtained by the LSMEANS command. The effect of diet, time, and interaction of diet × time were defined by the *F* test.

Ewe BW, BCS and BW of lambs were analyzed using the model $y_{ij} = \mu + D_i + b_j + e_{ij}$, in which μ = overall mean, D_i = fixed effect of diet, b_j = random effect of block, and e_{ij} = random error. The treatment means were obtained by the LSMEANS command. The treatment effect was defined by the *F* test. All analyzed variables were considered significant when $P < 0.05$.

3.4. RESULTS

The experimental diets did not affect the BW ($P = 0.52$) and BCS ($P = 0.81$) of ewes at 10th wk of lactation (Table 2). There was no interaction between diets and week on DMI ($P = 0.13$), nutrient intake, milk yield ($P = 0.41$), milk composition and FE of ewes (Table 2). The inclusion of narasin increased milk production ($P < 0.01$). Moreover, narasin did not affect the milk composition and, consequently, increased FCM ($P = 0.01$), FPCM ($P = 0.01$), fat ($P < 0.01$), protein ($P < 0.01$), lactose ($P = 0.04$), total solids ($P < 0.01$), and solids nonfat production ($P < 0.01$). The narasin inclusion increased the FE for milk ($P = 0.02$), FCM ($P = 0.02$) and FPCM ($P = 0.02$) compared with the control diet.

There was a time effect ($P < 0.01$) on intakes of DM, CP, NDF, ADF and ash, with a progressive increase, and the highest values found on wk 7 of lactation, followed by a decrease. There was a time effect ($P < 0.01$) on milk production and composition. The milk, FCM, FPCM, protein, lactose, total solids and solids nonfat production was greater on wk 4, followed by a progressive decrease until wk 10 of lactation ($P < 0.01$). The percentage of fat, protein, total solids and solids nonfat was greater on wk 10; however, the lactose percentage was greatest on wk 4 and 5. The FE for milk production, FCM and FPCM decreased during the lactation, with the greatest values observed on wk 3.

There was an interaction ($P < 0.01$) between diets and week for concentration of glucose (Figure 1). The narasin inclusion did not affect the plasma glucose concentration at the wk 3, 4, 5, 6 and 7; however, the ewes fed the N13 diet had a greater plasma glucose concentration ($P = 0.05$) than N0 at the wk 8, 9, and 10. There was no interaction ($P = 0.60$) between diets and week and diet effect ($P = 0.96$) for plasma urea; however, there was a time effect ($P < 0.01$) with the greater values observed on wk 9 of lactation (Figure 2).

The experimental diets did not affect the lamb ADG ($P > 0.05$), consequently, BW at weaning and after weaning was similar among treatments ($P > 0.05$; Table 3). However, there was an interaction ($P = 0.03$) between diets and week for initial concentrate DMI by the lambs

before the weaning. The lambs had the same initial concentrate DMI at the wk 3, 4, 5 and 6; however, lambs from N0 ewes had a greater ($P < 0.05$) initial concentrate DMI compared with N13 at the wk 7, 8, 9 and 10 (Figure 3). There was a time effect ($P < 0.01$) for ADG before and after weaning, with the greater values observed on wk 8 and 11, respectively. The initial concentrate DMI increased during the lactation of the ewes and after weaning.

3.5. DISCUSSION

3.5.1. Dry matter intake, body weight, milk yield and composition of ewes

The DMI was 2.27 and 2.26 kg/d for ewes fed N0 and N13 diets, respectively. These values are in accordance with those recommended by the NRC (2007) for 60 kg ewes rearing single lambs during the first 6 to 8 wk of lactation. Differently than monensin (Duffield et al., 2012), some research shows that the narasin inclusion on ruminant diets did not affect DMI in high forage diets (Silva et al., 2015; Polizel et al., 2018) and in high concentrate diets (Gobato et al., 2017; Polizel et al., 2016b) as in the present study. The literature has demonstrated that the inclusion of narasin in the diet has increased the feed efficiency of the animals with increase in performance, without reducing the DMI.

As there was no effect on DMI of the ewes during the experiment period, the consumption of NDF, ADF and Ash were equal for the animals of both experimental diets. In this experiment, the BW and BCS were similar during the experimental period, showing that the animals did not need to perform energy mobilization to maintain milk production.

Narasin improves milk production when compared with control diets. The apparent benefits of ionophore feeding in the lactation period are linked to a better glucose state, caused by increased production of propionate and better retention of nitrogen (McGuffey et al., 2001). The effect on FE for milk, FCM and FPCM of the N13 diet is due to the improved milk production of these animals and to the similar dietary intake of the N0 diet. Narasin is capable of changing rumen fermentation, especially to increase the molar proportion of propionate and decrease the acetate:propionate ratio (Polizel et al., 2018). The increase in propionic acid concentrations increases gluconeogenesis in hepatic tissue and improvement in energy

metabolism (Baird et al., 1980). It generates an improvement in the efficiency of retention of crude energy by the animal, since propionate is the main precursor of glucose for ruminants.

With the advancement of lactation, the epithelial cells of the mammary gland pass from the state of active secretion to the non-secretory state by the involution process, characterizing the decline of production at the end of lactation (Pulina and Nudda, 2002). There was an effect (Table 2) on the production of fat, protein, lactose, total solids and total dry solids since the production of these milk compounds accompanies milk production. There was no effect of dilution of the milk compounds when there was an increase in milk production of the animals during the experimental period. As described by Resende *et al*. (2008), the period of lactation, from a nutritional point of view, is one of the moments that should be more focused on females since they are faced with 3 distinct phases. In the first, shortly after birth of lamb, the ewe goes through a negative energy balance, since the milk production of this animal is increasing and its consumption has not yet reached the maximum potential, thus taking place the mobilization of body reserves. In the second phase, the energy balance is equal to zero, since milk production is already decreasing and the female has already reached the peak of DM consumption. In the last phase, the energy balance is positive, with replacement of the body reserves. Therefore, the present study demonstrated that narasin provided a greater energy supply to the animals, with this energetic gain destined to the production of milk and also of the milk compounds, without alterations in milk composition.

3.5.2. Blood metabolites.

The increase in blood glucose from wk 7 until 10 of lactation in ewes fed diets containing narasin can be explain by the energy balance of the ewes at the end of lactation, resulting in a decrease in milk production. The inclusion of narasin in ruminant diets increases molar proportion of propionate (Polizel et al., 2018) and propionate is the mainly glucose precursor in ruminants (Ellis et al., 2012), which at the end of lactation was shown at higher levels due to the lower glucose requirement of these animals.

There was no effect on plasma concentrations of urea during the experiment. This is due to the fact that there is a relation between plasma urea concentrations and the amount of energy consumed (Cardoso et al., 2010), since urea levels may be associated with protein levels in the

diet and also reflect the energy and protein ratio of the diet. In the present study, the treatments did not affect the consumption of DMI and protein, resulting in the same concentration of urea in the.

The main controlling factor of plasma urea concentrations is the formation of ammonia in the rumen, and the concentration of blood urea appears to reflect changes in rumen ammonia production. Thus, the concentration of urea in the blood is influenced by the extent to which the absorbed amino acids are oxidized and by the absorption of ammonia from the rumen, substantially reflecting the extent of nitrogen balance in the animal, considering both the requirements of ruminal microorganisms and of the host animal (Lima, 2013). Ionophores, such as monensin and narasin, are potential feed additives to decreases rumen ammonia, especially to inhibit deamination (Bergen et al., 1984), which could decrease blood urea concentration; however, this was not observed in the present study.

3.5.3. Lamb's performance

Even with the higher N13 milk production in relation to N0, there was no effect on ADG and BW of the lambs of both treatments. The ADG of lambs during lactation was higher than in one of our previous studies (Ferreira et al., 2018), using the same methodology but presented values close to those reported by Susin et al. (1995). The non-effect on ADG and BW of lambs can be explained by the higher DMI of sheep lambs fed N0. The consumption of food in the trough is inversely proportional to the amount of milk ingested (Foot, 1972; NRC, 2007) or it also results in the suppression of constant breastfeeding (Folman et al., 1966). There is a correlation between offspring milk consumption and growth rate of these lambs (Roda et al., 1984). This correlation is high during the first six weeks of lactation, and then declines and may become negative at the later stage of lactation (Peart, 1982).

The decline in the lactation curve is impacted by the decrease in milk demand by lambs due to increased solid food intake. Barnicoat *et al*. (1949) found that milk loses importance from wk 8 of age of lambs, as they increase grain and forage consumption. In the present study, the young lambs started to really increase consumption of solid foods at around d 21 of age.

Suckling lambs that receive the least amount of milk increase the intake of solid foods more quickly in early life than those lambs that receive the most amount of milk at that stage. A lamb meets it energy through increased food intake, and weight gain in this case is influenced by the consumption of solid foods during breastfeeding of these animals (Hatfield et al., 1995).

Therefore, lambs that had greater access to higher amounts of milk, as in the N13 treatment, did not consume a large amount of concentrate in creep feeding when compared to lambs with lower milk yield. The higher milk content available for lambs of narasin tended to supply the energy needs, reducing the need for diets with solids consumption as a function of the satiety generated by milk, reducing the need for concentrate consumption. After weaning, the consumption of starter grain of these animals was similar due to the absence of breast milk.

3.6. CONCLUSIONS

Milk production is known as one of the limiting factors for lamb growth. The narasin inclusion (13 mg/kg of DM) in the diet of lactating ewe's increased milk production without altering its composition, making the lamb less dependent on the initial concentrate, since its requirements for gain are mainly attendant by the ewes' milk yield. In addition, the inclusion of narasin increased blood glucose concentration after the peak of lactation, providing more energy to the ewes. The results obtained in the present study demonstrate the possible productive gain with the inclusion of narasin.

3.7. ACKNOWLEDGEMENTS

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3.8. LITERATURE CITED

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Table 1. Proportions of the ingredients and chemical composition of the experimental diets

Item	Diets[1]	
	N0	N13
Ingredients, %		
Hay "coastcross"	50.0	50.0
Soybean meal	8.6	8.6
Citrus pulp	18.2	18.2
Ground corn	18.2	18.2
Mineral mix[2]	1.5	1.5
Soybean oil	3.0	3.0
Urea	0.5	0.5
Narasin, mg/kg of DM	0	13.0
Chemical composition, %		
Dry matter, g/kg as-fed basis	88.5	88.5
Organic matter	80.5	80.6
Crude protein (CP)	13.9	14.3
Neutral detergent fiber (NDF)	46.0	46.1
Acid detergent fiber (ADF)	20.4	20.5
Ether extract (EE)	4.45	4.63
Ash	8.00	7.98
Non-fiber carbohydrates (NFC)	27.7	27.0

[1]N0 = diet without feed additive; N13 = diet containing 13 mg/kg of narasin of DM.

[2] Ca: 22%, P: 5.5%, Mg: 3.5%, S: 2.2%, Cl: 10.55%, Na: 7.0%, Mn: 1500 mg/kg, Fe: 500 mg/kg, Zn: 1550 mg/kg, Cu: 440 mg/kg, Co: 50 mg/kg, I: 40 mg/kg, and Se: 20 mg/kg.

Table 2. Body weight (BW), body condition (BCS), milk production and composition of the ewes fed experimental diets.

Item	Diets[1]		SEM[2]	*P*-value		
	N0	N13		Diet (D)	Time (T)	D × T
BW, kg						
wk 2	58.4	59.7	2.42	0.58	-	-
wk 10	57.8	59.4	2.11	0.52	-	-
BCS						
wk 2	2.83	2.88	0.07	0.62	-	-
wk 10	3.10	3.13	0.09	0.81	-	-
Intake, kg/d						
DM	2.27	2.26	0.10	0.93	<0.01	0.13
CP	0.30	0.32	0.02	0.23	<0.01	0.14
NDF	1.04	1.03	0.05	0.99	<0.01	0.18
ADF	0.46	0.46	0.02	0.80	<0.01	0.24
Ash	0.18	0.18	0.01	0.98	<0.01	0.29
Production, g/3h						
Milk	194	241	15.5	<0.01	<0.01	0.41
FCM	235	314	24.4	0.01	<0.01	0.48
FPCM	219	292	22.3	0.01	<0.01	0.49
Fat	16.5	22.4	1.83	<0.01	0.04	0.23
Protein	7.95	10.03	0.64	<0.01	<0.01	0.72
Lactose	9.33	11.14	0.97	0.04	<0.01	0.43

Total solids	35.2	45.9	3.38	<0.01	0.02	0.16
Solids, nonfat	19.1	23.5	1.57	<0.01	<0.01	0.38
Composition						
Fat, %	8.65	9.23	0.33	0.21	<0.01	0.98
Protein, %	4.13	4.16	0.10	0.79	<0.01	0.06
Lactose, %	4.78	4.66	0.06	0.19	<0.01	0.70
Total solids, %	18.5	19.0	0.23	0.25	<0.01	0.94
Solids, nonfat, %	9.88	9.73	0.09	0.17	<0.01	0.26
Urea, mg/dL	16.0	14.9	0.87	0.40	<0.01	0.91
Somatic cell count	1434	1668	334	0.28	0.41	0.54
Feed efficiency						
Milk/DMI	0.69	0.94	0.08	0.02	<0.01	0.28
FCM/DMI	0.85	1.20	0.10	0.02	0.01	0.57
FPCM/DMI	0.78	1.11	0.09	0.02	0.02	0.43

[1]N0 = diet without feed additive; N13 = diets containing 13 mg/kg of narasin of DM.

[2] Standard error of the means.

Table 3. Body weight, ADG and DMI of lambs from ewes fed experimental diets.

Item	Diets[1]		SEM[2]	*P*-value		
	N0	N13		Diet (D)	Time (T)	D × T
BW, kg						
wk 2	6.86	6.86	0.35	0.98	-	-
Weaning	22.1	21.2	1.03	0.35	-	-
After weaning[3]	26.3	25.7	1.11	0.60	-	-
ADG, g						
Before weaning	263	266	13.8	0.77	<0.01	0.23
After weaning[3]	296	294	24.1	0.95	<0.01	0.28
Starter DMI, g/d						
Before weaning	186	106	20.21	<0.01	<0.01	0.03
After weaning[3]	759	652	67.7	0.19	<0.01	0.30

[1] N0 = diet without feed additive; N13 = diets containing 13 mg/kg of narasin of DM.

[2] Standard error of the means.

[3] After weaning represents the average of 14 d after weaning.

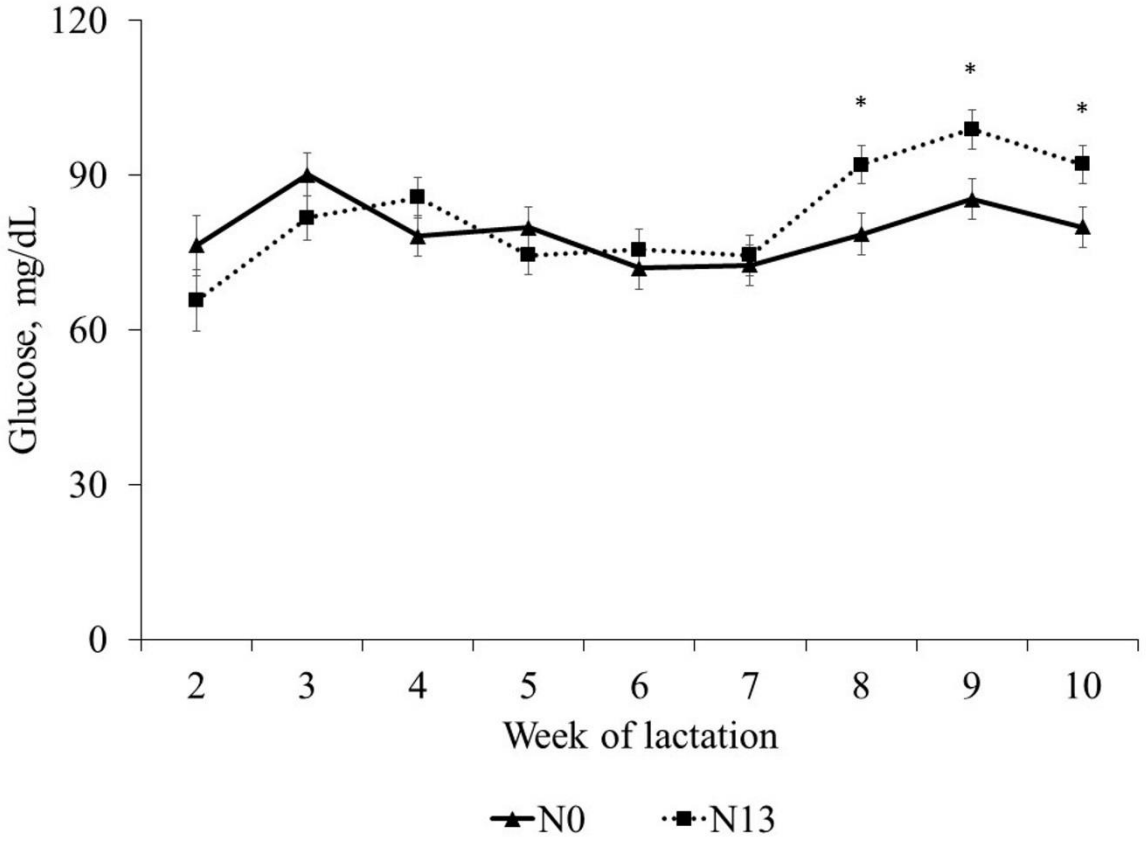

Figure 1. Blood glucose concentration in ewes fed the control diet (no additive; N0) or a diet containing 13 mg/kg of narasin of DM (N13) from samples collected on wk 2 to 10 related to lambing. There was a diet × time effect ($P = 0.01$) for blood glucose concentration. Diet containing narasin increased ($P < 0.01$) glucose concentration in ewes on wk 8, 9 and 10.

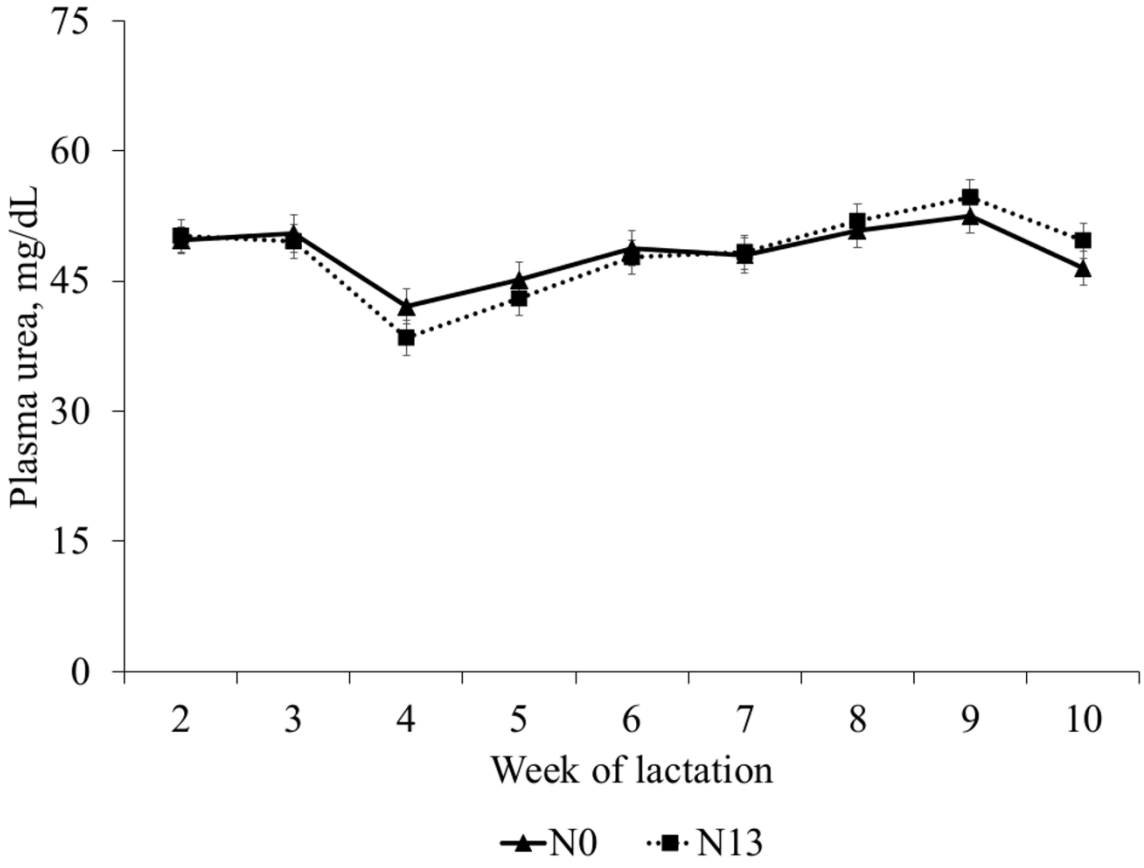

Figure 2. Plasma urea concentration in ewes fed the control diet (no additive; N0) or a diet containing 13 mg/kg of narasin of DM (N13) from samples collected on wk 2 to 10 related to lambing. There was no diet and week interaction (P = 0.60) and diet effect (P = 0.96) for plasma urea. There was a time effect ($P < 0.01$) with the greater values observed on wk 9 of lactation.

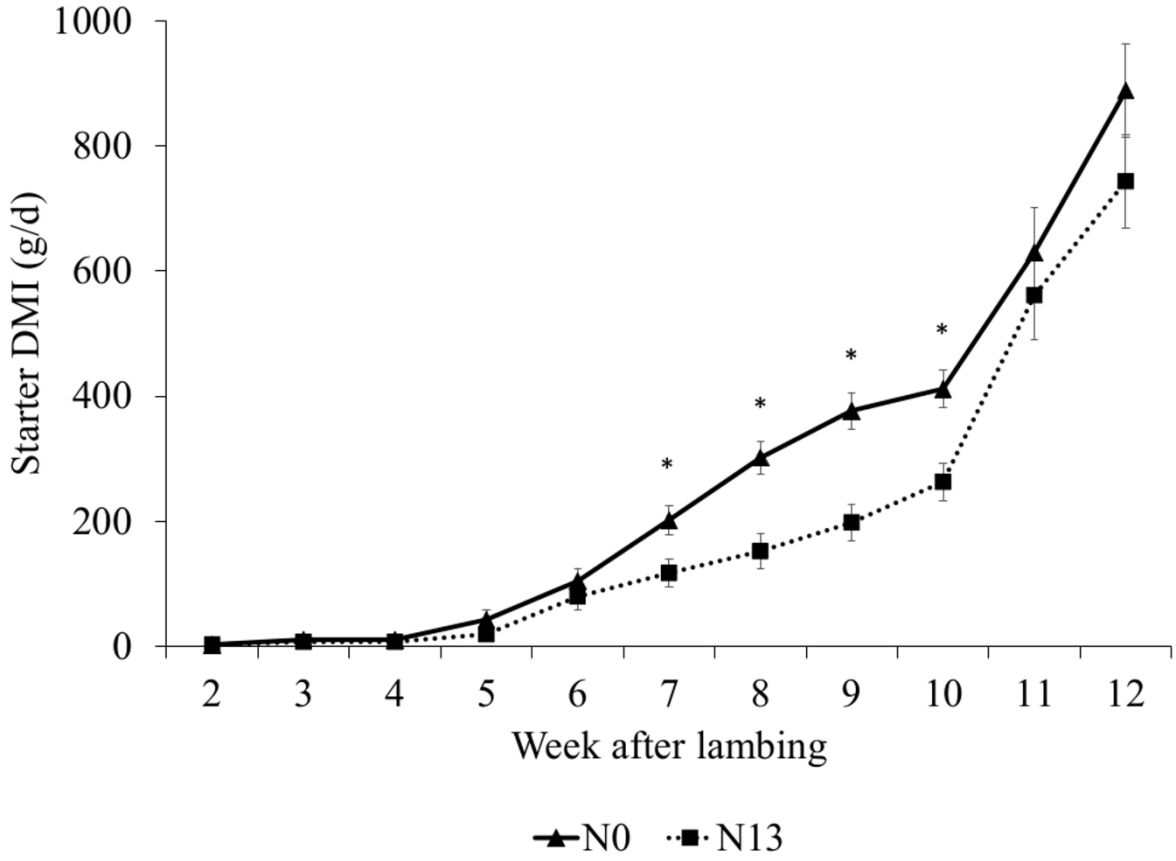

Figure 3. Starter DMI (g/d) of lambs from ewes fed the control diet (no additive; N0) or ewes fed diet containing 13 mg/kg of narasin of DM. There was a diet × time interaction ($P = 0.03$) for starter DMI before weaning. Lambs from ewes fed N13 had a lower starter DMI ($P < 0.02$) on wk 7, 8, 9 and 10 related to lambing.

4. NARASIN INCLUSION AND LEVELS OF NDF ON PERFORMANCE, CARCASS CHARACTERISTICS AND RUMINAL MORPHOMETRY OF FEEDLOT LAMBS

4.1. ABSTRACT

This trial aimed to evaluate the inclusion of narasin in diets with different levels of neutral detergent fiber (NDF) in finishing lambs dry matter intake (DMI), average daily gain (ADG), feed efficiency (FE), carcass characteristics, meat quality, rumen morphometrics fermentation parameters, cecum and feces content in feedlot lambs. Forty-four male lambs, with 24.24 ± 1.05 kg of initial body weight (BW) and 82.16 ± 1.03 days of age were assigned to a randomized complete block design. The treatments were: arranged in a 2 × 2 factorial design, which factor 1 was 20 or 25% of NDF content, based on DM and factor 2 was 0 (N0) or 13 (N13) mg/kg DM of narasin inclusion on diet. The animals were weight on 0d and 115d, and slaughtered after 115 days of confinement. Performance evaluations, carcass characteristics, ruminal and cecum parameters were evaluated. The NDF levels in the diets did not affect the ADG ($P = 0.27$). However, diet containing 25% NDF resulted in increase in lambs' DMI ($P = 0.04$) consequently, reduced the FE ($P < 0.01$). The inclusion of 13 mg/kg DM of narasin did not affect the performance of lambs ($P > 0.33$). Inclusion of 13 mg/kg DM of narasin increased SFT ($P < 0.01$), BWT ($P < 0.01$) and MS ($P = 0.04$). The inclusion of 20% NDF resulted in increased BWT ($P = 0.03$) and perirenal fat weight ($P = 0.05$). The inclusion of narasin reduced protein content ($P = 0.02$) and increased ether extract (EE) ($P < 0.01$) of the meat. There was interaction between the hay contents and the inclusion of narasin on the molar proportion of propionate ($P = 0.02$), isovalerate ($P = 0.01$), and the ac:prop ratio ($P < 0.01$). Inclusion of narasin reduced the molar ratio of acetate ($P < 0.01$) and increased ruminal pH ($P = 0.05$) and total SCFA concentration ($P < 0.01$) of lambs. Inclusion of 13 mg/ kg DM of narasin increased the propionate molar ratio ($P = 0.02$) and reduced the isobutyrate ratio ($P = 0.05$) and the ac:prop ratio ($P = 0.03$) on the cecal fermentation. The use of diets containing 25% NDF resulted in higher number of papillae in the ruminal epithelium per cm² ($P = 0.04$) and reduced keratin thickness ($P = 0.05$). The inclusion of narasin increased papilla height ($P = 0.04$), papillae surface area ($P = 0.04$), absorptive area per cm² ($P = 0.04$) and representativeness of papillae area in relation to the total absorptive area ($P = 0.02$). In addition, the inclusion of narasin reduced keratin thickness ($P = 0.02$). The inclusion of narasin in the diet of feedlot lambs, even without altering the final BW of the animals, caused great changes to occur in the carcass,

papillae histology, rumen, cecal and fecal fermentation of these lambs. The use of different levels of NDF in the lamb's diets provided the animals with different performances and rumen environments. Lambs with lower NDF levels were more efficient, however, it is necessary that adequate minimum NDF levels be established in order to promote a healthy rumen environment together with good animal performance.

Keywords: feed efficiency, cecal fermentation, propionate, feed additives.

4.2. INTRODUCTION

Brazilian sheep farming can be an alternative for the country's breeders through the reorganization and strengthening of the production chain (Martins et al., 2011). However, efforts are still needed to make evolution and for sheep farming to become an organized and productive chain in order to avoid seasonality of production and to achieve a good quality end product (Sorio and Rasi 2010; Raineri; et al.,2014). Currently, the demand from the consumer market for a better-quality product has led to intensification of the finishing of lambs to slaughter younger animals (Silva Sobrinho and Moreno 2009).

Thus, the intensive system is a very important alternative for the evolution of the productive chain, as it generates an increase in the stocking of the property and productive indexes, causing a faster return on the invested capital, reducing parasitic infestations (Carvalho et al. 2007), increase the stocking rate of ownership, improve feed herd conditions, providing a quality lamb in the period between harvest (Carvalho and Medeiros, 2010). However, with the reduction of the confinement period, the need for proper feed management is seen, as it is common in this type of system to date diets with a high level of concentrate, but at the same time it becomes necessary to monitor the levels fiber so that there is no impairment in animal performance (Santana, 2015). It is recommended by the NRC (1996) for beef cattle a minimum of 25% effective NDF to maintain an adequate rumen pH, thus allowing a higher fiber intake and microbial growth, better rumination and grain fermentation. However, the ideal NDF content for feedlot lambs is not yet known, aiming adequate ruminal health and good performance.

An alternative for the optimization of sheep breeding with tools that seek to improve the productive indexes of the herds in the properties, can be highlighted the additives that have been provided in the herds, either for pasture or feedlot animals, with the purpose of improving productive performance by positive stimulation of microbial activity (Bertipaglia, 2008). The main functions of ionophores are the increase of propionic acid concentration, reduction the proportion of acetic and butyric acid, reduction in methane and lactic acid production, thus promoting an improvement in the efficiency of energy use, as well as aid in the prevention of acidosis (Taylor, 2001).

The hypotheses of the present study were that the inclusion of higher NDF content in the diets would decrease the energy density of the diet, which may cause lower performance of animals during feedlot, and the use of narasin in diets would result in increased feed efficiency

by increasing ADG without changing DMI, increased carcass yield and meat quality, in addition to different responses regarding the use of ionophore depending on the content of NDF used in the diet. The use of narasin and lower NDF inclusions would result in improved rumen papilla development and changes in rumen, cecum and rectum fermentation processes.

The objective of this study was to evaluate the inclusion of narasin in diets with different levels of NDF in finishing lambs on performance, carcass characteristics, meat quality, rumen morphometrics and fermentation parameters of rumen, cecum and fecal content.

4.3. MATERIALS AND METHODS

This study was conducted at sheep confinement facility of the Sheep and Goat Intensive Production System, Department of Animal Science, "Luiz de Queiroz" College of Agriculture (**ESALQ**), University of São Paulo (**USP**), in Piracicaba, State of São Paulo, Brazil. All animals use procedures followed the guidelines recommended by the Animal Care and Use Committee at the same institution (protocol number **2019-21)**.

4.3.1. Animals and experimental design

Forty-four male lambs (*Dorper* × *Santa Inês*), with an initial body weight (BW) of 24.24 ± 1.05 kg and 82.16 ± 1.03 days old, were used in this study. The animals were kept indoors, in an individual tie-stall system, with a slatted floor, mineral box, free-choice access to a water and feed bunk.

The lambs were randomly allocated to 11 completely randomized blocks, defined according to age and initial BW. The dietary treatments were designed with 2 × 2 factorial arrangement: 20 or 25% of NDF content (DM basis) and 0 or 13 mg/kg DM of narasin (ZIMPROVA, Elanco Animal Health, Indianapolis, IN).

4.3.2. Feed management, collection of samples and methodologies

The experimental diets were formulated according to the National Research Council (2007). The metabolizable energy (ME) to the diets were estimated using the Small Ruminant Nutrition System, v. 1.8.6 (Cannas et al., 2004).

Brachiaria hay was coarsely chopped (DPM-4 mill, Nogueira®, Itapira, São Paulo Brazil; equipped with a 1-cm pore size sieve). Subsequently, chopped hay was added to the ground corn, soybean meal, urea, ammonia chloride, limestone and mineral premix and mixed by using a horizontal mixer (Lucato®, Limeira, São Paulo, Brazil), with a 500 kg capacity. Thirteen milligrams of narasin were added per kilogram of diet (as-fed basis). Feed were sampled weekly and frozen at -18°C for later analysis.

Individual amount of total mixed ration was weighed on an electronic scale accurate to 1 g (Marte® AC 10K, São Paulo, SP, Brazil) and offered, *ad libitum*, at 08:00 h, allowing daily refusal of up to 5%. The leftover was collected and weighed weekly in order to calculate DMI. Amounts of feed offered to lambs were calculated according to previous DMI, and adjustments were done when needed so that refused feed did not exceed 0.05 kg of daily intake.

4.3.3. Laboratory Analyses and calculations

Feeds and orts were sampled weekly, and frozen at -20°C for later analysis and to determine the DM intake. The lambs were weighed after solid fasting period of 14 hours at the beginning and at the end of the experiment (112 d) to calculated the average daily gain (ADG) and feed efficiency (FE).

The feed offers and leftover were dried at 55°C for 72 h and ground through a 1-mm screen in Wiley mill (Marconi, Piracicaba, São Paulo, Brazil). The dry matter (DM) was determined by drying at 105°C for 24 h, and ash concentration was obtained by incinerating the samples an oven at 550°C for 4 h (AOAC, 2012). Total nitrogen concentration was determined using a Leco TruMac® N (Leco Corporation, St. Joseph, MI, USA; AOAC, 2012). The crude protein (CP) was calculated by multiplying the total nitrogen by 6.25. The NDF was determined according to Van Soest et al. (1991) using α-amylase heat stable and sodium sulfite in an

Ankom 2000 System (Ankom Technology Corp., Fairport, NY, USA). The ADF was determined according Goering and Van Soest (1970) and ether extract (EE) according to AOAC (2012). Non fiber carbohydrates (NFC) of the diets were estimated according to the following equation: NFC (%) = 100% - (% NDF + % CP + % EE + % ash).

4.3.4. Slaughtering and carcass characteristics

At the end of the 115d feeding trial, all animals were weighed after a 16h fast to determine the ADG and FE (g of BW gain/kg of feed) and slaughtered following the Brazilian legislation. All the viscera were separated, identified and weighed. The carcass was weighed to obtain hot carcass weight (HCW) and hot carcass yield (HCY) at the time of slaughter. After 24 h a cold chamber (4°C), the carcass was weighed again to determine the cold carcass weight (CCW), chilled carcass yield (CCY), subcutaneous fat thickness (SFT) over the 12th rib, *Longissimus dorsi* muscle area (LM area), body wall thickness (BWT) and marbling score (MS) were obtained.

Carcasses were cooled in a cold chamber at 2°C for 24h, kidney and perirenal fat was removed from the carcass. Then, perirenal fat was separated from the kidneys and weighted using an electronic scale (Marte® AC 10K, São Paulo, SP, Brazil). The carcass was split along the spine and the left sides were separated into eight joints that were individually weight to estimate the proportion of the higherpriced joints (leg+chump+loin+ribs) as described by Santos-Silva et al (2002).

Approximately 15 cm samples of the LM area were removed of the carcass of each animal according to Bertoloni et al. (2019) to determine the chemical composition of the meat. The DM, ash, total N and ether extract content of the *Longissimus dorsi* meat were determined according to AOAC (2012).

4.3.5. Ruminal, cecal and fecal fluid harvesting

Content from rumen, cecum and rectum were collected for measurement of short-chain fatty acids (SCFA). A representative sample of ruminal and cecum content was collected from each animal, and immediately filtered through nylon cloth for a yield of approximately 100 mL of fluid, which was then used to measure the pH in a digital potentiometer (DIGIMED DM20, São Paulo, Brazil). Fecal samples obtained from each lambs (5 g/animal) were mixed with 5 mL of deionized water to evaluate the fecal pH (DIGIMED DM20), according to the methodology described by Channon, Rowe and Herd (2004). After the pH was measured, two aliquots of rumen, cecum and feces fluid were reserved, stored in plastic vials, and frozen at -18°C for subsequent analysis of SCFA according to Ferreira et al. (2016) and ammonia nitrogen (N-NH3) according to Chaney and Marbach (1962), adapted for microplate reader (BIO-RAD, Hercules, CA) using a 550 nm absorbance filter.

4.3.6. Histology of the rumen papillae

A 1-cm^2 fragment of each rumen was collect from the ventral sac and storage in 70% alcohol solution and submitted to histological analysis. Histological sections were stained with hematoxylin and eosin, embedded in paraffin wax, and sectioned according to the methodology described by Odongo et al. (2006).

For histological analysis, the Leica Qwin Image Analyzer program contained in the Leica light electron microscope were used. Histological measurements, such as papillae height, papillae width, papillae surface area and keratinized layer thickness were determined in 4 papillae per animal using computer –aided light 112 microscope image analysis.

The height of the papilla was defined as a straight line drawn from the base of the apex of the papilla. To measure the width and thickness of keratin were drawn straight in four distinct places of the papilla and an average was considered. The area was measured by circling all the computed parts and obtaining an average.

4.3.7. Statistical analysis

Statistical analyzes were performed using the MIXED procedure of SAS (2002). All data were tested for the presence of disparate information ("outliers") and normal distribution of residuals (Shapiro-Wilk test). The observation was considered outlier when standard deviation in relation to mean were less than -3 or more than +3.

The data were analyzed according to the procedure for linear mixed models (PROC MIXED). In the model, the effect of NDF content, narasin inclusion and interaction NDF content × narasin inclusion were considered fixed effect. The block effect was considered random effect. The averages of each treatment were obtained using the LSMEANS command. The effects were considered significant when $P \leq 0.05$.

4.4. RESULTS

4.4.1. Performance

There was no interaction between NDF levels and narasin in lambs performance. Adding narasin in diets did not affect the lamb's performance variables. The NDF levels in the diets did not affect the ADG ($P = 0.27$), resulting in the same final BW ($P = 0.33$). However, diet containing 25% NDF resulted in increase in lambs' DMI ($P = 0.04$), which reduced the FE ($P < 0.01$) compared to the diet with 20% NDF.

4.4.2. Carcass yield

There was no interaction between NDF levels and Narasin inclusion in variables. There was no treatment effect for slaughter weight, HCW, CCW, HCY, CCY and LM area (Table 6).

The N13 increased SFT ($P < 0.01$), BWT ($P < 0.01$) and MS ($P = 0.04$). The inclusion of 20% NDF resulted in increased BWT ($P = 0.03$) and perirenal fat weight ($P = 0.05$); however, there was no effect of NDF contents on SFT and DM (Table 6).

There was no interaction between NDF and N13 and effect of these factors on the weight of the cuts (Table 7).

NDF level and N13 did not affect moisture and ash content in lamb meat (Table 8). However, the N13 reduced crude protein ($P = 0.02$) and EE ($P < 0.01$) of the meat. Diets containing 20% NDF resulted in increased EE content ($P = 0.03$) in meat, however there was no effect on crude protein.

4.4.3. Ruminal Fermentation

There was interaction between the NDF level and the narasin on the molar proportion of propionate (P = 0.02), isovalerate (P = 0.01), and the acetate-to-propionate ratio (P < 0.01; Table 9).

The molar proportion of propionate was higher in lambs fed 20% NDF diet with the inclusion of N13 when compared to the 25% NDF and N0. The isovalerate molar ratio and acetate-to-propionate ratio was higher for the 25% NDF diet animals without the inclusion of N13. There was no interaction between NDF levels and narasin inclusion for the other variables.

Inclusion of narasin reduced the molar ratio of acetate ($P < 0.01$) and increased ruminal pH ($P = 0.05$) and total SCFA proportion ($P < 0.01$) of lambs. There was no effect of N13 on the other ruminal parameters evaluated.

There was an effect of NDF level on ruminal fermentation parameters, in which lambs fed a diet containing 25% NDF increased the molar ratio proportion of acetate ($P < 0.01$), isobutyrate ($P = 0.01$), isovalerate ($P < 0.01$), C2:C3 ratio and ruminal pH. In contrast, higher NDF inclusion reduced the molar ratio of valerate ($P = 0.01$).

4.4.4. Cecal Fermentation.

There was no interaction between NDF level and narasin inclusion on cecal fermentation parameters (Table 10).

Inclusion of 13 mg/ kg DM of narasin increased the propionate molar ratio ($P = 0.02$) and reduced the isobutyrate ratio ($P = 0.05$) and the C2:C3 ratio ($P = 0.03$). There was no effect of the N13 on the other cecal fermentation variables.

The inclusion of higher NDF content reduced the molar ratio of valerate (P < 0.01), however, no effects were observed on other cecal fermentation variables.

4.4.5. Fecal Fermentation.

There was interaction between NDF and narasin for the molar ratio of acetate ($P = 0.01$) and butyrate ($P = 0.05$; Table 11).

Diets with the inclusion of N13, the molar proportion of acetate was higher for the diet containing 20%NDF. On the other hand, the butyrate molar ratio was higher with the inclusion of ionophore in diets with 25% NDF compared to diets containing 20% NDF. There was no effect of NDF and N13 for the other variables evaluated.

4.4.6. Rumen morphometrics

There was no interaction between dietary NDF levels and the inclusion of narasin on the variables related to ruminal papillae morphology and histology of lambs (Table 7). The diets containing 25% NDF resulted in higher number of papillae in the ruminal epithelium per cm² ($P = 0.04$) and reduced keratin thickness ($P = 0.05$). There was no effect of NDF content on the other variables analyzed.

The inclusion of N13 did not affect the average number of papillae per cm², papilla width and absorptive tissue thickness. However, the inclusion of N13 increased papillae height ($P = 0.04$), papillae surface area ($P = 0.04$), ASA per cm² ($P = 0.04$) and representativeness of papillae area in relation to the total AA ($P = 0.02$). In addition, the inclusion of N13 reduced keratin thickness ($P = 0.02$; Table 12).

4.5. DISCUSSION

4.5.1. Performance

The same ADG observed for the animals that received the experimental diets is due to the fact that even the diet containing 20% NDF presented higher energy content (Cannas, 2004) during the experimental period. There was a higher intake of the animals that received the diet containing 25% NDF resulted in a similar intake of energy and consequently similar ADG.

Feed intake is regulated and limited by metabolic and/or physical requirements (Van Soest, 1994). The higher DMI of lambs in the 25% NDF diet may have been influenced by several factors. The first the lower energy level of diet because higher amounts of roughage may be induced an increase their DMI to supply energy demand. Turino et al. (2007) attributed the higher intake of DM as the increase in dietary fiber inclusion to the effect of dietary energy dilution, and this can happen as long as there is no physical limitation for intake (Mertens et al, 1994). Ferreira et al. (2011), studying the effect of inclusion of soybean hulls in the diet of lambs in place of corn, observed a linear increase over DMI. The authors attributes increased intake to decreased dietary energy density, once DM intake is negatively related to NDF concentration only when ruminal filling limits intake.

Another factor attributed to the higher DMI with 25% NDF was a higher fiber content that made the diet safer, which stimulated rumination, allowing an adequate ruminal environment, with less pH fluctuation (Mendes et al. 2010). High-energy diets increase risk of acidosis, which may compromise animal's performance and health (Mertens, 1996). High production animals have a higher demand for nutrients, especially energy. Thus, it is necessary to include concentrated to reach nutritional requirements, causing a reduction in the proportion of roughage in the diet (Mertens, 1997). However, an increase in the concentrate may induce a digestive disorder that compromise animal health, and a reduced production performance. The ruminal pH is influenced by feed and saliva amount, which has a high buffering power, due to the concentration of bicarbonate present in its constitution (Owens and Goetsch, 1988; Van Soest, 1994). Saliva flow is stimulating by chewing and rumination, resulting from reflexes initiated by physical stimuli of the coarse particles in the rumen wall (Harfoot, 1981; Hoover and Stokes, 1991).

Unlike the study by Polizel (2016b), reported a linear increase in the performance of lambs feedlot for 56 days with the inclusion of 0, 5, 10 and 15 mg of narasin, using a diet with a composition and protein content similar to the present study. However, the present study, using a confinement period of 112 days, did not observe an increase in lamb performance. Hypothetical that this narasin may have an effect only at the beginning of the confinement period and subsequently be diluted over time.

Fat also influences DMI, when lambs reach maturity, more fat was deposited, hampering to predict voluntary intake. Fatter animals had decrease in DMI, even on high-grain diets, where physical factors may be less important (Scharrer and Langhans, 1990). There was negative feedback on feed intake and adipose tissue, which can be explained by leptin. One leptin function is to maintain an adequate energy balance, however, if leptin secretion increases, either to energy excess in diet or by body fat, a decrease in intake (occurs because neuropeptide Y secretion) (Cassady, 2000). Even though there was no difference in final BW, lambs fed diets with 20% NDF showed higher fat deposition, as observed in the perirenal and meat fat content.

Another important factor to be highlighted was that the inclusion of the additive in the experimental diets did not affect the DMI. As reported by several authors, unlike monensin that reduces DMI (Duffield, 2012), narasin not affect DMI and minerals when included in the diet, either in diets containing high forage (Silva et al., 2015, Gobato et al., 2016, Polizel et al., 2016a, Polizel et al., 2018, Oliveira, 2018, Polizel et al., 2019) or concentrate (Polizel et al., 2016b).

4.5.2. Carcass yield

There are no reports in the literature on the effect of narasin inclusion on carcass characteristics and weight of lambs slaughtered in feedlot. The present study demonstrated that N13 was effective in increasing STF and BWT. The higher STF acts as a thermal insulator, preventing excessively rapid cooling of the carcass, which could result in shortening of sarcomeres, resulting in tougher meat (Silva Sobrinho et al., 2005). STF is influenced by sheep breed (Silva Sobrinho et al., 2005), composition (Diaz et al., 2002) and dietary energy density (Restle et al., 2001). Diets containing high concentrate it provides higher energy intake, resulting in carcass production with greater deposition of subcutaneous fat and meat with

greater juiciness (Cañeque et al., 1989). Preston and Willis (1974) described that more energetic diets resulted in higher fat carcasses when compared to medium and low energy diets.

In Brazil, ideal fat carcass thicknesses have not yet been determined, however, Osório and Osório (2001) suggest that they may vary from 2 to 5 mm according to carcass weight. In the present study, the inclusion of narasin provided values for an ideal fat thickness considered by these authors, with average values of 2.75 mm. Diets without the additive inclusion presented an average of 2.09 mm for STF, close to the limit of the values considered as ideal.

The BWT was influenced by the inclusion of narasin in the experimental diets, resulting in higher carcass levels of the animals that intake the additive, since this is an area of fat accumulation in some animals. Connect to that, marbling is an important feature because it is directly related to the sensory characteristics of meat (tenderness, flavor and juiciness), which is perceived and appreciated by consumers (Costa et al., 2002). Generally, marbling occurs in animals with high rates of weight gain (Di Marco, 1998). The greater amounts of energy intake by the animal, the greater the deposition of intramuscular fat, resulting in positive effects on the sensory qualities of meat (Ladeira and Oliveira, 2006). However, the composition and deposition of the tissues are not constant (Berg and Butterfield, 1976). At each stage of the animal's life, a type of tissue is deposited, where younger animals deposit more muscle, and after puberty, when they are heavier, begin to retain greater amounts of fat (Brody, 1945; Owens et al., 1995). After maturity, the growth of other tissues is practically nil, with the growth of only adipose tissue. (Owens et al., 1993).

According to Sanûdo et al. (1997), the higher levels of marbling lead to lower cooking loss and consequently to obtaining more succulent meat, because meat fat acts as a barrier against moisture loss in meat. In the present study, the inclusion of narasin increased the marbling score and the fat content of LM muscle, suggesting that the ionophore may have altered the energy efficiency of the animals, allowing greater fat deposition. There is evidence that intramuscular tissue adipocytes have a preference for glucose while subcutaneous adipocytes have a preference for acetate. Smith and Crouse (1987) observed that acetate contributed with 70 to 80% of carbons used for the synthesis of adipocytes from subcutaneous tissue, and with only 10 to 25% in adipocytes from intramuscular tissue. Most of the carbons used in the synthesis of intramuscular adipose tissue (50 to 75%) came from glucose.

Carcass yield is a trait directly related to meat production, varying according to intrinsic and / or extrinsic factors to the animal (Cunha et al., 2008). This index is an important parameter

in the evaluation of the animals, since it is directly related to the commercial value of the lambs, since it expresses the percentage relation between the carcass weight and the BW of the animals. Since in the present study there was no effect on carcass yields, the different NDF contents can be used in the lambs diet without any subsequent effect on the commercial value of the lamb and the carcass yield.

The similarity in the cuts yield, not influenced by the experimental diets, confirms the law of anatomical harmony (Boccard and Dumont, 1960), who verified that the cuts yields even for different slaughter weights do not vary greatly. Siqueira et al. (2001) explaining the similarity in cut yields by the atomic harmonic law, reported that even in carcasses of similar weight and fat amounts, virtually all body regions are in similar proportions, whatever the conformation of the genotypes considered. In the present study, the animals were slaughtered with the same BW at the end of the experiment, which may justify the absence of effect of the treatments on the weight of the cuts.

Perirenal fat was influenced by different levels of NDF in the diet, whose the animals receiving the diet 20% NDF, presented higher amount of perirenal fat. For Colomer-Rocher (1974), the rate of development of perirenal fat is similar to that of total carcass fat and may be an indicator of its general fattening state, where, this deposition also occurs with the maturity of the animal (Lawrence and Fowler, 2002). Higher level of concentrate in the diet increases the concentration of propionic acid in the rumen and decreases the C2:C3 ratio, resulting in higher energy availability in the form of glucose, which favors lipogenesis and consequent deposition of visceral fat (Kozloski, 2002). The greater production of propionate, instead of acetate or butyrate, causes greater energy efficiency, due to the greater supply of gluconeogenic, and decrease energy loss (Manella et al., 2003). Moreno et al. (2011) also reported increased perirenal and omental fat for lambs fed a higher concentrate diet. According to Prata (1999), sheep meat has around of 75% of moisture, 19% of protein, 4% of fat and 1.1% of mineral matter in centesimal composition. The meat of lambs that received the diet with higher concentrate level presented values similar to Prata (1999), Souza (2014) and Bertoloni et al (2020) for moisture, protein, fat and ashes in the present study. However, there was an effect of experimental diets on protein and fat content of meat. Studies have shown that narasin has the ability to alter rumen fermentation processes (Polizel et al., 2018; Miszura et al., 2018), having a direct relationship with the animal's energy metabolism. The present study is the first to report the effects of narasin on sheep meat composition, suggesting that the molecule is capable of altering the fat deposition in the meat, increasing the degree of marbling.

The 20% NDF diet also resulted in higher fat when compared to the 25% NDF diet. The inclusion of higher grain content in the diet results in increased amounts of easily digestible ruminal and intestinal carbohydrates, increasing energy availability for animal development (Theurer et al., 1999). Thus, the use of different levels of roughage and the inclusion of narasin becomes a strategy to improve sheep carcass parameters, aiming at a better quality for the consumer market.

4.5.3. Ruminal, cecal and fecal fermentation

The decrease in the acetate ratio and C2:C3 ratio with the decrease in roughage content in the diet is due to the reduction in the dietary NDF content, since fibrolytic bacteria produce more acetate, whereas amylolytic ones produce propionate (Blaxter, 1962). In general, decreasing the bulk: concentrate ratio also decreases the C2: C3 ratio (Annison and Armstrong, 1970). As in the present study, Fuller and Johnson (1981) also reported a decrease in C2: C3 ratio with the addition of ionophores in high concentrate diets, since one of the main functions of ionophores is the increase of propionic acid concentration, with reduction of acetic and butyric (Tayarol, 2001).

The increase in propionate molar proportion in the diet containing 25% NDF and the inclusion of narasin may be justified by possible changes in the population of microorganisms caused by ionophore, which generates an increase in the amount of gram-negative bacteria, thus favoring propionate-producing bacteria in the fermentation process (Dawson and Boling, 1984; Russell, 1987). The increase in propionate may also be due to changes in the metabolism of gram-negative bacteria, causing them to produce more propionate (Bergen and Bates, 1984, Stahl et al., 1988). Recent studies have demonstrated the ability of narasin to increase the propionate molar ratio and consequently the C2: C3 ratio (Polizel et al., 2018; Miszura et al., 2018).

Branched-chain fatty acids (BCFA) (isobutyric, isovaleric) are essential nutrients for fiber-degrading microorganisms and those that degrade non-fibrous carbohydrates (Bryant, 1995). After the absorption of these acids, the microorganisms synthesize the essential amino acids (valine, leucine and isoleucine). Cline et al. (1996) observed an increase in cellulose digestion by sheep receiving BCFA, where in the present study, the increase in the proportions

of isovalerate and isobutyrate in diets with higher hay content may be indicative of greater efficiency of fibrolytic bacteria in the degradation of fibers.

The addition of N13 increased the total SCFA in rumen fluid, as reported by Polizel et al. (2016c), when evaluating the effects of increasing concentrations of narasin (0, 8, 16, 24 and 32 mg / kg DM) on lambs fed high forage diets on ruminal fermentation. Diets containing high starch content result in higher fermentation rate causing ruminal pH to fall, favoring the development of lactic acid producing bacteria, resulting in increased lactic acid concentration in the rumen environment (Owens et al., 1998). Lactate has a medium acidification capacity when compared to the commonly produced rumen SCFA promoting greater effect on reducing rumen pH (Dawson et al., 1997). In this sense, the increase in the amount of fibrous carbohydrates that stimulate rumination results in an increase in rumen pH, minimizing the risk of acidosis (Owens et al., 1998). In the present study, it was observed that the increase from 20 to 25% NDF inclusion resulted in increase in rumen pH. In addition, the inclusion of narasin also resulted in changes in fermentative pressure and consequent increase in rumen fluid pH.

When added to the diet, ionophores decrease the population of lactate-producing bacteria and increase ruminal pH stability (Coe et al., 1999). In this study, the inclusion of 13 ppm narasin resulted in increased ruminal pH of lambs, regardless of the NDF content of the diets. In the literature, Nagaraja et al. (1987) observed that narasin was more effective in inhibiting lactic acid production than other additives. In addition, Wong et al. (1977) pointed out that narasin is approximately three times more effective than monensin in inhibiting ATPase. Thus, narasin becomes an alternative in ruminant nutrition when its objective is to control rumen microbiota, alteration of fermentative parameters and consequently productive gain.

The effects of narasin on cecal fermentation parameters have not been documented in the literature. The present study suggests that the action of narasin may directly or indirectly influence post-ruminal fermentation, with an increase in the proportion of propionate, a reduction in isobutyrate and an C2:C3 ratio. Studies evaluating monensin reported the possible effect of ionophores on intestinal fermentation. Mbanzamihigo et al. (1960) observed that in addition to changes in rumen fermentation, the use of monensin also resulted in alteration in cecal fermentation, where in vitro cecum propionate production was stimulated by monensin administration, while methanogenesis and acetate were reduced.

Marounek et al. (1990) in an in vitro study to evaluate the effect of monensin on cecum and colon contents of steers and cows observed an increase in propionic, butyric acid decline, without change in acetic acid. The effects observed in the present study suggest that narasin has made rumen fermentation more energy efficient, especially by increasing the molar proportion of propionate.

DeGregorio et al (1984) reported increased molar ratio of valerate in cecum with increased concentrate in sheep diet. In the present study, increased dietary concentrate content resulted in a higher molar ratio of valerate in cecal fermentation. Despite this effect, increasing the NDF inclusion from 20 to 25% had little effect on the cecal fermentation process, suggesting that more significant changes in the roughage: concentrate ratio would be necessary to observe changes on the cecum fermentation process. Throughout the gastrointestinal tract, as seen in the present study, fermentation rates change, as does the proportion of SCFA and pH. According to Van Soest (1994), ruminoreticular is the main site of action of symbiotic microorganisms on the organic matter of the diet. In ruminants, starch first undergoes microbial fermentation in the rumen, with consequent production of microbial cells and SCFA, and what is not transformed subsequently undergoes enzymatic digestion in the small intestine with glucose release (Waldo, 1973). According to Orskov (1986), starch can also be fermented in the large intestine with end products similar to rumen fermentation.

Starch that escapes this initial fermentation will either be digested in ID or will be fermented in the cecum-colon (Karr et al., 1996; Beever et al., 1972). However, the fermentation rate that occurs in cecum-colon content of ruminants is lower than ruminal content (Hungate et al., 1959), explaining the decrease in the effect of the studied variables on cecal fermentation in this study. However, even at lower than rumen fermentation values, it is known that the cecum-colon production of SCFA contributed about 10% of the ME absorbed daily by the ruminant (Siciliano-Jones and Murphy, 1989).

The inclusion of N13, as well as the use of different levels of NDF in the diet of feedlot lambs, were factors that influenced the fatty acid fermentation in different proportions along the TGI. The fecal fermentation parameters in the present study showed alterations regarding the molar proportion of acetate and butyrate, and the other variables studied were not affected by the experimental diets.

Experimental diets altered the fecal fermentation process, especially in relation to the molar proportion of ketogenic fatty acids. The increase in the butyrate molar ratio, and the

decrease in acetate with the inclusion of N13, is due to the fact that the reactions involved in the formation of acetate and butyrate from pyruvate are interrelated and all from Acetyl-CoA (Berchielli et al., 2006). Changes in fermentation may have been influenced by the addition of the additive, which possibly generated a change in the rumen microbiota, thereby modifying digestion and fermentation at different sites throughout the digestive system of these animals.

4.5.4. Rumen Morphometrics

As reported by Costa et al. (2003) the foods offered exert great influence both macroscopically and microscopically, interfering with the development of the rumen papillae. Since the SCFA produced is influenced by diet composition, it is important to note that diets containing higher concentrate inclusions (mainly starch) stimulate propionate production and higher roughage (fiber) inclusions stimulate acetate production. That is, the composition of the diet is directly related to morphological and histological issues of the ruminal epithelium. Diets with high fermentation rate provide greater rumen keratin layer thickness due to the higher vacuolation observed in the rumen keratinized stratum cells, causing an abrasive effect on the ruminal mucosa keratin. Thus, greater cellular vacuolization, that is, the increase in the size of the Dead cells that make up the layer, a fact that occurs when the death rate is higher than the rate of tissue regeneration.

The keratinized layer thus constitutes an important protective barrier, which hinders the entry of invading microorganisms and the action of aggressive substances to the epithelium. It can be observed in the present study the decrease in keratin thickness in diets with higher NDF content, that is, showing that this type of diet for feedlot lambs makes the rumen a less aggressive environment to the rumen epithelium. The use of narasin has also reduced keratin thickness due to the fact that the molecule has the ability to maintain higher rumen pH in abrupt dietary changes as reported by Polizel et al. (2017b).

The height, thickness and shape of the papillae depend on the energetic composition of the feed. For papilla-height growth to occur, a large demand for energy is required, which must be intake through the feed offered (Cavalcanti, 2014). As the inclusion of the ionophore provided a higher amount of propionate, it can be inferred that there was greater substrate availability for papillae growth. According to Lesmeister and Heinrichs (2004), papillary height

can be used as the main developmental parameter of the rumen epithelium, being the factor that best represents the effect of treatment on rumen development.

The surface area of the papillae, together with the NOP per cm², determines the total ASA, increasing this will result in greater absorption capacity of the ruminal epithelium, resulting in lower rumen accumulation of SCFA and greater pH stability (Wang et al., 2009). In this sense, the use of feed additives that increase the total ASA is of great interest, especially in diets containing high concentrate, resulting in beneficial effects on the rumen environment, as well as on the energy metabolism of the animal.

The NDF content of diets for feedlot lambs has an important effect on animal performance, ruminal fermentation, papilla morphology and histology. Both levels evaluated resulted in the same final weight at 112 days of confinement, however, with higher FE when 20% NDF was used. On the other hand, the inclusion of 25% NDF ensured increased rumen pH, which may connote safer fermentation. This fact was confirmed by the smaller papilla keratin thickness when higher NDF content was used in the diets.

Regarding the inclusion of narasin, although no effect on animal performance was observed, this was the first study to evaluate the effects of the molecule on rumen morphometrics. The observed results showed that the alteration caused by narasin in the rumen fermentation process favored the development and health of the papillae, being observed higher, height absorptive area, and smaller keratin thickness. These changes are of great importance for lambs fed high concentrate diets, especially in long term feedlots.

4.6. CONCLUSIONS

The inclusion of narasin in the diet of feedlot lambs, even without altering the final BW of the animals, caused great changes to occur in the carcass, papillae histology, rumen, cecal and fecal fermentation of these lambs, showing the great potential for using the molecule at this stage from creation.

The use of different levels of NDF in the lamb's diet provided the animals with different performances and rumen environments. Lambs with lower NDF levels were more efficient, however, it is necessary that adequate minimum NDF leves be established in order to promote a healthy rumen environment together with good animal performance.

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Table 4. Proportion and chemical composition of experimental diet ingredients (% in DM).

Item	Diets			
	20NFD		25NDF	
	N0	N13	N0	N13
Ingredientes				
Hay "*Brachiaria"*	10.0	10.0	20.0	20.0
Ground Corn	73.2	73.2	63.7	63.7
Soybean Meal	13.0	13.0	12.5	12.5
Urea	0.60	0.60	0.60	0.60
Ammonium chloride	0.50	0.50	0.50	0.50
Limestone	1.20	1.20	1.20	1.20
Mineral mix	1.50	1.50	1.50	1.50
Narasin. mg/kg de DM	0.00	13.0	0.00	13.0
Chemical composition				
DM, % on MM	88.4	86.8	88.4	87.8
Organic Matter	93.6	93.8	93.3	92.8
Crude Protein (CP)	17.5	17.2	17.6	17.1
Neutral detergent fiber (NDF)	20.0	20.0	25.0	25.0
Acid detergente fiber (ADF)	8.0	8.0	11.0	10.0
[3]Metabolizable energy (ME)	2.90	2.90	2.75	2.75

[1] 20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin. [2] Composition: Ca: 22%, P: 5.5%, Mg: 3.5%, S: 2.2%, Cl: 10.55%, Na: 7.0%, Mn: 1500 mg/kg, Fe: 500 mg/kg, Zn: 1550 mg/kg, Cu: 440 mg/kg, Co: 50 mg/kg, I: 40 mg/kg, and Se: 20 mg/kg. [3](Cannas et al., 2004).

Table 5. Performance of lambs fed diets with different levels of NDF with or without narasin.

Item	Diets[1]				SEM	*P*-value[2]		
	20NDF		25NDF			NDF	N	NDF×N
	N0	N13	N0	N13				
BW, kg								
Initial	24.25	24.15	24.17	24.26	1.06	0.93	0.97	0.66
Final	51.45	51.33	50.17	50.33	1.57	0.33	0.99	0.91
ADG, kg	0.243	0.244	0.232	0.233	0.01	0.27	0.91	0.97
DMI, kg/d	1.099	1.052	1.146	1.139	0.04	0.04	0.41	0.54
FE	0.221	0.231	0.203	0.206	0.01	<0.01	0.33	0.63

[1]20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin. [2]NDF =; N = ; NDF ×N = .

Table 6. Carcass yield of hay fed lambs and the inclusion of narasin.

Item	Diets				SEM	*P*-value		
	20% NDF		25% NDF			NDF	Narasin	NDF×N
	N0	N13	N0	N13				
Slaughter weight, kg	55.96	54.65	53.83	54.82	1.60	0.41	0.89	0.34
HCW, kg	29.46	29.69	29.14	28.77	0.96	0.41	0.93	0.70
HCY, %	53.28	54.22	54.05	52.52	0.67	0.46	0.63	0.06
CCW, kg	29.14	29.00	28.68	28.09	0.89	0.35	0.62	0.75
CCY, %	52.12	53.06	53.38	51.27	0.96	0.79	0.55	0.12
BWT, mm	17.34	21.08	16.37	19.40	0.79	0.03	<0.01	0.54
STF, mm	2.22	2.90	1.97	2.61	0.25	0.20	<0.01	0.93
LM area, cm^2	17.93	18.67	19.57	19.07	0.89	0.24	0.88	0.47
Marbling score	1.33	1.64	1.11	1.46	0.16	0.20	0.04	0.90
Perirenal fat	0.93	1.04	0.70	0.88	0.10	0.05	0.16	0.74

20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin ^{2}F = effect of contents NDF; N = effect of narasin inclusion; F×N = effect of interaction between NDF content and narasin inclusion, HCW = hot carcass weight, HCY = hot carcass yield, CCW = cold carcass yield, CCY = cold carcass yield, BWT = body wall thickness, STF = fat thickness subcutaneous, LM area = Loin eye area.

Table 7. Yield of carcass cuts of lambs fed with hay content and inclusion of narasin.

Item	[1]Diets				SEM	[2]*P*-value		
	20% NDF		25% NDF			NDF	Narasin	NDF×N
	N0	N13	N0	N13				
Carcass length , cm	66.25	65.95	66.10	65.54	0.70	0.69	0.54	0.85
Shank lenght , cm	40.56	39.84	40.20	40.46	0.45	0.76	0.58	0.26
Cuts weight, kg								
Neck	1.06	1.05	1.06	1.11	0.05	0.47	0.63	0.54
Pallete	2.44	2.47	2.46	2.34	0.06	0.33	0.45	0.21
Matambre	1.02	0.96	0.95	1.01	0.05	0.78	0.97	0.20
Rib	3.98	4.09	3.72	3.84	0.15	0.09	0.43	0.98
Shank	4.25	4.17	4.26	4.19	0.12	0.90	0.51	0.99
Tail	0.15	0.14	0.14	0.13	0.01	0.33	0.52	0.81
Loin	1.05	1.03	1.11	1.06	0.05	0.37	0.47	0.68

20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin, ;[2] [2]F = effect of contents NDF; N = effect of narasin inclusion; F×N = effect of interaction between NDF content and narasin inclusion

Table 8. Meat composition of hay fed lambs and the inclusion of narasin.

Item	[1]Diets				SEM	[2]*P*-value		
	20% NDF		25% NDF			NDF	Narasin	NDF×N
	N0	N13	N0	N13				
Moisture	73.78	73.61	74.12	75.12	0.53	0.07	0.41	0.25
Crude Protein	21.41	21.17	21.11	20.29	0.42	0.83	0.02	0.06
Eter Extract	3.10	3.89	2.52	3.34	0.25	0.03	<0.01	0.96
Ash	1.29	1.29	1.24	1.25	0.04	0.23	0.96	0.97

[1]20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin, ; [2] [2]F = effect of contents NDF; N = effect of narasin inclusion; F×N = effect of interaction between NDF content and narasin inclusion

Table 9. Ruminal fermentation parameters of lambs fed diets.

Item	[1]Diets				SEM	[2]*P*-value		
	20% NDF		25% NDF			NDF	Narasin	NDF× N
	N0	N13	N0	N13				
Molar proportion								
Acetate	50.54	45.83	60.59	49.40	1.79	<0.01	<0.01	0.07
Propionate	34.03	37.70	25.35	37.14	1.75	0.01	<0.01	0.02
Isobutirate	0.71	0.80	1.26	0.90	0.13	0.01	0.29	0.09
Butirate	8.03	11.06	8.31	8.82	1.32	0.46	0.18	0.34
Isovalerate	0.94	1.20	2.84	1.52	0.29	<0.01	0.06	0.01
Valerate	4.11	3.04	1.74	2.22	0.55	0.01	0.58	0.16
C2:C3	1.54	1.26	2.57	1.42	0.14	<0.01	<0.01	<0.01
Total, mM	97.28	114.46	75.04	116.22	8.62	0.20	<0.01	0.15
Ph	6.11	6.40	6.41	6.57	0.11	0.04	0.05	0.57

[1]20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin, ; [2]2F = effect of contents NDF; N = effect of narasin inclusion; F×N = effect of interaction between NDF content and narasin inclusion

Table 10. Parameters of cecal fermentation of lambs fed experimental diets.

Item	[1]Diets				SEM	[2]*P*-value		
	20% NDF		25% NDF			NDF	Narasin	NDF×N
	N0	N13	N0	N13				
Molar proportion								
Acetate	62.80	62.28	63.52	62.28	1.30	0.84	0.61	0.84
Propionate	19.45	21.76	19.32	21.09	0.60	0.64	0.02	0.75
Isobutirate	0.92	0.55	2.26	0.88	0.32	0.06	0.05	0.24
Butirate	12.19	12.87	12.54	13.79	0.70	0.52	0.33	0.77
Isovalerate	2.60	0.73	1.23	0.47	1.22	0.49	0.26	0.64
Valerate	2.00	1.99	1.16	1.49	0.14	<0.01	0.39	0.37
C2:C3	3.32	2.89	3.37	2.99	0.19	0.69	0.03	0.89
Total, mM	76.88	76.14	54.05	67.47	8.54	0.09	0.49	0.45
pH	6.07	6.07	6.47	6.14	0.14	0.08	0.19	0.22

[1]20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin, ; [2]F = effect of contents NDF; N = effect of narasin inclusion; F×N = effect of interaction between NDF content and narasin inclusion

Table 11. Fecal fermentation parameters of lambs fed experimental diets.

Item	[1]Diets				SEM	[2]*P*-value		
	20% NDF		25% NDF			NDF	Narasin	NDF×N
	N0	N13	N0	N13				
Molar proportion								
Acetate	62.18^{ab}	67.35^{a}	65.19^{ab}	61.19^{b}	1.77	0.37	0.74	0.01
Propionate	19.04	18.69	19.29	20.19	1.11	0.43	0.80	0.57
Isobutirate	0.85	0.84	0.80	0.81	0.08	0.63	0.99	0.92
Butirate	11.28^{ab}	9.82^{b}	11.61^{ab}	15.60^{a}	1.38	0.03	0.35	0.05
Isovalerate	0.95	0.88	0.83	0.90	0.07	0.49	0.99	0.37
Valerate	1.93	1.74	1.60	1.51	0.21	0.19	0.51	0.83
C2:C3	3.52	3.76	3.43	3.18	0.28	0.21	0.98	0.35
Total, mM	73.61	71.94	71.99	6..40	6.90	0.37	0.36	0.51
pH	6.20	6.19	6.13	6.35	0.09	0.58	0.22	0.18

[1]20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin, ; [2]F = effect of contents NDF; N = effect of narasin inclusion; F×N = effect of interaction between NDF content and narasin inclusion

Table 12. Morphology and histology of the ruminal papillae of lambs fed diets containing different levels of NDF with or without the inclusion of narasin.

Item	Diets				SEM	*P*-value		
	20% NDF		25% NDF			NDF	Narasin	NDF×N
	N0	N13	N0	N13				
Macroscopic variables								
N° of papillae/cm²	60.63	63.97	66.31	70.66	2.85	0.04	0.19	0.86
Microcopic variables								
Papillae height, mm	2.13	2.93	2.17	2.44	0.26	0.39	0.04	0.31
Papillae width, mm	0.43	0.40	0.39	0.41	0.02	0.39	0.66	0.12
Papillae surfasse area, mm²	0.92	1.26	0.92	1.02	0.10	0.22	0.04	0.24
[3]Papillae KLT, µm	21.25	18.98	19.34	18.22	0.65	0.05	0.02	0.39
ASA, cm²/cm² of rumen wall	2.03	2.50	2.10	2.30	0.16	0.69	0.04	0.41
Papillae area, % of ASA	55.34	63.46	56.26	61.16	2.20	0.80	0.02	0.55

20NDF= diet containing 20% of NDF on DM; 25NDF = diet containing 25% of NDF on DM; N0 =diet without narasin inclusion; N13 = Diet with 13 mg/kg DM of narasin , ; [2]F = effect of contents NDF; N = effect of narasin inclusion; F×N = effect of interaction between NDF content and narasin inclusion; [3]ASA = Absorptive surface area; KLT = Keranized layer thickness.

5. FINAL CONCLUSIONS

The inclusion narasin in the sheep diet, be it for ewes in lactation period, or for feedlot lambs proved to be a great tool for nutritional use, bringing great benefits and qualitative and quantitative changes in the production and performance of animals.

However, further studies are needed to understand the magnitude of the molecule, as well as its mode of action, harm and benefits of its use in the different stages of creation. Studies with narasin in the prepartum phase of ewes, especially in the final third of gestation, are necessary to find out what the molecule acts in relation to one of the most demanding phases for ewes. Likewise, studies on genomics, and composition of the fatty acid profile of sheep's milk fed with narasin is important to know what changes the inclusion of the additive causes in this compound.

Studies aiming not only at the performance of lambs, but also at the increase in milk production of ewes of specific breeds for milk production is interesting, aiming at the use of the molecule for an increase in milk production, and at the gain for the producer.

As for the inclusion of narasin in the feedlot of lambs, genomic studies are necessary for a better understanding of how changes in fermentation occur in the lambs' cecum. Studies on carcass and meat quality such as tenderness, color and flavor are interesting to find out if the molecule changes any of these parameters that are appreciated by the consumer market.

Regarding the inclusion of different levels of NDF for feedlot lambs, it is necessary to evaluate other levels of NDF, to know if lower levels, or even the non-inclusion of a source of roughage in the diet would be accepted for feedlot lambs. Confinement time is also of great importance in new studies, as it can influence the final response depending on the diet provided.

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