### Use Of Ginger (Zingiber Officinale Roscoe) In The Diet Of Confined Sheep

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#### Abstract:

The objective of this study was to evaluate the inclusion of ginger (0.0%, 0.2%, 0.4% and 0.6%) in the diet of sheep as a natural additive on the intake and digestibility of nutrients, in addition to ruminal parameters. Four mixed breed sheep with an initial body weight of 25 kg were used, distributed in a Latin square experimental design (4x4). The diets were balanced with 12.0% crude protein (isoprotein) and 69.5% total digestible nutrients (isoenergetic). The studied variables were subjected to analysis of variance and the observed differences were determined by regression analysis considering 5% significance. The mean values obtained for the intake values expressed in kg animal-1 day-1 for DM; OM; CP; EE, NDF; ADF; TCH and CNF were 0.64; 0.60; 0.058; 0.011; 0.29; 0.19; 0.51; and 0.22, respectively. The mean values of the nutrient digestibility coefficients were 51.59; 64.92; 55.97; 76.86; 39.73; 30.68; 52.54; and 69.50%, respectively, for DM, OM, CP, EE, NDF, ADF, TCH, and NFC. No interaction effect was observed for the pH of the ruminal fluid; however, the pH value differed in relation to time. The concentration of N-NH3 in the ruminal fluid differed. The inclusion of up to 0.6% ginger in the diet of confined sheep does not alter the values of intake and nutrient digestibility coefficient; however, the inclusion of 0.4% ginger allows a higher concentration of N-NH<sub>3</sub> in the ruminal fluid in the time interval of 2h12min after feeding, requiring the inclusion of rapidly fermented carbohydrates in the diet.

Key Word: Phytogenic additive; Ammonia; pH; Ruminal fermentation.

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#### I. Introduction

According to Moreira (2018), the use of antibiotics in animal feed has been the subject of heated debate. The main issue is the possible deposition of antibiotic residues in animal products (meat and milk) and the concern about the emergence of resistant bacteria that could reach the food chain and generate impacts on human health. According to Silva (2019), society in general, technicians and producers have been seeking alternatives to improve the quality of food, and consequently improve the performance of these animals, using more digestible foods, with the power to improve the ruminal environment and contribute to better absorption and use of food. The use of phytotherapeutic additives in animal feed can be a viable alternative, as it is a natural and low-cost resource, in addition to being healthier for consumers of animal products.

These are compounds derived from plants that, when incorporated into animal diets, can promote better performance and better quality of the final products. According to Koiyama (2012), the term phytogenic refers to phytogenesis, which in turn refers to the origin or formation of plants. Therefore, phytogenic additives are products originating from plants, also known as phytobiotics or nutraceuticals. They comprise a wide variety of herbs, spices, and derived products such as essential oils, oleoresins, and extracts. When added to animal diets, they can increase productivity, improve diet quality and hygiene conditions, and improve the quality of food derived from these animals.

According to Pasquale and Pimenta (2014), phytogenic additives can have antimicrobial action and comprise a wide range of substances. The antimicrobial action of plant extracts can be attributed to their lipophilic nature, making them capable of penetrating bacterial cells and disintegrating their cell membrane.

A study conducted by Moreira (2018) highlighted that the effectiveness of phytogenic additives is related to the type of diet provided to animals, the concentration of the compound in the diet, the amount consumed, the action on the gastrointestinal tract and the physiological state of the animal.

According to Oliveira (2018), ginger is also considered one of the most important and valued spices around the world, and also has high therapeutic potential in various pathologies, such as nausea, stomach pain, colds and hyperglycemia. Several properties of ginger have been studied in scientific experiments, with antiinflammatory, antiemetic and anti-nausea, antimutagenic, anti-ulcer, hypoglycemic and antibacterial activities having been described. As high-quality feeds such as soybeans and corn become more expensive, finding ways to improve the use of fibrous or low-digestible feeds has become more than necessary for raising small ruminants, in the same way that the search for additives that help to improve the efficiency of feed and, consequently, animal use has become extremely important (DELLAQUA, 2020).

Therefore, the aim was to evaluate the use of a phytogenic additive based on ginger (*Zingeber officinale* Roscoe) and its influence on nutrient intake and digestibility, in addition to ruminal parameters of confined sheep.

#### **II. Material And Methods**

#### Location

The experiment was conducted at the Pontes University Campus and at the Animal Metabolism Sector (SeMA) belonging to the Mato Grosso State University (UNEMAT) and at the UNEMAT Food Analysis and Animal Nutrition Laboratory (LAANA). The study was authorized by CEUA/UNEMAT under opinion 006/2021.

#### **Experimental design**

Four mixed-breed sheep with an average initial body weight of 20 kg were housed in individual metabolism cages. A Latin square  $(4 \times 4)$  experimental design was used with four animals, four periods, and four experimental diets with different levels of dehydrated ginger inclusion.

#### Experimental diets and animal management

The feeds used were sugarcane silage (roughage), ground corn grain, and soybean meal (Table 1).

|                     | Table 1: Chemical composition of experimental loous. |       |                                |      |       |       |       |       |       |  |  |
|---------------------|--|-------|--------------------------------|------|-------|-------|-------|-------|-------|--|--|
|                     |  |       | % of nutrients expressed in DM |      |       |       |       |       |       |  |  |
|                     | % DM   | OM    | CP                             | EE   | NDF   | ADF   | TC    | NFC   | TDN   |  |  |
| Sugarcane<br>Silage | 30.95  | 91.85 | 4.27                           | 0.84 | 66.52 | 46.96 | 86.74 | 20.22 | 58.70 |  |  |
| Corn grain          | 89.29  | 98.36 | 9.24                           | 3.30 | 15.90 | 10.93 | 85.82 | 69.92 | 86.00 |  |  |
| Soybean meal        | 90.29  | 93.21 | 51.52                          | 0.93 | 16.55 | 11.95 | 40.77 | 24.22 | 81.00 |  |  |
| Ginger              | 90.41  | 91 73 | 6.04                           | 0.30 | 13 56 | 4 54  | 85 40 | 71.84 | 75.00 |  |  |

Table 1: Chemical composition of experimental foods.

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; TCH: total carbohydrates; NFC: non-fibrous carbohydrates and TDN: total digestible nutrients.

Source: Prepared by the authors.

The treatments consisted of the following levels of inclusion of dehydrated ginger in the diets (% DM): 0.0%; 0.2%, 0.4% and 0.6% ginger. The experimental diets were composed of 60% sugarcane silage and 40% concentrate, balanced to be isonitrogenous (12.0% crude protein) and isoenergetic (69.0% total digestible nutrients), according to the recommendations of the NRC (2007), Table 2.

The sheep had access to water and mineral salt provided in individual rations. The DM supply of the experimental diets was ad libitum (ad libitum) so that there was approximately 10.0% of leftovers daily, which was provided twice a day.

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|                                    | Ginger Inclusion Levels % |       |       |       |  |  |  |
|------------------------------------|---------------------------|-------|-------|-------|--|--|--|
|                                    | 0.0%                      | 0.2%  | 0.4 % | 0.6%  |  |  |  |
| Sugarcane silage                   | 60.0                      | 60.0  | 60.0  | 60.0  |  |  |  |
| Ground corn grain                  | 26.5                      | 26.3  | 26.1  | 25.9  |  |  |  |
| Soybean meal                       | 13.5                      | 13.5  | 13.5  | 13.5  |  |  |  |
| Ginger                             | 0.0                       | 0.2   | 0.4   | 0.6   |  |  |  |
| Total                              | 100.0                     | 100.0 | 100.0 | 100.0 |  |  |  |
| Chemical composition               |                           |       |       |       |  |  |  |
| Dry matter (DM)                    | 54.42                     | 54.42 | 54.42 | 54.43 |  |  |  |
| Organic matter (OM) %              | 93.76                     | 9375  | 93.73 | 93.72 |  |  |  |
| Crude protein (CP) %               | 11.97                     | 11.96 | 11.95 | 11.95 |  |  |  |
| Ethereal extract (EE) %            | 1.51                      | 1.5   | 1.49  | 1.49  |  |  |  |
| Neutral detergent fiber (NDF) %    | 46.36                     | 46.36 | 46.35 | 46.35 |  |  |  |
| Acid detergent fiber (ADF) %       | 32.68                     | 32.67 | 32.66 | 32.64 |  |  |  |
| Total Carbohydrates (TCH) %        | 80.29                     | 80.29 | 80.29 | 80.29 |  |  |  |
| Non-fibrous carbohydrates (NFC) %  | 33.93                     | 33.93 | 33.94 | 33.94 |  |  |  |
| Total Digestible Nutrients (TDN) % | 68.95                     | 68.92 | 68.9  | 68.88 |  |  |  |

#### Experimental periods and data collection

The experiment lasted 60 days, divided into four experimental periods lasting 15 days, with 10 days for animal adaptation and 5 days for collecting leftovers, feces and urine, with the fifth day being used only for collecting ruminal fluid, in addition to measuring the total amount of food supplied daily per animal (sheep) and per treatment within each experimental period.

The consumption of DM and other nutrients in the diet was determined by the difference in the amount of diet supplied per animal per day minus the amount of leftovers per animal in the 24-hour period. A digital scale was used to measure the amount of food (diet) offered and the leftovers for the 24-hour period.

Three units of measurement were used to tabulate and present the average values of nutrient consumption, with nutrient consumption expressed in kilograms per day (kg day<sup>-1</sup>); percentage of body weight (% BW) and kilograms per kilogram of metabolic weight (kg (kg 0.75)<sup>-1</sup>). The consumption of the following nutrients was evaluated: dry matter (DM); organic matter (OM); crude protein (CP); ether extract (EE); neutral and acid detergent fiber (NDF and ADF, respectively); total carbohydrate (TCH) and non-fibrous carbohydrate (NFC).

The nitrogen content of the studied feeds and leftovers was obtained by the semi-micro-Kjeldahl method, using 6.25 as the conversion factor for CP; mineral matter (MM) and organic matter (OM) were determined by the muffle furnace incineration method at 600°C, according to Silva and Queiroz (2002).

The determination of neutral detergent fiber (NDF) in the feeds and leftovers was performed according to Van Soest et al. (1991). Chemical analysis of feces and urine was performed; however, these data were not used in the present study to determine nutrient intake, but rather to determine the nutrient digestibility coefficient and nitrogen balance of sheep fed ginger (phytogenic additive).

The determination of total carbohydrates (TCH) of food, leftovers and feces was obtained by the equation: TCH = MO - [EE + CP], the non-fibrous carbohydrate content (NFC) of food, leftovers and feces was determined by the equation: NFC = 100 - (CP + NDF + EE + MM), according to Sniffen et al. (1992).

A leather bag was adapted for total feces collection in each sheep. The feces of each animal were weighed daily in the morning and homogenized, with composite samples being taken, corresponding to 10% of their total weight. The samples were placed in plastic bags, identified by animal and experimental period and stored in a freezer at -10 oC, for later analysis. After the collection period, the food, leftover and feces samples were dried in an oven at 55°C for 72 hours, and processed in a knife mill using a 1 mm sieve, then homogenized in equal amounts, based on dry weight, to form composite samples of feces and animal leftovers period<sup>-1</sup> experimental diet<sup>-1</sup>.

On the last day of each collection period, ruminal fluid was collected. Five collections of ruminal fluid were performed per animal, at times zero (before feeding), 2, 4, 6 and 8 hours after the first feeding. For a better understanding of the graphs for determining the pH value and ammonia nitrogen concentration of the ruminal fluid, for each value of 0.1 on the x-axis (time) this corresponds to 6 minutes. Since the time scale is 60 minutes per hour and the scale on the x-axis is decimal.

A 12 mm diameter probe made of silicone suitable for this activity was used to collect ruminal fluid from the sheep. The total length of the probe is 100 cm, but only 25 to 30 cm were introduced into the animal's mouth until it reached the rumen and its contents (rumen fluid). The estimated time to perform this procedure was less than 3 minutes.

A vacuum pump with a pressure of 40 mm Hg, Tecnal TE3 brand, suitable for this type of collection, was used. Therefore, as the silicone probe has a thin diameter (12 mm) and the time used for collection was short (less than 3 minutes), a local anesthetic "Neodexa Spray" was used, which allowed the probe to pass through the initial channel of the esophagus without causing pain to the animal.

The animal did not undergo any surgical incision (cut) or any other type of cut (incision), only the introduction of the silicone probe into the mouth and into the rumen. Before the esophageal probe was introduced into the animal's mouth, it was lubricated with mineral oil (Nujol type) to facilitate its movement during the ruminal fluid collection procedure.

100 mL of ruminal fluid were collected from each animal so that when filtered through a double cotton cloth, approximately 80 mL remained. These were homogenized and the pH was measured with the potentiometer immediately after each collection of ruminal fluid. Then, approximately 50 mL of the ruminal fluid was transferred to a properly labeled bottle with 1 mL of  $H_2SO_4$  to stop fermentation. This sample of ruminal fluid was used to determine the concentration of ammoniacal nitrogen (N-NH<sub>3</sub>), following the recommendations of Vieira (1980).

#### Statistical analyses

The variables studied were interpreted using analysis of variance in the Statistical and Genetic Analysis System (SAEG) program (UNIVERSIDADE FEDERAL DE VIÇOSA, 1997). Normality testing was performed using ANOVA/SAEG. The differences observed for the ginger inclusion levels (0.0%; 0.2%; 0.4% and 0.6% of DM) were determined by regression analysis considering 5% significance.

For the observed pH and N-NH<sub>3</sub> values of the ruminal fluid (ruminal parameters), the experimental diets were arranged in a split-plot scheme, and the sampling times were arranged as subplots, which characterizes a factorial of 4 (ginger inclusion levels) x 5 (time in hours after the first feeding being zero; 2; 4; 6 and 8 hours).

#### **III. Result And Discussion**

The inclusion of ginger at levels of 0.0%; 0.2%; 0.4% and 0.6% in DM, in the diet of sheep did not alter (P>0.05) the consumption of DM, OM, CP and EE. It was observed that the inclusion of ginger in the diet of sheep provided an average value for the consumption of DM, OM, CP and EE expressed in kg animal<sup>-1</sup> day<sup>-1</sup> of 0.64; 0.60; 0.058 and 0.011, respectively (Table 3).

| Table 3. Average daily intake of dry matter (DMI), organic matter (OMI), crude protein (CPI), ether        |
|--|
| extract (EEI), neutral digestible fiber (NDF), acid digestible fiber (ADF), total carbohydrates (TCH), and |
| non-fiber carbohydrates (NFC) of sheep fed diets containing ginger inclusion.                              |

|  |       | Ginger incl |       | Regression | CV(0)    |         |
|--|-------|-------------|-------|------------|----------|---------|
|  | 0.0   | 0.2         | 0.4   | 0.6        | equation | C V (%) |
| DMI kg animal <sup>-1</sup> day <sup>-1</sup>  | 0.64  | 0.64        | 0.65  | 0.61       | Ý= 0.64  | 4.26    |
| DMI % BW                                       | 3.01  | 3.01        | 2.99  | 2.86       | Ý= 2.97  | 5.06    |
| DMI kg (kg <sup>0,75</sup> ) <sup>-1</sup>     | 0.065 | 0.065       | 0.065 | 0.061      | Ý= 0.064 | 4.92    |
| OMI kg animal <sup>-1</sup> day <sup>-1</sup>  | 0.6   | 0.6         | 0.61  | 0.57       | Ý= 0.60  | 4.06    |
| OMI % BW                                       | 2.81  | 2.83        | 2.81  | 2.68       | Ý= 2.79  | 3.77    |
| OMI kg (kg <sup>0,75</sup> ) <sup>-1</sup>     | 0.06  | 0.061       | 0.061 | 0.057      | Ý= 0.060 | 3.71    |
| CPO kg animal <sup>-1</sup> day <sup>-1</sup>  | 0.061 | 0.059       | 0.058 | 0.052      | Ý=0.058  | 11.28   |
| CPI % BW                                       | 0.28  | 0.28        | 0.27  | 0.24       | Ý= 0.27  | 10.98   |
| CPI kg (kg <sup>0,75</sup> ) <sup>-1</sup>     | 0.006 | 0.006       | 0.006 | 0.005      | Ý=0.006  | 13.83   |
| EEI kg animal <sup>-1</sup> day <sup>-1</sup>  | 0.011 | 0.011       | 0.011 | 0.01       | Ý= 0.011 | 7.99    |
| EEI % BW                                       | 0.051 | 0.051       | 0.05  | 0.045      | Ý=0.049  | 6.89    |
| EEI kg (kg <sup>0,75</sup> ) <sup>-1</sup>     | 0.001 | 0.001       | 0.001 | 0.001      | Ý= 0.001 | 0.9     |
| NDFI kg animal <sup>-1</sup> day <sup>-1</sup> | 0.29  | 0.29        | 0.29  | 0.28       | Ý= 0.29  | 6.16    |
| NDFI % BW                                      | 1.36  | 1.37        | 1.35  | 1.33       | Ý= 1.35  | 3.85    |
| NDFI kg (kg <sup>0,75</sup> ) <sup>-1</sup>    | 0.03  | 0.029       | 0.029 | 0.028      | Ý=0.029  | 4.34    |
| ADFI kg animal <sup>-1</sup> day <sup>-1</sup> | 0.19  | 0.19        | 0.2   | 0.19       | Ý= 0.19  | 7.49    |
| ADFI % BW                                      | 0.9   | 0.91        | 0.91  | 0.89       | Ý= 0.90  | 5.21    |
| ADFI kg (kg <sup>0,75</sup> ) <sup>-1</sup>    | 0.019 | 0.019       | 0.02  | 0.019      | Ý=0.019  | 5.37    |
| TCHI kg animal <sup>-1</sup> day <sup>-1</sup> | 0.51  | 0.51        | 0.52  | 0.49       | Ý= 0.51  | 3.92    |
| TCHI % BW                                      | 2.4   | 2.42        | 2.41  | 2.3        | Ý= 2.38  | 3.29    |
| TCHI kg (kg <sup>0,75</sup> ) <sup>-1</sup>    | 0.051 | 0.052       | 0.052 | 0.05       | Ý=0.051  | 3.24    |
| NFCI kg animal <sup>-1</sup> day <sup>-1</sup> | 0.22  | 0.22        | 0.23  | 0.21       | Ý= 0.22  | 7.01    |
| NFCI % BW                                      | 1.04  | 1.05        | 1.05  | 0.98       | Ý= 1.03  | 7.19    |
| NFCI kg (kg <sup>0,75</sup> ) <sup>-1</sup>    | 0.022 | 0.023       | 0.023 | 0.021      | Ý= 0.022 | 7.16    |

g (kg0.75)-1: grams per kilogram of metabolic weight; %BW: percentage of body weight; g animal-1 day-1: grams per animal per day; and %CV: coefficient of variation.

Source: Prepared by the authors.

In a study conducted by Ibrahim et al., (2022) significant differences were observed in weight gain, feed conversion, digestibility and reduced diet cost by up to 17% per kg of weight gain, in cattle diets with the inclusion of ginger powder in the diet at levels of 0, 250, 500 and 750 g/100 kg, respectively, with six animals per treatment, in a completely randomized design.

Since the inclusion of different levels of ginger in sheep diets did not change (P> 0.05) the consumption of DM, OM, CP and EE, the behavior (concentrations) of these nutrients consumed can be better observed in Figure 1, where we can notice a lower numerical value for the intake of nutrients with the diet containing 0.6% ginger. However, this reduction was not significant for the consumption of DM, OM, CP and EE.

Therefore, it is worth presenting the percentage reduction observed for the consumption of DM and OM (kg animal<sup>-1</sup> day<sup>-1</sup>) between the diet with 0.6% ginger in relation to the diets with 0.0 and 0.2% ginger, which presented a reduction of 4.69% and 5.00% in the consumption of DM and OM kg animal<sup>-1</sup> day<sup>-1</sup>, respectively (Table 3). This observation in the percentage reduction of DM and OM intake leads us to promote the idea that the inclusion of ginger in the diet could be greater than 0.6%, which could cause a greater amplitude of variation and, thus, would help to obtain a decreasing linear equation or a curve, with a probable maximum or minimum point.



Figure 1. Average values of dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) intake of sheep fed diets containing different levels of ginger. Source: Prepared by the authors.

Geron et al., (2013) and Geron et al., (2017) demonstrated that the inclusion of diets containing on average 12 to 14% CP and energy concentration close to 68 to 70% TDN and concentrated bulk ratio within the variation of 40 to 60% in the total diet, the DM and OM consumption range (kg animal<sup>-1</sup> day<sup>-1</sup>) of sheep produced in a tropical region in confinement was 0.50 to 0.69 kg DM animal<sup>-1</sup> day<sup>-1</sup> and 0.48 to 0.64 kg OM animal<sup>-1</sup> day<sup>-1</sup>, indicating that the data observed in the present study are within the variation observed for the consumption of sheep produced in confinement in the state of Mato Grosso.

A study carried out by Abo Bakr (2019) with ewes in late gestation fed with ginger powder and ginger essential oil demonstrated significant results in increasing milk production with the addition of 3 g of ginger. There was a decrease in ammonia concentration when 2 ml of ginger essential oil was used and an improvement in feed conversion of these sheep using 6 g of ginger powder added to the diet.

The inclusion of different levels of ginger in the sheep diets did not alter (P>0.05) the consumption of NDF; ADF; TCH and NFC expressed in kg animal<sup>-1</sup> day<sup>-1</sup>; % BW and g kg (0.75)-1. The mean values for the consumption of NDF; ADF, TCH and NFC expressed in kg animal-1 day-1 of the sheep fed the different levels of ginger were 0.29; 0.19; 0.51 and 0.22, respectively.

The inclusion of 0.0%; 0.2%; The addition of 0.4% and 0.6% ginger in sheep diets did not alter (P>0.05) the consumption of different carbohydrates (neutral detergent fiber – NDF, acid detergent fiber – ADF, total carbohydrate – TCH and non-fibrous carbohydrate – NFC). However, it is worth highlighting some percentage differences observed in the consumption of mainly TCH and NFC expressed in kg animal<sup>-1</sup> day<sup>-1</sup>, where it was observed that the diet with 0.6% ginger provided a percentage reduction of 3.92% and 4.55% in relation to the diets with 0.0% and 0.2% ginger, respectively.

The levels of 0.6% ginger in the experimental diets of sheep provided a numerical (percentage) reduction in the consumption of the carbohydrates evaluated in this study, but there was no statistically significant difference (Figure 2). This observation can be corroborated by the fact that if there is a percentage reduction in dry matter consumption, this fact may be reflected in other nutrients since, in the evaluation of food (rations), the organic compounds (proteins, carbohydrates and fats) added together total a high proportion of the DM of the food or diet.



Figure 2 – Consumption of NDF, ADF, CHT and CNF of sheep fed diets containing different levels of ginger.

Source: Prepared by the authors.

In general, pungency or spicy flavor is present in ginger, making it one of the main attributes of this condiment. According to the literature, its presence is directly related to the concentration of active ingredients such as gingerol and shadoal, and could somehow cause changes in DM intake and other nutrients. However, this hypothesis was not confirmed in the present study. This fact is an indication that reinforces the need for new studies using higher doses of ginger inclusion to evaluate whether it can alter nutrient intake and probably the ruminal environment.

Another fact that may have contributed to obtaining the observed data on intake of different nutrients in the present study is correlated with the levels of CP, TDN and the forage: concentrate ratio of the experimental diets, which were similar to each other (Table 2). This reinforces the hypothesis that there was a significant effect between the treatments, which would be directly correlated with the levels of ginger included in the diets. However, this was not observed. Therefore, new studies should be carried out to confirm the data obtained in the present study.

The inclusion of levels of 0.0%; 0.2%; 0.4% and 0.6% of ginger in sheep diets did not alter (p>0.05) the digestibility coefficient of DM, OM, CP, NDF, ADF; TCH and NFC. An average value for the digestibility coefficient (CD) of DM; OM; CP, NDF; ADF, TCH and NFC was 51.59; 54.92; 55.97; 76.86; 37.72; 30.54; 53.13 and 74.31%, respectively (Table 4).

# Table 4 – Average values of total digestibility coefficients (DC) of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid (ADF), total carbohydrate (TCH) and non-fibrous carbohydrate (NFC) of sheep fed diets containing different inclusion levels of

|           | ginger ( <i>Zing</i> ) | eber officinale F | koscoe) and c | coefficients of varia | tion (CV). |       |  |  |
|-----------|------------------------|-------------------|---------------|-----------------------|------------|-------|--|--|
|           |                        | Regression        | CV %          |                       |            |       |  |  |
|           |                        |                   |               |                       |            |       |  |  |
|           | 0.0                    | 0.2               | 0.4           | 0.6                   |            |       |  |  |
| DCSDM (%) | 51.84                  | 50.03             | 52.99         | 51.64                 | Ý= 51.59   | 10.91 |  |  |
| DCOM (%)  | 55.00                  | 54.09             | 55.32         | 55.27                 | Ý= 54.92   | 9.18  |  |  |
| DCCP (%)  | 58.28                  | 56.19             | 53.46         | 55.95                 | Ý= 55.97   | 9.66  |  |  |
| DCEE (%)  | 78.20                  | 79.13             | 76.77         | 73.37                 | Ý= 76.86   | 10.58 |  |  |
| DCNDF (%) | 38.57                  | 43.72             | 39.00         | 37.72                 | Ý= 39.75   | 13.03 |  |  |
| DCADF (%) | 30.83                  | 33.13             | 28.70         | 30.54                 | Ý= 30.68   | 20.43 |  |  |
| DCTCH (%) | 52.62                  | 51.55             | 53.04         | 53.13                 | Ý= 52.59   | 9.65  |  |  |
| DCNFC (%) | 70.91                  | 61.81             | 70.93         | 74.31                 | Ý= 69.50   | 10.95 |  |  |
|           |                        |                   |               |                       |            |       |  |  |

Source: Prepared by the authors.

Since there was no effect of including ginger in the sheep diet on nutrient intake. it was expected that the nutrient CD would not show any difference. since the diets were isoproteic and isoenergetic and had the same proportion of concentrate (35%).

A study carried out by Geron et al., (2016) to evaluate the inclusion of slow-release urea in the diet of sheep with a body weight of 30 kg, fed 65% corn silage and 35% concentrate, containing 14% CP and 69.5% TDN of the total diet. The authors observed a variation range for the DM and CP CD of 63.0 to 71.00% and 52.16 to 65.40%. respectively. Thus, we can observe that the data of the present study for the DM and CP CD were below those observed by Geron et al., (2016). Of course, the diet (ingredients and body weight of the animals) were different and this fact may contribute to the range of variation observed between studies.

Geron et al., (2013) when evaluating the different levels of concentrate in the feeding of sheep in a tropical region receiving rations with 12.5% CP and body weight of 20 kg. The authors observed that the average DM CD was 58.4% and the CP CD varied by 54.7%. thus the data of the present study were within the range observed by Geron et al., (2013).

In general. it can be observed that the levels used to evaluate the effect of using the natural additive – ginger in sheep feeding did not provide the necessary change in the ruminal fermentation environment, which may have contributed to obtaining the nutrient CD data in the present study. Thus, other experiments with higher levels of ginger inclusion are necessary to elucidate the capacity of this additive to improve digestion and change the ruminal environment. The inclusion of ginger levels of 0.0%; 0.2%; 0.4% and 0.6% in sheep diets did not alter (p>0.05) the pH values observed in the ruminal fluid (Table 5). This result can be partly explained by the fact that the experimental diets were isoproteic and isoenergetic. Furthermore, all diets presented a roughage:concentrate ratio of 60:40, which may have provided a similar ruminal environment for the different levels of ginger inclusion in the diets.

 Table 5. pH values of ruminal fluid from sheep fed diets containing inclusion levels of ginger as a function of collection times after morning feeding.

| *-                      |     |              |     | - <del>-</del> |         |
|-------------------------|-----|--------------|-----|----------------|---------|
| Collection time - hours |     | A.v.o.mo.co. |     |                |         |
|                         | 0.0 | 0.2          | 0.4 | 0.6            | Average |
|                         |     |              |     |                |         |

| Use Of Ginger | (Zingiber | Officinale Rosco | e) In The D | iet Of <b>(</b> | Confined | Sheep |
|---------------|-----------|------------------|-------------|-----------------|----------|-------|
|               |           |                  |             |                 |          |       |

| 0       | 6.94     | 6.91  | 6.96 | 6.96 | 6.94 |
|---------|----------|-------|------|------|------|
| 2       | 6.49     | 6.54  | 6.57 | 6.48 | 6.52 |
| 4       | 6.53     | 6.39  | 6.46 | 6.41 | 6.45 |
| 6       | 6.51     | 6.56  | 6.48 | 6.58 | 6.53 |
| 8       | 6.75     | 6.66  | 6.78 | 6.58 | 6.71 |
| Average | 6.64     | 6.61  | 6.65 | 6.68 | 6.63 |
|         | <b>D</b> | 11 /1 |      |      |      |

Source: Prepared by the authors.

Geron et al., (2016) and Geron et al., (2017) reported that the method of collecting ruminal fluid using an esophageal probe can interfere with pH values, due to contamination by saliva and consequently alkalinization of the ruminal fluid, especially at the first collection time.

In the present study, it was found that the collection of fluid using an esophageal probe before the morning feeding at midnight presented an average pH value of 6.94 for the different experimental diets. This fact may have occurred due to the methodology of collecting ruminal fluid and the smaller amount of food in the rumen, which may have favored contamination by saliva (buffer) of the ruminal fluid sample collected at this time. Furthermore, sensory stimuli such as the visualization and odor of food (ZEOULA et al., 2003) may have led to increases in ruminal ammonia concentrations (GERON et al., 2016) through increased salivation before feeding, which may have contributed to the result obtained of ruminal fluid pH close to 7.0 before morning feeding.

Another factor that corroborates the results obtained for the pH value of the ruminal fluid of sheep fed with different levels of inclusion of the phytogenic additive - ginger. is correlated with the high NDF content (>46%) and constant value of approximately 33% of non-fibrous carbohydrates (mainly starch). which may have favored adequate rumination and consequently the production of buffer (saliva).

However, the pH value of the ruminal fluid differed (p<0.05) in relation to time, for all experimental diets containing ginger. The behavior of the experimental diets containing different levels of ginger is demonstrated in Figure 3.



Figure 3 – pH value of the ruminal fluid for the different experimental diets containing ginger as a function of time after the first morning feeding. Source: Prepared by the authors.

The mean regression equation for the pH value of the ruminal fluid of sheep fed different levels of ginger was  $pH = 6.912054 - 0.216397x + 0.024319x^2$  ( $R^2 = 95.95\%$ ). The estimated mean minimum point for the pH value was 6.43 for the time of 4h24min after the morning feeding and the maximum point observed by the equation was 6.91 for the time of 0h after the morning feeding (Figure 4).





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The observed values of ammoniacal nitrogen (N-NH<sub>3</sub>) concentration in the ruminal fluid of sheep fed diets containing different levels of ginger are shown in Table 6.

## Table 6. Observed values of ammoniacal nitrogen (N-NH3) concentration in ruminal fluid expressed in mg/100 ml of ruminal fluid from sheep fed diets containing inclusion levels of ginger as a function of collection times after morning feeding.

| 0.0  | 0.2   | 0.4   |  |  |  |  |  |
|--|---|---|--|--|--|--|--|
|  | 0.2   | 0.4   | 0.6  |  |  |  |  |
| 36.75  | 38.24   | 43.66   | 34.04  | 38.17  |  |  |  |
| 43.58  | 40.69   | 50.05   | 37.98  | 43.07  |  |  |  |
| 43.05  | 43.14   | 44.28   | 36.93  | 41.85  |  |  |  |
| 41.74  | 38.76   | 39.73   | 35.53  | 38.94  |  |  |  |
| 30.45  | 34.30   | 32.90   | 29.75  | 31.85  |  |  |  |
| 1  | 2   | 3   | 4  | 38.78  |  |  |  |
| $^{1}$ N-NH <sub>3</sub> = 36.7125 + 4.5565625X - 0.66.937X <sup>2</sup> (R <sup>2</sup> = 96.66%) |   |   |  |  |  |  |  |
| $^{2}$ N-NH <sub>3</sub> = 38.035 + 2.460X - 0.36875X <sup>2</sup> (R <sup>2</sup> = 93.84%)       |   |   |  |  |  |  |  |
| ${}^{3}$ N-NH <sub>3</sub> = 44.8925 + 2.0075X - 0.450X <sup>2</sup> (R <sup>2</sup> = 91.38%)     |   |   |  |  |  |  |  |
| = 34.2225 + 2.2  | 273750X - 0.3531  | $25X^2 (R^2 = 97.31\%)$                               | ő)   |  |  |  |  |
|  | $\begin{array}{r} 36.75 \\ \hline 43.58 \\ \hline 43.05 \\ \hline 41.74 \\ \hline 30.45 \\ \hline 1 \\ \hline \\ = 36.7125 + 4.5 \\ H_3 = 38.035 + 2 \\ H_3 = 44.8925 + 2.3 \\ \hline \\ = 34.2225 + 2.3 \\ \hline \end{array}$ | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ | $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | $\begin{array}{c c c c c c c c c c c c c c c c c c c $ |  |  |  |

Source: Prepared by the authors.

The concentration of ammoniacal nitrogen (N-NH<sub>3</sub>) in the ruminal fluid was altered (p<0.05) with the inclusion of different levels of ginger in the sheep diet, as shown in Figure 5. In addition, there was an effect in relation to the time (hours) after the morning feeding. Thus, for each level of ginger inclusion, a regression equation was determined and consequently the maximum points for the N-NH<sub>3</sub> concentration were established at different times (Figure 5).

In general, the average N-NH<sub>3</sub> concentration of the ruminal fluid observed for sheep fed different levels of ginger showed an N-NH<sub>3</sub> value of 38.78 mg/100 mL of ruminal fluid. which remained above the optimal range of 15 to 23 mg/100<sup>-1</sup> mL for maximum ruminal fermentative activity (GERON et al., 2015; PEREIRA et al., 2009) and above the concentration of 5.0 mg/100<sup>-1</sup> mL established in the literature to not limit microbial growth (ZEOULA et al., 2003; PEREIRA et al., 2009). However, the N-NH<sub>3</sub> concentrations of the ruminal fluid for all experimental diets behaved quadratically (p<0.05) with convex curve behavior as a function of time (hours) after the first feeding (Figure 5).



Figure 5. Values observed for the concentration of ammoniacal nitrogen (N-NH<sub>3</sub>) expressed in mg/100 mL of ruminal fluid of sheep fed diets containing 0.0%; 0.2%; 0.4% and 0.6% ginger. Source: Prepared by the authors.

Statistical analysis of the variables indicated that the behavior of the N-NH<sub>3</sub> concentration of the ruminal fluid showed a difference (P<0.05) for the different levels of ginger. Thus, the average curve for the N-NH<sub>3</sub> concentration value does not represent the behavior of each treatment as a function of time. Therefore, as there was an effect (P<0.05) of the different levels of ginger on the N-NH<sub>3</sub> concentration of the ruminal fluid as a function of time after feeding the sheep, it was necessary to perform the unfolding of the regression equations for each level of ginger to observe which ones presented the highest concentrations of N-NH<sub>3</sub> (Figure 6).



Figure 6 – Colored line showing observed value and dotted line showing estimated value of ammonia nitrogen (N-NH<sub>3</sub>) concentration in ruminal fluid (mg/100 mL of ruminal fluid) of sheep fed different levels of ginger. Source: Prepared by the authors.

Thus, we can observe that for each level of ginger additive used in sheep feeding, there was (p<0.05) a distinct behavior. Thus, for the diet with 0.0% ginger, the point of maximum N-NH<sub>3</sub> concentration in the ruminal fluid was 44.60 mg/100 mL of ruminal fluid obtained at 3:30 am. For the diet with 0.2% ginger, the maximum N-NH<sub>3</sub> concentration in the ruminal fluid was 42.13 mg/10 mL of ruminal fluid at 3:18 am after feeding.

According to the mathematical estimate, the diet with 0.4% ginger presented a point of maximum N-NH<sub>3</sub> concentration of 47.13 mg/100 mL of ruminal fluid at 2:12 am after feeding. However, the diet with 0.6% ginger showed a maximum N-NH<sub>3</sub> concentration point of 37.88 mg/100 mL of ruminal fluid at 3h:12min after feeding (Figure 6).

These estimated data suggest that ginger altered (p<0.05) the behavior of the ammonia nitrogen (N-NH<sub>3</sub>) concentration of the ruminal fluid, indicating that the 0.4% ginger level allowed the highest ammonia concentration in the rumen, in a shorter time (hours) compared to the other ginger levels. In addition, it was observed that the pH value of the ruminal fluid of the diet with 0.4% ginger behaved similarly (p>0.05) to the other ginger levels in sheep feeding. Thus, it is evident that the inclusion of 0.4% ginger in sheep diets allowed for greater N-NH<sub>3</sub> availability in the rumen for the time interval of less than 2h30min after the sheep's morning feeding, which leads technicians. nutritionists and sheep producers to use sources of fast-available energy when including ginger as a phytogenic additive to obtain the greatest use of dietary nitrogen released in the form of ammonia (NH<sub>3</sub>) and/or ammonium (NH<sub>4</sub>) in the ruminal environment.

According to Geron et al., (2017). the individual breakdown of the pH and N-NH<sub>3</sub> equations of each diet allows for greater confidence and precision in relation to the estimated average curve and observed values. Thus becoming an important tool that helps technicians and nutritionists to formulate rations containing ingredients that can improve the use of dietary nitrogen (NH<sub>3</sub>) and thus improve the efficiency of fermentation and production of short-chain fatty acids.

#### **IV. Conclusion**

The inclusion of up to 0.6% ginger in the diet of confined sheep does not alter the values of consumption and digestibility coefficient of nutrients. However, the inclusion of 0.4% ginger allows for a higher concentration of ammonia nitrogen in the ruminal fluid in the time interval of 2h12min after feeding, requiring the inclusion of rapidly fermented carbohydrates in the diet.

Therefore, it is possible to evaluate new dosages of ginger inclusion in the diets of ruminant animals to validate the results observed in the present study.

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