

**Desempenho e parâmetros sanguíneos de novilhas mestiças Holandês/Zebu alimentadas  
com duas fontes de taninos**

**Performance and blood parameters of Holstein/Zebu crossbred heifers fed with two  
tannins sources**

**Parámetros de rendimiento y sangre de vaquillas cruzadas Holstein/Zebu alimentadas  
con dos fuentes de taninos**

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## Resumo

O objetivo da presente pesquisa foi avaliar o desempenho e os parâmetros sanguíneos de vacas em lactação alimentadas com uma dieta de duas fontes de taninos, à base de sorgo (tanino condensado) com concentrações crescentes de ácido tânico (taninos hidrolisáveis). O aumento dos níveis de ácido tânico em uma dieta à base de sorgo para cinco vacas mestiças da raça Holandesa / Zebu foi submetido a um delineamento experimental quadrado latino  $5 \times 5$ . Avaliar o efeito na ingestão de vaca, digestibilidade, produção de leite e parâmetros sanguíneos. Todas as vacas receberam 9,87 kg / MS de silagem de milho como volumoso e 6,38 kg de concentrado constituído por 2,58 kg / MS de sorgo moído 0,87 kg / MS de farinha de milho 1,32 kg / MS de farelo de soja 0,44 kg / MS de farelo de trigo 0,2 kg / MS de uréia e 0,18 kg / MS de mistura mineral. A dieta 1 (controle) continha sorgo com baixo teor de tanino e as demais dietas continham sorgo com alto teor de tanino. Os níveis de ácido tânico adicionados às dietas foram estabelecidos com base na quantidade de tanino condensado no sorgo com alto teor de tanino. Assim, as dietas 2, 3, 4 e 5 foram suplementadas com 1,5g (13% MS), 79,5g (2,6% MS), 157,5g (3,9% MS) e 235,5g (5,2% MS) de ácido tânico, totalizando 0,078, 0,156, 0,234 e 0,332 tanino total kg / dia, respectivamente, para avaliar o efeito na síntese de proteínas microbianas. As dietas continham 35 kg de silagem de milho (volumoso) e 6,40 kg de concentrado. O consumo natural e de matéria seca não diferiram significativamente entre os grupos, exceto o consumo de extrato etéreo (EE) que diferiu significativamente. A digestibilidade aparente do MS, proteína bruta, EE, fibra em detergente neutro, carboidrato total e carboidrato não fibroso não diferiram ( $P > 0,05$ ) com o aumento da suplementação de tanino na dieta. Os níveis de GOT aumentaram linearmente. Os níveis de glicose no sangue, triglicerídeos e colesterol (total, LDL e HDL) não diferiram significativamente. A hemoglobina mostrou uma diferença significativa. Não houve diferença significativa na uréia, creatinina e ácido úrico. Dietas com suplementação de duas fontes de taninos não causaram diminuição na ingestão ou digestibilidade alimentar dos animais. O nível de GOT mudou significativamente, mostrando comportamento linear, porém abaixo do nível de toxicidade, sem qualquer alteração nos outros parâmetros sanguíneos. A produção de leite diminuiu com o aumento da suplementação de taninos na dieta.

**Palavras-chave:** Ácido tânico; Ingestão; Digestibilidade; Hepatotoxicidade.

## Abstract

The present research objective was to evaluate the performance and blood parameters of lactating cows fed with a diet of two tannin sources, based on sorghum (condensed tannin)

with increasing concentrations of tannic acid (hydrolyzable tannin's). Increasing levels of tannic acid in a sorghum-based diet for five Holstein/Zebu crossbred lactating cows were subject to a 5 × 5 Latin square experimental design. To assess the effect on cow intake, digestibility, milk production, and blood parameters. All cows received 9.87 kg/DM of corn silage as roughage and 6.38 kg of concentrate consisting of 2.58 kg/DM of ground sorghum 0.87 kg/DM of cornmeal 1.32 kg/DM of soybean bran 0.44 kg/DM of wheat bran 0.2 kg/ DM of urea and 0.18 kg/ DM of mineral mixture. Diet 1 (control) contained low-tannin sorghum and the other diets contained high-tannin sorghum. The levels of tannic acid added to the diets were established based on the quantity of condensed tannin in high-tannin sorghum. Thus, diets 2, 3, 4 and 5 were supplemented with 1.5g (13.%DM), 79.5g (2.6%DM), 157.5g (3.9%DM) and 235.5g (5.2%DM) of tannic acid, totalling 0.078, 0.156, 0.234 and 0.321 total tannin kg/day respectively, to assess the effect on microbial protein synthesis. The diets contained 35 kg of corn silage (roughage) and 6.40 kg of concentrate. The natural and dry matter intake did not significantly differ between groups, except for ether extract (EE) intake that significantly differed. The DM apparent digestibility, crude protein, EE, neutral detergent fiber, total carbohydrate, and non-fibrous carbohydrate did not differ ( $P > 0.05$ ) with the increase in dietary tannin supplementation. The GOT levels increased linearly. The blood glucose, triglyceride, and cholesterol (total, LDL and HDL) levels did not significantly differ. Hemoglobin showed a significant difference. No significant difference in urea, creatinine and uric acid occurred. Diets using two tannins sources supplementation caused no decrease in the dietary intake or digestibility in the animals. The GOT level changed significantly, showing linear behavior, however below the toxicity level, without any change in the other blood parameters. Milk production decreased with the increase in dietary tannin supplementation.

**Keywords:** Tannic acid; Intake; Digestibility; Hepatotoxicity.

### **Resumen**

El objetivo de la presente investigación fue evaluar el rendimiento y los parámetros sanguíneos de las vacas lactantes alimentadas con una dieta de dos fuentes de tanino, a base de sorgo (tanino condensado) con concentraciones crecientes de ácido tánico (tanino hidrolizable). Los niveles crecientes de ácido tánico en una dieta a base de sorgo para cinco vacas lactantes cruzadas Holstein / Zebu fueron sometidas a un diseño experimental cuadrado latino 5 × 5. Evaluar el efecto sobre el consumo de vacas, la digestibilidad, la producción de leche y los parámetros sanguíneos. Todas las vacas recibieron 9.87 kg / DM de ensilaje de maíz como forraje y 6.38 kg de concentrado que consiste en 2.58 kg / DM de sorgo molido 0.87 kg / DM de harina de maíz 1.32 kg / DM de salvado de soja

0.44 kg / DM de salvado de trigo 0.2 kg / DM de urea y 0.18 kg / DM de mezcla mineral. La dieta 1 (control) contenía sorgo bajo en taninos y las otras dietas contenían sorgo alto en taninos. Los niveles de ácido tánico añadidos a las dietas se establecieron en función de la cantidad de tanino condensado en el sorgo con alto contenido de taninos. Por lo tanto, las dietas 2, 3, 4 y 5 se complementaron con 1,5 g (13% de MS), 79,5 g (2,6% de MS), 157,5 g (3,9% de MS) y 235,5 g (5,2% de MS) de ácido tánico, totalizando 0.078, 0.156, 0.234 y 0.321 kg de tanino total / día respectivamente, para evaluar el efecto sobre la síntesis de proteínas microbianas. Las dietas contenían 35 kg de ensilaje de maíz (forraje) y 6,40 kg de concentrado. La ingesta de materia natural y seca no difirió significativamente entre los grupos, excepto la ingesta de extracto de éter (EE) que difirió significativamente. La digestibilidad aparente de la MS, la proteína cruda, la EE, la fibra de detergente neutral, los carbohidratos totales y los carbohidratos no fibrosos no difirieron ( $P > 0.05$ ) con el aumento de la suplementación dietética de taninos. Los niveles de GOT aumentaron linealmente. Los niveles de glucosa en sangre, triglicéridos y colesterol (total, LDL y HDL) no difirieron significativamente. La hemoglobina mostró una diferencia significativa. No hubo diferencias significativas en urea, creatinina y ácido úrico. las dietas que utilizan dos suplementos de fuentes de taninos no causaron disminución en la ingesta dietética o digestibilidad en los animales. El nivel de GOT cambió significativamente, mostrando un comportamiento lineal, sin embargo por debajo del nivel de toxicidad, sin ningún cambio en los otros parámetros sanguíneos. La producción de leche disminuyó con el aumento de la suplementación de taninos en la dieta.

**Palabras clave:** Ácido tánico; Ingestión; Digestibilidad; Hepatotoxicidad.

## 1. Introduction

In recent decades, a series of chemical additives have been applied to ruminant nutrition to modulate ruminal fermentation and improve feed intake and efficiency. However, most supplements may not be used routinely due to toxicity problems in the host animals and microbial adaptation to these additives (Patra, 2011). Research studies aimed at exploiting bioactive phytochemical compounds, like tannins, to find positive effects on ruminal metabolism that can increase ruminants' production efficiency, especially the ones with selective antimicrobial properties (Patra & Saxena, 2011). Therefore, tannins are currently known for having beneficial and adverse effects depending on their type and chemical structure, source plant species, ingested quantity or concentration, and the species and specificities of each animal (Grabber, 2009; Mueller-Harvey, 2006).

The numerous benefits tannin's may bring to ruminants include improved use of dietary proteins, increased growth rates, live weight or wool, higher milk production,

increased fertility, improved animal wellbeing and health, through bloat prevention and reduced parasitic load (Mueller-Harvey, 2006) and anthelmintic purposes due to their ability to inhibit egg hatching and larval motility of gastrointestinal nematode parasites (Naumann et al. 2017).

Contrast diets with increasing concentrations of tannic acid were fed to dairy cows. In order to comprehend their performance and blood parameters in the attempt to ensure the cows health, all blood tests have been performed to ensure follow-up on possible metabolic changes in organs such as kidneys and liver, and changes in immune system metabolism. It was expected a reduction in blood cholesterol levels and perhaps triglycerides, however without the increase of creatinine or TGO and TGP, maintaining the consumption and improving protein metabolism and productivity.

The present research objective was to evaluate the performance and blood parameters of lactating cows fed with a diet of two tannin sources, based on sorghum (condensed tannin) with increasing concentrations of tannic acid (hydrolysable tannin's).

## **2. Methodology**

All practices performed in the present study involving the use of animals were approved by the Institutional Animal Care of the Ethics Commission in Animal Use of the Biotechnological Centre of Federal University of Paraiba (CEUA/BIOTEC/UFPB) (protocol number 072/2016). This experiment was conducted at the Cattle Farming Unit of the Animal Science Department of the Agricultural Science Centre/Federal University of Paraiba, Campus II, in the municipality of Areia/PB Brazil, from February to May (dry to rain season).

### **Animal and Management**

Five crossbred Holstein × Zebu heifers were used, the cows were approximately four years old (averaging  $420 \pm 30$  kg initial BW,  $450 \pm 35$  kg final BW,  $435 \pm 32,5$  kg average BW) and approximately 100 days of lactation, with an average initial production of  $18 \pm 4$  kg/day were used in a  $5 \times 5$  Latin square design. The cows were housed in individual 18 m<sup>2</sup> stalls with concrete floors and equipped with individual stalls feeders and water dispensers. Each experimental period lasted 20 d, with 15 d for diet adaptation and last 5 d for sample collection, for a total of 100 experimental days. Cows were weighed at the beginning and at the end of each experimental period.

Before starting the experiment, the cows were dewormed and treated for ectoparasites

with 3.5% Ivermectin. The adaptation period to the facilities, the experimental diets and the stabling totalled 10 d.

The experimental diet consisted as roughage and concentrate, at fixed roughage: concentrate ratio of 64:36 as a TMR. The diet was offered in equal amounts twice a day at 06.00 AM and 01.30 PM after milking. Every day, orts from each animal were removed and weighed to calculate the food (natural and dry matter) intake. To ensure ad libitum access to the roughage, voluntary forage intake was determined daily, and roughage was fed at 140% of the average intake for the previous 5 d. The corn silage was produced at the Agricultural Science Centre/Federal University of Paraiba, Areia/PB.

### Treatments

The experimental diet was formulated to meet the lactating demands according to recommendations from the NRC (National Research Council – NRC, 2001).

All cows received 9.87 kg/DM of corn silage as roughage and 6.38 kg of concentrate consisting of 2.58 kg/DM of ground sorghum, 0.87 kg/DM of corn meal 1.32 kg/DM of soybean bran, 0.44 kg/DM of wheat bran, 0.2 kg/ DM of urea and 0.18 kg/ DM of mineral mixture (Table 1). (Composition:  $\geq$  190 g/kg Ca, 60 g/kg P, 20 g/kg S, 20 g/kg Mg, 35 g/kg k, 70 g/kg Na, 15 mg/kg Co, 700 mg/kg Cu, 10 mg/kg Cr, 700 mg/kg Fe, 40 mg/kg I, 1600 mg/kg Mn, 19 mg/kg Se, 2500 mg/kg Zn, vitamin A 400000 IU/kg, vitamin D3 100000 IU/kg, vitamin E 2400 IU/kg; Bovigold® DSM) daily.

Diet 1 (control diet) contained BRS Ponta Negra cultivar sorghum (0.92% total condensed tannin in the DM) to provide the lowest possible amount of tannins. Diets 2, 3, and 4 contained A9904 cultivar sorghum (2.55% total condensed tannin in the DM), to provide the highest quantity of available tannins possible, in order to guarantee the tannin effects in animal metabolism.

The tannic acid levels added to the diets were established based on the analysis of the condensed tannin quantity in high-tannin sorghum. The levels of total condensed tannin (DM) in A9904 were calculated according to the HCl-butanol method (Hagerman & Butler, 1978) and the total condensed tannin in the DM in the control sorghum (BRS Ponta Negra cultivar) according to Terril et al. (1992) method, because of the different tannin content in each one.

**Table 1** - Chemical composition, dietary ingredients proportion, condensed tannin and hydrolysable tannin (tannic acid) concentration in experimental diets in kilogram (kg) of natural matter and nutritional composition of diets ingredients on a dry matter (%) basis

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	DM	CP	EE	NDF	MM	NFC	TC
	Kg					% DM						
Corn silage	35.00	35.00	35.00	35.00	35.00	28.2	8.0	2.2	55.1	4.8	33.6	84.8
Ground corn	1.00	1.00	1.00	1.00	1.00	86.7	9.0	4.3	14.0	1.2	74.5	80.6
Soybean meal	1.50	1.50	1.50	1.50	1.50	88.3	48.8	2.0	14.5	6.1	30.4	43.5
Wheat meal)	0.50	0.50	0.50	0.50	0.50	87.7	16.4	1.7	44.5	6.3	30.1	74.0
Urea	0.20	0.20	0.20	0.20	0.20	100	283	0.0	0.0	0.0	0.0	0.0
Sorghum A9904	0.00	3.00	3.00	3.00	3.00	85.2	10.9	2.7	14.0	3.3	73.9	82.4
Sorghum control	3.00	0.00	0.00	0.00	0.00	87.5	9.3	2.9	13.8	3.5	73.9	84.4
Mineral mix *	0.18	0.18	0.18	0.18	0.18	100	0.0	0.0	0.0	100	0.0	0.0
Tannic acid	0.00	0.0015	0.0795	0.158	0.236	100	0.00	0.00	0.00	0.00	0.00	0.00
Total	41.38	41.4	41.46	41.54	41.62							

Dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), mineral matter (MM), non-fibrous carbohydrate (NFC), total carbohydrate (TC). \*190 g/kg Ca, 60 g/kg P, 20 g/kg S, 20 g/kg Mg, 35 g/kg k, 70 g/kg Na, 15 mg/kg Co, 700 mg/kg Cu, 10 mg/kg Cr, 700 mg/kg Fe, 40 mg/kg I, 1600 mg/kg Mn, 19 mg/kg Se, 2500 mg/kg Zn, vitamin A 400000 IU/kg, vitamin D3 100000 IU/kg, vitamin E 2400 IU/kg.

Tannic acid (C<sub>76</sub>H<sub>52</sub>O<sub>46</sub>) P.A. purchased from Anidrol® laboratory products, Sao Paulo- Brazil, a purified powder product was added to prepare the increasing tannin doses. Predetermined proportions of powder tannic acid (based on the quantity of condensed tannin from sorghum A9904, 1.27, we've added, continuously, this same quantity, therefore, 2.6, 3.9, and 5.2, to find the final total percentages) were mixed with the concentrate on the day before trough feeding. Thus, diets 2, 3, 4 and 5 were supplemented with 1.5, 79.5, 157.5 and 235.5 g of tannic acid respectively, and provided an increase in the dietary total tannin percentages as outlined in Table 2.

**Table 2** – Percent (% DM basis) and kilogram (kg) contributions of condensed tannin from sorghum and powder tannic acid added

Diet	Sorghum Contribution CT (%)	Powder tannic acid. (HT) (%)	Sorghum CT Contribution in 3 kg	Tannic acid Addition in kg/day	Total tannin in diet (CT + HT) (%)	Total tannin (CT + HT) (kg/day)
Diet 1	0.46	0.00	0.0276	0.0000	0.46%	0.0276

Diet 2	1.27	0.03	0.0765	0.0015	1.30%	0.0780
Diet 3	1.27	1.33	0.0765	0.0795	2.60%	0.1560
Diet 4	1.27	2.63	0.0765	0.1575	3.90%	0.2340
Diet 5	1.27	3.93	0.0765	0.2355	5.20%	0.3120

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CT- Condensed tannin, HT- Hydrolysable tannin

Therefore, the following tannin sources were provided in the experimental diets: condensed tannins through both sorghum cultivars and hydrolysable tannins through tannic acid. Water was provided to the animal's ad libitum.

Experimental diets nutritional composition (concentrate plus roughage) (%DM) by laboratory analysis. Diet 1 (control diet) 36.85 DM, 15.55 CP, 1.99 EE, 38.66 NDF, 5.48 MM, 33.68 NFC and total tannin 0.46% and to Diets 2, 3, 4 and 5, 37.30 DM, 15.46 CP, 2.08 EE, 38.66 NDF, 5.67 MM 33.24 NFC and total tannin 1.30%, 2.60%, 3.90% and 5.2% respectively.

#### Experimental Procedures and Sample Collections

Each experimental period lasted 20 d, with 15 d for diet adaptation and last 5 d for sample collection, for a total of 100 experimental days. Cows were weighed at the beginning and at the end of each experimental period.

Dry matter intake: Feed intake was quantified from day 15 to 20 of each period. Representative samples of roughage and orts were collected daily, weighed and stored in plastic bags in a fridge, and blended manually at the end of each period to obtain pooled samples per treatment. Samples of roughage and orts were oven-dried (60°C) for 72 hours, weighed and ground in a Wiley mill (model 3; Arthur H. Thomas, Philadelphia, PA) to pass through a 2-mm screen. After that, half of each ground sample was ground again to pass through a 1-mm screen.

Faecal Collection: Faecal samples output were collected immediately after each spontaneous defecation from day 15 to 20 (day 1, 3 and 5) of each period and weighed and stored in aluminium plates, and oven-dried (55°C) for 72 hours, weighed and ground in a Wiley mill (model 3; Arthur H. Thomas, Philadelphia, PA) to pass through a 2-mm screen and blended manually at the end of each period to obtain pooled samples per treatment in proportion to the daily excretion to measure digestibility. After grinding, half of each ground sample was ground again to pass through a 1-mm screen.

**Milk samples:** Milk production was recorded daily by individual weighing (kg/day). Milk samples were collected twice daily (morning and afternoon) from day 15 to 20 of each period. Milk samples from the morning milking were stored in a plastic container and refrigerated for subsequent mixing with milk samples from the afternoon milking to form a composite sample/cow/day. Sterile plastic bottles with 100 and 200 mL capacities were used for the collections and then stored in a freezer at -20°C for subsequent analysis (no preservative was used).

**Blood samples:** From day 15 to 20 of each period (day 1, 3 and 5) was collected in the morning period, 4 hours after feeding the animals by jugular venepuncture. The blood was collected into 10-mL vacuolated tubes. Immediately after collection, the samples were cooled prior to the performance of a complete blood count (without clot activators). Plasma was separated from another portion of the collected blood (with clot activators) through centrifugation at 3000 rpm for 15 minutes within 45 minutes of collection. Plasma samples were freeze stored at -20°C for later analysis.

**Ruminal digestibility:** The feed, orts and faecal samples were stored, pre-dried in a forced air oven at 55°C for 72 hours and weighed for the digestibility assay. The samples were ground in a “Willey” mill with a 2-mm sieve. An in situ procedure using tissue non-tissue (TNT) bags (100 g/m<sup>2</sup>) was used to assess internal indicators in the feed and feces from a Holstein/Zebu ox with rumen cannulae. The TNT bags met the ratio of 20 to 25 mg of DM per cm<sup>2</sup> surface, including 0.9 g of feed and raw materials, 0.2 g of feces, 0.3 g of silage and 0.7 g of feed leftovers. The incubation time required to assess the indigestible dry matter (DMi) and indigestible neutral detergent fiber (NDFi) levels was 240 hours according to INCT-CA number F-009/1 (Detmann et al. 2012).

Once removed from the rumen, the bags were immediately immersed in ice water and washed several times until the water became clear; then the bags were pre-dried in the oven for approximately 48 hours at 60°C and in a non-ventilated oven (105°C) for 60 minutes, weighed and washed for the laboratory tests.

**Laboratory analysis:** The DM and nutrient intake estimates were performed by assessing the DM, organic matter (OM), CP, EE, MM, NDF, acid detergent fiber (ADF) and total carbohydrate (TCH) levels and the total digestible nutrient (TDN) intake according to Sniffen (1992). The NFC levels were experimentally calculated using the equation proposed by Hall et al. (1999) and digestibility test.

Pooled samples of each material ground through 1-mm sieves (roughage, feces, orts, and diets) were analyzed according to the standard analytical procedures of the Brazilian

National Institute of Science and Technology in Animal Science (INCT-CA; (Detmann et al. 2012) for DM (dried overnight at 105°C; method INCT-CA (Detmann et al. 2012) number G-003/1), ash (complete combustion in a muffle furnace at 600°C for 4 h; method INCT-CA number M-001/1). The crude protein (CP) and total nitrogen (N) levels (Kjeldahl procedure; method INCT-CA number N-001/1 (Detmann et al. 2012) and a correction factor of 6.25, ether extract was conducted in a reflux system (Soxtherm, Gerhardt, Germany) by Randall procedure; method INCT-CA number G-005/1 (Detmann et al. 2012).

The assessment of the neutral detergent fiber (NDF), neutral detergent insoluble nitrogen (NDIN) and neutral detergent insoluble protein (NDIP) levels was performed according to INCT - CA F-002/1, by Detmann et al. (2012).

$$\text{TCH} = 100 - (\% \text{CP} + \% \text{EE} + \% \text{Ash})$$

$$\text{NFC} = \% \text{TCH} - \% \text{NDF}_{\text{cp}}, \text{ with NDF corrected for ash and protein.}$$

$$\text{TDNI} = (\text{DCPI} + \text{DNFCI} + \text{NDFI} + (\text{DEEI} \times 2.25))$$

$$\% \text{TDN} = (\text{TDNI} / \text{DMI}) \times 100$$

Where TDNI is the total digestible nutrient intake, DCPI is the digestible CP intake, DNFCI is the digestible non-fibrous carbohydrate intake, NDFI is the NDF intake, DEEI is the digestible EE intake and DMI is the DM intake.

The urea, uric acid, creatinine, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), glucose, triglycerides, total cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins (LDL) analyses were performed using blood collected into vacuolated tubes (with clot activators). The blood analyses were performed in the Automatic Biochemistry Analyzer LABMAX 240 (2231.110.111).

The 4% fat-corrected milk production (FCMP) was calculated using the formula of Gaines 1928 proposed by the NRC (National Research Council – NRC, 2001) as follows:

$$\text{FCMP (4\%)} = [(\text{MP} \times 0.4) + (\% \text{Fmilk} \times 0.15)].$$

$$\text{MP} = \text{kg milk produced}$$

$$\% \text{Fmilk} = \% \text{milk fat}$$

The milk total nitrogen levels were assessed using the method of Kjeldahl 2000 with the correction factor adapted for milk of 6.38 (Randall procedure; method INCT (Detmann et al. 2012) -CA number G-005/1).

The method of Folch (1957) was used to assess the milk fat. The milk allantoin analysis was performed at the Milk Laboratory of the Department of Animal Science of UFRPE according to Chen and Gomes (1992).

The experimental diets were analysed in the Animal Nutrition Laboratory of the

CCA/DZ/UFCP to determine the DM, MM, EE and CP contents according to Detmann et al. (2012). The assessment of NDF was performed according to the methods by Detmann et al. (2012), and gross energy was assessed by sample oxidation in a bomb calorimeter. The non-fibrous carbohydrate (NFC) and total digestible nutrient (TDN) values were calculated according to the NRC (2001).

Statistical analyses: Statistical analyses were performed using the MIXED procedure of SAS 9.4 (SAS, 2010) according to a  $5 \times 5$  Latin square design including the fixed effect of treatment and the random effects of cows and experimental period. Statistical significance was considered at  $P \leq 0.05$ . The equation of the statistical model was  $y_{ijk} = m + l_i + c_j + tk(ij) + e_{ijk}$ , where  $y_{ijk}$  = value observed at the experimental unit which received the k treatment (at line i and column j); m = effect of the general mean;  $l_i$  = line i effect;  $c_j$  = column j effect;  $tk(ij)$  = k treatment effect applied at line i and column j;  $e_{ijk}$  = random error (residue). At the analysis, the coefficients were analysed by the minimum square method. It was applied the Normality test (Kolmogorov). The Delta was always the same (1).

### 3. Results and discussion

Food intake and digestibility: The voluntary intake of forage and concentrate did not differ ( $P \leq 0.05$ ) between treatments, in organic (OM) or dry (DM) matter (Table 3).

**Table 3-** Average intake and the intake as a function of the live weight in the experimental diets.

Variable	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P- value	
							L	Q
	Intake (kg/ dia <sup>-1</sup> )							
NM	40.6	40.3	41.2	40.9	40.0	1.64	0.80	0.37

DM	14.8	14.4	14.9	14.8	14.1	1.14	0.57	0.48
CP	2.1	2.1	2.1	2.0	2.1	0.10	0.79	0.21
EE*	0.4	0.3	0.4	0.4	0.4	0.01	0.01*	0.25
NDF	7.6	7.2	7.7	7.0	7.5	0.64	0.72	0.55
TCH	10.8	10.1	10.9	9.8	10.7	1.14	0.65	0.35
NFC	5.0	4.7	5.0	4.6	4.9	0.39	0.89	0.20
TDN	8.1	8.9	8.5	7.6	8.4	-	-	-
Intake (%BW)								
DM	3.4	3.3	3.5	3.5	3.3	0.30	0.63	0.45
NDF	1.8	1.7	1.8	1.6	1.7	0.17	0.80	0.48
CP	0.5	0.5	0.5	0.5	0.5	0.03	0.88	0.45

\*Significantly different ( $P < 0.05$ ). Natural matter (NM), dry matter (DM), crude protein (CP), \*ether extract (EE)  $y = -0.0075x + 0.4035$  ( $R^2 = 0.1541$ ), neutral detergent fiber (NDF), total carbohydrate (TCH), non-fibrous carbohydrate (NFC), total digestible nutrients (TDN), Standard error mean (SEM), probability (P), quadratic (Q), linear (L).

The DM, NDF and CP intakes as a function of live weight did not differ, possibly because the intake in absolute terms was not significantly affected, with initial body weight of  $420 \pm 30$  kg and final BW  $450 \pm 35$  kg. Thereby no decrease in intake occurred.

Sorghum is a widely produced cereal in the world, it's an excellent energy source for animal nutrition. It is found in its composition, phenolic compounds such as tannin, which is the most profuse compound in plants after cellulose, hemicellulose and lignin, it is soluble in water, acetone and alcohol. In plants, they may be found as condensed tannins or hydrolysable tannins. The simplest form of hydrolysable tannin, the tannic acid, can be degraded by the ruminal microorganisms.

Makkar et al. (1995) reported that the presence of tannins in sorghum grain was associated with a low intake of DM, which was not observed in this experiment. Tannins form complexes with salivary glycoproteins (Goel et al. 2005), thereby decreasing the voluntary intake (Chang, 1994; Chung et al. 1998; Monteiro et al. 2005), reducing feed efficiency (Chung et al. 1998) in ruminants and non-ruminants and decreasing protein, DM, carbohydrate and lipid digestibility (Chang 1994; Monteiro et al. 2005), metabolizable energy (Chung et al. 1998), weight gain and milk production (Mueller-Harvey, 2006).

The CP (crude protein), NDF (neutral detergent fiber), TCH (total carbohydrate), NFC (non-fibrous carbohydrate) and TDN (total digestible nutrient) intakes did not significantly differ ( $P \leq 0.05$ ) with the increase in the dietary tannin concentrations. To all nutrients tested, only the EE (ether extract) intake was linearly affected by the tannin concentrations ( $P < 0.05$ ) among the study diets according to the equation  $y = -0.0075x + 0.4035$  ( $R^2 = 0.1541$ ).

The DM, NDF and CP intakes as a function of live weight did not differ ( $P \leq 0.05$ ). The average body live weight, feed efficiency, milk protein percentage and 4% fat-corrected milk production did not differ ( $P \leq 0.05$ ) with the increase in dietary tannins, as outlined in Table 4.

**Table 4-** Mean body weight, feed efficiency, milk production, fat (%), protein (%) and 4% fat-corrected milk production of cows fed sorghum and increasing concentrations of tannic acid.

Variable	Diet	Diet	Diet	Diet	Diet	SEM	P- value	
	1	2	3	4	5		L	Q
Body live weight (kg)	431.2	433.6	437.8	449.2	456.0	10.11	0.96	0.70
Feed efficiency	1.1	1.1	1.1	1.0	1.1	0.09	0.41	0.85
Milk production (kg/day)*	15.7	16.0	15.8	15.4	14.7	0.62	0.01*	0.07
Total fat (%)*	3.3	3.2	3.8	3.7	3.7	0.44	0.04*	0.55
Crude protein (%)	3.5	3.5	3.3	3.6	3.3	0.35	0.66	0.73
Milk production 4%	14.0	14.0	15.3	14.7	14.2	1.34	0.61	0.23

\*Significantly different. \*Milk production,  $y = -0.249x + 16.271$  ( $R^2 = 0.6677$ ). \*Total fat,  $y = 0.138x + 3.114$  ( $R^2 = 0.5939$ ), Standard error mean (SEM), probability (P), quadratic (Q), linear (L).

Palatability is often based on astringency associated with CT-protein complexes formed from proteins in saliva; thus, the greater the protein bound by CT, the greater the astringency and the lower the palatability. However, not all CT bind protein equally (Naumann et al., 2017).

Grainger et al. (2009) observed an average DM (dry matter) intake ranging from 17.4 to 12.8 kg/day when testing dietary supplementation with purified condensed tannins in lactating cows, which accounted for an average tannin intake ranging from 163 g/day to 244 g/day. In this experiment, the tannin intake (condensed and tannic acid) ranged from 27 g/day to 321 g/day without affecting the intake significantly.

Even though the literature has reported some negative effects to animal feed with tannins, including decreased feed palatability, for the tested nutrients, only the EE (ether extract) intake was linearly affected by the tannin concentrations ( $P \leq 0.05$ ) among the studied diets according to the equation  $y = -0.0075x + 0.4035$  ( $R^2 = 0.1541$ ).

The negative tannin effect is dose dependent. The provided tannin type must be chemically determined to understand why mammals intoxicate when ingesting tannins. The hydrolysable tannin present in tannic acid, which was provided to the cows in this experiment, consists primarily of gallic acid (Getachew et al. 2008).

### Milk production

The original milk yield (kg/day) 15.71, 15.96, 15.80, 15.41 and 14.74 had a linear effect ( $P \leq 0.05$ ), with a reduction of 249g for each percentage unit of tannin in the diet, with regression equation,  $y = -0.249x + 16.271$ ,  $R^2 = 0.6677$ , decreasing on average 0.350 kg of milk as the tannin inclusion was increased. The milk production corrected for 4% fat (14.05, 14.00, 13.30, 14.68 and 14.19 kg/cow/day) and the dry matter intake (14.75, 14.40, 14.92, 14.80 and 14.08 kg/cow/day) did not differ ( $P \leq 0.05$ ) significantly between diets. The percentage of milk fat increased linearly ( $P \leq 0.05$ ) according to the following regression equation:  $y = 0.138x + 3.114$  ( $R^2 = 0.5939$ ).

### Ruminal digestibility

The apparent digestibility of DM, OM, CP, EE, NDF, TCH and NFC did not differ ( $P \leq 0.05$ ) regardless the dietary tannin, as outlined in Table 5. The maximum tannin dose provided (321 g) had no effect on the dietary nutrients digestibility.

**Table 5-** Apparent digestibility coefficients (%) of DM, OM, CP, EE, NDF, TCH and NFC in the experimental diets

Variable	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P- value	
							L	Q
DM	63.8	62.3	64.1	62.4	60.2	4.49	0.27	0.53
OM	64.2	63.2	64.0	62.5	61.3	4.54	0.33	0.74
CP	58.6	56.8	61.7	52.0	54.2	4.30	0.16	0.59
EE	67.2	64.2	71.7	66.4	64.1	6.14	0.65	0.28
NDF	54.0	52.6	57.7	54.1	54.6	6.11	0.63	0.28
TCH	66.0	63.4	64.3	60.2	63.7	6.25	0.36	0.58
NFC	91.3	89.8	87.1	85.2	90.7	16.20	0.68	0.39

Dry matter (DM), organic matter (OM) crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), total carbohydrate (TCH), non-fibrous carbohydrate (NFC), Standard error mean (SEM), probability (P), quadratic (Q), linear (L).

### Plasma metabolites

All blood tests were performed to ensure cow health and to monitor possible metabolic changes in organs, including kidneys and liver, and changes in cholesterol metabolism and the immune system. The GOT (glutamate oxalacetate transaminases or aspartate transaminase

(AST)) levels showed significant differences ( $P \leq 0.05$ ) among the blood parameters tested (Table 6).

**Table 6-** Blood parameters and reference levels of cows fed sorghum and different levels of tannic acid.

Variable	Reference Value	Diet					SEM	P- value	
		1	2	3	4	5		L	Q
GPT U/L	0 – 38	30.6	29.7	32.9	39.6	31.6	5.36	0.14	0.25
GOT U/L*	0 – 132	72.3	77.3	72.6	84.6	76.5	3.67	0.01*	0.14
Urea mg/dL	23 – 58	56.1	50.9	54.1	51.3	52.9	10.98	0.69	0.68
Creatinine mg/dL	1.0 – 2.0	1.1	1.1	1.2	1.2	1.1	0.09	0.99	0.55
Glucose mg/dL	45 – 75	49.7	47.2	44.6	44.4	49.9	5.39	0.76	0.06
Triglycerides mg/dL	0 – 14	9.6	10.6	7.4	10.1	25.3	16.56	0.21	0.23
Uric Acid	-- --	1.0	0.9	0.9	0.8	0.9	0.18	0.31	0.39
Total cholesterol mg/dL	80 – 120	151.28	136.3	131.7	160.6	121.7	21.11	0.26	0.69
HDL	-- --	50.3	46.5	48.1	52.5	41.2	8.04	0.30	0.38
LDL	-- --	13.9	13.4	14.0	14.2	14.6	2.66	0.58	0.77
Erythrocytes $10^6$ U/L	5 – 10	5.2	4.9	5.0	5.1	5.2	0.40	0.57	0.31
Hemoglobin mg/dL*	8 – 15	10.4	9.5	7.9	9.8	10.3	1.70	0.97	0.05
Hematocrit %	24 – 46	22.1	20.2	17.1	21.5	21.6	3.69	0.95	0.08
Leukocytes U/L	4 – 12	11.3	8.2	10.2	10.6	11.1	1.70	0.39	0.07
Platelets $10^5$ U/L	1 – 8	48.6	61.9	42.4	34.2	52.6	12.83	0.49	0.50

Benchmarks for adult cattle according to Kaneko *et al.* (1997). \*Significantly different based on analysis of variance and regression at a 5% significance level. Glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT),  $*y = 1.5654 + 71.957 (R^2 = 0.2464)$ , hemoglobin  $*y = 0.4395x^2 - 2.6458x + 12.705 (R^2 = 0.6844)$ , high-density lipoproteins (HDL), low-density lipoproteins (LDL), Standard error mean (SEM), probability (P), quadratic (Q), linear (L).

The GOT levels increased linearly ( $P \leq 0.05$ ), with the highest media at 84.59 U/L. The blood glucose, triglyceride and cholesterol (total, LDL and HDL) levels did not significantly differ ( $P \leq 0.05$ ). Hemoglobin showed a significant difference ( $P \leq 0.05$ ) with quadratic behavior in which the maximum point was 10.44 mg/dL and the minimum point was 7.94 mg/dL.

No significant difference ( $P \leq 0.05$ ) in urea, creatinine and uric acid occurred. Table 6 outlines the means of the digestibility coefficients according to the diets. Tannins may also reduce the apparent digestibility of feed nitrogen (Grainger *et al.* 2009; Waghorn, 2008). However, no significant differences were found in this study.

The blood plasma biochemical composition reflects the metabolic state of animal tissues, which allows the tissue damage evaluation, organ function disorders, animal

adaptation to nutritional and physiological challenges and specific or nutritional metabolic imbalances (González & Scheffer 2002). These analyses enable the physiological changes assessment caused by dietary tannins in lactating cows. During the experiment time we didn't observe any change in blood parameters, which may indicate that no clinical damage was caused by tannins.

Total cholesterol remained high in all diets and LDL was low compared with HDL, which most likely indicated a weak liver fat effect. So, the milk fat may be primarily derived from acetates produced in the rumen.

The main hemoglobin function is oxygen transportation from the lungs to the body. Hemoglobin is present in red blood cells, and it helps to some extent with nutrient transport to cells and collects toxic substances, including carbon dioxide, for subsequent release from the organism. Hemoglobin analysis is used to identify diseases such as anemia. According to Kaneko et al. (1997), the normal hemoglobin levels of cows range from 9 to 15 mg/dL; thus, the differences in hemoglobin levels found between treatments were within the normal range for cows.

In this experiment, at the highest dietary dose of tannic acid (235.5 g/day), 560 mg of tannic acid per kg/LW was provided to the cow with the lowest weight ( $\pm$  420 kg). GOT and GPT (glutamate pyruvate transaminases or alanine transaminase (ALT)) are enzymes present in the liver and other organs, which, at high levels, indicate liver diseases. The GOT levels increased linearly ( $P \leq 0.05$ ). Despite this performance, the GOT levels at the highest dose of tannic acid were 84.59 U/L, which was still within the normal blood GOT levels for cows according to Kaneko et al. (1997), which range up to 132 U/L.

#### **4. Conclusion**

Diets using two tannins (hydrolysable and condensed) sources supplementation didn't decrease the dietary intake or affect the animals digestibility.

The GOT level changed significantly, showing linear behaviour, however below the toxicity level, with no change in the other blood parameters.

Milk production decreased with the increase in dietary tannin supplementation.

We recommend more studies using two tannin sources.

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