Ciência Rural, Santa Maria, v.54:09, e20230367, 2024
http://doi.org/10.1590/0103-8478cr20230367
ISSNe 1678-4596
ANIMAL PRODUCTION

Effect of using tannin extract from *Acacia mearnsii* on intravaginal temperature, production and milk composition of Holstein cows in a subtropical environment

Laura Valadão Vieira[1*] Kauani Borges Cardoso[1] Jordani Borges Cardoso[1] Jéssica Halfen[1] Antônio Amaral Barbosa[1] Edgard Gonçalves Malaguez[1] Cássio Cassal Brauner[1] Marcio Nunes Corrêa[1] Eduardo Schmitt[1] Francisco Augusto Burkert Del Pino[1]

[1]Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC), Universidade Federal de Pelotas (UFPel), 96160-000, Capão do Leão, RS, Brasil. E-mail: lauravaladaovieira@gmail.com. [*]Corresponding author.

ABSTRACT: This study evaluated the effect of tannin extract from *Acacia mearnsii* on intravaginal temperature, production and milk composition of Holstein cows in a subtropical environment. The study period was 29 days and 20 multiparous Holstein cows were selected with an average milk production of 50 ± 6 kg/day and 147 ± 83 days in milk. The cows were separated into two treatments: Control (n = 10) did not receive dietary supplementation. Tannin (n = 10) received Condensed Tannin (Tanac S.A., Montenegro, RS, Brazil) at a dose of 0.19% dry matter (40 g/cow/day). Animals were monitored for milk production and composition as well as intravaginal temperature. The environmental temperature and humidity index (THI) were monitored. The mean THI was 72.39 ± 0.69. The mean intravaginal temperature was 39.17 ± 0.14 °C in the Tannin treatment and 39.03 ± 0.14°C in the Control treatment (P > 0.05). The animals in the Tannin treatment exhibited an intravaginal temperature of 39.2 °C at THI 69.38, and the Control treatment exhibited the same temperature at THI 70.16 (receiver operating characteristic (ROC); P < 0.01). Milk production was higher in the Tannin treatment than in the Control treatment (51.38 ± 0.90 kg/day and 49.94 ± 1.05 kg/day, respectively; P < 0.01). The protein content in milk was higher in the Tannin treatment than in the Control treatment (3.20 ± 0.01% and 3.04 ± 0.01%; respectively; P < 0.01). The results of this study suggested that tannin extract from *Acacia mearnsii* can attenuate heat stress and its negative effects on animal production.
Key words: additive, nutrition, milk production.

Efeito do uso de extrato de tanino de *Acacia mearnsii* na temperatura intravaginal, produção e composição do leite de vacas Holandês em ambiente subtropical

RESUMO: Este estudo avaliou o efeito do extrato de tanino de *Acacia mearnsii* na temperatura intravaginal, na produção e na composição do leite de vacas Holandesas mantidas em ambiente subtropical. O período de estudo foi de 29 dias e foram selecionadas 20 vacas multíparas Holandesas com produção média de leite de 50 ± 6 kg/dia e 147 ± 83 dias em leite. As vacas foram separadas em dois tratamentos: Controle (n = 10) não recebeu suplementação alimentar. Tanino (n = 10) recebeu Tanino Condensado (Tanac S.A., Montenegro, RS, Brasil) na dose de 0,19% de matéria seca (40 g/vaca/dia). Os animais foram monitorados quanto à produção e composição do leite, bem como à temperatura intravaginal. O índice de temperatura e umidade ambiental (ITU) foi monitorado. O ITU médio foi de 72,39 ± 0,69. A temperatura intravaginal média foi de 39,17 ± 0,14 °C no tratamento Tanino e 39,03 ± 0,14 °C no tratamento Controle (P > 0,05). Os animais do tratamento Tanino exibiram temperatura intravaginal de 39,2°C no ITU 69,38, e o tratamento Controle exibiu a mesma temperatura no ITU 70,16 (característica operacional do receptor (ROC); P < 0,01). A produção de leite foi maior no tratamento Tanino do que no tratamento Controle (51,38 ± 0,90kg/dia e 49,94 ± 1,05kg/dia, respectivamente; P < 0,01). O teor de proteína no leite foi maior no tratamento Tanino do que no tratamento Controle (3,20 ± 0,01% e 3,04 ± 0,01%; respectivamente; P < 0,01). Os resultados deste estudo sugerem que o extrato de tanino de *Acacia mearnsii* pode atenuar o estresse térmico e seus efeitos negativos na produtividade animal.
Palavras-chave: aditivo, nutrição, produção de leite.

INTRODUCTION

Heat stress affects dairy cows worldwide, particularly in regions with tropical and subtropical climates (ZARINA et al., 2021). The incidence will likely increase over time considering the growing production indices of dairy herds and the rising environmental temperature, which can increase by 1.5 °C by 2030 (BECKER et al., 2020). Therefore, heat stress constitutes a significant challenge for farmers as it hinders the animals' productivity, fertility, conception rate, immunity, and health (AKBAR et al., 2021).

Heat stress can occur when the ambient temperature exceeds 25 °C or when the Temperature Humidity Index (THI) exceeds 68 (LIU et al., 2019). In this case, the body temperature of dairy cows

Received 07.07.23 Approved 01.14.24 Returned by the author 04.12.24
CR-2023-0367.R2
Editors: Rudi Weiblen Henrique Mendonça Nunes Ribeiro Filho

reaches 39.2 °C or more (LIANG et al., 2013). This causes behavioral changes, including decreased dry matter intake (DMI), as well as reduced degradation of fat reserves occurs to minimize the production of metabolic heat (BAUMGARD & RHOADS, 2013).

Heat stress positively regulates the expression of leptin, acting on the hypothalamus, which induces a reduction in dry matter intake to decrease heat production (SLIMEN et al., 2015). The decline in consumption may be the cause of the increase in muscle proteolysis; however, it cannot be excluded that muscle catabolism might occur independently of reduced consumption, serving as an adaptive mechanism to heat (RHOADS et al., 2013; SLIMEN et al., 2015). Increasing muscle protein degradation at the expense of lipolysis serves as a means to minimize endogenous heat production (SLIMEN et al., 2015). The amino acids resulting from this process are useful for glucose production through gluconeogenesis and can act as an energy source for metabolic pathways involved in maintaining thermoregulation (WHEELOCK et al., 2010).

Moreover, providing a source of protein seems appropriate to aid in maintaining thermoregulation during stressful periods. Several dietary additives are being investigated to alleviate heat stress. While not studied for this purpose to date, we believed that tannins, owing to their mode of action, could be considered an alternative ingredient to mitigate the adverse effects of heat stress.

Tannins are polyphenolic compounds commonly found in angiosperms and dicots, including species such as *Acacia* and *Eucalyptus*. Particularly, tannin extracted from the bark of Black Acacia, *Acacia mearnsii*, comprises a colloidal structure containing a high number of non-toxic phenolic dihydric nuclei. Its extraction process involves aqueous leaching of the bark. Chemically, acacia tannin represents a blend of complex substances, primarily condensed polyphenols, particularly flavan-3-4-diol, along with other compounds like sugars and hydrolyzable gums. These compounds are categorized as hydrolyzable or condensed based on their chemical makeup (MCMAHON et al., 2000; MIN & SOLAIMAN, 2018). Condensed tannins, specifically, are widely used as dietary supplements for ruminants due to their ability to bind proteins and enhance their bioavailability (PATRA & SAXENA, 2011).

Historically, tannins have been widely used in the pharmaceutical and leather industry. Recently, these compounds have gained visibility in animal nutrition, initially as anti-helminthics and to reduce methanogenesis (VIEIRA et al., 2020). Also, condensed tannin from *Acacia mearnsii* was efficient in improving the absorption of amino acids in steers (ORLANDI et al., 2015). The use of condensed tannins to alleviate heat stress in cattle is poorly understood and the outcomes derived from incorporating condensed tannins into the ruminant diet exhibit variability, largely contingent upon the dosage and extraction source of the additive (FONSECA et al., 2023).

Based on the information above we hypothesized that dietary supplementation with tannin extract from *Acacia mearnsii* could potentially increase amino acid bioavailability for gluconeogenesis, and therefore energy availability to aid in thermoregulation of high-produced dairy cows in a subtropical climate. Therefore, this study evaluated the effect of tannin extract from *Acacia mearnsii* on the intravaginal temperature of high-producing Holstein cows as well as milk production and composition.

MATERIALS AND METHODS

Animals and housing

Twenty multiparous Holstein cows with an average milk production of 50 ± 6 Kg/day and 147 ± 83 days in lactation (DIM) were selected for a 29-day study (14 days of adaptation and 15 days of trial period). The animals were housed in a freestall system and milked three times per day at 4:30 AM, 12:30 PM, and 6:30 PM at a farm in southern Brazil (27° 42' 39" S 51° 45' 39).

This study was conducted during summer, and summers in the region are characterized by high temperatures. According to the Köppen-Geiger classification system, the region has a subtropical humid climate (MORENO, 1961). During the study, a meteorological station installed at the dairy farm recorded an average THI of 72.39 ± 0.69. According to LIU et al. (2019), THI above 68 is considered critical for dairy cows.

The animals were clinically evaluated weekly throughout the experimental period. All cows had access to food and water *ad libitum*.

Experimental design

The 20 cows selected for this study were divided into two homogeneous treatments, totaling 10 animals/treatment. These animals were selected using DIM and productivity data. Throughout the study period, the animals were fed a consistent basal diet. One group received an additional tannin extract sourced from *Acacia mearnsii* (Weibull AQ, Tanac, S.A., Montenegro, RS, Brazil), constituting the Tannin treatment, while the control group received the basal diet alone. The Weibull AQ product used in this study contained 72% condensed tannin based on dietary dry matter. All product specifications were provided by Tanac S.A., Montenegro, RS, Brazil.

The diet was devised by the farm's nutritionist following the guidelines stipulated in the NRC (2001), adhering to the nutritional criteria for a cow averaging 50 kg/day of milk production across two lactations. The cows in the Control treatment had an average DIM of 146.5 ± 22.52 and an average milk production of 49.77 ± 2.20 kg, while the cows in the Tannin treatment had an average DIM of 147 ± 31.42 and an average milk production of 49.68 ± 1.70 kg. The p value for DIM P = 0.98 and P value for production P = 0.99.

The diet, consisting of 64% roughage and 36% concentrate, was formulated as a total mixed ration (TMR). Roughage sources included corn silage, oats silage, and cottonseed, while a commercial feed served as the concentrate. The TMR was administered daily following the morning milking routine. The tannin extract from *Acacia mearnsii* was supplemented by mixing it with the TMR, being applied both directly as a top dressing and thoroughly mixed in the feeder. The dosage of tannin extract was calculated based on an estimated daily Dry Matter Intake (DMI) of 21 kg per cow. Gradually, over a 14-day adaptation period, the tannin extract dosage was increased to 0.19% (40 g/cow/day) of the dietary Dry Matter (DM). To ensure the complete intake of the tannin extract, the animals were gradually restrained using headlocks in their feedbunks and released only after complete consumption. The tannin extract dose was calculated in agreement with the study of ORLANDI et al. (2015).

Dietary samples

Samples of the diet were collected weekly for chemical analysis to subsequently determine the total crude protein (CP) and ether extract content. The Soxhlet extractor was used following the Weende method (AOAC, 2000) for ether extract extraction. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted using the VAN SOEST et al. (1991) method (AOAC, 2000). Additionally, the organic matter (OM) content of the TMR was calculated as OM = DM – ashes (AOAC, 2000).

The chemical composition of the feeds was as follows:

Corn silage: 37% dry matter, 3.55% dry matter ash, 8% dry matter crude protein, 2.60% dry matter ether extract, 55% dry matter neutral detergent fiber, and 30% dry matter acid detergent fiber.

Oats silage: 40% dry matter, 9.81% dry matter ash, 10% dry matter crude protein, 3.35% dry matter ether extract, 66% dry matter neutral detergent fiber, and 46% dry matter acid detergent fiber.

Cottonseed: 90.10% dry matter, 4.20% dry matter ash, 23.50% dry matter crude protein, 22% dry matter ether extract, 50.30% dry matter neutral detergent fiber, and 40.10% dry matter acid detergent fiber.

Concentrate: 89.31% dry matter, 9.96% dry matter ash, 32.89% dry matter crude protein, 8.50% dry matter ether extract, 20% dry matter neutral detergent fiber, and 15% dry matter acid detergent fiber. The concentrate included ground corn grain (21.53% of dry matter), soy oil (1.15%), protected fat (1.10%), dried distillers grains (24.26%), soybean meal (46.01%), sodium bicarbonate (2.25%), dicalcium phosphate (2.30%), magnesium oxide (0.24%), vitamin premix (0.24%), sodium chloride (0.69%), and sulfur (0.24%).

TMR: 50.70% dry matter, 6.80% dry matter ash, 17.67% dry matter crude protein, 6.30% dry matter ether extract, and 32.73% dry matter acid detergent fiber.

THI and vaginal temperature measurements

The instrument to measure temperature and humidity (Instrutemp®, São Paulo, Brazil) was installed inside the freestall next to the animal bedding. Temperature and humidity data were recorded every 10 minutes during the entire experimental period. The software EasyWeather® was used to retrieve the temperature and humidity data. The THI was calculated using the following formula: THI = ((1.8 × external temperature + 32) – ((0.55 – 0.0055 × external humidity) × (1.8 × external temperature – 26))) (NRC, 2001).

The temperature data logger (Thermochron ibutton, KY, USA) was attached to an intravaginal progesterone implant to register the cows' internal body temperature. The implants contained low progesterone concentrations, similar to the methodology used by MOHAMED et al. (2021). All implants were autoclaved before usage.

Each temperature data logger was implanted on day 16, removed after 4 days, and then reimplanted on day 25 and removed on day 29 of the experimental period. The data logger registered the internal temperature of the cows every 30 minutes. Thermodata Viewer (Thermodata Pty Ltd®, BigCommerce, Australia) was used to retrieve the internal temperature data.

During each evaluation period, the internal temperature was measured for four consecutive days, which is similar to SCANAVEZ et al. (2017). The cows were considered under heat stress when the internal temperature reached 39.2 °C or higher (LELES et al., 2017).

Milk production and quality

Individual milk production was monitored daily during each milking using the records from the milking machine (Gea®, Renânia do Norte-Vestfália, Germany). Milk samples were obtained during the morning, afternoon, and evening milking on days 14, 21, and 29 in sterile 50 mL flasks containing Bronopol (2-bromo-2 nitropropano-1-3 diol). Samples were analyzed for protein content, lactose, fat, and milk urea nitrogen using infrared spectrometry (ISO 9622/ IDF 141).

Statistical analysis

The animals were considered experimental units and data normality was verified using the Shapiro-Wilk test.

Non-parametric data, such as internal body temperature, were evaluated using the Wilcoxon-Mann-Whitney test. The THI data as well as milk production and composition were analyzed using repeated measurements in the SAS software (SAS Institute Inc., Cary, EUA, 2016). The animal was considered a random effect, whereas the treatment, timepoint, and interaction between them, were considered fixed effects.

Considering the statistical model:

$$Yijk = \mu + Ti + Aj + Pk + TPik + \varepsilon ijk, Yijk = \mu + Ti + Aj + Pk + TPik + \varepsilon ijk \quad \text{(Equation 1)}$$

In which Yijk represents the continuous variable; µ represents the general mean; Ti is the fixed effect of treatment (i = Control X Tannin); Aj is the random effect of the animal (j = 1–20); P is the fixed effect of time (k = 1– 29); Tpik is the fixed effect of the interaction between treatment "i" and time "k." Additionally, εijk is the random error associated with each observation.

The receiver operating characteristic (ROC) (THOMPSON & ZUCCHINI, 1989) analysis was used to evaluate the THI required to elevate the intravaginal temperature above physiological levels. For all variables, the differences were considered statistically significant when P < 0.05.

RESULTS

The THI was > 68 for 23/29 experimental days. The mean THI during the entire study period was 72.39 ± 0.69 (78.64–64.82; Figure 1). Intravaginal temperature was not different between Tannin and Control (P > 0.05). The mean intravaginal temperature was 39.17 ± 0.14 °C and 39.03 ± 0.14 °C in Tannin and Control, respectively (Figure 1).

The ROC analysis was used to determine at which THI point cows in different treatments manifested heat stress. It was established in the test that animals with an intravaginal temperature greater than 39.2 °C were in heat stress. The THI point that maximized sensitivity and specificity in the Control treatment was > 69.38 (Sensitivity = 72.85; this value represents that approximately 73% of the cows were truly in heat stress when the THI exceeded 69.38). In the Tanin treatment, the THI point that maximized sensitivity and specificity was > 70.16 (Sensitivity = 69.21; this value represents that approximately 69% of the animals were truly in heat stress when THI exceeded 70.16) (Figure 2). The values of P < 0.0001 in curves A and B indicate that the ROC curve was able to significantly diagnose true positive cases, that is, animals that were truly under heat stress (Figure 2).

Milk production was higher for animals in the Tannin than for those in the Control. The concentration of fat (P = 0.79), lactose (P = 0.28), total solid matter (P = 0.99), and MUN (P = 0.23) concentration in milk was similar among treatments. Protein concentrations in milk were higher for cows in the Tannin than for those in the Control (P < 0.01) (Table 1).

DISCUSSION

As mentioned previously, to our knowledge, condensed tannins or the tannin extract from *Acacia mearnsii* have not yet been deeply studied as potential heat stress mitigators. Despite this, evidence indicates that condensates of tannins from *Acacia mearnsii* contribute to forming a tannin-protein complex resistant to ruminal degradation (ORLANDI et al., 2015). We consider this effect crucial in reducing the incidence and mitigating the adverse effects of heat stress on animal performance. This mechanism limits protein digestibility in the rumen while enhancing amino acid absorption in the small intestine (COSTA et al., 2021).

Despite reductions in dry matter consumption or alterations in energy balance, cows experiencing heat stress exhibit increased muscle catabolism (RHOADS et al., 2013; SLIMEN et al., 2015). These mechanisms serve adaptively to diminish or prevent elevations in body temperature (BAUMGARD & RHOADS, 2013). The breakdown of muscle proteins likely occurs to meet elevated

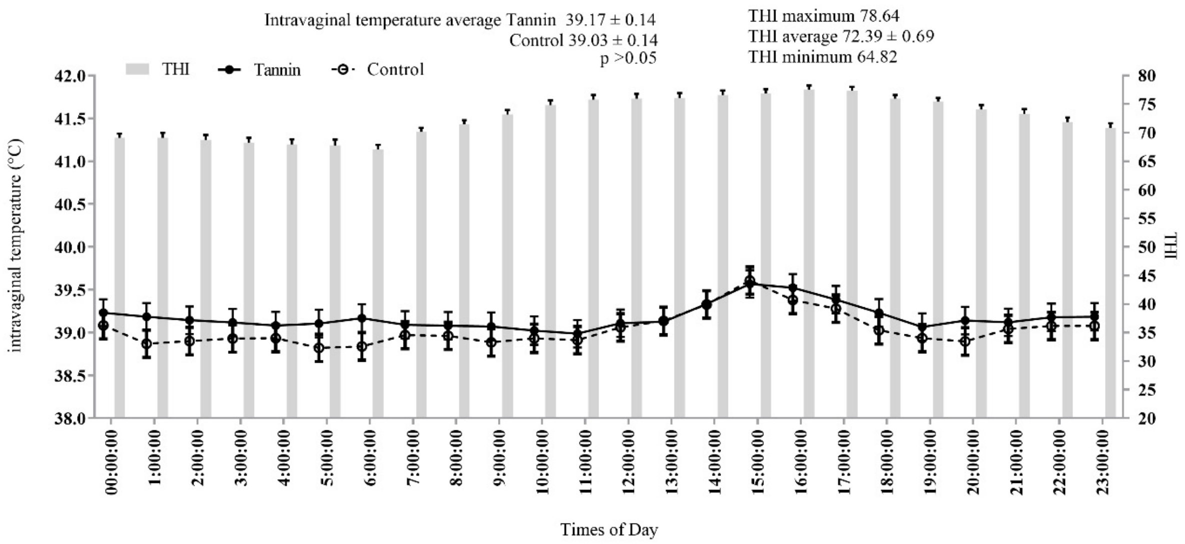

Figure 1 - Intravaginal temperature and THI. Data are presented as mean ± standard error. Tannin treatment (supplemented with condensed tannins at 0.19% of the dry matter in the diet); control treatment; THI, temperature, and humidity index. The bars represent the mean THI/hour during the study.

energy demands and ensure effective thermoregulation (RÍUS et al., 2019). Consequently, additional protein supplementation becomes imperative for animals in warmer climates, such as those in this study, wherein the Temperature-Humidity Index (THI) consistently surpassed the critical threshold (68) for most of the study period (LIU et al., 2019). In this study, all cows exhibited body temperatures above the physiological limit of 39.2 °C (LIANG et al., 2013). Previous research has demonstrated that THI > 68 increases the body temperature of dairy cows (LEIVA et al., 2017; CHANG-FUNG-MARTEL et al., 2021). In this study, cows in the Control exhibited body temperature ≥ 39.2 °C at THI 69.38, whereas those in the Tannin only exhibited this temperature at THI 70.16. These data indicate that animals supplemented with condensed tannins had improved thermotolerance compared to those in the Control. Nonetheless, the mean intravaginal temperatures were observed in the characterized heat stress (REJEB et al., 2016).

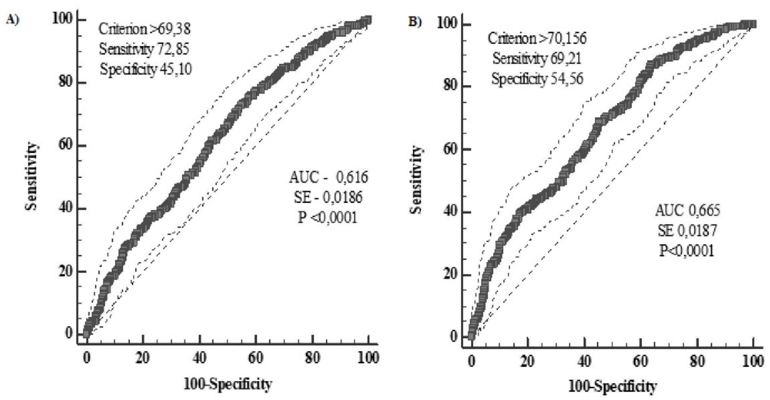

Figure 2 - Receiver operating characteristic (ROC) analysis. A) Animals in the Control treatment (Criterion > 69.38; AUC = 0.616; SE = 0.0186; P < 0.0001; Sensibility = 72.85; Specificity = 45.10) e B) animals in the Tannin treatment (animals supplemented with condensed tannin at 0.19% the dietary dry matter) (Criterion > 70.16; AUC= 0.665; SE = 0.0187; P < 0.0001; Sensibility = 69.21; Specificity = 54.56).

Table 1- Mean ± standard error (SD) of production and composition of milk from cows in both treatments.

Item	---------------------------------Treatment-------------------------------				P-value
	Tannin[1]	SD	Control[2]	SD	Treatment
Milk production Kg/day	51.83	0.90	49.94	1.05	<0.01
Fat (%)	3.81	0.30	3.70	0.44	0.79
Lactose (%)	4.75	0.00	4.69	0.00	0.28
Crude protein (%)	3.20	0.01	3.04	0.01	<0.01
Total solids (%)	12.46	0.26	12.51	0.33	0.82
MUN[3] (mg/dl)	20.46	1.12	18.01	1.32	0.23

[1]Tannin treatment (supplemented with condensed tannins at 0.19% the dry matter), [2]Control treatment, [3]milk urea nitrogen

The results in this study are consistent with those of another study from our group (CARDOSO et al., 2023). In that study, the maximum THI for heat stress was 63.66 for control animals and 64.06 for animals supplemented with 150 g of condensed tannins/cow/day (CARDOSO et al., 2023). Therefore, our research demonstrated that condensed tannins are efficient at attenuating heat stress even when provided at a small dose, as observed in this study. It is a fact that heat stress represents an increase in energy demand for thermoregulation (JI et al., 2020), and it is known that to meet this demand, the animal mobilizes muscle proteins, which generates less metabolic heat than lipomobilization (RÍUS et al., 2019). Therefore, one of the strategies to mitigate heat stress is to provide proteins with lower ruminal degradability (DAS et al., 2016; CONTE et al., 2018). Therefore, our study suggested that tannin extract from *Acacia mearnsii* can support thermoregulation, which we believe is due to the formation of the tannin-protein complex resistant to ruminal digestion.

When provided in high doses, tannins can have negative effects such as decreasing the palatability of the diet and the digestibility of the fiber, which can compromise the productivity of the animals (VIEIRA et al., 2020). In our previous research, animals supplemented with 150 g of condensed tannins/cow/day did not exhibit a reduction in productivity (CARDOSO et al., 2023), which differed from the results in the current study. The effects of condensed tannins on milk production are variable and likely depend on the source from which the tannins were extracted as well as the dose. Other studies demonstrated that tannin utilization does not affect or reduce milk production (CARDOSO et al., 2023; OLIVEIRA et al., 2023; ORLANDI et al., 2020). The study by GRAZZIOTIN et al. (2020) demonstrated an increase in milk production. Nonetheless, in that study, the authors evaluated a mixture of condensed and hydrolyzable tannins. In our study, the effects on milk production were likely due to the dose (40 g/cow/day) and the attenuation of heat stress which reduces milk production (TAO et al., 2020).

The animals in the Tannin exhibited a higher protein concentration in milk than those in the Control. This result highlights the notion that condensed tannins increase amino acid absorption in the intestine. Nonetheless, this did not compromise the protein balance in the diet considering that MUN was similar between treatments.

Other studies showed that animals experiencing heat stress exhibit a reduction in the protein concentration of milk. Additionally, the proteins in the protein-tannin complex may have low ruminal absorption (GRAZZIOTIN et al., 2020). Nevertheless, this did not occur in this study since heat stress was attenuated and the concertation of protein in milk increased only in the Tannin animals.

Condensed tannins possess the ability to create complexes with proteins that contain essential amino acids like leucine, valine, and isoleucine. This interaction likely enhances amino acid absorption in the small intestine (MIN et al., 2001). Such an effect can potentially elevate protein content in milk and mitigate the detrimental impact of heat stress, including muscle degradation. Although, this study did not quantify these specific amino acids, exploring this mechanism of action for condensed tannins should be a focus in future research endeavors. The outcomes of this study serve as a foundation to support future investigations into the mechanisms and efficacy of tannin extract from *Acacia mearnsii* in alleviating heat stress. With the projected increase in global temperatures and the imperative to enhance dairy herd productivity to meet escalating food demands worldwide, research of this nature becomes increasingly vital (RAUW et al., 2020).

CONCLUSION

In conclusion, supplementing Holstein dairy cows in a subtropical environment with tannin extract from *Acacia mearnsii* at a rate of 40g/cow/day could serve as an effective strategy to alleviate heat stress. This supplementation potentially resulted in a reduction of intravaginal temperature, particularly at higher Temperature-Humidity Index (THI) levels compared to the control group. Additionally, cows treated with tannins exhibited increased milk production and milk protein levels.

ACKNOWLEDGEMENTS

The authors would like to thank the Universidade Federal de Pelotas (UFPel), the Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for funding this project, as well as for granting research scholarships.

DECLARATION OF CONFLICT OF INTEREST

The authors have not stated any conflicts of interest.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All procedures were supervised and approved by the Ethics Committee for Animal Experimentation of the Universidade Federal de Pelotas (UFPel) registered under number 23110.013916 / 2020-30.

REFERENCES

AKBAR, M. S. et al. Heat stress and its management in dairy cattle: Current scenario in South Asia. **Pakistan Journal of Agricultural Research**, v.34, n.2, p.407-413, may. 2021. Available from: <https://www.cabidigitallibrary.org/doi/pdf/10.5555/20210473972>. Accessed: Mar. 13, 2023. doi: 10.17582/journal.pjar/2021/34.2.407.413.

AOAC - Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, **Gaithersburg, MD, USA**. 2020. Methods 925.10, 65.17, 974.24, 992.16. Available from: <https://www.aoac.org/>. Accessed: Mar. 13, 2023.

BAUMGARD, L. H.; RHOADS JUNIOR, P. R. Effects of heat stress on postabsorptive metabolism and energetics. **Annual Review of Animal Biosciences**, v.1, p.311-337, 2013. Available from: <https://www.annualreviews.org/doi/abs/10.1146/annurev-animal-031412-103644>. Accessed: Mar. 13, 2023. doi: 10.1146/annurev-animal-031412-103644

BECKER, C. A. et al. Invited review: Physiological and behavioral effects of heat stress in dairy cows. **Journal of dairy science**, v.103, n.8, p.6751-6770, aug. 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030220303829>. Accessed: Mar. 18, 2023. doi: 10.3168/jds.2019-17929

CARDOSO, K. et al. Internal temperature and THI tolerance level of cows supplemented with *Acacia mearnsii* tannins, Ottawa, Canadá. In: **American Dairy Science Association-ADSA**, Ottawa, Canadá, 2023. Available from: <https://www.adsa.org/Portals/0/SiteContent/Docs/Meetings/PastMeetings/2023_ADSA_Program.pdf. (Posters w1767W)>. Accessed: Dec. 02, 2023.

COSTA, E. I. de S. et al. Intake, nutrient digestibility, nitrogen balance, serum metabolites and growth performance of lambs supplemented with *Acacia mearnsii* condensed tannin extract. **Animal Feed Science and Technology**, v.272, p.14744, feb. 2021. Available from: <https://www.sciencedirect.com/science/article/pii/S0377840120306489>. Accessed: Oct. 12, 2023. doi: 10.1016/j.anifeedsci.2020.114744.

CHANG-FUNG-MARTEL, J. et al. Negative relationship between dry matter intake and the temperature-humidity index with increasing heat stress in cattle: a global meta-analysis. **International Journal of Biometeorology**, v.65, n.12, p.2099-2109, july. 2021. Available from: <https://link.springer.com/article/10.1007/s00484-021-02167-0>. Accessed: Mar. 18, 2023. doi: 10.1007/s00484-021-02167-0.

CONTE, G. et al. Feeding and nutrition management of heat-stressed dairy ruminants. **Italian Journal of Animal Science**, v.17, n.3, p.604-620, jan. 2018. Available from: <https://www.tandfonline.com/doi/full/10.1080/1828051X.2017.1404944>. Accessed: Mar. 18, 2023. doi: 10.1080/1828051X.2017.1404944.

DAS, R. et al. Impact of heat stress on health and performance of dairy animals: A review. **Veterinary World**, v.9, n.3, p.260-268, mar. 2016. Available from: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4823286/>. Accessed: Abr. 10, 2023. doi: 10.14202/vetworld.2016.260-268

FONSECA, N. V. B. et al. Additive tannins in ruminant nutrition: an alternative to achieve sustainability in animal production. **Sustainability**, v.15, n.5, p.1-11, feb. 2023. Available from: <https://www.mdpi.com/2071-1050/15/5/4162>. Accessed: Apr. 20, 2023. doi: 10.3390/su15054162.

GRAZZIOTIN, R. C. B. et al. Altered rumen fermentation patterns in lactating dairy cows supplemented with phytochemicals improve milk production and efficiency. **Journal of Dairy Science**, v.103, n.1 p.301-312, jan. 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030219309956>. Accessed: Apr. 10, 2023. doi: 10.3168/jds.2019-16996.

ISO 9622:2013 (IDF 141): Milk and liquid milk products. **Guidelines for the application of mid-infrared spectrometry**. Available from: <https://www.iso.org/obp/ui/en/#iso:std:56874:en>. Accessed: Apr. 10, 2023.

JI, B. et al. A review of measuring, assessing and mitigating heat stress in dairy cattle. **Biosystems Engineering**, v.199, p.4-26, nov. 2020. Available from: <https://www.sciencedirect.com/science/

article/pii/S1537511020302026>. Accessed: Oct. 12, 2023. doi: 10.1016/j.biosystemseng.2020.07.009.

LEIVA, T. et al. Supplementing an immunomodulatory feed ingredient to modulate thermoregulation, physiologic, and production responses in lactating dairy cows under heat stress conditions. **Journal of Dairy Science**, v.100, n.6, p.4829-4838, jun. 2017. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030217303296>. Accessed: Apr. 10, 2023. doi: 10.3168/jds.2016-12258.

LELES, J. S. et al. Heat Stress and Body Temperature in Brown Swiss Cows Raised in Semi-Arid Climate of Ceará State, Brazil. **Acta Scientiae Veterinariae**, v.45, p.1-8, 2017. Available from: <http://www.redalyc.org/articulo.oa?id=289053641037>. Accessed: Apr. 11, 2023.

LIANG, D. et al. Influence of breed, milk production, season, and ambient temperature on dairy cow reticulorumen temperature. **Journal of dairy science**, v.96, n.8, p.5072-5081, aug. 2013. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030213004360>. Accessed: Apr. 15, 2023. doi: 10.3168/jds.2012-6537.

LIU, J. et al. Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: A novel idea for monitoring and evaluation of heat stress—A review. **Asian-Australasian journal of animal sciences**, v.32, n.9, p.1332-1339, jan. 2019. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6722315/>. Accessed: Oct. 12, 2023. doi: 10.5713/ajas.18.0743

MCMAHON, L. R. et al. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. **Canadian Journal of Plant Science**, v.80, n.3, p.469-485, july 2000. Available from: <https://cdnsciencepub.com/doi/abs/10.4141/p99-050>. Accessed: Oct. 12, 2023. doi: 10.4141/P99-050.

MIN, B. R. et al. The effect of condensed tannins in Lotus corniculatus upon reproductive efficiency and wool production in ewes during autumn. **Animal Feed Science and Technology**, v.92, n.3, p.185-202, aug. 2001. Available from: <https://www.sciencedirect.com/science/article/pii/S0377840101002589>. Accessed: Apr. 15, 2023. doi: 10.1016/S0377-8401(01)00258-9.

MIN, B. R.; SOLAIMAN, S. Comparative aspects of plant tannins on digestive physiology, nutrition and microbial community changes in sheep and goats: A review. **Journal of Animal Physiology and Animal Nutrition**, v.102, p.1181-1193, 2018. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/jpn.12938>. Accessed: Oct. 12, 2023. doi: 10.1111/jpn.12938

MOHAMED, R. H. et al. Investigations on the vaginal temperature, cycle stages, and steroid hormone concentrations during the breeding season in camels (Camelus dromedarius). **Veterinary world**, v.14. n.5, p.1102–1108, may. 2021. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8243658/>. Accessed: Apr. 13, 2023. doi: 10.14202/vetworld.2021.1102-1108.

MORENO, J. A. Clima do Rio Grande do Sul. In: MORENO, J. A. **Boletim Geográfico do Rio Grande do Sul**, 1961, p.49-83.

NRC, **Nutrient Requirements of Dairy Cattle**. 7th rev. ed. Natl. Acad. Sci., Washington, DC, 2001. 405p.

OLIVEIRA, L. N. et al. Effect of low dietary concentrations of *Acacia mearnsii* tannin extract on chewing, ruminal fermentation, digestibility, nitrogen partition, and performance of dairy cows. **Journal of Dairy Science**, v.106, n.5, p.3203-3216, may. 2023. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030223001546>. Accessed: Nov. 01, 2023. doi: 10.3168/jds.2022-22521.

ORLANDI, T. et al. Digestibility, ruminal fermentation and duodenal flux of amino acids in steers fed grass forage plus concentrate containing increasing levels of *Acacia mearnsii* tannin extract. **Animal Feed Science and Technology**, v.210, p.37-45, dec. 2015. Available from: <https://www.sciencedirect.com/science/article/pii/S0377840115300213>. Accessed: Oct. 12, 2023. doi: 10.1016/j.anifeedsci.2015.09.012.

ORLANDI, T. et al. Effect of using *Acacia mearnsii* tannin extract as a feed additive on nutritional variables and productive performance in dairy cows grazing a temperate pasture. **Animal Science Journal**, v.91, n.1, p.e13407, jun. 2020. Available from: <https://onlinelibrary.wiley.com/doi/epdf/10.1111/asj.13407>. Accessed: Nov. 01, 2023. doi: 10.1111/asj.13407.

PATRA, A. K.; SAXENA, J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. **Journal of the Science of Food and Agriculture**, v.91, p.24-37, 2011. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/jsfa.4152>. Accessed: Oct. 12, 2023. doi: 10.1002/jsfa.4152.

RAUW, W. M. et al. Perspectives for the sustainability of swine production in relation to climate change and new food resources. **Journal of the Science of Food and Agriculture**, v.100, n.9, p.3575-3586, feb. 2020. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/jsfa.10338>. Accessed: Apr. 19, 2023. doi: 10.1002/jsfa.10338.

REJEB, M. et al. A complex interrelationship between rectal temperature and dairy cows' performance under heat stress conditions. **Open Journal of Animal Sciences**, v.6, n.01, p.24-30, jan. 2016. Available from: <https://www.scirp.org/pdf/OJAS_2016011515000634.pdf>. Accessed: Mar. 22, 2023. doi: 10.4236/ojas.2016.61004.

RÍUS, A. G. Invited Review: Adaptations of protein and amino acid metabolism to heat stress in dairy cows and other livestock species. **Applied Animal Science**, v.35, p.39-48, 2019. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1080744619300051>. Accessed: Apr. 18, 2023. Accessed: Mar. 20, 2023. doi: 10.15232/aas.2018-01805.

RHOADS, R. P. et al. 2011 and 2012 early careers achievement awards: metabolic priorities during heat stress with an emphasis on skeletal muscle. **Journal of Animal Science**, v.91, n.6, p.2492-2503, june 2013. Available from: <https://academic.oup.com/jas/article-abstract/91/6/2492/4717023?login=true>. Accessed: Oct. 12, 2023. doi: 10.2527/jas.2012-6120.

SCANAVEZ, A. L. A. et al. Association between 4-day vaginal temperature assessment during the dry period and performance in the subsequent lactation of dairy cows during the warm season. **Journal of Animal Science**, v. 95, no. 12, p.5208-5217, dec. 2017. Available from: <https://academic.oup.com/jas/article-abstract/95/12/5208/4772033?login=true>. Accessed: Abr, 14, 2023. doi: 10.2527/jas2017.1620.

SLIMEN, I. B. et al. Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. **Journal of animal physiology and animal nutrition**, v.100, n.3, p.401-412, aug. 2016. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/jpn.12379>. Accessed: Oct. 12, 2023. doi: 10.1111/jpn.12379.

TAO, S et al. Impact of heat stress on lactational performance of dairy cows. **Theriogenology**, v.150, p.437-444, july 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S0093691X20301606>. Accessed: Apr. 17, 2023. doi: 10.1016/j.theriogenology.2020.02.048.

THOMPSON, M. L.; ZUCCHINI, W. On the statistical analysis of ROC curves. **Statistics in medicine**, v.8, p.1277-1290, 1989. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/sim.4780081011>. Accessed: Oct. 12, 2023. doi: 10.1002/sim.4780081011.

VAN SOEST, P. J et al. Methods for dietary fiber, neutral detergent fiber, nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, n.10, p.3583-3597, oct. 1991. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030291785512>. Accessed: Nov. 14, 2023.

VIEIRA, L. V. et al. Use of tannins as a nutritional additive for ruminants. **Arquivos de Ciências Veterinárias e Zoologia da UNIPAR**, v.23, n.1cont, p.1-13, oct. 2020. Available from: <https://revistas.unipar.br/index.php/veterinaria/article/view/8040/3977>. Accessed: Mar. 10, 2023. doi: 10.25110/arqvet.v23i1cont.2020.8040.

WHEELOCK, J. B. et al. Effects of heat stress on energetic metabolism in lactating holstein cows, **Journal of Dairy Science**, v.93, n.2 p.644–655, 2010. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030210715071>. Accessed: Apr. 20, 2023. doi: 10.3168/jds.2009-2295.

ZARINA, A. Impact of heat stress on Dairy Animals. In: NARAYANAN, M.K.; PHAND, S.; BEENA, V.; HARIKUMAR, S.; ZARINA, **A Climate Smart Dairying in the Context of Global Warming**, 2021. p.49-59.