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Nutritional Strategies with Tannins and Sunflower Oil: Impacts on Milk Yield. Fatty Acid Profile. and Oxidative Stability in Dairy

Michele Regina Sippert
Universidade Estadual de Maringa
Francilaine Eloise de Marchi
Universidade Estadual de Maringa
João-Luiz Prati Daniel
Universidade Estadual de Maringa
Jesus Alberto Cardozo Osorio
Universidad del Tolima
Jakeline Fernandes Cabral
Universidade Estadual de Maringa
Fabio Seiji Dos Santos
Universidade Estadual de Maringa
Magali Soares dos Santos Pozza
Universidade Estadual de Maringa
Maximiliane Alavarse Zambom
Universidade Estadual do Oeste do Parana
Altair Antonio Valloto
Federal University of Parana: Universidade Federal do Parana
Julio Cesar Damasceno
Universidade Estadual de Maringa
Rogério Aleson Dias Bezerra
Universidade Estadual de Maringa
Alexandre Menezes Dias
Universidade Federal de Mato Grosso do Sul
Luís Carlos Vinhas Ítavo
Universidade Federal de Mato Grosso do Sul
Geraldo Tadeu Santos
gtsantos50@gmail.com

Universidade Estadual de Maringa https://orcid.org/0000-0003-3720-5790

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Abstract

This study evaluated the effects of supplementing the diet of lactating dairy cows with condensed tannins from black wattle and/or sunflower oil. The impacts on milk yield. composition. oxidative status. fatty acid profile. and urinary purine derivatives were investigated. Eight cows received different diets over adaptation and data collection periods. Sunflower oil supplementation reduced milk fat content and 3.5% fat-corrected milk yield. Although condensed tannins were expected to protect polyunsaturated fatty acids (PUFAs) from ruminal biohydrogenation. antioxidant concentrations in milk did not support this effect. Tannins had limited influence on the milk fatty acid profile but were associated with reduced milk urea nitrogen (MUN) levels, suggesting effects on nitrogen metabolism. The inclusion of sunflower oil led to milk enriched with PUFAs. conjugated linoleic acid (CLA). and C18:1 cis-9. However. condensed tannins alone did not significantly enhance PUFA protection or modify fat composition. In summary. while sunflower oil positively influenced milk fatty acid quality, it negatively impacted milk fat content. Condensed tannins affected nitrogen use efficiency but failed to protect PUFAs from biohydrogenation. These findings contribute to improving dietary strategies for lactating cows to enhance milk quality and metabolic efficiency.

INTRODUCTION

Manipulating ruminal fermentation can enhance the utilization of high-quality feeds or by-products by ruminants. Specifically, modifying fermentation through feed additives is a strategy to improve animal health by reducing metabolic disorders (Dos Santos et al. 2010; Plaizier et al. 2018) and optimizing nutrient utilization (McCann et al. 2017). Feed additives can also enhance final products such as milk and meat by extending shelf life. improving flavor and naturally increasing bioactive compounds like antioxidants.

Tannins are secondary plant compounds with varying molecular weights. classified into hydrolyzable and condensed tannins. Condensed tannins from the bark of black wattle (*Acacia mearnsii*) were originally used in the leather industry but have recently gained attention as additives for ruminants. poultry, and swine. In ruminants, these tannins may improve nitrogen utilization, alter the ruminal environment, and reduce methane emissions (Denninger et al. 2020). In dairy cows, they have been associated with increased concentrations of conjugated linoleic acid (CLA) in milk (Benchaar et al. 2008; Cieslak et al. 2012).

However, high doses of condensed tannins can negatively affect animal performance. A major concern is their potential to reduce dry matter intake (DMI), thereby limiting milk production. Avilla et al. (2020) reported optimal digestibility at around 12 g/kg of dry matter without reducing DMI, though milk protein, particularly casein, was decreased. Other studies (e.g., Benchaar et al. 2008; Orlandi et al. 2015; Henke et al. 2017; Aguerre et al. 2016) have shown mixed effects on digestibility, nitrogen utilization, and milk production depending on tannin type and dosage.

Sunflower seeds or oil are typically used to increase dietary energy density (Palmquist and Jenkins, 2017), improving energy balance and nutrient efficiency (Leite et al. 2017; Rufino et al. 2018). oil is particularly rich in polyunsaturated fatty acids (PUFAs), especially linoleic acid (C18:2), which accounts for about 57% of its total fatty acid content. Including PUFAs in dairy cow diets improves the milk fatty acid profile, notably by increasing CLA content (Shingfield et al. 2013).

However, PUFA-rich milk is more prone to lipid oxidation, which may impair sensory qualities and reduce shelf life. Combining antioxidant sources with oil in the diet may help mitigate these effects (Dos Santos et al. 2014). While Lynch et al. (2005) demonstrated that pasteurized milk with high CLA and vaccenic acid levels (increased by 7.5 and 8 times, respectively) did not exhibit increased oxidized flavors in sensory evaluations, they concluded that it is feasible to produce dairy products with enhanced natural content of VA and cis-9, trans-11 CLA without compromising flavor.

The objective of this study was to evaluate the effects of including condensed tannins from *Acacia mearnsii* as a source of polyphenols and sunflower oil as a source of PUFAs in the diet of dairy cows. The impacts on milk yield, composition, oxidative status, fatty acid profile, and purine derivatives in urine were assessed.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethics Committee on Animal Use in Experimentation of the State University of Maringá, Paraná, Brazil (Protocol No. 4305280720). Eight primiparous lactating dairy Holstein cows, with an average body weight of 485 ± 37 kg and approximately 160 ± 35 days in milk, were used. The cows were housed in individual tie-stall facilities and milked twice daily, at 6:00 a.m. and 4:00 p.m.

A 4 × 4 double Latin square design with a 2 × 2 factorial arrangement was employed, testing the presence or absence of condensed tannins and sunflower oil. The experiment consisted of four periods of 24 days each, with 18 days for adaptation and 6 days for sample collection. Cows were fed twice daily (8:00 a.m. and 4:00 p.m.), and feed allowances were adjusted to ensure 10% refusals. The forage-to-concentrate ratio was 60:40. All diets were isonitrogenous and formulated to meet the nutritional requirements of lactating cows, following NRC (2001) guidelines (Table 01 and Table 2).

Insert Table 1 and Table 2 here

Milk and Urine Sampling and Analysis

Daily milk yield was recorded individually. Milk yield corrected to 3.5% fat was calculated using the equation proposed by Sklan et al. (1992). Composite milk samples (morning and afternoon milkings) were collected on days 20 and 21 and preserved with bronopol in plastic bottles. These were sent to the Paraná Association of Dutch Cattle Breeders (APCBRH) for analysis of fat, protein, lactose, total solids,

milk urea nitrogen (MUN), and somatic cell count (SCC). Additional samples were frozen for later analysis of milk fatty acid profile, oxidative stability, and total antioxidant capacity.

Milk fat was extracted by centrifugation as described by Murphy et al. (1995) and esterified according to ISO 5509 (1978). Fatty acid methyl esters were quantified by gas chromatography. Lipid oxidation was assessed by measuring primary oxidation products, determined through conjugated diene hydroperoxides (CD) following Kiokias et al. (2006), and secondary oxidation products via thiobarbituric acid-reactive substances (TBARS) according to King (1962). Antioxidant capacity was measured using ABTS and DPPH assays, as described by Singleton and Rossi (1965).

To assess microbial protein synthesis, spot urine samples were collected approximately four hours after morning feeding on days 22 and 23. Samples were filtered, acidified, and refrigerated for later analysis of uric acid, creatinine, and allantoin. Milk allantoin was also quantified, following the methodology of Chen and Gomes (1992). Microbial nitrogen synthesis was estimated using the equations described by Verbic et al. (1990).

Statistical Analysis

Statistical analyses were conducted using SAS software (Statistical Analysis System, version 9.4, 2001), employing the MIXED procedure. Data were subjected to analysis of variance (ANOVA), and treatment means were compared using Tukey's test at a 5% significance level. Variables related to milk production, composition, MUN, urinary purine derivatives, and milk antioxidants were analyzed using the following model:

$$Y=\mu+Ai+Dj+Pk+Eijky$$

where:

- μ = overall mean
- Ai = random effect of animal (i = 1 to 4)
- Dj_ = fixed effect of diet (j = 1 to 4)
- Pk = fixed effect of period (k = 1 to 4)
- Eijky = residual error

RESULTS

Table 3 presents data on milk yield, composition, and somatic cell count (SCC) of dairy cows fed diets with or without condensed tannins (TN) and sunflower oil (SO).

No significant differences in uncorrected milk yield (kg/cow/day) were observed among the four treatments. Average milk yields were: 16.32, 15.63, 15.86, and 15.43 kg/day for CON, SO, TN, and SO+TN treatments, respectively. However, when milk yield was corrected to 3.5% fat, diets containing sunflower

oil (SO and SO+TN) showed reduced production compared to the control and TN-only treatments. A similar pattern was observed for milk fat content, which was negatively affected by the inclusion of sunflower oil.

Fat-corrected milk yield (3.5% FCM) was significantly reduced in treatments containing sunflower oil. The SO and SO+TN diets, containing 2.31% and 2.36% DM of sunflower oil, respectively, also led to increased formation of the trans-10, cis-12 CLA isomer (C18:2).

Milk protein content was also negatively affected by sunflower oil supplementation compared to the CONT and TN treatments. Protein content averaged 3.44 g/100 g for treatments without sunflower oil and 3.31 g/100 g for those with sunflower oil ($P < 0.007$).

Lactose content (g/kg) was not influenced by treatment, remaining stable between 43.8 and 45.2 g/kg. Milk urea nitrogen (MUN) was significantly reduced ($P < 0.0001$) by sunflower oil supplementation, consistent with the observed reduction in milk protein. In contrast, TN did not significantly affect MUN.

Insert Table 3 here

No significant changes in TBARS (thiobarbituric acid reactive substances, mmol/kg fat) were detected across treatments (Table 4). Although plasma antioxidant activity was not assessed, conjugated diene concentrations increased significantly in the presence of sunflower oil ($P < 0.0001$), indicating decreased oxidative stability of milk fat. TN did not provide a protective effect against this outcome.

Insert Table 4 here

Antioxidant capacity assessed by ABTS and DPPH assays was not significantly affected by the inclusion of TN or OG (Table 4).

Insert Table 4 here

Table 5 presents the fatty acid profile of milk fat. Diets containing sunflower oil led to increases in several fatty acids: C18:1 n9c, C18:2 n6t, C18:2 cis-9, trans-11 CLA, and C18:2 trans-10, cis-12 ($P < 0.0001$). However, the combination of TN and OG negatively affected C18:3 n3 levels ($P < 0.0013$). TN alone resulted in minimal changes, with a modest increase in C18:2 n6c ($P < 0.0142$).

Insert Table 5 here

Table 6 summarizes the saturated (SFA), unsaturated (UFA), and polyunsaturated fatty acid (PUFA) contents of milk fat. Medium-chain fatty acids (MCFA) and saturated fatty acids were predominant. However, the inclusion of sunflower oil reduced short-chain (SCFA) and medium-chain fatty acids while increasing UFA and PUFA content. The most notable increase was in CLA content compared to treatments without sunflower oil. In contrast, the presence of TN had no significant effect on the sum of SCFA, MCFA, long-chain fatty acids (LCFA), SFA, UFA, PUFA, total CLA, or the SFA/PUFA ratio.

Insert Table 6 here

Table 7 shows the urinary purine derivative concentrations. Overall, purine derivatives were unaffected by the treatments, except for uric acid and allantoin. Average urine output was 21.92 L/day, with no significant differences among treatments.

Microbial protein synthesis (g/day) and microbial nitrogen production (g/day) tended to increase in OG treatments ($P = 0.0718$ and $P = 0.0900$, respectively), while TN treatments showed no effect ($P = 0.7285$).

Insert Table 7 here

Creatinine concentrations were unaffected by any treatment, averaging 6.44 mg/dL. Uric acid concentrations were higher ($P < 0.0104$) in cows receiving sunflower oil, while allantoin was higher in cows receiving TN ($P < 0.0504$). However, the ratios of allantoin to uric acid, uric acid to creatinine, and (allantoin + uric acid) to creatinine remained unchanged.

DISCUSSION

Milk production of cows under the CON, OG, TN, and OG + TN treatments did not differ significantly and was higher than the values reported by Thanh et al. (2023a), whose average was 13.15 kg/cow/day. However, when milk production was corrected to 3.5% fat, the treatments that included sunflower oil (OG) showed lower production than the CONT and CT groups. Ha et al. (2023) similarly observed no effects of grape seed oil and tannin extract on the milk yield or milk fat content of dairy goats. Thanh et al. (2023b) also reported reduced milk fat in cows fed diets containing flaxseed oil, sunflower oil, or crude tuna oil. These findings are consistent with the hypothesis that supplementation with oils rich in polyunsaturated fatty acids (PUFAs) can disrupt ruminal fermentation and fiber digestibility, reduce acetate production and consequently limit the synthesis of short- and medium-chain fatty acids in milk.

Moreover, diets rich in sunflower oil—as used in the OG and OG + TN treatments—are known to increase conjugated linoleic acid (CLA), which downregulates genes involved in lipid synthesis in the mammary gland (Bauman et al. 2001; Harvatine and Bauman, 2006). Thus, the lower milk yield corrected for 3.5% fat in the OG and OG + TN treatments is attributed to the inclusion of sunflower oil. These diets, with sunflower oil comprising 2.31% and 2.36% of dry matter (DM), respectively, also led to higher levels of the C18:2 isomer trans-10, cis-12 CLA, which is negatively correlated with milk fat percentage (Bauman et al. 2001; Kadegowda et al. 2007).

In contrast, Figueiroa et al. (2013) found no significant differences in milk yield corrected to 4% fat when sunflower seeds, with ether extract levels between 65.3 and 71.7 g/kg DM, were included in the cows' diet. However, in their study, milk fat content in sunflower seed treatments ranged from 2.63% to 3.18%, values lower than those observed in this experiment under OG and OG + TN treatments.

Milk protein levels were also negatively affected by the presence of sunflower oil compared to CONT and TN treatments. Protein levels ranged from 3.44 g/100 g in treatments without OG to 3.31 g/100 g in those with OG ($P < 0.007$), aligning with results from Figueiroa et al. (2013). Likewise, Thanh et al. (2023a) reported reduced protein content in milk from cows fed 2.5% soybean oil or a blend of soybean and tuna oil. Lactose content (g/kg) was unaffected by treatments, remaining stable between 43.8 and 45.2, in agreement with Rufino et al. (2018), although some studies (e.g., Muniz et al. 2022; Thanh et al. 2023a) report slightly higher values.

Milk urea nitrogen (NUL) was significantly reduced ($P < 0.0001$) with the inclusion of OG, mirroring the trend seen with protein levels. However, Rufino et al. (2018) did not observe this effect in Jersey cows despite using similar ether extract levels in diets with sunflower oil. Conversely, the presence of TN had no significant effect on NUL, possibly due to the tannin type or dosage (10 g/kg DM) being insufficient to affect ruminal fermentation or protein metabolism. Urea is synthesized in the liver from ammonia, which is derived from amino acid catabolism and recycled ruminal nitrogen. Higher dietary protein degradability typically leads to more ammonia production, elevated serum and milk urea levels, and increased nitrogen losses in urine (Figueiredo-Paludo et al. 2025).

Although TBARS concentrations were not significantly altered, plasma antioxidant activity—indicative of systemic antioxidant status—did not show a clear relationship. A higher level of conjugated dienes was observed with SO inclusion, indicating decreased milk fat stability. NT treatments did not exert a protective effect. Matthaiou et al. (2014) suggested that antioxidant supplementation has minimal impact unless dietary antioxidant levels are notably low. ABTS and DPPH antioxidant markers were unaffected by NT and SO. However, Lynch et al. (2005) found that milk high in CLA and vaccenic acid had acceptable sensory properties despite elevated oxidation products.

The main rationale for including oilseeds such as sunflower oil in dairy cow diets is to enhance dietary energy density and nutrient utilization (Palmquist and Jenkins 2017; Leite et al. 2017; Rufino et al. 2018). Sunflower oil, a PUFA source rich in linoleic acid (C18:2; ~57% of total FA), improves milk fatty acid profiles by increasing CLA content (Shingfield et al. 2013). In this study, SO inclusion significantly increased unsaturated fatty acids (C18:1 n9c, n9t; C18:2 n6t) ($P < 0.0001$), while C18:2 n6c remained unchanged. An interaction effect ($P < 0.0013$) was found for C18:3 n3, which increased with OG and decreased with NT.

While inclusion of oilseeds improves milk FA profiles, it also heightens susceptibility to lipid oxidation, potentially compromising flavor and shelf life. Antioxidants in the diet may mitigate this risk (Dos Santos et al. 2019), although TN did not exhibit such protective effects here. Prior studies (e.g., Benchaar et al. 2008; Cieslak et al. 2012) reported increased CLA with TN, but our data did not confirm this. Similarly, Toral et al. (2011) found no significant changes in milk PUFA when combining tannin extract with sunflower oil in ewes.

Our data showed significant increases in C18:1 n9c, C18:2 n6t, C18:2 cis-9, trans-11, and C18:2 trans-10, cis-12 (all $P < 0.0001$) with SO. However, combining TN with SO negatively affected C18:3 n3 levels ($P <$

0.0013). TN treatments resulted in only minor changes to milk FA, with a significant increase in C18:2 n6c ($P < 0.0142$).

As shown in Table 6, short-chain (SCFA) and medium-chain fatty acids (MCFA), along with saturated fatty acids (SFA), were dominant in milk fat. Sunflower oil supplementation reduced SCFA and MCFA levels while increasing unsaturated FA (UFA and PUFA), particularly CLA, compared to non-supplemented groups. TN supplementation did not significantly influence SCFA, MCFA, LCFA, SFA, UFA, PUFA, CLA, or the SFA/PUFA ratio.

SCFA and MCFA are synthesized de novo in the mammary gland from acetate and beta-hydroxybutyrate, via acetyl-CoA carboxylase and fatty acid synthase (Chilliard et al. 2000; Chilliard and Ferlay 2004). Stearoyl-CoA desaturase (Δ9-desaturase) introduces cis-double bonds, contributing significantly to the synthesis of C18:2 cis-9, trans-11 and other monounsaturated FA (Shingfield and Wallace 2014).

Urine production is influenced by factors like body weight, milk yield, diet, water intake, and temperature. Pina et al. (2006) reported 14.21 L/day in cows fed cottonseed meal, while this study observed an average of 21.92 L/day. Using the Nennich et al. (2006) equation, estimated urine volumes were 25.32, 23.69, 24.82, and 23.55 L/day for CONT, SO, TN, and SO + TN, respectively—closely matching actual values.

Microbial protein and nitrogen synthesis tended to increase with OG ($P < 0.0718$ and $P < 0.0900$), but not with TN ($P < 0.7285$), consistent with Avila et al. (2020). Creatinine levels averaged 6.44 mg/dL and were unaffected by treatment—higher than values reported by Conceição et al. (2019) for Girolando (1.47 mg/dL) and Holstein (1.64 mg/dL) cows. Uric acid increased ($P < 0.0104$) with SO, while allantoin increased with TN ($P < 0.0504$), but ratios of Allantoin/Uric Acid, Uric Acid/Creatinine, and Allantoin+Uric Acid/Creatinine remained stable. Despite suggestions that TN can enhance nitrogen use and rumen modulation (Naumann et al. 2017, Poli et al. 2025), our results showed limited changes in urinary purine derivatives.

CONCLUSION

The inclusion of sunflower oil (SO) reduced 3.5% fat-corrected milk yield and decreased milk fat and protein concentrations. NUL levels were also lower in milk from cows fed SO. Tannin (TN) supplementation did not effectively protect PUFAs from ruminal biohydrogenation and had minimal impact on milk fat composition. Nonetheless, the milk fatty acid profile was altered by the experimental diets. SO supplementation enriched milk with PUFA, MUFA, and C18:1 cis-9, while increasing PUFA, LCFA, and total CLA, and decreasing SCFA and MCFA. Milk from cows fed SO and TN did not exhibit enhanced antioxidant capacity; in contrast, SO increased conjugated diene concentrations, suggesting reduced milk stability. SO altered purine derivatives, and TN increased allantoin and protein synthesis, while SO decreased uric acid synthesis.

Declarations

ETHICS STATEMENT

This study was conducted in accordance with ethical standards. The experimental protocol was approved by the Ethics Committee on the Use of Animals in Experimentation of the State University of Maringá, PR, (nº 4305280720).

ARTIFICIAL INTELLIGENCE STATEMENT

During the preparation of this work, the authors used ChatGPT to assist in the organization and linguistic revision of the manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CONFLICT OF INTEREST

The authors have not stated any conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, M.R.S., G.T.D.S., J.L.P.D., F.E.D.M.; Methodology, M.R.S., G.T.D.S., J.L.P.D., M.A.Z., L.C.V.I.; Formal analysis, Investigation, M.R.S., F.E.D.M., R.A.D.B., J.A.C.O., J.F.C., M.A.Z., M.S.S.P.; Writing-original draft preparation, M.R.S., F.S.S., L.C.V.I., A.M.D., J.C.D., M.A.Z., M.S.S.P.; Writing-review and editing, G.T.D.S., F.S.S., M.S.S.P., M.A.Z, M.R.S., J.C.D., A.A.V., A.M.D., J.F.C.; Supervision, G.T.D.S., A.A.V., L.C.V.I., R.A.D.B., J.A.C.O., A.A.V.; Project administration, M.R.S., G.T.D.S., F.S.S., J.C.D.; Funding acquisition, G.T.D.S, M.R.S., F.S.S., A.A.V. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY DECLARATION

Not applicable.

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Tables

Table 1 to 7 are available in the Supplementary Files section.

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