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Composition, nitrate and nitrite levels, and antioxidant activity of milk from agroecological and conventional systems

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Abstract

Agroecological dairy production systems are gaining attention worldwide. However, few studies have compared the quality of bovine milk produced in these systems with that produced in conventional dairy production systems. In view of the different farming practices, it was expected that milk quality would differ between systems. This study aimed to compare the quality of milk produced by cows raised in the two systems. Twelve conventional farms and 10 agroecological farms were evaluated. Milk samples were analysed for chemical composition, somatic cell count, nitrate and nitrite levels, and total antioxidant capacity (TAC), which was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Mean somatic cell counts (SSC) were high for both agroecological systems (2.31 SCC/mL (log10)) and conventional systems (2.42 SCC/mL (log10)). Nitrate levels of milk in agroecological and conventional systems were 0.15 ± 0.03 and 0.17 ± 0.02 mg/L, and nitrite levels were 0.05 ± 0.03 and 0.07 ± 0.02 mg/L. The DPPH radical scavenging activity levels were 6.31 ± 0.55% and 6.64 ± 0.44% for agroecological and conventional systems. According to the ABTS method, TAC values were 6.63 ± 0.28% and 6.48 ± 0.20% for agroecological systems and conventional systems. Thus, although these systems adopted different farming practices, no significant differences were observed in milk composition, SCC, nitrate, nitrite, and TAC.

Keywords: chemical composition, dairy cow, milk urea nitrogen, nitrogen metabolism, somatic cell count
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Introduction

Milk composition can be influenced by environmental factors and management practices (Jenkins & McGuire, 2006; Pragna *et al.,* 2017). Changes in human behaviour have led to a search for food from sustainable production systems such as agroecology (Assis, 2006). In the agroecological system, the use of more rustic resilient breeds is evidenced. Rustic animals are defined by management with low input, which can affect milk composition considerably and contrasts with modern conventional dairy farming based on intensive production and high-yield breeds (Poulsen *et al.,* 2019).

Milk contains important macro- and micronutrients that are sources of energy and nutrition for humans (Yilmaz-Ersan *et al.,* 2018), such as proteins, minerals, and vitamins (Jenkins & McGuire, 2006). Chemical composition and SCC play important roles in milk quality (Cinar *et al.,* 2015; Sharma *et al.,* 2011). It is crucial to monitor the presence of subclinical mastitis (inflammation of the mammary gland) in cows (Silva Junior *et*

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al., 2019), because the disease affects milk yield and composition negatively (Ogola *et al.,* 2007; Auldist *&* Hubble*,* 1998).

Mastitis may not only increase SCC in milk, but may promote the release of free radicals, decreasing TAC (Yang & Li, 2015). Milk contains several compounds with antioxidant potential, including vitamins A, C and E, carotenoids, uric acid, conjugated linoleic acids, catalase, superoxide dismutase, glutathione peroxidase, proteins and amino acids (Pihlanto, 2006; Yilmaz-Ersan *et al.*, 2018).

The antioxidant potential of milk can be affected by diet (Lindmark-Månsson & Åkesson, 2000), because dietary antioxidants can be transferred to it (Havemose *et al.*, 2004). According to some studies, the levels of antioxidants in milk rise as the amount of green fodder in cow diet is increased (Grega *et al.,* 2005; La Terra *et al.,* 2010). Castillo *et al.* (2013) argued that sustainable agricultural systems might influence milk TAC.

The diet may also affect the nitrate and nitrite levels in milk (Radzymińska *et al.,* 2008). The levels of nitrate and nitrite in raw milk have also been associated with place of origin (Zbikowski *et al.,* 2000), that is, the physical and geographical characteristics of dairy farms. But these compounds are also associated with health problems (Nag, 2010). In humans, nitrate may react with secondary amines in the gastrointestinal tract, forming potentially carcinogenic products (Chamandust *et al.,* 2016; Hord *et al.*, 2009). When ingested at high levels, nitrite can cause methaemoglobin formation in human beings (Cockburn *et al.,* 2013).

This study aimed to compare the chemical composition, SCC, nitrate and nitrite levels, and TAC of milk from agroecological and conventional systems.

Material and Methods

All experimental procedures were in accordance with the standards of the Animal Ethics Committee (CEUA) of the State University of Maringá (Protocol no. 3103111018). A total of 148 samples of raw milk were collected from 22 family farms in northern and western Paraná State, southern Brazil. Ten dairy systems adopted agroecological farming practices, whereas 12 farms implemented conventional farming practices. Agroecological systems were located in the municipalities of Palotina, Paranacity, Marechal Cândido Rondon, Maripá, Jandaia do Sul and Mandaguaçu. These dairy farms performed agroecological management in an integrated manner, ensuring sustainability with minimal environmental impacts (Nunes *et al.,* 2017), promoting family farming, and preserving natural resources. Agroecological systems do not use pesticides or grain from farms that grow genetically modified crops, use chemical fertilizers or apply monoculture. Conventional systems were located in Iguaraçu, Palotina, Iguatemi, Maringá, and Nova Santa Rosa. Farms were randomly selected from lists provided by the Paraná Institute of Technical Assistance and Agricultural Extension (EMATER) and the Centre for Support and Promotion of Agroecology (CAPA). Farm visits were conducted from October 2018 to October 2019.

Milk samples were collected aseptically. Briefly, teats were washed and dried with paper towels or clean cloths. The first jets of milk were discarded, and a representative sample of the milking process was collected and homogenized. For farms that collected milk directly into tanks, milk samples were obtained at the end of milking. Sampling was performed according to the usual teat hygiene and milking practices adopted at each farm to avoid disrupting the routine of milkers and to minimize animal stress.

Samples to determine TAC, nitrate and nitrite were kept frozen until use. Samples collected to determine SCC, milk urea nitrogen, protein, fat, lactose, and total solids were stored in bronopol preservative and sent for analysis to the Central Laboratory of the Paraná Herd Analysis Programme (PARLPR), Holstein Breeders' Association of Paraná State (APCBRH), Curitiba, Brazil. Milk composition was analysed in 33 samples from agroecological systems and 107 from conventional systems. For TAC analysis, 40 milk samples from agroecological systems and 105 milk samples from conventional systems were used. Nitrate and nitrite levels were assessed in 41 samples from agroecological systems and 107 samples from conventional systems. Fewer samples of agroecological milk were collected because fewer agroecological farms were included in the study and because agroecological farms had smaller herds than conventional farms.

The reagents DPPH, ABTS, and *N*-(1-naphthyl) ethylenediamine dihydrochloride were purchased from Sigma–Aldrich® (Ribeirão Preto, São Paulo, Brazil). Potassium persulfate was obtained from Acros Organics (New Jersey, USA), cadmium granules (pure metal) from Dinâmica (Indaiatuba, São Paulo, Brazil), and ethyl alcohol (99.5%), methyl alcohol (100%), and sulfanilic acid from Synth (Diadema, São Paulo, Brazil). α-Naphthol was purchased from INLAB (São Paulo, São Paulo, Brazil), and sodium hydroxide and zinc sulfate from FMaia (Belo Horizonte, Minas Gerais, Brazil).

The TAC and nitrate were determined with an electronic UV-Vis spectrophotometer (Evolution 300, Thermo Fisher Scientific, Massachusetts, USA). Nitrite determination was performed using a VersaMax molecular devices microplate reader (Sunnyvale, CA, USA). Chemical composition and urea assays were

performed on a Bentley FTS infrared spectrometer (Bentley Instruments®, Minnesota, USA). The somatic cell count was estimated by flow cytometry with an electronic counter (Somacount FCM, Bentley Instruments®).

Milk fat, lactose, protein, total solids, and milk urea nitrogen levels were determined according to ISO 9622/IDF 141 (IDF, 2012), using the infrared method. Flow cytometry to determine SCC was performed according to ISO 13366-2/IDF 148-2 (IDF, 2006) and somatic cell count data (SCC/mL) were transformed to $\log_{10}$.

Milk nitrate levels were quantified by the method of Cortas & Wakid (1990), with the modifications proposed by Teixeira *et al.* (2014). Briefly, milk deproteinization was achieved by adding 75 mmol/L $ZnSO_4$ and 55 mmol/L NaOH. After 10 min of rest, samples were centrifuged at 3000 rpm for 15 min. Then samples were reduced with cadmium granules for 90 min, and nitrate was determined by reading the absorbance at 545 nm. Quantification was performed by comparison against a standard curve, obtained in the concentration range of 0 to 2 mg/L, with a coefficient of determination (R^2) of 0.99850.

Nitrite was determined by the classical Griess method (Griess, 1879) as described by Wood *et al.* (1967). Deproteinization of milk was carried out according to Cortas & Wakid (1990), with some adaptations. One mL aliquot of milk was mixed with 1 mL of 75 mM zinc sulphate and 1 mL of 55 mM sodium hydroxide. Samples were left to rest for 10 min and were centrifuged at 6000 rpm and 4 °C for 30 min. Subsequently, the supernatant was filtered through a 0.22 µm polyethersulfone membrane filter and used for analysis. Deproteinized samples were treated with sulfanilamide solution for 10 min, *N*-1-naphthyl ethylenediamine dihydrochloride solution was added, and the microplate was read at 540 nm.

The TAC was assessed by measuring the elimination of DPPH and ABTS radicals. DPPH radical scavenging activity was measured according to Li *et al.* (2009). For this, 100 µL of extract was mixed with 1900 µL of a methanol solution containing 0.06 mM DPPH and kept in the dark for 30 min. The absorbance was measured at 515 nm, and TAC was calculated as:

$$DPPH\ inhibition = [1 - (A_t/A_0)] \times 100$$

where A_0 is the initial absorbance of the sample and A_t is the absorbance of the sample after 30 min.

The ABTS activity was determined following Fellegrini *et al.* (1999) with modifications reported by Dong *et al.* (2015). The absorbance of the ABTS solution was measured at 734 nm. TAC was calculated as:

$$ABTS\ activity = [1 - (A_t/A_0)] \times 100$$

where A_0 is the initial absorbance of the sample and A_t is the absorbance of the sample after 6 min.

Statistical analysis was performed using the MIXED procedure of SAS version 9.3, according to the model:

$$Y_{ij} = \mu + \tau_i + \alpha_j + e_{ij}$$

with $\alpha_j \approx N(0, \sigma^2_\alpha)$ and $e_{ij} \approx N(0, \sigma^2_e)$, where Y_{ij} is the observed value, μ is the general average, τ_i is the fixed effect of treatments (i = conventional and agroecological systems), α_i is the random effect of the animal, e_{ij} is the residual error, N indicates a normal distribution, and σ^2_α and σ^2_e are the variances associated with the random effects of animal and residual variance, respectively. Comparison of means was performed using Tukey's test. Values of $P \leq 0.05$ were considered to denote statistical significance, and values of $P \leq 0.10$ were considered to represent trends.

Results and Discussion

There were no significant differences between management systems in milk physicochemical composition or SCC (Table 1). The levels of milk fat, protein, and total solids observed in this study were similar to those reported by Doska *et al.* (2012), namely 3.40 g/100 g of fat, 3.13 g/100 g of protein, and 12.0 g/100 g of total solids. These similarities probably arose because the dairy cows received an adequate diet, with their nutritional and production needs being met regardless of the system. Most of the animals used in this study were Holstein-Frisian or 50% Holstein-Frisian, with medium to low milk production. Milk lactose levels did not differ significantly between agroecological (4.18%) and conventional systems (4.29%) (Table 1) and were considered low. Low lactose levels may occur because of mastitis (Reis *et al.,* 2013), because there is a loss of lactose from milk through paracellular pathways that are upregulated during mastitis, which can be identified by high SCC levels (Auldist & Hubble, 1998). Lactose concentration may also be altered in extreme and unusual feeding situations (Jenkins & McGuire, 2006).

Table 1 Chemical composition of milk from cows managed under agroecological and conventional dairy production systems

Item	Agroecological dairy production systems (n = 33)	Conventional dairy production systems (n = 107)	*P*-value
Fat, g/100 g	3.41 ± 0.45	3.24 ± 0.37	0.7722
Protein, g/100 g	3.26 ± 0.09	3.31 ± 0.06	0.7021
Urea nitrogen, mg/dL	12.60 ± 1.72	11.33 ± 1.41	0.5734
Lactose, g/100 g	4.18 ± 0.13	4.29 ± 0.10	0.5196
Total solids, g/100 g	11.79 ± 0.57	11.83 ± 0.46	0.9625
Somatic cell count, log_{10}	2.31 ± 0.17	2.42 ± 0.12	0.5983

n: number of milk samples

Lactose levels are correlated with protein and fat levels, because their biosynthesis occurs in the same cells in the mammary gland (Rajčević *et al.,* 2003). Park *et al.* (2007) observed that milk from cows with mastitis had low lactose contents. Parity number also influences milk lactose levels, and lactose concentration decreases with increasing parity number (Miglior *et al.,* 2006). Heck *et al.* (2009) investigated seasonal variations in the chemical composition of Holstein milk (*n* = 52) and observed lactose contents of 4.46–4.55 g/100 g. In the present study, the lack of significant differences in lactose levels between management systems was possibly because cows were subject to similar handling and feeding practices.

Similar SCC values were observed between agroecological and conventional milk samples. Farm management and hygiene practices are essential to ensure udder health (Firth *et al.,* 2019) and avoid increasing SCC at herd level. Factors such as interactions between infectious agents, poor management practices, genetics, and the environment may promote an increase in SCC (Hisira *et al.,* 2019). Machado *et al.* (2000) evaluated 7941 milk samples and found a mean SCC of 641 000 cells/mL, lower than the mean values observed in the current study for both systems.

The milk urea nitrogen contents in this study were in accordance with literature values (Kananub *et al.,* 2018; Bondan *et al.,* 2019). One study, investigating the effects of fertility on milk urea nitrogen of milk from more than 1200 cows, reported a mean milk urea nitrogen of 12.6 mg/dL (Rajala-Schultz *et al.,* 2001), similar to the values observed here for both systems. In another experiment, the least square mean milk urea nitrogen concentration was 12.4 mg/dL (SD = 3.01; *n* = 1,138) (Jonker *et al.,* 2002).

Table 2 shows nitrate and nitrite levels in milk from agroecological systems and conventional systems. No significant differences in nitrate or nitrite concentrations were observed between systems. The values observed here were lower than those reported by Santos *et al.* (2005). The authors evaluated raw milk (*n* = 45) from cows in conventional systems and agroecological systems in the Central Depression Region of Rio Grande do Sul, Brazil, and found nitrate and nitrite levels of 6.65 ± 0.84 mg/L and 1.76 ± 0.17 mg/L, respectively.

Table 2 Nitrate and nitrite levels in milk from cows managed under agroecological and conventional production systems

Item	Agroecological dairy production systems (n = 41)	Conventional dairy production systems (n = 107)	*P*-value
Nitrate, mg/L	0.15 ± 0.03	0.17 ± 0.02	0.6392
Nitrite, mg/L	0.05 ± 0.03	0.07 ± 0.02	0.6116

n: number of milk samples.

According to the Joint FAO/WHO Expert Committee on Food Additives, the acceptable daily intakes of nitrate and nitrite are 0–3.7 mg/kg bodyweight and 0–0.07 mg/kg bodyweight, respectively. The levels of nitrate and nitrite in milk from both management systems were within a good safety margin. For instance, a six-month-old child weighing 7.5 kg would need to drink 15 L of conventional system milk daily to reach nitrite intake levels that could compromise health.

The DPPH and ABTS antioxidant activities did not differ significantly (P >0.05) between agroecological and conventional system milk samples (Table 3). The DPPH scavenging activity of milk from both systems was lower than the value (8.75%) reported in a previous study (Jafari et al., 2017) and ABTS scavenging activity was lower than that found by Yilmaz-Ersan et al. (2018) for raw milk (21.48%). Given the benefits of antioxidants to human health (reduction or prevention of various types of cancer, cardiovascular disease, neurological disease, arteriosclerosis, and ageing-related disease) (Jafari et al., 2017), high antioxidant activities could add value to milk (Castillo et al., 2013).

Table 3 Antioxidant activity of milk from cows managed under agroecological and conventional production systems

Item	Agroecological dairy production system (n = 40)	Conventional dairy production system (n = 105)	*P*-value
DPPH, %	6.31 ± 0.55	6.64 ± 0.44	0.6537
ABTS, %	6.63 ± 0.28	6.48 ± 0.20	0.6829

n: number of milk samples; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

Supplementation of diets with natural antioxidants can enhance the antioxidant potential of milk produced by cows. Milk antioxidant activity can also be increased by supplementation with phytochemicals Khan *et al.,* 2019). The lack of difference in TAC observed in the present study can because cows from the two management systems had similar diets with similar antioxidant levels.

Conclusion

No significant differences were observed in the composition of milk, nitrate, nitrite, SCC or TAC between the agroecological and conventional production systems. These similarities probably resulted from the cows having received an adequate diet that met their nutritional and production needs, and the implementation of similar hygienic practices during milking. The levels of nitrite and nitrate were in accordance with satisfactory standards for consumption. Thus, the choice of milk from agroecological and conventional systems could depend on the preference of consumers for the way in which their milk is produced.

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Authors' Contributions

MF-P conceived and designed the experiment, performed the research, conducted the experiment, collected the samples and data, carried out laboratory and statistical analysis, and wrote the manuscript. MSSP performed the research, methodology, and supervision. JACO collected the samples and data and performed the statistical analysis. LCVÍ and JAH conducted the laboratory and statistical analyses. RCSJ performed the research, laboratory analysis and wrote the manuscript. FSS interpreted the results, and wrote, reviewed and edited the manuscript. GTS conceived and designed the experiment, performed the research and supervision and wrote the manuscript.

Conflict of Interest Declaration

The authors declare there is no conflict of interest for the current manuscript.

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