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Chemical composition and fatty acid profile in organic milk from dairy cows fed with microalgae (*Schizochytrium limacinum*)

Composição química e perfil de ácidos graxos no leite orgânico de vacas leiteiras alimentadas com microalgas (Schizochytrium limacinum)

Composición química y perfil de ácidos grasos en la leche orgánica de vacas lecheras alimentadas con microalgas (Schizochytrium limacinum)

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Neiva Carneiro
ORCID: https://orcid.org/0000-0003-1712-8628
Universidade do Oeste de Santa Catarina, Brazil
E-mail: neivacarneiromelo@gmail.com
Wilson Zacaron
ORCID: https://orcid.org/0000-0003-0338-9712
Universidade do Oeste de Santa Catarina, Brazil
E-mail: wilsonzacaron@outlook.com
Gabriel Rossato
ORCID: https://orcid.org/0000-0003-3909-7009
Universidade do Oeste de Santa Catarina, Brazil
E-mail: gabrielrossato30@gmail.com
Gabriela Solivo
ORCID: https://orcid.org/0000-0003-4769-7821
Universidade do Oeste de Santa Catarina, Brazil
E-mail: gabriellasolivo@gmail.com
Renata Bolzan Falk
ORCID: https://orcid.org/0000-0002-7852-132X
Federal University Santa Maria, Brazil
E-mail: renatabfalk@gmail.com
Roger Wagner
ORCID: https://orcid.org/0000-0002-5943-4909
Federal University Santa Maria, Brazil
E-mail: rogerwag@gmail.com
Aleksandro S. Da Silva
ORCID: https://orcid.org/0000-0001-5459-3823
Universidade do Estado de Santa Catarina, Brazil
E-mail: aleksandro.silva@udesc.br
Claiton André Zotti
ORCID: https://orcid.org/0000-0002-6845-9454
Universidade do Oeste de Santa Catarina, Brazil
E-mail: claiton.zotti@unoesc.edu.br

Abstract

Our aim was to determine whether microalgae (*Schizochytrium limacinum*) supplementation affects daily production, composition and fatty acid profile of organic milk. Eight lactating cows were kept in pasture and divided in two groups: those fed corn cob as a supplement twice a day during milking (CTL) and those fed corn cob mixed with 100 g of microalgae (ALG) per cow daily. Microalgae did not affected daily milk production and composition, but a tendency of milk fat reduction was observed. The level of stearic acid in the milk of cows fed ALG was significantly lowered 2.46-fold, whereas levels of elaidic acid and conjugated linoleic acid were significantly elevated by 3.3-fold and 1.8-fold, respectively. A significantly greater PUFA:MUFA ratio was observed in ALG treatment, while the MUFA:saturated fatty acid ratio showed a tendency to increase (P=0.073). Microalgae rich in omega-3 fatty acids successfully enrich organic milk without negatively affecting productivity or composition. Consumers could be attract to increase the intake of omega 3 polynsaturated fat from organic milk. These results could support nutritionist and farmers decision to feed microalgae to dairy cattle since it is economically viable.

Keywords: Polyunsaturated fatty acids; Milk enrichment; Nutraceutical; Lipid supplementation.

Resumo

Nosso objetivo foi determinar se a suplementação de microalgas (*Schizochytrium limacinum*) afeta a produção diária, composição e perfil de ácidos graxos do leite orgânico. Oito vacas em lactação foram mantidas a pasto e divididas em

dois grupos: as alimentadas com sabugo de milho como suplemento duas vezes ao dia durante a ordenha (CTL) e as alimentadas com sabugo de milho misturado com 100 g de microalgas (ALG) por vaca diariamente. As microalgas não afetaram a produção e composição diária do leite, mas foi observada uma tendência de redução da gordura do leite. O nível de ácido esteárico no leite de vacas alimentadas com ALG foi significativamente reduzido em 2,46 vezes, enquanto os níveis de ácido elaídico e ácido linoléico conjugado foram significativamente elevados em 3,3 vezes e 1,8 vezes, respectivamente. Uma proporção significativamente maior de PUFA: MUFA foi observada no tratamento com ALG, enquanto a proporção de MUFA: ácido graxo saturado mostrou uma tendência a aumentar (P = 0,073). Microalgas ricas em ácidos graxos ômega-3 enriquecem com sucesso o leite orgânico sem afetar negativamente a produtividade ou composição. Os consumidores podem ser atraídos para aumentar a ingestão de gordura polinsaturada ômega 3 do leite orgânico. Esses resultados podem apoiar a decisão de nutricionistas e agricultores de alimentar o gado leiteiro com microalgas, uma vez que é economicamente viável.
Palavras-chave: Ácidos graxos poliinsaturados; Enriquecimento de leite; Nutracêutico; Suplementação lipídica.

Resumen

Nuestro objetivo fue determinar si la suplementación con microalgas (Schizochytrium limacinum) afecta la producción diaria, la composición y el perfil de ácidos grasos de la leche orgánica. Ocho vacas lactantes se mantuvieron en pastoreo y se dividieron en dos grupos: las alimentadas con mazorca de maíz como suplemento dos veces al día durante el ordeño (CTL) y las alimentadas con mazorca de maíz mezclada con 100 g de microalgas (ALG) por vaca al día. Las microalgas no afectaron la producción y composición diaria de la leche, pero se observó una tendencia a la reducción de la grasa de la leche. El nivel de ácido esteárico en la leche de las vacas alimentadas con ALG se redujo significativamente 2,46 veces, mientras que los niveles de ácido elaídico y ácido linoleico conjugado se elevaron significativamente 3,3 y 1,8 veces, respectivamente. Se observó una relación PUFA: MUFA significativamente mayor en el tratamiento con ALG, mientras que la relación MUFA: ácidos grasos saturados mostró una tendencia a aumentar (P = 0,073). Las microalgas ricas en ácidos grasos omega-3 enriquecen con éxito la leche orgánica sin afectar negativamente la productividad o composición. Los consumidores podrían verse atraídos por aumentar la ingesta de grasas polinsaturadas omega 3 de la leche orgánica. Estos resultados podrían respaldar la decisión de nutricionistas y agricultores de alimentar con microalgas al ganado lechero, ya que es económicamente viable.
Palabras clave: Ácidos grasos polinsaturados; Enriquecimiento de la leche; Nutracéutico; Suplementación lipídica.

1. Introduction

Interest in organic products has been increasing worldwide because of recent consumer concerns regarding food safety as well as because of environmental degradation caused by the use of chemicals that leave residues (Dragincic et al., 2015). In addition to increasing consumer demand for products with proven quality and less use of chemicals and pesticides, the demand for enriched products possessing additional health benefits is a growing niche market. The Brazilian National Health Surveillance Agency (ANVISA) defines enriched or fortified foods as those to which nutrients are added for the purpose of enhancing nutritional value, either by quantitatively replenishing nutrients lost during processing or by supplementation to levels higher than normal. Milk enrichment with concentrated sources of omega 3 (ω3) fatty acids differentiate the product on the market. In this sense, the demand for products with higher levels of polyunsaturated fatty acids (PUFA), especially in developed countries among upper classes, has been growing (Zymon et al., 2014). Omega-3 PUFAs have been studied in terms of their effects on brain structure and function as well as on overall health. They also protect against cardiovascular disease and enhance the immune system (Ghasemi Fard et al., 2019).

Fish oil is an excellent source of essential ω3 PUFA, especially eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA) (Kolanowski and Laufenberg, 2006); however, per capita fish consumption in Brazil is only 9.5 kg, compared to a world average of 20 kg (FAO, 2018). Therefore, the strategy of enriching animal products with ω3 PUFA can be an alternative to achieve recommended intake levels of 250 to 500 mg per day (Kus and Mancini-Filho, 2010).

Milk production from the predominant intake of fresh forage increases unsaturated FA content, which is beneficial to health when compared to milk from cows kept in feedlots (Vahmani et al., 2013a). Nevertheless, milk fat is practically devoid of ω3 PUFA, specifically EPA and DHA (Vahmani et al., 2013b). A source of ω3 PUFA that has been widely researched in the last decade, heterotrophic microalgae (produced by bioreactors), are sources primarily of DHA, and are used as a nutraceutical (Del-Campo et al., 2007) to enrich cow's milk (Moran et al., 2019). However, the use of microalgae in an organic milk production system have not been reported in the literature. Therefore, our objective was to determine whether microalgae supplementation affects milk yield as well as chemistry composition and fatty acid profiles in milk of lactating cows kept in an organic production system.

2. Methodology

The study was carried out on a farm with organic production in the municipality of Quilombo in Santa Catarina. All procedures used were approved by the Animal Use Ethics Committee - CEUA of the University of Western Santa Catarina - UNOESC under protocol 74/2017.

Eight lactating multiparous Jersey cows, with average weight of 374 ± 48 kg, average yield of 8.9 ± 1.5 liters, were allocated in blocks according to milk production and days in milk production of 151.4 ± 32.9. They were subsequently divided into control treatment (CTL n = 4 cows) and microalgae treatment (ALG n = 4 cows). The collections were performed in two periods of 14 days each, with daily microalgae offered from the first day of each period. From the 12th to the 14th day, data were collected.

During the study, the cows grazed on Giant Missionary Grass *(Axonopus catarinensis Valls)* and Tifton (*Cynodon* spp.) (Table 1) in a rotational grazing system for 21 hours a day, with free access to water. The cows were milked twice a day (07h00 and 18h00) and received four kilos of corn cob per cow per day, divided into two kilos during each milking.

Table 1. Chemical composition of grass (mixture of giant missionary grass and Tifton) and corn cob fed to lactating cows.

Item	Feed	
	Grass	Corn cob
DM ($g.kg^{-1}$ as fed)	224.5	871.6
Ash ($g.kg^{-1}$ DM)	105.3	70.0
CP ($g.kg^{-1}$ DM)	100.8	74.9
NDF ($g.kg^{-1}$ DM)	730.7	308.2
ADF ($g.kg^{-1}$ DM)	447.3	141.3

Source: Authors.

The control treatment consisted in the supply of corn row supplementation, and the experimental treatment included 100 g of microalgae (*Schizochytrium Limacinum*) (All-G Rich™ *Schizochytrium limacinum* CCAP 4087/2; Alltech, Inc.) per cow per day (ALG treatment) added to the corn cob. Considering that the amount of supplemented, corn cob was small. At every milking, each cow received 50 g of microalgae.

Samples of grazed forage and corn cob were collected at the beginning, as well as on days 12, 13, and 14 of the experimental period. The forage was collected in the paddocks where the animals remained during their day, simulating the degree of grazing. Then, all analyses were performed at the Animal Nutrition Laboratory of University of Western of Santa Catarina (UNOESC). To perform chemical composition analyses, dry matter (DM) (930.15), crude protein (CP) (954.01) and ash (942.05) were measured as described by Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were performed as described by Van Soest (1963), in polyester bags (Komarek, 1993), in which the samples were dried in an autoclave at 110 ºC for 40 min (Senger et al., 2008) in a sequential method.

Milk production was recorded on day zero of the experiment before the start of microalgae supplementation. Milk samples were taken on days zero, 12, 13, and 14 of each experimental period.

Individual milk samples were collected during six consecutive milking for the final three days of each period (days 12, 13 and 14) in equal proportions in the morning and afternoon milking, then divided into two cups. One flask was refrigerated at 4 ºC and subsequently sent to the laboratory of the Paranaense Association of Holstein Cattle Breeders to determine the chemical composition (fat, protein, defatted dry extract and lactose). The methodology used for milk composition analysis was infrared, according to International Dairy Federation - IDF, Standard 141 Second Edition 2013-09-15. The second cup was kept in a freezer at –20 ºC and sent to the laboratory of the Federal University of Santa Maria (UFSM) for analysis of fatty acid composition, using gas chromatography according to the method of Bligh and Dyer (1959). The results were expressed as a percentage of the total area of the chromatograms considering carbon-chain equivalent size correction factors and the ester-to-acid conversion factor. The FA composition of the corn cob and grass (Table 2) and of microalgae (Table 3) are presented below.

Table 2. Fatty acid composition[1] of native pasture composed of giant mission grass (*Axonopus Catarinensis Valls*) and Tifton (*Cynodon* spp.).

	Pasture	Corn cob
Fatty acids	Fatty acids g/100 g total FA	
C16:0	60.17	24.39
C18:0	5.59	3.06
C18:1n9c	n.d	37.88
C18:2n6c	14.72	34.67
C18:3n3	19.52	n.d

[1]Total fatty acids 3.14% and 3.40%, respectively for pasture and corn cob. n.d: not detected. Source: Authors.

The cows were considered experimental units and were randomly distributed in a randomized block design. The data were analyzed using Proc Mixed SAS (SAS University Edition), using the sampling days after the beginning of supplementation (12, 13, and 14 days) as repeated measures for milk FA production and overall milk composition. The model included fixed effect of treatment, time in days and interaction between treatment and day, with period and animal within period as random effect. There were no response variables with significant interactions between treatments and days. Mean comparisons between treatments were analyzed using the T-test (α = 0.05).

Table 3. Chemical Composition of Microalgae Supplement.

Variables	Microalgae Supplement[1]
Composition	
Dry matter (%)	97.4±0.1
Organic matter (% DM)	96.2±0.1
Crude protein (% DM)	15.6±0.2
Non-fibrous carbohydrates (% DM)	15.3±2.1
Neutral detergent fiber (% DM)	2.0±1.8
Ethereal acid hydrolysis extract (% DM)	63.2±2.7
Fatty acids- FA (% DM)	33.2±3.0
Fatty acids g/100 g total FA	
<C16	5.74±0.01
C16:0	52.58 ± 0.36
C18:0	1.41 ± 0.01
C18:1	0.13 ± 0.01
C18:2 cis-9. cis-12	Not found
C18:3 cis-9. cis-12. cis-15	0.03±0.01
C20:4 cis-5. cis-8. cis-11. cis-14	0.08±0.01
C20:5 cis-5. cis-8. cis-11. cis-14. cis-17	0.41±0.01
C22:5 cis-4. cis-7. cis-10. cis-13. cis-16	6.31±0.06
C22:6 cis-4. cis-7. cis-10. cis-13. cis-16. cis-19	29.98±0.28
ω6	6.56±0.07
ω3	30.50±0.29

[1]All-G-Rich, Alltech. Source: Authors.

3. Results and Discussion

The addition of microalgae did not change milk production (P = 0.787), protein (P = 0.203), lactose (P = 0.701), or total solids levels in milk (P = 0.112); however, it tended to reduce fat content (P = 0.084) without affecting production of milk constituents (Table 4).

Table 4. Production and composition of milk from cows consuming a control diet supplemented with Schizochytrium limacinum.

	Treatments[1]			P-value		
	CTL	ALG	EPM	Trt	Day	Trt*Day
Milk production, L/day	8.88	9.16	0.289	0.787	0.657	0.833
Fat, %	4.31	3.38	0.168	0.084	0.351	0.498
Fat, kg/d	0.345	0.321	0.011	0.553	0.354	0.791
Protein, %	3.36	3.14	0.048	0.203	0.026	0.07
Protein, kg/d	0.271	0.303	0.011	0.437	0.536	0.131
Lactose, %	4.29	4.22	0.049	0.701	0.528	0.401
Lactose, kg/d	0.349	0.406	0.014	0.281	0.617	0.812
Total solids, %	12.89	11.63	0.241	0.112	0.193	0.241

[1] CTL: without microalgae; ALG: microalgae (50 g fed twice daily at milking). Source: Authors.

The concentrations of palmitic acid, palmitoleic acid, elaidic acid, and conjugated linoleic acid (CLA) in milk fat were increased with microalgae (Table 5). Conversely, there were significant reductions in the proportions of stearic acid and oleic acid, without differences on composition of others fatty acids.

Table 5: Effect of *Schizochytrium limacinum* supplementation on the concentration of major fatty acids and biologically relevant fatty acids in organic milk.

	Treatments[1]			P-value		
Fatty acids. g/100 g of total	CTL	ALG	EPM	Trt	Day	Trt*Day
Butyric - C4	5.70	8.25	0.93	0.12	0.79	0.85
Caproic - C6	0.80	0.95	0.06	0.46	0.15	0.73
Caprylic - C8	0.65	0.71	0.04	0.59	0.21	0.78
Capric - C10	1.92	2.16	0.15	0.62	0.25	0.83
Lauric - C12	2.70	2.98	0.15	0.45	0.23	0.85
Meristic - C14	14.60	14.44	0.42	0.90	0.58	0.62
Myristoleic- C14:1	0.42	0.41	0.03	0.62	0.52	0.55
Pentadecanoic - C15	1.07	1.10	0.03	0.26	0.52	0.65
Palmitic - C16	37.50	41.75	0.73	0.01	0.28	0.66
Palmitoleic - C16:1	0.69	0.94	0.04	0.02	0.59	0.81
Margaric - C17	0.51	0.55	0.02	0.65	0.66	0.31
Stearic - C18	13.97	6.146	0.90	<0.0001	0.53	0.80
Elaidic - C18:1 n9 trans	2.587	8.631	0.73	<0.0001	0.16	0.17
Oleic - C18:1 n9 cis	15.70	9.48	0.91	0.01	0.87	0.96
Linoleic - C18:2 n6 cis	0.52	0.51	0.02	0.92	0.22	0.73
α- Linoleic - C18:3 n3	0.19	0.15	0.01	0.12	0.12	0.46
Conjugated Linoleic, CLA	0.48	0.87	0.06	0.01	0.35	0.74
AGS	76.79	76.56	0.83	0.91	0.66	0.78
MUFA	19.32	19.44	0.91	0.95	0.39	0.52
PUFA	1.18	1.59	0.08	0.04	0.58	0.60
PUFA/MUFA[2]	5.97	8.45	0.45	0.01	0.90	0.12

PUFA/SFA[3]	1.56	2.12	0.13	0.07	0.70	0.63
MUFA/SFA[4]	25.51	26.06	1.55	0.87	0.54	0.70

[1]CTL: without microalgae; ALG: microalgae (with microalgae 50 g fed twice daily at milking).
[2]Polyunsaturated fatty acids/monounsaturated fatty acids. [3]Polyunsaturated fatty acids/saturated fatty acids. [4]Monounsaturated fatty acids/saturated fatty acids. Source: Authors.

As expected, total PUFAs in milk fat significantly increased (P = 0.04), as well as their proportion with respect to MUFAs (P = 0.01); there was a tendency to increase the ratio of MUFA to SFA (P = 0.07) without significantly altering SFA, MUFA, or MUFA/SFA.

The animals remained in pasture and received microalgae mixed with corn during milking. Reduction in diet acceptability when microalgae are supplied to lactating cows has been reported in the literature (Franklin et al., 1999; Lamminen et al., 2017); nevertheless, in our study, all corn supplements were ingested by cows in both treatments. No significant differences for inclusion of microalgae (91.8 g/cow/day) on daily production, milk solids content or production was also observed by Da Silva et al. (2016). Similar results were also observed with supplementation of protected microalgae, without change in dry matter intake or milk yield, while milk fat percentage was reduced (Vahmani et al., 2013b). In previous studies, the inclusion of microalgae and marine polyunsaturated fatty acids reduced milk fat content (Boeckaert et al., 2007; Sinedino et al., 2017).

The reduction in fat content, even in grazing cows, may be related to the effect of PUFA on fiber digestion. Polyunsaturated fatty acids alter rumen biohydrogenation, increasing the concentration of trans fatty acids with 18-carbon, mainly derivatives of linoleic acid metabolism (Barletta et al., 2016) and CLA. As a result, de novo synthesis of short chain fatty acids is inhibited, leading to reduction in milk fat content and increased proportions of long-chain fatty acids (Angulo et al., 2012). Franklin et al. (1999) observed that the percentage of fat in the milk of cows fed microalgae was lower than in the milk of cows fed control diets.

Large accumulations of vaccenic acid and reductions of stearic acid in rumen fluid (Boeckaert et al., 2007) and in milk (Moate et al., 2013) have been reported. In the present study, microalgae supplementation caused a significant reduction in stearic acid content (2.46-fold) and levels of vaccenic acid and conjugated linoleic acid in milk fat were 3.3- and 1.8-fold greater, respectively. This may have been due to the complete inhibition of ruminal biohydrogenation, characterized by conversion of stearic acid to vaccenic acid by group B bacteria, mainly *Fusocillus* (Harfoot and Hazlewood, 1997). Marine lipids inhibit the saturation of vaccenic acid and other PUFA metabolism products, presenting substantial potential to modulate the final stage of biohydrogenation (Jeyanathan et al., 2016). In fact, EPA and DHA supplementation may reduce the extent of biohydrogenation and increase the production of trans fatty acids, CLA and PUFA that reach the gut, especially in cattle, where biohydrogenation kinetics are lower and more incomplete than those of sheep (Toral et al., 2018).

Saturated fatty acids are associated with cardiovascular diseases, and PUFA consumption is associated with the reduction of these problems because they lower cholesterol and blood pressure as well as correlating with development and function of the brain (Bentsen, 2017). These findings suggest that increasing PUFA levels in milk of cows supplemented with microalgae is a beneficial effect (Franklin et al., 1999; Sinedino et al., 2017; Moran et al. 2018).

Cows kept in a grazing system had milk contents of SFA reduced while CLA increased (Vahmani et al., 2013b). Lamminen et al. (2019) observed that the concentrations of ω3 fatty acids in milk fat were higher in diets with microalgae than in those with soybean meal, while the opposite occurred with SFAs. Furthermore, α-linolenic acid and PUFA concentrations tended to be higher in microalgae diets.

Despite the ability to enrich milk using PUFA in the diet, increased levels of EPA and DHA in milk have been observed at low concentrations (Moran et al., 2018). This phenomenon may be explained by the extensive ruminal biohydrogenation undergone by PUFA, especially EPA, DHA and their precursor, linolenic acid. Furthermore, the low affinity for phospholipids (such as EPA and DHA) associated with HDL (high density lipoproteins) for the lipoprotein lipase enzyme in the mammary gland contributes to the low transfer rate from diet to milk Chilliard et al. (2007). Unfortunately, gas chromatographic analyses used to analyze FA were not able to determine the concentration of DHA and EPA, limiting the ability to elucidate the potential for the microalgae to enrich milk with these essential fatty acids.

Organic production even with potential for growth faces severe restrictions, including lack of trained technicians to guide producers. Almost all organic milk produced is diluted in conventional whole milk tanks. There is restriction in terms of ingredients suitable for use in the organic production system, as well as an absence of government policies aimed at encouragement of production and development of a consumer market. Even so, with limited access to few farms that operate organic milk production systems, the use of microalgae rich in ω3 fatty acids proved to be an adequate method of enriching animal products, respecting the requirements of organic production and making available to consumers a product with nutraceutical characteristics.

4. Conclusion

Cows kept in organic management system and fed diets supplemented with microalgae maintain production and composition of milk, with greater polyunsaturated fatty acid and conjugated linoleic acid contents.

Further studies on microalgae supplementation to dairy cows should focus on its effects on immunologic and antioxidative status, which are desirable especially in fresh cows.

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