Fasolo E, Peretti E, Tomaluski CR, Silva AD, Bottari NB, Baldissera MD, Farina R, Pereira W, Silva AS & Zotti CA. (2020). Effect of differing amounts of microalgae (Schizochytrium limacinum) added via milk on performance and antioxidant capacity of dairy calves. ***Research, Society and Development***, 9(**7**): 1-22, e647974467.

Efeitos de diferentes doses de microalgas marinhas (Schizochytrium limacinum) via leite sobre o consumo, desempenho e capacidade antioxidantes de bezerras leiteiras

Effect of differing amounts of microalgae (Schizochytrium limacinum) added via milk on performance and antioxidant capacity of dairy calves

Efecto de diferentes cantidades de microalgas (Schizochytrium limacinum) agregadas a través de la leche sobre el rendimiento y la capacidad antioxidante de los terneros lecheros

Recebido: 13/05/2020 | Revisado:19/05/2020 | Aceito:22/05/2020 | Publicado:30/05/2020

Eliana Fasolo
ORCID: https://orcid.org/0000-0003-2920-7497
Curso de Zootecnia, Universidade do Oeste de Santa Catarina, Brasil
E-mail: elianafasolo@hotmail.com
Evandro Peretti
ORCID: https://orcid.org/0000-0002-3910-2579
Curso de Zootecnia, Universidade do Oeste de Santa Catarina, Brasil
E-mail: evandroperetti2009@hotmail.com
Cristiane Regina Tomaluski
ORCID: https://orcid.org/0000- 0001-7363-3931
Curso de Zootecnia, Universidade do Oeste de Santa Catarina, Brasil
E-mail: cris-cristiane20@live.com
Anielen Dutra da Silva
ORCID: https://orcid.org/0000- 0003-2320-8720
Programa de Bioquímica Toxicológica, Universidade Federal de Santa Maria, Brasil
E-mail: anielen.dutra@gmail.com
Nathieli B. Bottari
ORCID: https://orcid.org/0000-0003-0294-9650
Programa de Bioquímica Toxicológica, Universidade Federal de Santa Maria, Brasil
E-mail: nathieli_bb@hotmail.com

Matheus Dellaméa Baldissera

ORCID: https://orcid.org/ 0000-0002-3280-8528

Programa de Farmacologia, Universidade Federal de Santa Maria, Brasil

E-mail: matheusd.biomed@yahoo.com.br

Renan Farina

ORCID: https://orcid.org/0000-0001-9464-7443

Medicina Veterinária, Instituto Federal de Santa Catarina, Brasil

E-mail: renanfarinavet@yahoo.com.br

Wanderson Pereira

ORCID: https://orcid.org/ 0000-0002-3080-1285

Medicina Veterinária, Instituto Federal de Santa Catarina, Brasil

E-mail: wanderson.pereira@ifc.edu.br

Aleksandro Schafer da Silva

ORCID: https://orcid.org/0000-0002-6940-6776

Departamento de Zootecnia, Universidade do Estado de Santa Catarina, Brasil

E-mail: aleksandro_ss@yahoo.com.br

Claiton André Zotti

ORCID: https://orcid.org/0000-0002-6845-9454

Mestrado em Sanidade e Produção Animal, Universidade do Oeste de Santa Catarina, Brasil

E-mail: claiton.zotti@unoesc.edu.br

Resumo

Objetivou-se avaliar se a adição de diferentes níveis de microalgas marinhas (Schizochytrium limacinum) via leite tem efeitos positivos sobre saúde e desempenho de bezerras leiteiras. Foram utilizadas 24 bezerras da raça holandesa, distribuídas em delineamento inteiramente casualizado: sem adição de microalgas, 6 gramas de microalga por dia, 12 gramas de microalga por dia e 18 gramas de microalga por dia fornecidas via leite. Foram realizadas duas coletas de sangue, aos 24 e 42 dias de vida, para avaliar enzimas antioxidantes: glutationa S-transferase (GST), catalase (CAT), superóxido dismutase (SOD) e espécies reativas ao oxigênio (ERO). A suplementação de quantidades crescentes de microalgas não alterou a ingestão de matéria seca (IMS) e desempenho (P>0,05). Aos 21 dias de vida, houve aumento linear para proteína (P = 0,03), globulina (P = 0,006) e SOD (P

= 0,05). Observou-se redução linear para ERO (P = 0,001) e quadrática para imunoglobulina de cadeia pesada (IgG) (P = 0,02) quando as doses de microalgas aumentaram. Próximo ao desmame, aos 42 dias, a inclusão de microalgas aumentou de forma linear os níveis de globulina sérica (P = 0,003), GST (P = 0,002) e SOD (P = 0,02). Por outro lado, atividade enzimática da CAT apresentou efeito quadrático (P = 0,05). A suplementação com microalgas via leite não altera a ingestão e desempenho de bezerras leiteiras. No entanto, o uso como aditivo alimentar na dose de 6 g ao dia é recomendada devido à estimulação das enzimas antioxidantes e redução do níveis séricos de radicais livres.

Palavras-chave: Bezerras; Desempenho; Sistema antioxidante; Sistema imune.

Abstract

This study evaluated the effect of different amounts of marine microalgae (Schizochytrium limacinum) via milk on performance, serum constituents and antioxidant indicators of dairy calves. Twenty-four Holstein calves were distributed to one of four treatments (six replicates) according to a completely randomized design: a control treatment (without microalgae), or microalgae supplementation at 6, 12 and 18 grams of microalgae per day supplied via milk. Two blood samples were taken, at 21 and 42 days of the experiment, to measure activities of antioxidant enzymes: glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD) as well as levels of reactive oxygen species (ROS). The supplementation of increased amount of microalgae did not influence dry matter intake (DMI) and performance (P>0.05). At the 21 days of life, there was a linear increase in serum protein (P = 0.03), globulin (P = 0.006) and in SOD activity (P = 0.05). Linear reduction in ROS (P = 0.001) and a quadratic effect for heavy immunoglobulin G (IgG) (P = 0.02) were observed as the microalgae amounts increased. Close to weaning, at 42 days, serum globulin (P = 0.003), GST (P = 0.002) and SOD (P = 0.02) linearly increased with the inclusion of microalgae. Conversely, enzymatic activity of CAT showed a quadratic effect (P = 0.05). Supplementation of microalgae via milk did not influence intake and performance dairy calves. However, the use of microalgae as a feed additive to dairy calves at amount of 6 g/d is recommended due to its stimulation of antioxidant enzymes and reduction on serum levels of free radicals.

Keywords: Dairy calf; Development; Antioxidant system; Immune system.

Resumen

Este estudio evaluó el efecto de diferentes cantidades de microalgas marinas (Schizochytrium limacinum) a través de la leche sobre el rendimiento, los componentes del suero y los indicadores antioxidantes de los terneros lecheros. Veinticuatro terneros Holstein se distribuyeron a uno de cuatro tratamientos (seis repeticiones) de acuerdo con un diseño completamente al azar: un tratamiento de control (sin microalgas) o suplementos de microalgas a 6, 12 y 18 gramos de microalgas por día suministrados a través de la leche. Se tomaron dos muestras de sangre, a los 21 y 42 días del experimento, para medir las actividades de las enzimas antioxidantes: glutatión S-transferasa (GST), catalasa (CAT), superóxido dismutasa (SOD), así como los niveles de especies reactivas de oxígeno (ROS). La suplementación de una mayor cantidad de microalgas no influyó en la ingesta de materia seca (DMI) y el rendimiento (P> 0.05). A los 21 días de vida, hubo un aumento lineal en la proteína sérica (P = 0.03), la globulina (P = 0.006) y en la actividad de SOD (P = 0.05). Se observó una reducción lineal en ROS (P = 0.001) y un efecto cuadrático para la inmunoglobulina G pesada (IgG) (P = 0.02) a medida que aumentaron las cantidades de microalgas. Cerca del destete, a los 42 días, la globulina sérica (P = 0.003), GST (P = 0.002) y SOD (P = 0.02) aumentaron linealmente con la inclusión de microalgas. Por el contrario, la actividad enzimática de CAT mostró un efecto cuadrático (P = 0.05). La suplementación de microalgas a través de la leche no influyó en la ingesta y el rendimiento de los terneros lecheros. Sin embargo, se recomienda el uso de microalgas como aditivo alimenticio para terneros lecheros en una cantidad de 6 g por día debido a su estimulación de enzimas antioxidantes y la reducción de los niveles séricos de radicales libres.

Palabras clave: Ternero lechero; Desarrollo; Sistema antioxidante; Sistema inmune.

1. Introduction

The breeding phase is the most critical period in dairy cattle farming. During this period, there are high costs incurred, and the animals are susceptible to environmental changes. The provision of adequate nutrition during this period guarantees adequate performance, in addition to improving immune functions that reduce morbidity and mortality rates. Liquid diet is one of the components most responsible for the cost of raising dairy calves, representing at least 70% of the variable costs (Bittar et al., 2016). This fact further emphasizes the importance of seeking alternatives that intensify gains and that

mitigate losses during this phase. Additives such as microalgae, have substances with high biological value, including polyunsaturated fatty acids (PUFA), proteins, pigments, antioxidants, vitamins, and minerals (Christaki et al., 2011). These characteristics have made microalgae attractive nutritional supplements for human and animal food, because polyunsaturated fatty acids found in microalgae cannot be synthesized by humans or other animals, and must be included in the diet (Simopoulos, 2002).

Microalgae added to ruminant feed enriched milk (Franklin et al., 1999; Sinedino et al., 2017) and meat (Phelps et al., 2016; Ponnampalam et al., 2016) with PUFA, in addition to improving the performance and fertility of lactating cows (Moran et al., 2017; Sinedino et al., 2017), and weight gain of cattle (Gutierrez et al., 2016) and confined sheep (Sucu et al., 2016). Because microalgae are rich in PUFA (Meale et al., 2014), identification of the proper inclusion amounts the diet is essential, because reduce feed intake in ruminants (Urrutia et al., 2016; Díaz et al., 2017) may impair antioxidant activity and growth potential. Supplementation of microalgae via milk in pre-ruminants remains poorly investigated (Schimek et al. 2016); nevertheless, it might be a way of microalgae consumption that does not reduce the solid feed intake, which is essential for the development and maturation of pre-stomachs (Diao et al., 2019).

The antioxidant status of suckling calves varies according to the liquid diet, especially with respect to levels of free radicals, with less antioxidant capacity being observed as a result of providing milk replacer (Abuelo et al., 2014) that does not appear to be observed when whole milk is provided (Ranade et al., 2014). Whole milk for calves is more common than milk replacer on Brazilian farms; therefore, even though the antioxidant capacity of whole milk has been reported, our hypothesis was that the addition of microalgae may provide benefits via antioxidant supplementation. Our objective was to evaluate the effect of different amounts of marine microalgae (Schizochytrium limacinum) via milk on performance, serum constituents and antioxidant indicators of dairy calves.

2. Materials and Methods

2.1. Ethics committee

The experiment was carried out in a commercial farm, under the approval of the Ethics Committee on the Use of Animals of the University of the West of Santa Catarina, protocol number CEUA 24/2018.

2.2. Animals and experimental model

Twenty-four Holstein female calves (33.8 ± 4.6 kg) were randomly distributed in individual stalls toa one of four treatments (six replicates) according to a completely randomized design: a control treatment (without microalgae), or microalgae supplementation at 6, 12 and 18 grams of microalgae per day supplied via milk. Calves received 10% of birth weight of colostrum and after the third day they started receiving five liters of whole milk twice a day (splitted in two meals) until weaning at 45 days of age.

The microalgae were supplied via milk during the morning meal. In our study, marine microalgae, *Schizochytrium limacinum* (ALL-G RICH ™, Alltech, Lexington, KY, USA) were used with the following composition: dry matter content (g/kg): 974 ± 1.0; fatty acids (g/ 100 g total fatty acids): 33.2 ± 3.0; C16:0 52.58 ± 0.36; 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; ω-6: 6.56 ± 0.07; ω-3: 30.50 ± 0.29. In addition to whole milk, starter and hay (*Cynodon* spp.) were also provided from the second and third days, respectively. Feed intake of hay and starter and orts were measured weekly.

Chemical analyses of starter and hay (Table 1) were performed at the Animal Nutrition Laboratory of University of Western of Santa Catarina (UNOESC).

Table 1. Chemical composition of starter and hay supplied to calves.

Composition	Starter	Hay
DM (%)	90.2	90.4
CP (% DM)	23.7	10.1
NDF (% DM)	27.7	68.2
ADF (% DM)	5.98	34.8
Ash (% DM)	9.41	8.65

DM: dry matter; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber. Source: Authors.

To perform chemical composition analyses, dry matter (DM) (930.15), crude protein (CP) (954.01) and ash (942.05) were measured as described by the Association of Official Analytical Chemists (AOAC, 1990).

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were performed as described by Van Soest et al. (1991), in polyester bags (Komarek, 1993), in which the samples were subjected to autoclaving at 110 ºC for 40 min (Senger et al., 2008) in a sequential method.

2.3. Sampling

At birth, the animals were weighed on a scale, and the withers height (distance from base of the front feet to the withers) and heart girth (circumference of the chest) measurements of the calves (Khan et al., 2011) were recorded weekly.

Two blood samples per animal from each treatment were performed, via jugular puncture, the first at 21 days of life and the second at 42 days. For this purpose, vacuum tubes containing sodium citrate and EDTA were used as an anticoagulant. An aliquot of the blood was used to determine the hematocrit, with the remainder of the sample being centrifuged at 2000 x *g*, for 20 minutes, at 4 ºC to obtain plasma. After centrifugation of the tube with EDTA, 1 mL plasma was stored in Eppendorf tubes for analysis of the

proteinogram, GST activity and ROS levels. The blood collected in tubes with sodium citrate was frozen to assess the activities of CAT and SOD.

2.4. Pro- and antioxidant variables

Serum ROS levels were measured using the method described by Ali et al. (1992). Volumes of 10 µL of serum were incubated with 12 µL of dichlorofluorescein for 1 mm at 37 °C for 1 h in the dark. Fluorescence was determined using 488 nm for excitation and 520 nm for emission. The results are expressed in U DCF/mg protein.

SOD activity was determined by spectrophotometry in whole blood by inhibiting the rate of adrenochrome formation by autocatalysis (McCord and Fridovich, 1969), and the result was expressed as U SOD/mg of protein.

CAT activity was measured according to Aebi (1984) in whole blood, which determines the rate of decomposition of H_2O_2 in water and oxygen. Specific CAT activity was expressed in U CAT/mg of protein.

Activity of GST in plasma was analyzed by spectrophotometry (340 nm) using the modified method of Habig et al. (1974), using 20 µL of serum mixed with 0.1 M of potassium phosphate buffer, 100 mM glutathione (GSH) and 100 nM 2,4-dinitrochlorobenzene (CDNB), used as a substrate. The results were expressed in µmol CDNB/min/mg protein.

2.5. Proteinogram

For protein fractionation, polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE) was performed, according to a technique suggested by Fagliari et al. (1998) using a mini gel (10 x 10 cm). The gel was stained with Coomassie Blue and photographed to identify and quantify protein fractions using the Labimage 1D software (Loccus Biotechnology). A standard containing fractions with molecular weight between 10 and 250 KD (Kaleidoscope, BIORAD) was used as reference for the identification of protein fractions.

2.6. Statistical analysis

Calves were considered experimental units and were randomly distributed in a completely randomized design. The data obtained were analyzed using the statistical program SAS University Edition.

Orthogonal polynomial contrasts were computed for linear and quadratic effects of microalgae supplementation levels. Tukey's test was used to compare means, with the means being estimated using the least squares method and standard error of the mean, adopting a significance level of 5%.

3. Results

3.1. Performance

Increasing amounts of microalgae added via milk did not significantly alter (P> 0.05) the intake of concentrate and hay, even when considering intake according to the metabolic live weight (Table 2), with the average starter intake of 44.7 g kg $PV^{0.75}$/day and hay of 11.9 g kg $PV^{0.75}$/day.

Table 2. Consumption of concentrate and hay in dry matter during the experimental period calculated by metabolic weight.

	Treatment[1]					P-value	
Variable	0	6	12	18	SEM	L	Q
DMI of starter g/day	851.7	1109	925	1079	69.9	0.49	0.75
g/Kg $^{0.75}$/ day	39.4	47.2	42.1	50.33	2.9	0.35	0.97
DMI of hay g/day	220.7	357.6	253.9	222.5	0.67	0.74	0.21
g/Kg $^{0.75}$/day	10.0	15.9	11.4	10.4	1.2	0.80	0.27
Total DMI (g/kg$^{0.75}$/day total)*	50.1	63.5	53.2	60.2	3.5	0.47	0.60

[1]Amount of microalgae fed to calves 0, 6, 12 or 18 grams/day.SEM: Standard Error of the Mean; L: Linear; Q: Quadratic; DM: Dry Matter; DMI: Dry matter intake; * Means transformed by the square root. Source: Author.

Calves had the same initial weight, withers height, and thoracic perimeter at the beginning of the trial. Weight, withers height, and initial chest circumference at weaning did not differ significantly between treatments. The addition of microalgae via milk did not significantly influence average body weight at weaning, daily weight gain in the first 21 days, from 21 to 42 days, and from birth to weaning (Table 3).

Table 3. Weight, height of withers (HW), thoracic perimeter (TP), performance and average daily gain (ADG) of suckling calves at initial and weaning.

	Treatments[1]					P-value	
Variable	0	6	12	18	SEM	L	Q
Initial body weight (kg)	32.7	37.8	32.7	32.6	0.97	0.53	0.19
Initial HW (cm)	73.8	76.4	72.4	73	0.81	0.37	0.53
Initial TP (cm)	72.4	74.6	71.6	71.4	0.85	0.45	0.51
Body weight at weaning (kg)	60.0	63.4	59.7	60.3	1.31	0.83	0.46
HW at weaning (cm)	84.6	86.1	82.8	84.3	0.68	0.56	0.29
TP at weaning (cm)	88.6	91.1	87.8	87.8	0.77	0.90	0.43
ADG 0 to 21 days (kg/d)	0.50	0.37	0.52	0.49	0.03	0.49	0.25
ADG 21 to 42 days (kg/d)	0.67	0.57	0.60	0.64	0.02	0.79	0.20
Overall ADG (kg/d)	0.61	0.54	0.63	0.64	0.02	0.18	0.20

[1]Amount of microalgae fed to calves 0, 6, 12 or 18 grams/day. SEM: Standard error of mean. P-Value (P<0.05); L: Linear; Q: Quadratic effects. Source: Authors.

3.2. Proteinogram

At the 21 days of life, there was a linear increase in serum protein (P = 0.03) and globulin (P = 0.006) and a quadratic effect for heavy immunoglobulin G (IgG) (P = 0.02) with the inclusion of microalgae, with no significant difference (P > 0.05) for albumin, ceruloplasmin, and light-chain IgG (Table 4).

Table 4. Levels of total protein, albumin, globulin and proteinogram in calves fed with microalgae on days 21 and 42 of experiment.

	Treatment[1]					P-value	
Variable	0	6	12	18	SEM	L	Q
			21 days				
Protein (g/dL)	6.54	5.96	7.41	7.98	0.30	0.03	0.29
Albumin (g/dL)	2.60	2.20	2.43	2.26	0.08	0.34	0.52
Globulin (g/dL)	3.94	3.76	4.97	5.72	0.27	0.006	0.31
Ceruloplasmin (g/dL)	0.44	0.36	0.39	0.31	0.04	0.44	0.95
Heavy-chain IgG (g/dL)	0.40	2.27	0.96	0.99	0.21	0.76	0.02
Light-chain IgG (g/dL)	1.66	1.93	0.98	1.18	0.25	0.34	0.95
			42 days				
Protein (g/dL)	6.91	7.03	7.82	7.52	0.17	0.10	0.54
Albumin (g/dL)	2.80	2.58	2.58	2.38	0.09	0.17	0.98
Globulin (g/dL)	4.11	4.45	5.24	5.15	0.16	0.003	0.42
Ceruloplasmin (g/dL)	0.32	0.38	0.64	0.52	0.07	0.19	0.57
Heavy-chain IgG (g/dL)	1.21	1.11	1.03	1.39	0.17	0.78	0.53
Light-chain IgG (g/dL)	0.97	1.91	1.14	1.89	0.22	0.31	0.82

[1]Amount of microalgae fed to calves 0, 6, 12 or 18 grams/day.SEM: Standard error of mean. P-Value ($P<0.05$); L: Linear; Q: Quadratic effects. Source: Authors.

Close to weaning, at 42 days, serum globulin concentrations increased ($P = 0.003$) linearly with the inclusion of microalgae. No other parameter of the proteinogram changed with increased inclusion of microalgae.

3.3. Oxidant and antioxidant status

At 21 days of life, there was a linear reduction in ROS (P = 0.001) and a linear increase in SOD activity (P = 0.05) with the inclusion of microalgae, with no effect (P> 0.05) on the activities GST or CAT (Table 5).

Table 5. Biomarkers of pro- and antioxidant status in animals that received different levels of *Schizochytrium* sp. expressed in two collections during the experiment: glutathione S-transferase (GST: µmol CDNB/min/mg protein), oxygen-reactive species (ROS: U DCF/mg protein), catalase (CAT: U CAT/mg protein) and superoxide dismutase (SOD: U SOD/mg protein).

	Treatment[1]					P-value	
Variable	0	6	12	18	SEM	L	Q
			21 days				
Plasma GST	51.9	55.4	60.1	56.3	3.03	0.24	0.26
Plasma ROS	2130.8	1589.5	1176.4	1027	196.7	0.001	0.35
Blood CAT	3.69	3.64	3.67	3.52	0.10	0.36	0.68
Blood SOD	34.9	35.4	36.9	40.4	1.84	0.05	0.44
			42 days				
Plasma GST	49.5	47.7	64.4	63	3.42	0.002	0.94
Plasma ROS	1563.5	1074.8	1301.7	1501.3	171.8	0.96	0.06
Blood CAT	3.43	3.67	3.63	3.45	0.09	0.99	0.05
Blood SOD	26.8	40.3	37.6	42.5	11.8	0.02	0.26

[1]Amount of microalgae fed to calves 0, 6, 12 or 18 grams/day. P-Value (P <0.05); SEM: Standard Error of the Mean; L: Linear; Q: Quadratic; GST: glutathione peroxidase; ROS: Reactive Oxygen Species; CAT: Catalase; SOD: Superoxide Dismutase.
Source: Authors.

A linear increase was observed in the activities of GST (P = 0.002) and SOD (P = 0.02) after the inclusion of microalgae after 42 days of experiment. The enzymatic activity of CAT showed a quadratic effect (P = 0.05) and the ROS levels (P = 0.06) tended to a quadratic response with the inclusion of microalgae.

4. Discussion

Similar to the DMI and performance observed in the present trial, the addition of 4 and 6 g of microalgae (S. limacinum) via milk did not significantly change DMI, body weight, or daily gain, both in pre- and post-weaning (Schimek et al. 2016). The addition of increasing amounts (0, 2, 6, and 25 g/d of microalgae/day) of microalgae (Spirulina platensis) via milk also did not significantly change the parameters of performance and DM consumption during the lactation period (Heidarpour et al. 2011), demonstrating that the addition of this microalgae had no direct effect on growth during the experimental period.

Because of the scarcity of data with supplementation of microalgae via milk in the diet of calves, its use by mixing in the concentrate has been researched in various species and categories of ruminants, especially the use of microalgae as a protein source (Lodge-Ivey et al., 2014; Rjiba-Ktita et al., 2019). In feedlot, the use of microalgae as a partial substitution of energy sources such as corn and soybean hull tended to increase the average daily gain and there was a linear increase in DMI with the increase in the inclusion of microalgae in the diet (Van Emon et al., 2015). For confined lambs, the addition of 5 g/d of microalgae increased the average daily weight gain, without altering the DMI (Sucu et al., 2017). Higher doses of microalgae (up to 3% of the DM of the diet) did not change the DMI and average daily gain in lambs (Meale et al., 2014). Some reasons for changes in the DMI of the diet when microalgae are used were summarized by Altomonte et al. (2018). The authors highlight the type of microalgae and its composition, the reduction in fiber digestibility and the change in rumen fermentation due to the PUFA content. Despite the high digestibility found in diets with microalgae mixed with the concentrate (Van Emon et al. 2015), it appears that the offer of this ingredient via milk does not benefit the performance of pre-ruminant animals. Nevertheless, the use of microalgae via whole milk and their metabolism in pre-ruminants remain unknown.

At 21 days of experiment, a significant increase in plasma levels of protein and globulin was observed in animals that received increasing doses of microalgae, which may

represent a positive effect on animal health. Globulins are a heterogeneous group of serum proteins that include lipoproteins, acute phase proteins and immunoglobulins, and are typically classified as alpha, beta or gamma (Tothova et al., 2016).

According to this author, decreased levels of globulin are related to liver damage, hemorrhages, and immune deficiency. Based on the increase in globulin levels observed in this study, seems that the inclusion of microalgae does not cause liver damage and can stimulate immune responses. In addition, the increase in serum albumin levels can also be considered an indicator of liver health, because albumin is exclusively synthesized by the liver, and its decrease indicates liver damage (Carvalho and Machado, 2018). As an example, a study conducted by Murthy et al. (2005) and Riad et al. (2019) reported that the addition of carotenoids from Spirulina platensis increased serum levels of proteins and albumin, conferring hepatoprotective activity in Wistar rats.

We expected that, due to the linear increase in globulins, there would also be a linear increase in heavy chain immunoglobulins as observed in other studies in calves (Tomasi et al., 2018; Volpato et al., 2018). Instead, a quadratic effect on day 21 was observed, where only the lowest dose (6 grams) increased levels of heavy-chain IgG. Heavy-chain IgGs are immunoglobulins A, M, G, and E, that are active in humoral immunes response (Volpato et al., 2018).

No differences on light-chain IgG and ceruloplasmin levels among treatments suggests that increases in globulins might be related to specific immunoglobulins or acute phase proteins such as C-reactive protein, haptoglobin, and amyloid, among others, all of which play important roles in the immune system in terms of restoration of homeostasis and the reduction of microbial growth before the animals acquire natural immunity (Murata et al., 2004).

Analyses of these variables individually could explain the increase in globulins observed here; previous studies showed that microalgae in animal feed stimulate the immune system (Riccio and Lauritano, 2019). For example, a group of goats supplemented with Spirulina had their immunoglobulin concentrations and cell-mediated immune responses against PHA-p increased (Yadav and Kumar, 2018). Considering the differences between S. limacinum and others microalgaes, the concentration of albumin and immunoglobulins of dairy suckling calves were also not affected when Spirulina platensis was fed (Ghattas et al., 2019).

In the present study, the inclusion of microalgae improved serum antioxidant status of dairy calves. This may represent an important effect in improving animal health status. Serum levels of ROS, one of the most harmful free radicals to the body, were significantly lower on day 21 in calves receiving microalgae, suggesting a lower production or better ability to eliminate this free radical, as observed by Sharma et al. (2019) in fish supplemented with the Ascochloris spp.

In general, the activity of two important antioxidant defense system enzymes was significantly increased after inclusion of microalgae, i.e., SOD and GST. According to Basu et al. (2009), GST plays an important role in the defense of cells against oxidative stress because it acts to reduce levels of hydrogen peroxide, while high concentrations are associated with a greater risk of oxidative imbalance and production of free radicals. Conversely, the increase in the enzymatic activity of SOD represents an improvement in the process of dismutation of hydrogen superoxide to hydrogen peroxide (Abreu and Cabelli, 2010). Sources of PUFA provide large amounts of antioxidants, reducing oxidation by inhibiting lipid peroxidation (Prisacaru, 2016).

Antioxidants slow or prevent oxidative damage, blocking oxidation reactions and offering protection to membranes and other parts of cells. These facts suggest that the addition of microalgae as a source of PUFA promoted improvements in the animals' antioxidant systems, health and could help calves during the transition period of weaning and post-weaning periods. Due to the potential of microalgae to improve calves antioxidant system, further studies should focus on the use of microalgae at critical window of development as in the pre-weaned calves time.

5. Final Considerations

The addition of S. limacinum microalgae does not influence dry matter intake, weight gain, and development in calves, regardless of inclusion.

The use of microalgae as a feed additive to dairy calves at amount of 6 g/d is recommended due to its stimulation of antioxidant enzymes and reduction on serum levels of free radicals, as well as providing an increase in globulins, important in the immune response of calves.

Conflict of Interest

There is no perceived conflict of interest.

Financial Support and Acknowledgments

We thank to the Programa de Mestrado em Sanidade e Produção Animal da Unoesc and Alltech for donating the product for research.

References

Abreu IA & Cabelli DE (2010). Superoxide dismutases—a review of the metal-associated mechanistic variations. *Biochimica et Biophysica Acta (BBA)- Proteins and Proteomics*, *1804*(2), 263-74.

Abuelo Á, Pérez-Santos M, Hernández J & Castillo C (2014). Effect of colostrum redox balance on the oxidative status of calves during the first 3 months of life and the relationship with passive immune acquisition. *The Veterinary Journal*, *199*(2), 295-9.

Aebi, H. (1984). [13] Catalase in vitro. In *Methods in enzymology* (Vol. 105, pp. 121-126). Academic Press.

Ali SF, LeBel CP & Bondy SC (1992). Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. *Neurotoxicology*, *13*(3), 637-48.

Altomonte I, Salari F, Licitra R & Martini M (2018). Use of microalgae in ruminant nutrition and implications on milk quality–A review. *Livestock Science*, *214*, 25-35.

Association of Official Analytical Chemists (1990). *Official methods of analysis of AOAC International*.

Basu TK, Temple NJ & Garg ML. (Eds.) (1999). *Antioxidants in human health and disease*. CABI Pub..

Bittar CMM, Ferreira LS & Silva JTD. (2016). Sucedâneos lácteos para bezerras leiteiras. *Cadernos Técnicos de Veterinária e Zootecnia (UFMG)*, (81), 56-74.

Carvalho JR & Machado MV (2018). New insights about albumin and liver disease. *Annals of Hepatology*, *17*(4), 547-60.

Christaki E, Florou-Paneri P & Bonos E. (2011). Microalgae: a novel ingredient in nutrition. *International Journal of Food Sciences and Nutrition*, *62*(8), 794-9.
Diao Q, Zhang R & Fu T (2019). Review of Strategies to Promote Rumen Development in Calves. *Animals*, *9*(8), 490.

Díaz MT, Pérez C, Sánchez CI, Lauzurica S, Cañeque V, González C & La Fuente J (2017). Feeding microalgae increases omega 3 fatty acids of fat deposits and muscles in light lambs. *Journal of Food Composition and Analysis*, *56*, 115-23.

Fagliari JJ, Santana AE, Lucas FA, Campos E & Curi PR (1998). Constituintes sangüíneos de bovinos recém-nascidos das raças Nelore (Bos indicus) e Holandesa (Bos taurus) e de bubalinos (Bubalus bubalis) da raça Murrah. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 253-62.

Franklin ST, Martin KR, Baer RJ, Schingoethe DJ & Hippen AR (1999). Dietary marine algae (Schizochytrium sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. *The Journal of Nutrition*, *129*(11), 2048-54.

Ghattas TA, Dawoud EN, Mahrous AF, Elgabry EA (2019). Effect of *Spirulina platensis* supplementation on growth, some biochemical and immunological parameters in suckling calves. *Egyptian Veterinary Medical Association*, *79*:443–60.

Gutierrez BH, Alvarez E, Arrizon AA, Carrasco R, Salinas-Chavira J & Zinn RA (2016). Influence of high-oil algae biomass as a feed intake and growth-performance enhancer in feedlot cattle during period of high ambient temperature. *Journal of Applied Animal Research*, *44*(1), 118-20.

Habig WH, Pabst MJ & Jakoby WB (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, *249*(22), 7130-39.

Heidarpour A, Fourouzandeh-Shahraki AD & Eghbalsaied S (2011). Effects of Spirulina platensis on performance, digestibility and serum biochemical parameters of Holstein calves. *African Journal of Agricultural Research*, *6*(22), 5061-65.

Khan MA, Weary DM & Von Keyserlingk MAG. (2011). Hay intake improves performance and rumen development of calves fed higher quantities of milk. *Journal of Dairy Science*, *94*(7), 3547-53.

Komarek AR (1993). A filter bag procedure for improved efficiency of fiber analysis. *Journal of Dairy Science*, *76*(suppl 1), 250-9.

Lodge-Ivey SL, Tracey LN & Salazar A (2014). Ruminant Nutrition Symposium: The utility of lipid extracted algae as a protein source in forage or starch-based ruminant diets. *Journal of Animal science*, *92*(4), 1331-42.

McCord, J. M., & Fridovich, I. (1969). Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). *Journal of Biological chemistry*, *244*(22), 6049-6055.

Meale SJ, Chaves AV, He ML & McAllister TA (2014). Dose–response of supplementing marine algae (Schizochytrium spp.) on production performance, fatty acid profiles, and wool parameters of growing lambs. *Journal of Animal Science*, *92*(5), 2202-13.

Moran CA, Fusconi G, Morlacchini M & Jacques KA (2017). Changes in docosahexaenoic acid (DHA) content in longissimus dorsi and backfat tissue of finishing pigs given diets containing 1% heterotrophically grown algae during the last 28 days. *Journal of Animal Science*, *95*, 59.

Murata H, Shimada N & Yoshioka M (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. *The Veterinary Journal*, *168*(1), 28-40.

Murthy KC, Rajesha J, Swamy MM & Ravishankar GA (2005). Comparative evaluation of hepatoprotective activity of carotenoids of microalgae. *Journal of Medicinal Food*, *8*(4), 523-8.

Phelps KJ, Drouillard JS, O'Quinn TG, Burnett DD, Blackmon TL, Axman JE ... & Gonzalez JM (2016). Feeding microalgae meal (All-G Rich™; Schizochytrium limacinum CCAP 4067/2) to beef heifers. II: Effects on ground beef color and palatability. *Journal of Aanimal Science*, *94*(9), 4030-4039.

Ponnampalam, E. N., Burnett, V. F., Norng, S., Hopkins, D. L., Plozza, T., & Jacobs, J. L. (2016). Muscle antioxidant (vitamin E) and major fatty acid groups, lipid oxidation and retail colour of meat from lambs fed a roughage based diet with flaxseed or algae. *Meat Science*, *111*, 154-160.

Prisacaru, A. E. (2016). Effect of antioxidants on polyunsaturated fatty acids–review. *Acta Scientiarum Polonorum Technologia Alimentaria*, *15*(2), 121-129.

Ranade, R., Talukder, S., Muscatello, G., & Celi, P. (2014). Assessment of oxidative stress biomarkers in exhaled breath condensate and blood of dairy heifer calves from birth to weaning. *The Veterinary Journal*, *202*(3), 583-587.

Riccio, G., & Lauritano, C. (2020). Microalgae with Immunomodulatory Activities. *Marine Drugs*, *18*(1), 2.

Riad, W. A., Elsadany, A. Y., & EL-diahy, Y. M. (2019). Effect of Spirulina platensis Microalga Additive on Performance of Growing Friesian Calves. *Journal of Animal and Poultry Production*, *10*(2), 35-40.

Rjiba-Ktita, S., Chermiti, A., Valdés, C., & López, S. (2019). Digestibility, nitrogen balance and weight gain in sheep fed with diets supplemented with different seaweeds. *Journal of Applied Phycology*, *31*(5), 3255-3263.

Senger, C. C., Kozloski, G. V., Sanchez, L. M. B., Mesquita, F. R., Alves, T. P., & Castagnino, D. S. (2008). Evaluation of autoclave procedures for fibre analysis in forage and concentrate feedstuffs. *Animal Feed Science and Technology*, *146*(1-2), 169-174.

Sharma, S., Shah, E., Davla, D., Dixit, G., Patel, A., & Kumar, A. K. (2019). Effect of microalga-based diet on oxidative stress enzymes of African catfish, Clarias gariepinus. *International Aquatic Research*, *11*(4), 377-387.

Schimek, D., Ziegler, B., Ziegler, D., & Chester-Jones, H. (2016). 1215 Performance and health of calves pre-and post-weaning when fed milk replacer supplemented with algae. *Journal of Animal Science*, *94*(suppl_5), 583-584.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy*, *56*(8), 365-379.

Sinedino, L. D., Honda, P. M., Souza, L. R., Lock, A. L., Boland, M. P., Staples, C. R., ... & Santos, J. E. (2017). Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. *Reproduction*, *153*(5), 707-723.

Sousa, I., Gouveia, L., Batista, A. P., Raymundo, A., & Bandarra, N. M. (2008). Microalgae in novel food products. *Food Chemistry Research Developments*, 75-112.

Sucu, E., Udum, D., GÜNEŞ, N., CANBOLAT, Ö., & Filya, I. (2017). Influence of supplementing diet with microalgae (Schizochytrium limacinum) on growth and metabolism in lambs during the summer. *Turkish Journal of Veterinary and Animal Sciences*, *41*(2), 167-174.

Tomasi, T., Volpato, A., Pereira, W. A. B., Debastiani, L. H., Bottari, N. B., Morsch, V. M., ... & Da Silva, A. S. (2018). Metaphylactic effect of minerals on the immune response, biochemical variables and antioxidant status of newborn calves. *Journal of Animal Physiology and Animal Nutrition*, *102*(4), 819-824.

Tothova, C., Nagy, O., & Kovac, G. (2016). Serum proteins and their diagnostic utility in veterinary medicine: a review. *Veterinární Medicína*, *61*(9), 475-496.

Urrutia, O., Mendizabal, J. A., Insausti, K., Soret, B., Purroy, A., & Arana, A. (2016). Effects of addition of linseed and marine algae to the diet on adipose tissue development, fatty acid profile, lipogenic gene expression, and meat quality in lambs. *PloS one*, *11*(6).

Van Emon, M. L., Loy, D. D., & Hansen, S. L. (2015). Determining the preference, in vitro digestibility, in situ disappearance, and grower period performance of steers fed a novel algae meal derived from heterotrophic microalgae. *Journal of Aanimal Science*, *93*(6), 3121-3129.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *Journal of Dairy Science*, *74*(10), 3583-3597.

Volpato, A., Da Silva, A. S., Crecencio, R. B., Tomasi, T., Fortuoso, B. F., Ribeiro, M. P., ... & Morsch, V. M. M. (2018). A prophylactic protocol to stimulate the immune response also controls infectious disease and, consequently, minimizes diarrhea in newborn heifers. *Microbial Pathogenesis*, *121*, 262-268.

Yadav, J., & Kumar, R. (2019). Effect of Spirulina (Spirulina platensis) Supplementation on Nutrient Intake, Digestibility and Rumen Fermentation Pattern in Barbari Goats. *Animal Nutrition and Feed Technology*, *19*(2), 267-273.

Percentage of contribution of each author in the manuscript

Eliana Fasolo – 20%

Evandro Peretti - 10%

Cristiane Tomaluski – 10%

Anielen Dutra da Silva – 5%

Nathieli B. Bottari – 5%

Matheus Dellaméa Baldissera – 5%

Renan Farina – 5%

Wanderson Pereira – 10%

Aleksandro Schafer da Silva - 10%

Claiton André Zotti - 20%