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Homeopathic immunological for dairy calves and its effects on metabolism, immune reactions, serum antioxidant responses, and growth performance

Imunológico homeopático para bezerros leiteiros e seus efeitos no metabolismo, reações imunes, respostas antioxidantes séricas e desempenho de crescimento

Inmunología homeopática para terneros lecheros y sus efectos sobre el metabolismo, las reacciones inmunitarias, las respuestas antioxidantes séricas y el rendimiento del crecimiento

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Maksuel Gatto de Vitt
ORCID: https://orcid.org/0000-0002-3989-8554
Universidade do Estado de Santa Catarina, Brasil
E-mail: mak-witt@hotmail.com
Aline Luiza do Nascimento
ORCID: https://orcid.org/0000-0002-6242-2028
Universidade do Estado de Santa Catarina, Brasil
E-mail: alineluizan@outlook.com
Rafael Vinicius Pansera Lago
ORCID: https://orcid.org/0000-0001-6059-7249
Universidade do Estado de Santa Catarina, Brasil
E-mail: rafael.lago@edu.udesc.br
Raissa Alves de Carvalho
ORCID: https://orcid.org/0000-0002-5047-6571
Instituto Federal de Santa Catarina, Brasil
E-mail: raissaalvescarvalho@ifc.br
Wanderson A. B. Pereira
ORCID: https://orcid.org/0000-0002-3080-1285
Instituto Federal de Santa Catarina, Brasil
E-mail: wanderson.pereira@ifc.edu.br
Aleksandro Schafer da Silva
ORCID: https://orcid.org/0000-0002-9860-1933
Universidade do Estado de Santa Catarina, Brasil
E-mail: aleksandro.silva@udesc.br

Abstract
This study determined whether adding an immunological homeopathic product (Imunorgan®) to the calf diet during the first months of life improves immune reactions, antioxidant responses, metabolism, and growth. The 12 calves with an average of 36±4 kg and 30 days of life, were divided into a control (n=6) and treatment (n=6) group who received the homeopathic product in milk during the first 30 days and subsequently in the concentrate (providing 10 g/day of the immunological homeopathic, based on *Arsenicum album, Baptisia tinctoria*, *Calcarea carbonica, Iodum purum, Medicago sativa, Mercurius solubilis, Nux vomica, Pyrogenium*, and sucrose vehicle). The animals in the treatment group had more significant body weight gain from 1 to 30 days of the experiment ($p \leq 0.05$). There was a treatment effect on hematologic results, with hemoglobin concentration showing major treatment x day interaction on day 90 ($p \leq 0.05$). Leukocyte, neutrophil, and lymphocyte counts were higher in the treatment group ($p \leq 0.05$). The albumin concentration in the treatment group animals was lower than in the control group ($p \leq 0.05$). The globulin concentration was higher in the treatment group ($p \leq 0.05$). There was a treatment x day interaction for the concentration of protein thiols on days 30, 60, and 90, with higher serum levels in calves in the treatment group ($p \leq 0.05$). Serum levels of reactive oxygen species were lower in calves of the treatment group ($p \leq 0.05$). The animals of the treated group showed a higher concentration of IgA. During the experiment, levels of ceruloplasmin, haptoglobin, transferrin, acid glycoprotein, and light-chain IgG also changed (treatment x day interaction). These findings suggest that the homeopathic product stimulated immunological responses.
Keywords: Dairy cattle; Performance; Immunity.

Resumo
Este estudo determinou se a adição de um agente homeopático imunológico (Imunorgan®) à dieta de bezerros durante os primeiros meses de vida melhora as reações imunológicas, respostas antioxidantes, metabolismo e crescimento. Os 12 bezerros com 36±4 kg de média e 30 dias de vida, foram divididos em grupo controle (n=6) e tratamento (n=6) que receberam o produto homeopático no leite durante os primeiros 30 dias e posteriormente no concentrado (fornecendo

10 g/dia do homeopático imunológico, baseado em *Arsenicum album, Baptisia tinctoria, Calcarea carbonica, Iodum purum, Medicago sativa, Mercurius solubilis, Nux vomica, Pyrogenium* e veículo de sacarose). Os animais do grupo tratamento tiveram ganho de peso corporal mais significativo de 1 a 30 dias do experimento (p≤0,05). Houve efeito do tratamento nos resultados hematológicos, com concentração de hemoglobina mostrando maior interação tratamento x dia no dia 90 (p≤0,05). As contagens de leucócitos, neutrófilos e linfócitos foram maiores no grupo de tratamento (p≤0,05). A concentração de albumina nos animais do grupo tratamento foi menor do que no grupo controle (p≤0,05). A concentração de globulina foi maior no grupo de tratamento (p≤0,05). Houve interação tratamento x dia para a concentração de tióis proteicos nos dias 30, 60 e 90, com níveis séricos mais elevados nos bezerros do grupo tratamento (p≤0,05). Os níveis séricos de espécies reativas de oxigênio foram menores nos bezerros do grupo de tratamento (p≤0,05). Os animais do grupo tratado apresentaram maior concentração de IgA. Durante o experimento, os níveis de ceruplasmina, haptoglobina, transferrina, glicoproteína ácida e IgG de cadeia leve também mudaram (interação tratamento x dia). Esses achados sugerem que o produto homeopático estimulou respostas imunológicas.
Palavras-chave: Gado leiteiro; Atuação; Imunidade.

Resumen
Este estudio determinó si agregar un agente homeopático inmunológico (Imunorgan®) a la dieta de los terneros durante los primeros meses de vida mejora las reacciones inmunológicas, las respuestas antioxidantes, el metabolismo y el crecimiento. Los 12 terneros, con un promedio de 36 kg y 30 días de vida, se dividieron en grupo control (n=6) y tratamiento (n=6) quienes recibieron el producto homeopático en leche durante los primeros 30 días y posteriormente en el concentrado (aportando 10 g/día del homeopático inmunológico, a base de sobre Arsenicum album, Baptisia tinctoria, Calcarea carbonica, Iodum purum, Medicago sativa, Mercurius solubilis, Nux vomica, Pyrogenium y vehículo de sacarosa). Los animales en el grupo de tratamiento tuvieron una ganancia de peso corporal más significativa de 1 a 30 días del experimento (p≤0.05). Hubo un efecto del tratamiento sobre los resultados hematológicos, mostrando la concentración de hemoglobina más grande interacción tratamiento x día en el día 90 (p≤0.05). Los recuentos de leucocitos, neutrófilos y linfocitos fueron mayores en el grupo de tratamiento (p≤0,05). La concentración de albúmina en los animales del grupo tratamiento fue menor que en el grupo control (p≤0.05). La concentración de globulina fue mayor en el grupo de tratamiento (p≤0.05). Hubo interacción tratamiento x día para la concentración de tioles proteicos en los días 30, 60 y 90, con niveles séricos más altos en los terneros del grupo de tratamiento (p≤0.05). Los niveles séricos de especies reactivas de oxígeno fueron menores en los terneros del grupo de tratamiento (p≤0.05). Los animales del grupo tratado mostraron una mayor concentración de IgA. Durante el experimento, los niveles de ceruloplasmina, haptoglobina, transferrina, glicoproteína ácida e IgG de cadena ligera también cambiaron (interacción tratamiento x día). Estos hallazgos sugieren que el agente homeopático estimuló las respuestas inmunológicas.
Palabras clave: Ganado lechero; Actuación; Inmunidad.

1. Introduction

Calf breeding is critical to dairy cattle systems because it seeks to improve the herd and replace discarded animals (Barkema et al., 2015). Animal replacement is expensive because of the low value of discarded animals, while the investment costs associated with replacement animals are high (De Vries & Marcondes, 2020). Comparing breeding and external acquisition of spare animals, breeding on the farmer is the lowest cost option, where the animals are already adapted to the farm's environment (Leroy et al., 2016). The focus on breeding calves is meant to decrease mortality and disease incidence during their early life, doubling birth weight at weaning and achieving sexual maturity early, which is economically feasible (Ring et al., 2018). Pre-delivery, colostrum production, navel disinfection, and milk intake are critical for calf breeding and development (Ferrazza et al., 2018). Maternal loss occurring after birth creates enormous challenges for the calf. To reduce this impact, passive immunity through the intake of colostrum in the first hours of life is essential and helps to boost the immune system to combat environmental and pathogenic agents (Hurley & Theil, 2011). In the first three months of life, there is a concern regarding stimulating animals' immune responses; for this reason, there are several commercially available products for this purpose.

Homeopathic remedies with immunological properties are commercially available and contribute to company revenue. Despite the lack of scientific studies, homeopathic remedies are increasingly common in animal production because they improve animal performance and help combat diseases (Doehring & Sundrum, 2016). Homeopathy is based on the theory that agents possess healing properties during the preparation of an "active" component due to the transfer of this biological activity from the active component to the diluent or even by the transformation of the diluent. In other words, the higher the

dilution and succussion, the more potent the healing power (Lees et al., 2017). Some of these agents are developed to boost immunity and inhibit disease progression (Şenel, 2019). Homeopathic products can be produced from vegetables, minerals, and animals (Cermin et al., 2019) and act as preventive and curative agents. The literature presents many types of research using homeopathy in animals in this context. However, there are few high-quality randomized in vivo experiments involving veterinary homeopathy (Mathie & Clausen, 2014). Therefore, the present study aimed to determine whether adding an immunological homeopathic to the diet of dairy calves can stimulate immunity and antioxidant responses, modulate metabolism, and favor growth.

2. Methodology

The project was approved by Committee for the Use of Animals in Research (CEUA) of the University of the State of Santa Catarina (UDESC), under protocol number 2877070619.

2.1 Homeopathic product

We used a commercially-available product (Imunorgan®, Organica Homeopatia, Chapecó, Santa Catarina, Brazil). The homeopathic was prepared using a centesimal scale (CH = centesimal Hahnemannian) and was formulated based on *Arsenicum album* (potency 30 CH), *Baptisia tinctoria* (potency 30 CH), *Calcarea carbonica* (potency 30 CH), *Iodum purum* (potency 30 CH), *Medicago sativa* (potency 30 CH), *Mercurius solubilis* (potency 30 CH), *Nux vomica* (potency 30 CH), *Pyrogenium* (potency 30 CH), and vehicle (sucrose q.s.p - 10 kg). The commercial product is marketed to animals, and the dose of 10 grams per calves per day was used according to the manufacturer's instructions.

2.2 Animals, installation, and feeding

The experiment was carried out in an experimental station in southern Brazil (Guatambú, Santa Catarina). The animals were housed in a wooden shed covered with fiber cement tiles, with side shade screens and a perforated floor. The animals were placed in 12 individual stalls containing a feeder and a drinking trough. Sanitary management was carried out daily, including cleaning the drinking troughs and the removal of feces.

The 12 Jersey calves (females), with an average weight of 36±4 kg and 30 days of life, were purchased from a local rural breed producer (27° 4' 20" South; 53° 9' 29" West). All calves were daughters of the same bull, therefore, they had genetic similarity. The animals received colostrum on the farmer where they were born during the first six hours of life and were transported to the experimental station of the university when they were between 15 and 20 days old. The pre-experiment feed consisted of cow's milk (whole milk) and concentrate. In the first 30 days of the experiment (corresponding to 30 to 60 days of life), the animals were fed with cow's milk (4 L/animal/day), concentrate, and hay ad libitum. The withdrawal of milk from the diet was gradual and occurred between the 30th and 35th days, reducing the amount of whole milk per day until the milk was suspended from feeding on the 36th day of the experiment (the 66th day of life). From that date, the supplied concentrate and hay were made available ad libitum. The milk was obtained from a herd with a predominance of Jersey breed animals; the hay provided was Tifton 85 (Cynodon spp.); the concentrate contained 22% protein, formulated based on ground corn, soybean meal, wheat bran, and vitamin-mineral premix according to the nutritional requirements of this phase of life (NRC 2001; Table 1).

Table 1 - Feed supplied to calves during the suckling (Stage I: 1–30 days) and post-suckling (Stage II: 36 to 90) phases.

Feed	Days 1 to 30	Days 36 to 90
Cow milk[1]	4.0 (L/animal/d)	-
Hay[2]	Ad libitum	Ad libitum
Concentrate[3]	Ad libitum	Ad libitum

Note 1: Fresh whole milk purchased from the farm with a herd of Jersey cows.
Note 2: The hay used in our experiment was Tifton. Based on the amount of hay available, it was estimated that, in the first 30 days of the experiment, the average consumption per animal was 200 g/animal/day; between days 31 and 60, it was 450 g/animal/day; in the final 30 days of the study, consumption was on average 900 g/animal/day.
Note 3: The ingredients used in the production of the concentrate were: ground corn grain (260 g/kg), soybean bran (260 g/Kg), wheat bran (180 g/Kg), rice bran (150 g/kg), soybean meal micronized low fiber (100 g/kg), whey permeate (5 g/kg), whey powder (5 g/kg), and vitamin-mineral premix (40 g/kg).
Source: Authors.

2.3 Experimental design

The calves were randomly divided into two groups, where each animal was considered an experimental unit. The control group (n = 6) were animals that received milk or concentrate without the homeopathic product, with only sucrose as a vehicle at the same dose (10 g/animal/day). The treatment group (n = 6) were animals that ingested 10 g/animal/day of the homeopathic immunological agent (Imunogan®, Organica Homeopatia), made available once a day through the feed (milk or concentrate). In the milk, the product was diluted and supplied to the animals through bottles, in this way the animal consumed the entire product, when the liquid diet period ended, the homeopathic product was inserted next to the concentrate, which was consumed in its entirety.The experiment lasted 90 days, with 30 days of liquid (cow's milk – 4 L/animal/day) and solid diet (concentrate ad libitum) between days 30 and 35 of the feed transition period (the amount of milk was gradually reduced over time). Between days 36 and 90, the animals were fed only solid feeds (concentrate and chopped Tifton hay). Milk was provided twice a day at 08:00 and 17: 00 at 37 ºC, while concentrate and hay were provided ad libitum. The feed composition is detailed in Table 2.

2.4 Calves performance

The calves were weighed on a digital scale (Linear®, Chapecó, Brazil) on days 1, 15, 30, 60, and 90 of experimental period. The weighing occurred in the morning with the animals fasting. Concentrate consumption was evaluated on the same days as weighing because it was provided ad libitum, based on the amount of concentrate ingested per animal.

2.5 Sample collection

Blood collection was performed on days 1, 15, 30, 60, and 90 of the experimental period. Samples were collected from the jugular vein using two vacuum tubes, with ethylenediamine tetraacetic acid anticoagulant (EDTA K2 K3 Na2 Plastic Blood Sample Collection Tube Medical Consumables®, China) and without anticoagulant (Serum Vacuum Blood Collection Tubes®, China). The samples were stored in a thermal box at 10 ºC during transport to the laboratory which lasted 1 hour. Subsequently, samples without anticoagulants were centrifuged (Mylabor®, Daiki, São Paulo, Brazil) at 865 g for 10 minutes to obtain serum, while samples with anticoagulants were used in the blood count analysis.

Table 2 - Chemical composition analyzed of the feed consumed by the Jersey calves during the experiment.

Feed	Chemical composition (g/kg)
Cow milk	
Fat	62.3
Lactose	47.1
Protein	39.8
Total solid	149.2
Concentrate	
Dry matter (g/kg)	922
Crude protein (g/kg)	216
Ether extract (g/kg)	26.4
NDF (g/kg)	189
ADF (g/kg)	91.5
Ash (g/kg)	45.0
Hay	
Dry matter (g/kg)	897
Crude protein (g/kg)	136
Ether extract (g/kg)	15.7
NDF (g/kg)	376
ADF (g/kg)	77.1
Ash (g/kg)	93.8

Note: NDF = Neutral detergent fiber; ADF = Acid detergent fiber. Source: Authors.

2.6 Laboratory analysis

2.6.1 Feed analysis

Milk composition analysis was performed using semi-automatic equipment (Milk Meter® devices, Germany). The levels of fat, protein, lactose, and total solids were determined; results expressed in g/kg.

The analyses of samples were pre-dried for 72 h at 55ºC, milled (Willey mill, 1mm) and subjected to analysis for determination of dry matter content (DM, 930.15), crude protein (CP, 984.13) was obtained by multiplying the result by 6.25 and the result was expressed in g/kg DM, ash (MM, 942.05) g/kg DM, according to Silva and Queiroz (2002). The NDF (NDFom, method number 2002.04) and ADF (ADFom, method number 973.18) contents were analysed according to Van Soest (1994) expressed exclusive of residual ash and without amylase. The results are displayed in Table 2.

2.6.2 Hemogram

The counts of erythrocytes and leukocytes and hemoglobin concentration were measured on a semi-automatic blood cell counter (model CELM CC530, São Paulo, Brazil). Subsequently, the leukocyte differential was calculated by reading stained blood smears using a Rapid Panotic kit (Laborclin Ltda, São Paulo, Brazil). The hematocrit was measured using microcapillary tubes centrifuged at 14000 rpm for 5 minutes.

2.6.3 Serum biochemistry analysis - metabolism

According to the manufacturers' recommendations, total serum protein, albumin, urea, cholesterol, and glucose levels were measured using specific commercial kit to each variable (Gold Analisa Diagnóstica Ltda®, Belo Horizonte, Minas Gerais, Brazil) and semi-automatic equipment (BIO PLUS 2000®, Bioplus Produtos para Laboratórios Ltda, Baruiri, Brazil). Globulin levels were calculated using the equation: globulin = total proteins – albumin (Kaneco et al., 2008).

2.6.4 Oxidative status

Serum glutathione S-transferase (GST) activity was measured based on the method described by Habig et al. (1974) and was expressed as U GST/mg of protein. Levels of protein thiols (PSH) were evaluated according to Sedlak and Lindsay (1968), and the results were expressed as nmol SH/mg of protein. Serum superoxide dismutase (SOD) activity was evaluated spectrophotometrically as described by Marklund and Marklund (1974), based on superoxide anion inhibition in the presence of pyrazolyl. Enzyme activity was expressed as units SOD/mg of protein.

Serum lipid peroxidation was measured as the amount of thiobarbituric acid reactive substances (TBARS) according to Jentzsch et al. (1996). The reaction was read using a spectrophotometer at 535 nm. The results were expressed as nmoles malondialdehyde/ml of serum. The determination of reactive oxygen species (ROS) was based on the technique described by Ali et al. (1992). The samples were diluted to 1:10 with 10 nM Tris (pH 7.4), and 5 µL of dichlorofluorescein diacetate (DCFH-DA) was added in methanol over 15 minutes at 37 ºC to form non-fluorescent dichlorofluorescein (DCFH). Reactive oxygen species (ROS) formation was determined using a DCF standard curve in methanol (0.05–1.0 µM). The results were expressed as U DCF/mg protein. All the analyzes were performed in triplicate biochemistry laboratory of the institution.

2.6.5 Serum electrophoresis

Polyacrylamide gel electrophoresis containing sodium dodecyl sulfate was performed according to the technique described by Fagliari et al. (1998) using mini-gels (10 x 10 cm) to measure serum acute-phase proteins and immunoglobulin levels. The gels were stained with Coomassie blue and were 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 a reference. Electrophoresis allowed the measurement of IgA, heavy-chain IgG, light-chain IgG, ceruloplasmin, acidic glycoprotein, haptoglobin, and transferrin.

2.6.6 Feces microbiological analysis

Stool samples collected on day 30 were placed in isothermal boxes and analyzed within two hours. Subsequently, 1 ml of the 104 dilutions of each sample was inoculated on 3M™ Petrifilm™ plates for E. coli and total coliform counts. The results were expressed as colony-forming units per g (CFU/g).

2.7 Statistical analyses

All data were analyzed using SAS's 'MIXED procedure' (SAS Inst. Inc., Cary, NC, USA; version 9.4), with the Satterthwaite approximation to determine the denominator degrees of freedom for the fixed effects test. The body weight gain, average daily gain, and concentrate intake were tested for fixed effect of treatment using the animal (treatment) as a random effect. The body weight data and blood results were analyzed as repeated measures and tested for fixed effects of treatment, day, and treatment × day using the animal (treatment) as a random effect. The d 1 results were included as an independent covariate. The d 1 results were removed from the data set for these variables to generate the average per treatment but were maintained as a covariate. The first-order autoregressive covariance structure was selected according to the lowest Akaike information criterion. Means were separated using the PDIFF method, and all results were reported as LSMEANS followed by SEM. The significance was defined for the effects of treatment and treatment × day interaction when $p \leq 0.05$ and tendency when $p > 0.05$ and ≤ 0.10.

3. Results

3.1 Calf performance

The results of performance are presented in Table 3. There was no treatment effect and interaction between treatment x day for body weight, average daily gain, or concentrate consumption ($p > 0.05$). Weight gain was significant in the calves of the treatment group in the period from 1 to 30 days of the experiment ($p \leq 0.05$; Table 3).

Table 3 - Body weight and feed intake (mean and standard error of the mean - SEM) of Jersey calves that received immunological homeopathic product.

Items[1]	Treatments[2]		SEM	*P* – values	
	Control (n = 6)	Treated (n = 6)		Treat	Treat × Day
Body weight (kg)				0.29	0.49
d 1	36.1	36.0	1.60		
d 15	44.4	46.9	1.60		
d 30	49.8	53.7	1.60		
d 60	68.8	69.7	1.60		
d 90	80.3	84.3	1.60		
Body weight gain (kg)					
d 1 to 30	13.8	17.7	1.34	**0.05**	-
d 30 to 90	30.1	30.7	1.53	0.80	-
d 1 to 60	44.3	48.3	2.12	0.18	-
Average daily gain (kg)					
d 1 to 90	0.49	0.54	0.02	0.16	-
Concentrate intake (kg DM)					
d 1 to 15	3.68	3.41	0.78	0.78	-
d 15 to 30	7.70	7.05	1.76	0.76	-
d 30 to 60	39.1	42.9	2.22	0.22	-
d 60 to 90	45.1	42.5	2.71	0.49	-
d 1 to 90	95.58	95.86	2.09	0.86	-

[1]Treatments included calves that received homeopathic product (treatment) and animals that did not receive the product (control).
[2]The d 1 results were removed from the data set to generate the average per treatment in the statistical analysis.
[a-b]Within a row: differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).
Source: Authors.

3.2 Hematology

Table 4 describes the hematologic results. Erythrocyte, hematocrit, monocyte, and eosinophil counts did not differ significantly between treatments ($p > 0.05$). The hemoglobin concentration had a treatment x day effect, with superior results on day 90, higher in the treatment group animals than in control ($p \leq 0.05$). Leukocyte, neutrophil, and lymphocyte counts were higher in the treatment group ($p \leq 0.05$). There was a tendency for higher leukocyte counts on days 30 and 90 ($p \leq 0.10$) when the animals consumed the homeopathic.

3.3 Serum biochemistry

The results of serum biochemistry are displayed in Table 5. There was no treatment effect or interaction between treatment x day for total protein, glucose, cholesterol, and urea concentration ($p > 0.05$). The albumin concentration in the treatment group animals was lower than in the control group ($p \leq 0.05$). Globulin concentration was higher in the group of calves that ingested the homeopathic product ($p \leq 0.05$), constituting a treatment effect.

Table 4 - Hemogram (mean and standard error of the mean - SEM) of Jersey calves that received immunological homeopathic product.

Items	Treatments[1]		SEM	*P* – values	
	Control (n = 6)	Treated (n = 6)		Treat	Treat × Day
Erythrocytes (x10^6 µL)				0.49	0.19
d 1	7.72	8.05	0.28		
d 15	7.54	7.95	0.28		
d 30	7.68	7.85	0.28		
d 60	7.65	7.40	0.28		
d 90	7.71	8.30	0.28		
Average[2]	7.60	7.89	0.23		
Hematocrit (%)				0.55	0.23
d 1	34.3	35.4	1.21		
d 15	32.4	33.9	1.21		
d 30	32.3	33.1	1.21		
d 60	30.5	29.5	1.21		
d 90	31.2	33.8	1.21		
Average[2]	31.5	32.6	1.04		
Hemoglobin (g/dL)				0.25	**0.05**
d 1	8.65	8.74	0.29		
d 15	8.42	9.09	0.29		
d 30	8.58	8.88	0.29		
d 60	8.25	8.32	0.29		
d 90	8.55^b	9.63^a	0.29		
Average[2]	8.44	8.99	0.34		
Leukocytes (x10^3 µL)				**0.02**	**0.07**
d 1	5.01	5.05	0.55		
d 15	5.73	7.03	0.55		
d 30	5.60^b	8.24^a	0.55		
d 60	5.65	6.78	0.55		
d 90	7.25^b	8.81^a	0.55		
Average[2]	6.06^b	7.71^a	0.37		
Neutrophils (x10^3 µL)				**0.08**	0.59
d 1	1.71	1.79	0.41		
d 15	1.59	2.12	0.41		
d 30	1.86	2.92	0.41		
d 60	1.70	1.71	0.41		
d 90	1.56	2.72	0.41		
Average[2]	1.67b	2.38a	0.29		
Lymphocytes (x10^3 µL)				**0.03**	0.23
d 1	3.11	3.06	0.32		
d 15	3.79	4.56	0.32		
d 30	3.58	4.91	0.32		
d 60	3.58	4.73	0.32		
d 90	5.16	5.57	0.32		
Average[2]	4.04b	4.94a	0.22		
Monocytes (x10^3 µL)				0.79	0.41
d 1	0.18	0.20	0.05		
d 15	0.32	0.25	0.05		
d 30	0.15	0.23	0.05		
d 60	0.31	0.24	0.05		
d 90	0.31	0.41	0.05		

Average[2]	0.27	0.28	0.04		
Eosinophils (x10^3 µL)				0.86	0.25
d 1	0.02	0.01	0.03		
d 15	0.01	0.08	0.03		
d 30	0.01	0.01	0.03		
d 60	0.04	0.10	0.03		
d 90	0.20	0.11	0.03		
Average[2]	0.07	0.07	0.01		

[1]Treatments included calves that received homeopathic product (treatment) and animals that did not receive the product (control).
[2]The d 1 results were removed from the data set to generate the average per treatment in the statistical analysis.
[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).
Source: Authors.

Table 5 - Serum biochemistry (mean and standard error of the mean - SEM) of Jersey calves that received immunological homeopathic product.

Items	Treatments[1]		SEM	*P* – values	
	Control (n = 6)	Treated (n = 6)		Treat	Treat × Day
Albumin (g/dL)				**0.04**	0.69
d 1	4.06	3.94	0.43		
d 15	4.64	3.41	0.43		
d 30	3.91	2.91	0.43		
d 60	4.54	3.81	0.43		
d 90	4.66	4.56	0.43		
Average[2]	4.46[b]	3.66[a]	0.22		
Globulin (g/dL)				**0.05**	0.14
d 1	5.62	4.82	0.68		
d 15	3.15	4.27	0.68		
d 30	3.77	6.17	0.68		
d 60	3.45	4.09	0.68		
d 90	4.04	4.82	0.68		
Average[2]	3.74[b]	4.74[a]	0.33		
Total protein (g/dL)				0.81	0.73
d 1	9.71	8.74	0.78		
d 15	7.81	7.66	0.78		
d 30	7.71	9.06	0.78		
d 60	8.01	7.87	0.78		
d 90	8.74	9.36	0.78		
Average[2]	8.22	8.38	0.32		
Glucose (mg/dL)				0.65	0.29
d 1	108	92.4	8.95		
d 15	108	102	8.95		
d 30	112	116	8.95		
d 60	90.1	99.6	8.95		
d 90	78.8	108	8.95		
Average[2]	100	104	6.64		
Cholesterol (mg/dL)				0.70	0.69
d 1	100	97.3	14.1		
d 15	88.0	104	14.1		
d 30	132	111	14.1		
d 60	95.2	77.9	14.1		
d 90	108	108	14.1		
Average[2]	106	100	8.68		
Urea (mg/dL)				0.49	0.72
d 1	24.3	22.9	2.11		
d 15	21.6	23.9	2.11		
d 30	24.1	25.6	2.11		
d 60	22.8	21.8	2.11		
d 90	24.8	28.9	2.11		
Average[2]	23.5	24.9	1.49		

[1]Treatments included calves that received homeopathic product (treatment) and animals that did not receive the product (control).
[2]The d 1 results were removed from the data set to generate the average per treatment in the statistical analysis.
[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).
Source: Authors.

3.4 Oxidative status

The results of oxidative status are displayed in Table 6. There was no treatment effect or interaction between treatment x day for TBARS levels and GST activity ($p > 0.05$). However, there was an interaction between treatment x day on days 30, 60, and 90 for the PSH levels in the treatment group, which presented a higher concentration of PSH ($p \leq 0.05$). ROS levels showed lower serum concentrations in the treatment calves than in the control group ($p \leq 0.05$).

3.5 Serum electrophoresis

The acute-phase protein and immunoglobulin results are displayed in Table 7. A treatment effect was observed only for IgA concentration, presenting higher levels in the treatment group. Interactions between treatment x day were observed for the concentration of IgA, ceruloplasmin, acid glycoprotein, and light-chain IgG ($p < 0.05$). There was also an interaction tendency for haptoglobin and transferrin ($p < 0.10$). The animals in the treated group presented higher concentrations of IgA on days 15 and 90; levels of ceruloplasmin were lower on day 90; transferrin concentration was higher on day 90; concentration of acid glycoprotein showed higher levels on day 15 but lower on day 60 than the control ($p \leq 0.05$). Light-chain IgG levels were higher on day 15 ($p \leq 0.05$). Heavy-chain IgG showed no significant difference between treatments and interaction ($p > 0.05$).

Table 6 - Oxidative response (mean and standard error of the mean - SEM) of Jersey calves that received the immunological homeopathic product.

Items	Treatments[1]		SEM	*P* – values	
	Control (n = 6)	Treated (n = 6)		Treat	Treat × Day
TBARS (nmol MDA/mL)				0.19	0.46
d 1	23.8	24.9	1.97		
d 15	21.1	20.7	1.97		
d 30	15.7	22.7	1.97		
d 60	22.6	21.9	1.97		
d 90	17.1	19.4	1.97		
Average[2]	18.9	21.3	1.26		
GST (µmolCDNB/min/mg proteinPSH (g/dL)				0.28	0.91
d 1	244	249	10.4		
d 15	232	250	10.4		
d 30	245	257	10.4		
d 60	245	247	10.4		
d 90	212	229	10.4		
Average[2]	233	247	8.96		
PSH (nmol SH/mg protein)				**<0.01**	**0.02**
d 1	62.5	64.6	3.35		
d 15	66.5	66.1	4.09		
d 30	52.6[a]	71.5[a]	3.35		
d 60	55.0[b]	78.8[a]	3.66		
d 90	51.1[b]	61.3[a]	3.35		
Average[2]	56.1[b]	69.5[a]	1.74		
ROS (U DCFH/mg protein)				**0.04**	0.56
d 1	5.63	5.66	1.25		
d 15	6.72	5.00	1.25		
d 30	28.1	24.1	1.25		
d 60	21.9	21.7	1.25		
d 90	20.1	19.1	1.25		
Average[2]	16.5[a]	15.1[b]	0.49		
SOD (U SOD/mg of protein)				0.71	0.54
d 1	3.84	2.21	0.23		
d 15	3.37	2.17	0.19		
d 30	3.74	3.61	0.19		
d 60	5.11	5.65	0.21		
d 90	6.08	5.33	0.20		
Average[2]	4.57	4.19	0.18		

[1]Treatments included calves that received homeopathic product (treatment) and animals that did not receive the product (control).
[2]The d 1 results were removed from the data set to generate the average per treatment in the statistical analysis.
[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).
Source: Authors.

Table 7 - Serum electrophoresis (mean and standard error of the mean - SEM) of Jersey calves that received the immunological homeopathic product.

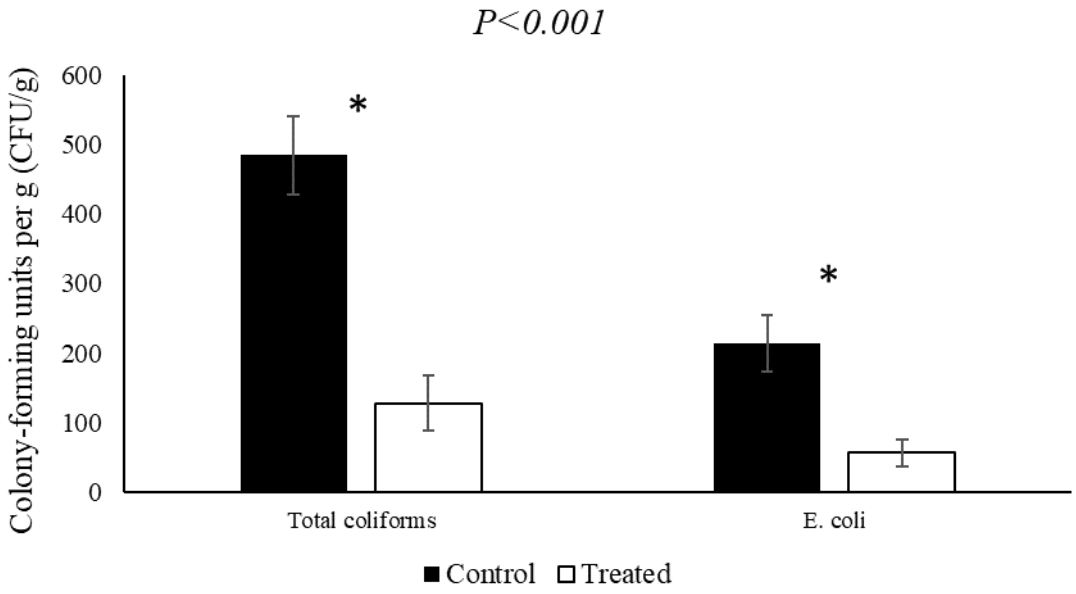
Items	Treatments[1]		SEM	*P* – values	
	Control (n = 6)	Treated (n = 6)		Treat	Treat × Day
IgA (g/dL)				**0.04**	**0.01**
d 1	1.01	1.18	0.04		
d 15	0.73[b]	1.10[a]	0.04		
d 30	0.82	0.88	0.02		
d 60	0.69	0.78	0.03		
d 90	0.75[b]	0.98[a]	0.02		
Average[2]	0.75[b]	0.94[a]	0.02		
Ceruloplasmin (g/dL)				0.77	**0.05**
d 1	0.61	0.45	0.05		
d 15	0.44	0.51	0.04		
d 30	0.53	0.45	0.05		
d 60	0.36	0.36	0.02		
d 90	0.77[a]	0.48[b]	0.03		
Average[2]	0.48	0.45	0.03		
Transferin (g/dL)				0.95	**0.10**
d 1	0.68	0.71	0.04		
d 15	0.66	0.65	0.02		
d 30	0.76	0.60	0.03		
d 60	0.52	0.60	0.04		
d 90	0.68[b]	0.81[a]	0.02		
Average[2]	0.66	0.67	0.03		
IgG-heavy chain (g/dL)				0.26	0.13
d 1	0.81	0.82	0.02		
d 15	1.06	1.15	0.03		
d 30	0.95	1.11	0.02		
d 60	0.76	0.75	0.02		
d 90	1.04	1.02	0.02		
Average[2]	0.95	1.01	0.02		
Haptoglobin (g/dL)				0.21	**0.07**
d 1	0.63	0.52	0.05		
d 15	0.47	0.32	0.05		
d 30	0.57	0.60	0.04		
d 60	0.60	0.56	0.03		
d 90	0.94[a]	0.77[b]	0.03		
Average[2]	0.65	0.56	0.04		
Acidic glycoprotein (mg/dL)				0.90	**0.03**
d 1	0.53	0.60	0.02		
d 15	0.68[b]	0.84[a]	0.02		
d 30	0.57	0.51	0.01		
d 60	0.42[a]	0.24[b]	0.02		
d 90	0.53	0.58	0.03		
Average[2]	0.55	0.54	0.02		
IgG-light chain (g/dL)				0.12	**0.01**
d 1	0.87	0.76	0.03		
d 15	0.26[b]	0.51[a]	0.02		
d 30	0.37	0.43	0.03		
d 60	0.44	0.41	0.02		
d 90	0.52	0.56	0.02		
Average[2]	0.40	0.48	0.02		

[1]Treatments included calves that received homeopathic product (treatment) and animals that did not receive the product (control).
[2]The d 1 results were removed from the data set to generate the average per treatment in the statistical analysis.
[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).
Source: Authors.

3.6 Feces microbiological analysis

The total coliform and *E. coli* counts were lower in the feces of animals from the treatment group (i.e., of the calves that consumed the homeopathic; Figure 1).

Figure 1 - Count of total coliforms and *Escherichia coli* in the feces of calves that consumed the homeopathic product for 30 consecutive days. Note: Results in mean and standard error of the mean – SEM.



Source: Authors.

4. Discussion

Weight gain was a primary finding in our study; it may be associated with the presence of the *Medicago sativa* homeopathic, a plant containing a compound that promotes the release of digestive enzymes and increases the number of beneficial bacteria for feed digestibility (Upadhaya et al., 2021). This compound may act with components in *Baptisia tinctoria*, known for its ability to neutralize gram-negative bacteria (Sandoval et al., 1998).

The higher number of neutrophils and lymphocytes may be related to the consumption of homeopathic immunological, which seek to stimulate physiological defense mechanisms against invaders (Shahabi, 2021), similar to what occurred in dogs that ingested homeopathic present in the feed (Marchiori et al., 2019). In the current study, the higher leukocyte count is a consequence of higher lymphocyte counts, which may be related to elevated serum globulins. The rapid response immune proteins (known as acute-phase proteins) and immunoglobulins such as IgA rose in the circulation of calves since serum IgA production depends on B lymphocytes in the bone marrow (Kerr, 2001). Nevertheless, it is critical to clarify that most of the IgA in the serum of our Jersey cows likely came from submucosal plasma cells in the gastrointestinal or respiratory tracts or from plasma cells in the bone marrow. IgA is the most prevalent antibody subclass at mucosal and participates in mucosal defense. Its potential as a therapeutic monoclonal antibody is less well known. Nevertheless, IgA possesses anti-inflammatory, and non-inflammatory functions that can be exploited in various immunotherapeutic strategies (Bakema and Egmond, 2011) as for homeopathy. Researchers proposed how IgA may contribute to the establishment and maintenance of beneficial interactions with the microbiota (Pabst and Slack, 2020); however, in the current study with Jersey calves, we were unable to make this statement because our findings allow us to say only that treated calves had higher serum IgA and lower fecal *E. coli* and that there may be a relationship between these findings.

The other globulins quantified on serum electrophoresis were higher in the serum of the calves from the homeopathic group. However, these differences occurred in one or two periods of analysis. It is impossible to establish a consistent behavior during the entire experimental period; this finding may be related to the feed transition period undergone by the animals. It is worth noting the higher concentration of heavy-chain IgG on day 15, a critical moment for calf production (45 days of age) (Fischer-Tlustos et al., 2020). Additionally, the higher levels of ceruloplasmin, acid glycoproteins, and haptoglobin can be a response to the consumption of the homeopathic produced using the plant *Nux vomica*, which includes strychnine and brucine and directly induces a physiological response in the liver (Downs et al., 2021), where these proteins are produced and metabolized.

The increase in PSH was an excellent result for calf health, indicating greater serum antioxidant responses. We cannot explain the mechanism that led to the higher serum concentration of this variable; however, we believe that it contributed to modulating oxidant and antioxidant responses, explaining why the calves had more significant weight gain. ROS showed a treatment effect, probably related to elevated thiol levels. These effects on antioxidant responses in calves who consumed the homeopathic may have an indirect relationship with the ingredients of *Arsenicum album* (Banerjee et al., 2008; Kundu et al., 2000; Mallick et al., 2003) and *Mercurius solubilis* (Oliveira et al., 2011), which promote metabolic balance. The combination of ingredients may have strengthened the antioxidant action because homeopathic remedies act as antioxidants (Jyothilakshmi et al., 2014).

5. Conclusion

The homeopathic immunological agent consumed by calves stimulates the production of neutrophils and lymphocytes and increases globulin levels. IgA is among the main altered globulins in this study. The homeopathic product favored weight gain in calves fed with supplemented milk during the first 30 days of the study.

Ethics committee

The Committee for the Use of Animals in Research (CEUA) of the University of the State of Santa Catarina (UDESC) approved the project under protocol number 2877070619.

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