



Milk supplemented with dried seaweed affects the systemic innate immune response in preweaning dairy calves

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ABSTRACT

Intact seaweed or seaweed extracts are used as feed supplements to improve the gut microbiome in young animals. Seaweeds provide functional polysaccharides, and they are a good source of vitamins, minerals, and phenolic compounds, all of which are relevant for immune system development. However, literature on the effects of dried seaweed supplementation on immune system development is limited, especially in calves. This experiment aimed to study the effect of feeding milk supplemented with *Ulva lactuca*, *Ascophyllum nodosum*, or *Saccharina latissima* on the systemic immune status of preweaning dairy calves. Forty male Holstein calves with birth body weight 41 ± 4 kg and plasma Brix percentage $\geq 8.7\%$ at d 2 after birth were used in this study. Calves were fed 4 L of cow milk twice a day (total 8 L/d). From d 2 to d 28, calves in the control group ($n = 10$) received milk without seaweed supplementation. Over the same period, experimental calves received milk supplemented with *Ulva lactuca* (SW1; $n = 10$), *Ascophyllum nodosum* (SW2; $n = 10$), or *Saccharina latissima* (SW3; $n = 10$). Dried and ground seaweeds were offered at a daily allowance of 50 g/8 L of milk (i.e., approximately 5% inclusion rate on a dry matter basis). Blood samples were collected from a jugular vein on d 2, 4, 7, 14, 21, and 28 after birth. Plasma concentrations of total protein, albumin, immunoglobulins, and acute-phase proteins (i.e., serum amyloid A, fibrinogen, and haptoglobin) were measured. We detected no differences in average daily gain, plasma immunoglobulins, albumin, or total protein. However, the contrast analysis revealed that plasma concentrations of fibrinogen (SW1 and SW2) and serum amyloid A (SW2 and SW3) were significantly higher in the seaweed groups compared with the control group.

We also found a tendency for high plasma haptoglobin in the seaweed groups (SW1 and SW2) compared with the control group. Differences in acute-phase protein concentrations could be partially explained by the large differences in micromineral intake between control and seaweed-supplemented calves. Feeding milk supplemented with dried seaweed increased plasma concentrations of variables related to the innate immune response in preweaning dairy calves.

Key words: immune response, *Ulva lactuca*, *Ascophyllum nodosum*, *Saccharina latissima*

INTRODUCTION

Raising unhealthy dairy calves has negative consequences for animal performance and welfare, increases the use of antibiotics, and could have negative long-term consequences for milk yield (Heinrichs and Heinrichs, 2011). Calves are born with an immature immune system (Weaver et al., 2000; Hernández-Castellano et al., 2018b). In addition, cow placenta (i.e., syndesmochorial placenta) does not allow the sufficient transfer of immune components such as immunoglobulins from dam to calf (Godden, 2008). Hence, susceptibility to infectious diseases is relatively high during the first month of life in dairy calves. One of the most promising strategies for enhancing health and performance in livestock species is the use of alternative feed supplements with prebiotic effects, such as extracts from herbs, plants, and cereals, among others (Markowiak and Slizewska, 2018). In this way, marine macroalgae (i.e., seaweed) have been proposed as an alternative functional feed ingredient for livestock (Morales-de la Nuez et al., 2014; Rajauria, 2015; Makkar et al., 2016).

Seaweeds are classified into 3 major groups: brown seaweeds (Phaeophyceae), red seaweeds (Rhodophyceae), and green seaweeds (Chlorophyceae; Makkar et al., 2016). They contain a wide variety of complex polysaccharides (e.g., fucoidans and β -glucans), antioxidants, and other bioactive compounds (e.g., pigments, polyphenols; Lynch et al., 2010; Okolie et al., 2017; Øverland et al., 2019). They are also high in minerals

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(I, K, Ca, Mg, P, Fe, and Zn) and vitamins (C, B₁, B₂, and E; Holdt and Kraan, 2011; Makkar et al., 2016; Corino et al., 2019), improving their value as functional feed ingredients (Rajapakse and Kim, 2011; Øverland et al., 2019). Microminerals such as Se, Cu, Zn, and Fe are cofactors of enzymes and can stimulate the immune response through increased antioxidant status, enhancing phagocytosis, and the proliferation and differentiation of macrophages, lymphocytes, and other immune cells (Puertollano et al., 2011).

Seaweeds are also rich in phenols, phlorotannins, pigments, and tocopherols (vitamin E), which are known for their antioxidant potential (Peinado et al., 2014; Corino et al., 2019). Thus, supplementation with a brown seaweed extract in weaned piglets enhanced blood serum antioxidant status (Wan et al., 2016, 2017). Furthermore, feedlot steers grazing on pasture treated with an *Ascophyllum nodosum* extract (Tasco-Forage; Acadian Seaplants Ltd., Dartmouth, NS, Canada) showed increased serum vitamin E concentrations and monocyte phagocytic activity (Allen et al., 2001a).

Based on the above, this study aimed to evaluate the effects of feeding milk supplemented with dried and ground seaweed from 3 algae species on systemic immune response in preweaning dairy calves. We hypothesized that feeding milk supplemented with seaweed during the first 28 d of life would enhance the development of immune response in dairy calves.

MATERIALS AND METHODS

The present study was conducted in compliance with the Danish Ministry of Justice Law No. 474 (May 15, 2014) concerning animal experimentation and the care of experimental animals.

Harvesting and Processing the Seaweed

Three different seaweeds were used in this experiment. The green seaweed *Ulva lactuca*, commonly known as sea lettuce, was harvested from the wild at Mariager Fjord (Denmark; 56.41413° N, 10.11560° E) in June 2018; it was air-dried and ground to a screen size of 0.8 mm. The brown seaweed *A. nodosum* (Thorverk HF, Reykhólar, Iceland), commonly known as knotted wrack, was harvested from the wild at Breiðafjörður bay (Iceland; 65.27781° N, 22.22921° W, and 65.10371° N, 22.36481° W) in July 2018; it was air-dried and ground to a screen size of 0.2 mm. The brown seaweed *Saccharina latissima*, commonly known as sugar kelp, was cultivated and harvested close to Horsens fjord (Denmark; 55.813822° N, 10.112930° E) in May 2018; it was air-dried and ground using a screen size of 0.8 mm.

Table 1. Proximate composition (g/kg of DM unless otherwise noted) of dried and ground seaweeds *Ulva lactuca* (SW1), *Ascophyllum nodosum* (SW2), and *Saccharina latissima* (SW3)

Item	SW1	SW2	SW3
DM (g/kg of fresh matter)	927.7	932.4	939.6
Crude ash	482.0	295.5	399.0
CP	127.5	91.0	121.5
Crude fat	13.0	30.0	15.0
NDF	129.7	344.7	216.7
ADF	73.7	189.2	79.9

Chemical Composition of the Seaweeds

The DM content of the 3 seaweeds was determined by drying the samples in a forced air oven at 60°C for 48 h. Nitrogen content was determined by the Dumas method using a Vario MAX CN (Elementar Analyses system GmbH, Hanau, Germany) as described by Hansen (1989). Crude protein was calculated as nitrogen content $\times 5$ (Angell et al., 2016). Crude ash content was measured by combustion of the sample for 6 h at 525°C. Crude fat content was determined by Soxhlet extraction with petroleum ether (Soxtec 2050; Foss Analytical, Hillerød, Denmark) after hydrolyzing with HCl as described by Stoldt (1952). Concentrations of NDF and ADF were determined as described by Mertens (2002) using a Fibertec M6 System (Foss Analytical). The proximate chemical compositions of the seaweeds are shown in Table 1.

Mineral Composition of the Seaweeds, Milk, and Calf Starter

Mineral concentrations of the dried seaweeds (Ca, Mg, P, K, Na, Cu, Fe, Mn, Zn, Se, Co) were determined using an X-series II inductively coupled plasma mass spectrophotometer (Thermo Electron Corporation, Bremen, Germany) equipped with a Meinhard nebulizer and a Peltier cooled quartz impact bead spray chamber at 3°C.

For analysis of mineral concentrations in cows' milk (Ca, Mg, P, K, Na, Cu, Fe, Mn, Zn, Se, Co) we sent samples to Eurofins Steins Laboratorium (Vejen, Denmark). However, concentrations of P and several microminerals (Cu, Fe, Mn, Zn, Se, Co) were below the detection limit offered by the laboratory. Because of low variation in the mineral composition of milk from dairy cows, we obtained the concentrations of these minerals from previous studies performed at the Department of Animal Science (Aarhus University, Denmark) by Hermansen et al. (2005), Jensen et al. (2012), and Bovenhuis et al. (2016) on Holstein dairy cows. Mineral concentrations (Ca, Mg, P, K, Na, Cu,

Table 2. Average daily feed intakes (g of DM/d per calf) and mineral concentrations in milk and calf starter for control calves (CON) and calves supplemented with *Ulva lactuca* (SW1), *Ascophyllum nodosum* (SW2), or *Saccharina latissima* (SW3)

Item	CON	SW1	SW2	SW3	NRC ²
Milk intake	1,047.6	1,043.6	1,051.7	1,047.6	
Seaweed intake	—	47.3	46.6	47.0	
Starter intake	35.6	60.9	35.0	33.9	
Macromineral concentration ¹					
Ca (g/100 g of DMI)					
Milk	0.90	1.13	1.07	1.09	0.95
Calf starter	1.32	1.32	1.32	1.32	0.70
Mg (g/100 g of DMI)					
Milk	0.07	0.16	0.11	0.11	0.10
Calf starter	0.20	0.20	0.20	0.20	0.10
P (g/100 g of DMI)					
Milk	0.67	0.65	0.65	0.66	0.76
Calf starter	0.39	0.39	0.39	0.39	0.45
K (g/100 g of DMI)					
Milk	0.90	0.93	0.96	1.12	1.12
Calf starter	1.17	1.17	1.17	1.17	0.65
Na (g/100 g of DMI)					
Milk	0.22	0.42	0.36	0.37	0.38
Calf starter	0.17	0.17	0.17	0.17	0.15
Micromineral concentration ¹					
Cu (mg/kg of DM)					
Milk	0.57	0.86	0.81	0.82	0.1–1.1
Calf starter	9.77	9.77	9.77	9.77	10.0
Fe (mg/kg of DM)					
Milk	2.27	209.6	87.5	142.0	3.00
Calf starter	251.5	251.5	251.5	251.5	50.0
Mn (mg/kg of DM)					
Milk	0.12	68.5	3.01	39.2	0.2–0.4
Calf starter	60.4	60.4	60.4	60.4	40.0
Zn (mg/kg of DM)					
Milk	30.9	30.5	30.2	32.1	15–38
Calf starter	65.0	65.0	65.0	65.0	40.0
Se (mg/kg of DM)					
Milk	0.07	0.52	0.17	0.22	0.02–0.15
Calf starter	0.32	0.32	0.32	0.32	0.30
Co (mg/kg of DM)					
Milk	0.00	0.07	0.10	0.10	0.004–0.008
Calf starter	0.24	0.24	0.24	0.24	0.10

¹Values for milk calculated based on analyzed (Ca, Mg, K, Na) or reference (P, Cu, Fe, Mn, Zn, Se, Co) values for whole milk mineral composition from Danish Holstein cows and analyzed mineral composition of seaweed.

²Recommendations of the National Research Council (NRC, 2001).

Fe, Mn, Zn, Se, Co) in the calf starter were analyzed at Eurofins Steins Laboratorium. Mineral concentrations in milk (with or without seaweed supplementation) and calf starter are shown in Table 2.

Animals

In this experiment, 40 male Holstein calves were used. Within 2 h after birth, calves were fed 4 L of good-quality colostrum (i.e., Brix percentage $\geq 22.0\%$). Calves remained with their dams for at least 12 h after birth, in compliance with the legal framework in Denmark. During this time, calves had access to the dam's colostrum. The good-quality colostrum fed within 2 h

after birth was obtained from the experimental farm's colostrum bank, stored at -20°C . Before feeding, colostrum was thawed in a water bath at 40°C for 20 min. Only calves with birth BW 41 ± 4 kg and blood plasma Brix percentage $\geq 8.70\%$ (i.e., approximate plasma IgG concentration ≥ 10 g/L) at d 2 after birth were used in this study. All calves were housed in individual pens (150 cm \times 150 cm) bedded with straw and had ad libitum access to fresh water, hay, and calf starter. Calf starter residuals were weighed and recorded once per week. Calf health status was monitored from birth until the end of the experimental period (d 28 after birth). Health status monitoring included inspection for diarrhea, coughing, and fever.

Seaweed Feeding and Experimental Treatments

After colostrum feeding, all calves were blocked according to birth order (4 calves per block). Each block contained 1 control calf and 3 experimental calves. Calves in the control group (**CON**; $n = 10$) were fed 4 L of cow's milk from the bulk tank ($40.0 \pm 2^\circ\text{C}$; 5.00% lactose, 3.50% protein, 4.00% fat, 13.5% TS) twice daily (i.e., 0630 and 1730 h) without seaweed supplementation. Calves in the *U. lactuca* group (**SW1**; $n = 10$) were fed 4 L of cow milk from the bulk tank twice daily, supplemented with 25 g of dried ground *U. lactuca*. Calves from the *A. nodosum* group (**SW2**; $n = 10$) were fed 4 L of cow milk from the bulk tank twice daily, supplemented with 25 g of dried ground *A. nodosum*. Calves from the *S. latissima* group (**SW3**; $n = 10$) were fed 4 L of cow milk from the bulk tank twice daily, supplemented with 25 g of dried ground *S. latissima*. All calves were milk-fed using a nipple bucket. To prevent deposition of the dried, ground seaweed on the walls and bottom of the nipple bucket, the seaweed was continuously mixed into the milk using a manual hand mixer, until the full 4 L of milk was consumed. Milk refusals were recorded 10 min after each feeding: twice per day during the experimental period.

Calves in the SW1, SW2, and SW3 groups received the seaweed supplementation from d 1 until the end of the experimental period (d 28 after birth). Calves were weighed weekly from d 1 to d 28 to calculate ADG. Individual milk, seaweed, and calf starter intakes were also recorded (Table 2).

Blood Sample Collection

Blood samples were collected from a jugular vein into sodium heparinized tubes (9 mL) at d 2, 4, 7, 14, 21, and 28 after birth. All blood samplings were performed within 3 h after the morning feeding. After collection, blood samples were placed on ice and then centrifuged at $2,000 \times g$ for 15 min at 4°C . The resulting plasma was aliquotted and stored at -20°C .

Variables Measured in Plasma

Plasma concentrations of IgG, IgM, and IgA were determined by ELISA (E11-118, E11-101, and E11-131 respectively; Bethyl Laboratories Inc., Montgomery, TX) according to the manufacturer's guidelines. All intra-assay coefficients of variation (**CV**) were $\leq 5\%$, and inter-assay CV were $\leq 6\%$.

Plasma total protein concentrations were determined using an ADVIA 1800 analyzer (Siemens Medical Solutions, Erlangen, Germany). Albumin concentrations in plasma were measured by bromocresol green-binding

and spectrophotometric determination at 596 nm (ADVIA 1800; Siemens Medical Solutions). Fibrinogen concentrations in plasma were measured by a particle-bound polyclonal rabbit anti-fibrinogen antibody (Q 0122; DakoCytomation, Glostrup, Denmark), followed by a turbidimetric assay (ADVIA 1800; Siemens Medical Solutions). A spectrophotometric assay was used to analyze plasma haptoglobin concentrations, following the manufacturer's guidelines (TP-801; Tridelta Developments Ltd., Kildare, Ireland). For the above-mentioned analyses, all intra-assay CV were $\leq 3\%$ and inter-assay CV were $\leq 4\%$. Serum amyloid A (**SAA**) concentrations in plasma were determined by ELISA (TP-802; Tridelta Developments Ltd.) according to the manufacturer's guidelines. All intra-assay CV for SAA were $\leq 5\%$, and inter-assay CV were $\leq 6\%$.

Statistical Analysis

Statistical analyses were performed using R 3.6.1 (R Core Team, 2019). The effects of seaweed supplementation on milk intake, calf starter intake, ADG, and plasma variables were analyzed using a linear mixed model. The model was fitted with REML, and the "lme" function from the "nlme" package was used (Pinheiro et al., 2019). The effect of seaweed supplementation, age, and their interaction were considered as fixed effects, and calf nested within block was considered as a random effect. Plasma IgA concentration at d 2 after birth was used as a covariate in the model during statistical analysis of plasma IgA concentrations. The residual error was assumed to be independent with constant variance, and to be normally distributed. Concentrations of IgG, IgM, IgA, SAA, fibrinogen, and haptoglobin were \log_{10} -transformed to comply with the model assumptions (i.e., variance homogeneity and normality of residuals).

Least squares means and standard error of mean were obtained using the emmeans package of R (Lenth et al., 2020). Differences between least squares means were evaluated using Tukey's method for comparing a family of 8 estimates. The contrast function was used to test the general effect of the supplementation of each seaweed (i.e., SW1, SW2, or SW3) against the CON group. Statistical significance was set as $P \leq 0.05$, and tendencies were set as $0.05 < P \leq 0.10$.

RESULTS

Calf Performance and Health

In this study, 3 calves (i.e., 1 calf from SW2 and 2 calves from SW3) had very low plasma IgG concentrations throughout the experimental period (i.e.,

2.00–7.70 g/L), on average, 4 to 8 times lower than the rest of the experimental calves. Plasma IgG concentration (indirectly measured using a Brix refractometer) was one of the major criteria used to select calves for the study. Furthermore, average plasma IgM and IgA concentrations in these calves were 2 to 6 times and 6 to 25 times lower, respectively, than the rest of the calves used in this study. Because of the high deviation in these acquired immune parameters, these 3 calves were considered outliers and excluded from the statistical analysis.

We detected no differences in ADG between SW1, SW2, SW3, and CON calves throughout the experimental period ($P = 0.85$). The ADG of SW1, SW2, SW3, and CON calves were 0.93 ± 0.03 , 0.90 ± 0.04 , 0.90 ± 0.04 , and 0.93 ± 0.03 kg/day, respectively. We found no diarrhea in the SW1 and SW2 groups. Two calves in the CON group had diarrhea, but they recovered completely after being treated with an electrolyte solution for 5 d. One calf in the SW3 group showed loose and thin feces for 2 d. During that time, we detected no changes in rectal temperature in that calf, so it was not treated with electrolytes.

Plasma Immunoglobulins

We observed no differences in plasma IgG concentrations (Table 3) between the SW1, SW2, SW3, and CON groups ($P = 0.56$), and none of the studied groups showed differences in plasma IgG concentrations during the experimental period ($P = 0.57$). Similarly, we observed no differences in plasma IgM concentrations (Table 3) between the SW1, SW2, SW3, and CON groups ($P = 0.27$), and none of the studied groups showed differences in plasma IgM concentrations during the experimental period ($P = 0.48$). Plasma IgA concentrations (Table 3) tended to be affected by seaweed supplementation ($P = 0.09$). However, average plasma IgA concentrations in the SW1, SW2, and SW3 calves were not different from that of the CON calves ($P > 0.05$), and we observed no differences in plasma IgA

concentrations between the 4 treatment groups during the experimental period ($P = 0.57$).

Plasma Proteins and Acute-Phase Proteins

Total protein concentrations in plasma (Table 3) were not different between the CON group and the SW1, SW2, and SW3 groups ($P = 0.17$), and we observed no differences in total protein concentrations for any of the studied groups throughout the experimental period ($P = 0.36$). We observed no differences in plasma albumin concentrations (Table 3) between the SW1, SW2, SW3, and CON groups ($P = 0.67$), but we did find a tendency for an interaction effect for seaweed supplementation and age ($P = 0.07$). Seaweed supplementation tended to affect plasma fibrinogen concentrations (Figure 1A) in the experimental calves ($P = 0.06$). Contrast analyses revealed higher plasma fibrinogen concentrations in SW1 and SW2 calves than in CON calves at d 14 of life ($P < 0.05$; Figure 1A). In addition, SW2 calves tended to have higher plasma fibrinogen concentrations than CON calves at d 21 of life ($P = 0.10$), and these differences were significant at d 28 ($P = 0.03$) (Figure 1A). Plasma haptoglobin concentrations (Figure 1B) tended to be affected by seaweed supplementation ($P = 0.09$). Contrast analyses confirmed this tendency at d 14, when SW1 and SW2 calves tended to have higher plasma haptoglobin concentrations than CON calves ($P = 0.10$). Plasma SAA concentrations (Figure 1C) were also affected by seaweed supplementation ($P = 0.03$). Contrast analyses showed that SW2 and SW3 calves had higher SAA concentrations than CON calves at d 14 of life ($P < 0.05$).

DISCUSSION

In the current study, none of the dried seaweed supplements enhanced ADG. Similar results were observed by Michiels et al. (2012) in piglets fed dried *A. nodosum* from d 4 to 28 of life.

Table 3. Average concentrations of plasma total protein, albumin, and immunoglobulins in control calves ($n = 10$) and calves supplemented with *Ulva lactuca* (SW1; $n = 10$), *Ascophyllum nodosum* (SW2; $n = 9$), or *Saccharina latissima* (SW3; $n = 8$)

Variable	Treatment				SEM	Fixed effects (P -value) ¹			Contrast (P -value) ²		
	Control	SW1	SW2	SW3		T	D	T \times D	C1	C2	C3
Total protein (g/L)	63.2	67.3	68.5	65.8	1.88	0.171	0.001	0.361	0.318	0.151	0.722
Albumin (g/L)	31.9	32.5	31.6	31.8	0.49	0.665	0.001	0.067	0.769	0.955	0.997
IgG (g/L)	20.3	25.3	25.7	24.0	3.51	0.559	0.001	0.572	0.685	0.645	0.865
IgM (g/L)	0.76	0.93	0.96	0.66	0.16	0.270	0.001	0.482	0.752	0.667	0.929
IgA (g/L)	0.15	0.20	0.22	0.21	0.03	0.091	0.001	0.566	0.403	0.175	0.149

¹D = age effect; T = seaweed supplementation effect; T \times D = interaction of seaweed supplementation and age.

²C1 = control vs. SW1 calves; C2 = control vs. SW2 calves; C3 = control vs. SW3 calves.

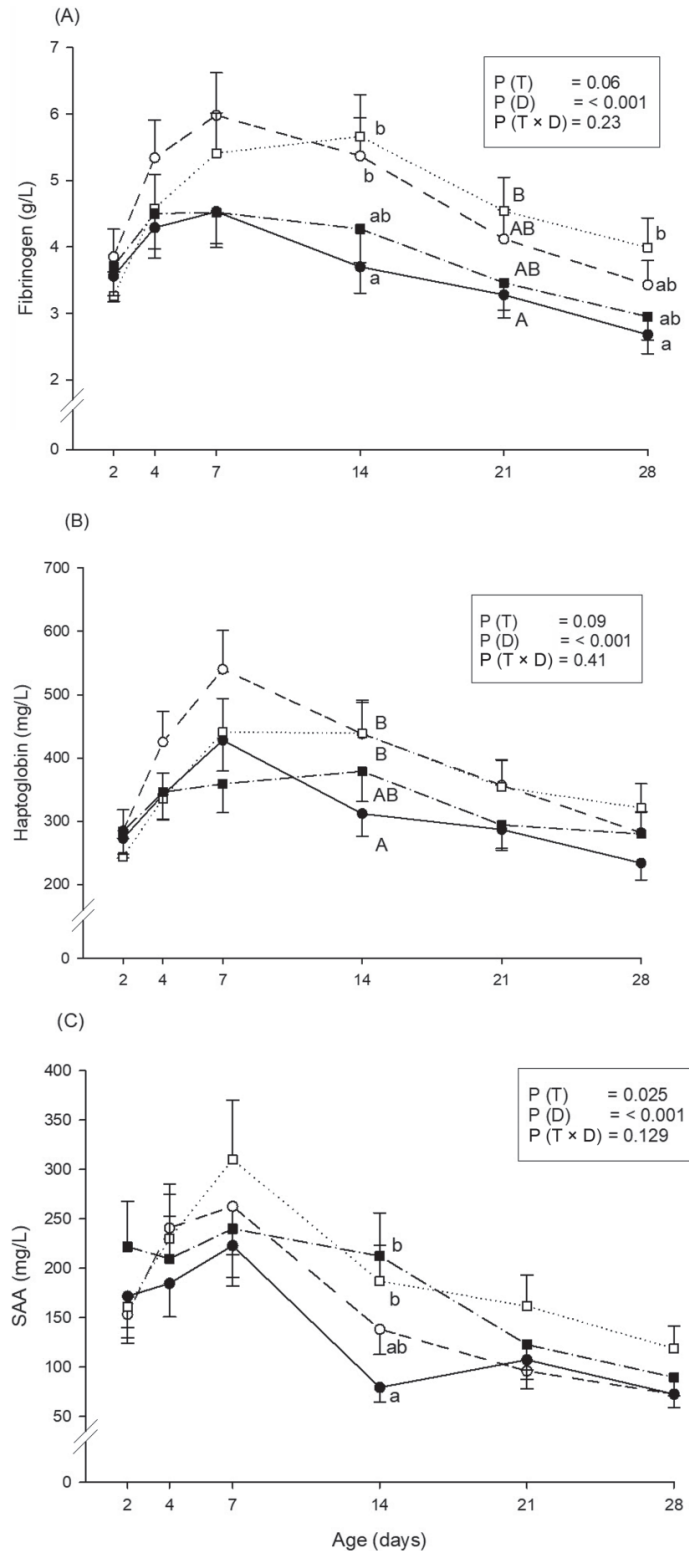


Figure 1. Concentrations of (A) plasma fibrinogen, (B) haptoglobin, and (C) serum amyloid A (SAA) in control calves (n = 10; black circles) and calves supplemented with *Ulva lactuca* (n = 10; white circles), *Ascophyllum nodosum* (n = 9; white squares), and *Saccharina latissima* (n = 8; black squares) during the experimental period. Values are expressed as mean \pm SEM. Lowercase letters (a, b) indicate significant differences between treatment groups within the experimental day ($P < 0.05$). Uppercase letters (A, B) indicate tendencies for differences between treatment groups within the experimental day ($0.05 < P \leq 0.10$). D = age effect; T = seaweed supplementation effect; T \times D = interaction of seaweed supplementation and age.

During the first weeks of life, dairy calves are highly dependent on maternal transfer of passive immunity via colostrum for protection against pathogens (Godden, 2008; Hernández-Castellano et al., 2018a). However, this passive immunization declines rapidly following the first week after birth (Hulbert and Moisés, 2016). During this early stage of life, the immune system is not fully developed in calves and the self-production of immune components such as IgG is still low (Chase et al., 2008; Hulbert and Moisés, 2016). Therefore, proper functioning of the innate immune system is highly relevant for protecting dairy calves against infectious agents, especially during the first month of life (Hulbert et al., 2011; Hulbert and Moisés, 2016). Innate immune mechanisms, including acute-phase proteins (**APP**), play an essential role in both the health status and the healing process (Grimble, 2001).

In the present study, none of the supplemented seaweeds affected plasma Ig concentrations during the first month of life. However, despite its lack of effect on the adaptive immune system, seaweed supplementation enhanced plasma concentrations of fibrinogen (SW1 and SW2 groups), haptoglobin (SW1 and SW2 groups), and SAA (SW2 and SW3 groups). These APP are commonly used as biomarkers of the innate immune response (Eckersall and Bell, 2010; Samarasinghe et al., 2020). During acute inflammation in cattle, blood SAA concentrations increase faster than fibrinogen, although fibrinogen concentrations stay elevated for longer (Eckersall, 2008). Increased SAA and fibrinogen concentrations might positively influence innate immune status by stimulating the migration, adhesion, and tissue infiltration of monocytes, macrophages, and neutrophils, and by increasing the efficiency of phagocytosis through opsonization (Eckersall and Bell, 2010; Ceciliani et al., 2012). Similarly, elevated plasma haptoglobin could play an important role in binding free hemoglobin, reducing free iron in plasma (i.e., antibacterial effect) and oxidative damage (Ceciliani et al., 2012).

Elevation of an APP concentration by less than 2-fold is considered a minor response (Khalil and Al-Humadi, 2020). In addition, the elevated plasma fibrinogen, SAA, and haptoglobin concentrations in the present study were much lower than those observed in inflammatory and infectious processes (Gänheim et al., 2003; Gänheim et al., 2007; Eckersall and Bell, 2010). This finding was also in agreement with visual clinical observations, including the incidence of diarrhea, rectal temperature, and coughing, which indicated that calves in all 4 experimental groups were healthy. The moderate increases in SAA, fibrinogen, and haptoglobin concentrations might indicate a more active innate immune response in the calves supplemented with seaweed, especially in those from the SW2 group.

Seaweeds are commonly characterized by a high concentration of minerals (Holdt and Kraan, 2011; Corino et al., 2019), which could explain the considerable amounts of ash in the 3 seaweeds used in this study. As can be seen in Table 2, the concentration of macrominerals in cow's milk without added seaweeds (i.e., milk fed to the CON group) was slightly lower than recommended by the NRC (2001). However, cow's milk supplemented with seaweeds (SW1, SW2, SW3) had slightly higher macromineral concentrations than the NRC recommendations (NRC, 2001), except for P and K (Table 2). Except for Zn, all seaweed groups had higher micromineral intakes through milk than the CON group (Table 2). Furthermore, the seaweed groups had higher Fe, Mn, Se, and Co concentrations in milk than those recommended by the NRC (2001). Microminerals such as Cu, Zn, Se, and Fe play an essential role in modulation of the immune system. Seaweeds are also a good source of tocopherols, although brown seaweeds contain considerably higher levels of α -, β -, γ -, and δ -tocopherols than red and green seaweeds (Gupta and Abu-Ghannam, 2011; Rajapakse and Kim, 2011; Øverland et al., 2019).

High mineral and tocopherol content in seaweeds may partly explain the higher plasma concentrations of fibrinogen, haptoglobin, and SAA observed in calves supplemented with seaweeds compared to those from the CON group. According to Gombart et al. (2020), microminerals (i.e., Zn, Fe, Cu and Se) play an important synergistic role in the immune response and can often be offered in higher amounts than the recommended dietary allowances. For instance, Cu stimulates neutrophil and monocyte responses and increases leukocyte counts in the bloodstream (Bordignon et al., 2019). Furthermore, Cu is essential for the proper function of macrophages (Gombart et al., 2020). In addition, tocopherols, Se, Cu, Zn, Mn, and Fe are cofactors of enzymes such as superoxide dismutase, peroxidases, and catalase, which are involved in the prevention of oxidative stress and tissue damage (Allen et al., 2001b; Wells et al., 2017). Based on these facts, it seems that some of the effects observed in this study could have been due to the increased micromineral supply in the diets of the calves supplemented with seaweeds.

Brown seaweeds, including *A. nodosum*, are a good source of fucoidans, which act as functional polysaccharides (Fitton et al., 2015, 2019). Fucoidans can bind with several pattern-recognition receptors on macrophages (e.g., toll-like receptor-4 and cluster of differentiation-14), which eventually stimulate the production of pro-inflammatory cytokines (Ale et al., 2011; Fitton et al., 2015; Okolie et al., 2017), enhancing the innate immune response (Fitton et al., 2015). Kupffer cells represent 80 to 90% of the fixed macrophages and

constitute the first macrophage population outside the gut to have close contact with both the antigens and the nutrients transported by the hepatic portal vein (Eckel and Ametaj, 2016). Furthermore, Kupffer cells secrete APP such as haptoglobin and SAA, which act as inflammatory mediators (Eckel and Ametaj, 2016). As shown by Neyrinck et al. (2007), dietary supplementation with brown seaweed extract containing β -glucan stimulated the function of Kupffer cells in the livers of male Wistar rats. The present study did not investigate the direct biomarkers for determining the functionality of macrophages in liver, but the increased plasma APP concentrations we observed might indicate improved activity of these cells. Furthermore, Kim et al. (2011) observed higher serum haptoglobin concentrations after an immune challenge in calves fed a β -glucan extract compared to a control group. These authors suggested that the efficient production of haptoglobin after a vaccine challenge is beneficial against incoming pathogens (Kim et al., 2011). However, McDonnell et al. (2019), suggested that increased plasma haptoglobin concentrations in calves fed a seaweed extract (β -glucan supplement from *Laminaria* spp.) compared with control calves might indicate an increased acute inflammatory response against weaning stress.

Based on our results, it is difficult to speculate which specific nutrients or other bioactive compounds (e.g., functional polysaccharides, tocopherols, or phlorotannins) present in these seaweeds influenced higher concentrations of innate immune proteins to a major or minor degree. The observed results should be interpreted cautiously considering the physiologically healthy status of the calves in our study. The responses observed in plasma APP appeared to be an effect of enhanced efficiency in production of these proteins in the seaweed-supplemented calves. However, they could also be an effect of increased inflammatory response in the calves against stressors around birth process, adjustments to post-natal life, or both. Further research would be beneficial for understanding the exact mechanisms behind the observed results and the possible deviations with an immune or vaccine challenge.

CONCLUSIONS

The current study demonstrated that milk supplemented with dried seaweeds (*Ulva lactuca*, *Ascophyllum nodosum*, or *Saccharina latissima*) did not affect ADG or variables related to the adaptive immune system (IgG, IgM, IgA) in preweaning dairy calves. Furthermore, total protein and albumin concentrations in plasma were also not affected. However, seaweed supplementation increased fibrinogen, haptoglobin, and SAA concentra-

tions in plasma around d 14 of life, indicating increased activity of the innate immune system.

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