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Effects of Supplementing Brown Seaweed By-products in the Diet of Holstein Cows during Transition on Ruminal Fermentation, Growth Performance and Endocrine Responses

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ABSTRACT: This study was conducted to examine the effects of supplementing brown seaweed by-products (BSB) in the diet of ruminants on ruminal fermentation characteristics, growth performance, endocrine response, and milk production in Holstein cows. In Experiment 1, the effects of different levels (0%, 2%, and 4% of basal diet as Control, 2% BSB, 4% BSB, respectively) of BSB were evaluated at 3, 6, 9, 12, and 24 h *in vitro* batch culture rumen fermentation. The pH tended to be higher for the higher level of BSB supplementation, with the pH at 12 h being significantly higher ($p<0.05$) than that of the control. The concentration of ammonia nitrogen was lower at 3, 9, 12, and 24 h incubation ($p<0.05$) compared with the control, and tended to be low at other incubation times. Volatile fatty acid concentration appeared to be minimally changed while lower values were observed with 4% BSB treatment at 24 h ($p<0.05$). In Experiment 2, effects of levels (0%, 2%, and 4%) of BSB on growth performance, endocrine responses and milk production were studied with Holstein dairy cows during transition. Dry matter intake, daily gain and feed efficiency were not affected by BSB supplementation. The concentration of plasma estrogen for the control, 2% BSB and 4% BSB after three months of pregnancy were 55.7, 94.1, and 72.3 pg/mL, respectively ($p = 0.08$). Although the differences of progesterone levels between BSB treatments and the control were minimal, the concentration in 4% BSB treatment increased to 157.7% compared with the initial level of the study. Triiodothyronine and thyroxine levels were also higher after both three months and eight months of pregnancy than the initial level at the beginning of the study. In addition, BSB treatments during one month after delivery did not affect daily milk yield and composition. In conclusion, the present results indicate that supplementation of BSB did not compromise ruminal fermentation, and animal performance at lower levels and hence may have potential to be used as a safe feed ingredient in dairy cows. (**Key Words:** Brown Seaweed By-products, Growth Performance, Fermentation, Blood metabolites, Holstein Cows)

INTRODUCTION

Brown seaweed (*Undaria pinnatifida*) is rich in a polysaccharide of alginic acid which has been demonstrated to suppress cholesterol absorption, and discharge heavy metals as well as act to prevent artery hardening (Kimura et al., 1996; Amano et al., 2005; Yoon et al., 2008). Acid water-soluble polysaccharide of fucoidan in the brown seaweed has shown effects of anti-coagulation of blood (Nishino et al., 2000), anti-tumor, anti-cancer activity (Haroun-Bouhedja et al., 2002; Maruyama et al., 2003) and anti-oxidation (Allen et al., 2001) and these benefits in part result from increasing thrombin, Factor Xa, urokinase-type plasminogen activator and tissue plasminogen activator. In addition, alginate oligomer, which is a degradation product of alginates by an enzyme in brown seaweed, in a large quantities can directly and indirectly influence the activation of immune system in the body by increasing cytokine secretion (Iwamoto et al., 2003) and improving the

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bioavailability of zinc (Yonekura and Suzuki, 2003). Brown seaweed also contains a large amount of minerals with higher contents of iodine (I) and calcium (Ca) compared with other foods (Kaufmann et al., 1998).

Brown seaweed is in the diets of humans in Asian countries including Korea, Japan and some part of China, but because only the leaves of brown seaweed is commercially available a vast amount of stems and roots remain as seaweed by-products. On the other hand, many countries with coastline often have problems with seaweed, and there is a potential to use brown seaweed and its by-products for other purposes such as animal diets or a fertilizer. Studies to use seaweeds as a feed were attempted earlier (Kaufmann et al., 1998; Fleurence, 1999; Bach et al., 2008; Holdt and Kraan, 2011). However, studies with dairy cows are limited to only the effects of supplementing brown seaweed by-products (BSB) on *in vitro* fermentation characteristics, milk production and milk composition with little information being available for its effects on growth, reproduction and milk production after parturition in dairy cows. In the present study therefore, the effects of BSB on fermentation characteristics in the rumen have been examined *in vitro*, and then the influence of adding BSB in the diet of Holstein dairy cattle on animal performance, endocrine responses, and milk production were investigated.

MATERIALS AND METHODS

The present study consisted of two experiments. In Experiment 1, the effect of different levels of BSB on fermentation characteristics *in vitro* was examined, while in Experiment 2 the influence of supplementing BSB on growth, endocrine responses, and milk production of Holstein cow was studied.

Experiment 1: Effect of different levels of BSB on fermentation characteristics in the rumen *in vitro*

Experimental design and diets: The study involved an examination of fermentation characteristics in the rumen using *in vitro* batch culture in triplicate with supplementation of BSB at various levels, namely 0% (control), 2% (2% BSB), and 4% (4% BSB) of dry matter, having replaced hay in the basal diets. Basal diets consisted of a mixture with commercial concentrates and hay approximately in the ratio of 35:65 as incubation substrates (Tables 1 and 2).

Preparation of rumen inoculum and incubation procedure: Rumen inoculum used was obtained from a Holstein cow equipped with a permanent ruminal cannula. Once being taken, rumen contents were transferred to a laboratory using a thermos bottle kept at 39°C, immediately after which the rumen content was filtered with four layers cheesecloth to remove feed particles, and stored in a constant temperature bath maintained at 39°C. The filtered rumen fluid was mixed with McDougal's buffer (McDougall, 1948) bubbled with CO_2 in 1:1 ratio, and used as a rumen inoculum. First, 0.25 g of substrate was placed in a 120 mL serum bottle, into which 25 mL of rumen inoculum was added under anaerobic conditions. The bottles were plugged with a butyl-rubber stopper and aluminum cap, and were incubated in a shaking incubator (SI-600R, Lab. Companion, Seoul, Korea) maintained at 39°C, 120 rpm, where the incubation times were 0, 3, 6, 9, 12, and 24 h. After completion of each incubation time, the pH was measured immediately using a pH meter (inoLab pH Level 1, Weilheim, Germany), and supernatant was sampled after centrifugation at 3,000 rpm for 15 min, and stored at –20°C for the analyses of ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA). For VFA analysis, 4 mL of supernatant was added to 1 mL of 25% HPO_3 and stored at –72°C.

Sample analysis: Dry matter (DM), crude protein, ether extracts, organic matter, Ca and phosphorus of basal diets

Table 1. Chemical composition of feeds used in Experiments 1 and 2 (% of dry matter unless otherwise stated)

Item	Diets		
	Concentrate	Tall fescue	BSB
Crude protein	13.50	14.21	8.37
Ether extract	2.00	3.47	0.87
Crude fiber	10.00	26.16	11.53
Crude ash	15.00	6.24	35.84
Ca	0.90	0.14	1.05
P (mg/100 g)	0.50	0.02	0.24
I (mg/100 g)	-	-	7.8
Total digestible nutrients	68.00	51.00	43.20

BSB, brown seaweed by-products.

Table 2. Ingredient and chemical composition of experimental diets used in Experiments 1 and 2 (% of dry matter unless otherwise stated)

Item	Control	2% BSB	4% BSB
Ingredient			
Concentrate	36.49	37.17	35.09
Tall fescue hay	63.51	60.93	61.47
BSB	0	1.90	3.44
Total	100.0	100.0	100.0
Chemical composition			
Dry matter	89.57	89.50	89.52
Crude protein	13.95	13.84	13.76
Ether extract	2.89	2.83	2.82
Neutral detergent fiber	52.14	51.46	51.76
Acid detergent fiber	27.53	27.07	27.34
Ca	0.45	0.47	0.47
P	0.33	0.33	0.33
I (ppm)	-	1.35	2.70
Total digestible nutrients	57.15	57.12	56.64

BSB, brown seaweed by-products.

and BSB used were analyzed according to the method of AOAC (1995), and acid detergent fiber and neutral detergent fiber were analyzed according to the method of Van Soest et al. (1991). Analysis of ammonia-N was determined by using a spectrophotometer (Optizen 1142H, MECASYS Co., Ltd, Daejeon, Korea) after color fixation in the same quantities for phenol and alkali according to Chaney and Marbach (1962). An analysis of VFA was made with gas chromatography (VARIAN CP-3800, Varian Inc., Walnut Creek, CA, USA) according to the method by Erwin et al (1961) with a fused silica capillary column (30 m×0.32 mm, 0.5 μm film thickness, HP Innowax, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with flame ionization detector.

Experiment 2: Effect of supplementing BSB on growth, endocrine status, and milk production of Holstein dairy cows

Experimental design and animals: Initially, twenty-one Holstein heifers (average body weight 419 kg) were randomly divided into 3 groups, consisting of control, 2% (2% BSB) and 4% (4% BSB) supplementation of BSB by replacing a relatively similar portion of tall fescue based on nutritional requirements (Tables 1 and 2), and the experiment was conducted for 12 months at the research farm of Seoul National University, Korea. All experimental procedures were in accordance with the "Guidelines for Care and Use of Experimental Animals of Konkuk University".

Feeding management: All heifers were offered feed based on their nutritional requirements with the target body weight gain at 0.7 kg/d, depending on their body weights and weight gains as shown by National Research Council (2001). Concentrates mixed with BSB were offered twice a day at 8:30 AM and 16:00 PM, and tall fescue was fed at 65% of DM intake with water and mineral blocks. Chemical composition of BSB was same as the one used in Experiment 1 (Tables 1 and 2). Body weight was measured at 9:00 AM at intervals of 2 months and at the end of the study. Animal performance in terms of daily gain and feed intake was measured for 9 months. Two months after beginning the experiment, artificial insemination of heifers was implemented by a specialized inseminator after confirmation of estrus. Most heifers gave birth to calves, and milk samples were collected (see below for the sampling procedure). However, 2 animals from the control and 1 animal from 2% BSB and 4% BSB fail to give a birth and hence were omitted from blood and milk sampling.

Sample collection and analysis: Ten-mL of blood was collected from the jugular veins in a heparin-treated vacuum tube (BD Vacutainer Systems Preanalytical Solutions, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 9:00 AM before the start of the experiment, on 120th day and 240th day of pregnancy. After collection, the plasma was separated by centrifugation (4°C, 3,000 rpm, 15 min), and stored at –70°C until further analysis. Milk production was recorded daily by automatic recording with ALPRO SYSTEM (Alfa-Laval Agri., Peterborough, Ontario, Canada), and 30 days after parturition the milk was analyzed for milk protein, milk fat, lactose and solid-not-fat (SNF) using Milkoscan-133B (Foss, Hilleroed, Denmark), and the number of somatic cells was determined by using Fossomatic-300 (Foss).

Analysis of total estrogen, progesterone, triiodothyronine (T3) and thyroxine (T4) in blood was as follows; Total estrogen was measured by radioimmunoassay (RIA) method using DA Total Estrogen kit (ICN Biomedicals, Irvine, CA, USA), and progesterone measured using CoAT-A-OUNT Progesterone kit (Diagnostic Products Corporation, Los Angeles, CA, USA). T3 and T4 were determined by RIA method using RIA-mat-T3 and RIA-mat-T4 kits (Byk-Sangtec Diagnostica, Dietzenbach, Germany).

Calculation and statistical analysis

All the results for Experiments 1 and 2 were subjected to analysis of variance with the level of BSB as the main effect using SAS version 8.1 (SAS Institute Inc., Cary, NC, USA) (SAS Institute Inc., 2000). Mean values for each treatment were further tested by Duncan's multiple range test, and the significance was declared when $p<0.05$.

RESULTS AND DISCUSSION

Experiment 1: Effect of different levels of BSB on fermentation characteristics in the rumen *in vitro*

The pH *in vitro* changed very little, ranging from 6.20 to

Table 3. Effects of dietary BSB supplementation on *in vitro* pH and ammonia nitrogen (NH_3-N) concentration in Experiment 1

Incubation time (h)	Treatments[1]			SEM	p-value
	Control	2% BSB	4% BSB		
pH					
3	6.62	6.60	6.63	0.009	0.546
6	6.51	6.51	6.53	0.008	0.535
9	6.39	6.37	6.39	0.007	0.460
12	6.26[b]	6.30[a]	6.30[a]	0.005	0.028
24	6.22	6.20	6.22	0.014	0.747
NH_3-N (mg/dL)					
3	7.66[a]	6.44[a,b]	5.05[b]	0.368	0.072
6	4.94	4.44	4.66	0.241	0.711
9	6.74[a]	6.98[a]	3.44[b]	0.497	0.047
12	8.00[a]	6.71[a,b]	4.74[b]	0.485	0.085
24	18.10[a]	14.03[b]	10.43[c]	0.570	0.005

BSB, brown seaweed by-products; SEM, standard error of the mean.
[1] All values represent the mean of triplicates.
[a,b,c] Means in the same row with different superscripts differ ($p<0.05$).

6.63 when BSB was supplemented at different levels (Table 3). The pH above 6.00 at 24 h of incubation in all treatments indicates replacing tall fescue with BSB does not cause any unfavorable fermentative environment for microorganisms. In the case of ammonia-N concentrations, supplementation of BSB at 4% showed a lower ($p<0.05$) ammonia-N at 3, 9, 12, and 24 h of incubation as compared with the control.

Ammonia-N values above 5 mg/dL in the rumen are known to be optimal for microbial growth (Satter and Slyter, 1974), although there are large discrepancies among studies (i.e., 1.4 mg/Dl [Schaefer et al., 1980] to 19.4 mg/dL [Mehrez et al., 1977]). The large quantities polysaccharides present in brown seaweed exist in structural carbohydrates including alginic acid, fucoidan and laminaran, and consists of heteropolysaccharides such as D-mannuronic acid and its isomer of L-guluronic acid, which account for most of the cell walls of Phaeophyceae, including brown seaweed (Beresford et al., 2000; Klinkenberg et al., 2001). These polysaccharides can be easily degraded by the rumen microbes and act as an energy for the microbes in the rumen (Greenwood et al., 1983; Beresford et al., 1999). Hence, reduction in ammonia-N with supplemented BSB is likely to occur due to synchronization between energy and N for the growth of rumen microorganisms. Concentrations of VFA were not different except for 24 h of incubation where total VFA concentration was lower ($p<0.05$) when 4% BSB was supplemented (Table 4). It is unclear why VFA concentration with 4% BSB diet at 24 h was lower than that in the other dietary treatments. One might speculate that DMD (dry matter degradability) is lower with 4% BSB; however, it is difficult to conclude this as we did not obtain results on DMD.

From the results of the *in vitro* study, the BSB supplementation did not change ruminal pH with little variation at high level of concentrate in the diets, and may

Table 4. Effects of dietary BSB supplementation on *in vitro* volatile fatty acids concentration in Experiment 1

Incubation time (h)	Treatments[1]			SEM	p-value
	Control	2% BSB	4% BSB		
Acetate (mM)					
3	45.47	48.30	46.02	1.434	0.8253
6	53.29	52.39	55.48	0.972	0.5745
9	62.51	56.41	52.96	1.884	0.0697
12	62.58	60.98	62.97	1.607	0.8789
24	71.47[a]	77.47[a]	62.40[b]	2.408	0.0057
Propionate (mM)					
3	11.78	12.78	12.75	0.473	0.7730
6	14.52	14.64	16.20	0.430	0.3446
9	17.69	15.21	15.58	0.536	0.0738
12	17.95	17.64	19.47	0.646	0.4907
24	20.70[a,b]	23.69[a]	19.15[b]	0.797	0.0357
Butyrate (mM)					
3	5.99	6.59	6.14	0.326	0.8504
6	7.57	7.16	7.90	0.210	0.5047
9	9.60	8.09	7.26	0.483	0.1133
12	9.65	9.09	9.25	0.407	0.8683
24	11.45[a]	12.06[a]	8.97[b]	0.551	0.0116
Valerate (mM)					
3	0.69	0.74	0.64	0.046	0.7955
6	0.83	0.76	0.81	0.022	0.4254
9	1.05	0.90	0.72	0.065	0.0827
12	1.06	1.00	0.97	0.050	0.7851
24	1.43[a]	1.40[a]	1.08[b]	0.072	0.0043
Total volatile fatty acids (mM)					
3	65.26	69.94	66.99	2.324	0.8332
6	77.70	76.39	82.01	1.645	0.5073
9	92.82	82.26	77.87	3.033	0.0753
12	93.33	90.64	94.55	2.740	0.8526
24	108.41[a]	118.14[a]	93.97[b]	3.941	0.0089

BSB, brown seaweed by-products; SEM, standard error of the mean.

[1] All values represent the mean of triplicates. [a, b] Means in the same row with different superscripts differ ($p<0.05$).

Table 5. Effects of dietary BSB supplementation on growth performance for 9 months in Holstein heifers in Experiment 2

Item	Control	2% BSB	4% BSB	SEM	p-value
Initial body weight (kg)	419.1	419.0	419.1	8.85	0.9997
Final body weight (kg)	612.7	610.3	611.7	17.46	0.9927
Weight gain (kg)	193.6	191.3	192.6	6.35	0.9928
Daily weight gain (kg/d)	0.70	0.70	0.70	0.023	0.9963
Feed intake (kg/d)	10.58	10.36	10.49	-	-
Tall fescue	6.78	6.38	6.45	-	-
BSB	0	0.18	0.36	-	-
Concentrate	3.80	3.78	3.58	-	-
Growth efficiency (%)	6.62	6.76	6.68	-	-

BSB, brown seaweed by-products; SEM, standard error of the mean.

show potential to improve N metabolism in the rumen without a particular influence on the generation of VFA for other than 24 h of fermentation. Thus it was not considered that supplementation of BSB in the diets of ruminants had an unfavorable influence on the fermentation characteristics in the rumen.

Experiment 2: Influence of adding BSB on growth, endocrine response, and milk production of Holstein dairy cows during transition

Animal performance: Animal performance in terms of daily gain and feed intake of Holstein cows is presented in Table 5. Daily gains with an average weight of 419 kg for a total of 275 days were approximately 0.7 kg/d for all three treatments as expected, indicating that addition of BSB did not influence daily gains. Alginic acids composing cell walls of seaweeds have been reported to have characteristics of forming viscous gel so that palatability preference is lowered, accompanied by a reduction in feed intakes (Beresford et al., 2000). Franklin et al. (1999) also reported that DM intake was significantly reduced as a result of daily supplementation of seaweeds for milking cows. On the other hand, the levels of supplement in the present study are not considered to have major impact on DM intake, as similar results were observed between treatments where total DM intakes were 10.58, 10.36, and 10.49 kg for the control, 2% BSB and 4% BSB, respectively. However, as this experiment was conducted with a group feeding regime and the results were presented with a mean value of all animals within a treatment, statistical analysis of the feed intake was not provided.

Endocrine responses: Influences of adding BSB on various hormones in blood are given in Table 6. The pattern of estrogen before and after pregnancy indicated a normal pattern of estrogen. In terms of both estrogen and progesterone, no particular differences per stage were indicated among treatments. T3 and T4 are hormones synthesized in the thyroid glands, where I is an important precursor for the synthesis. Pattanaik et al. (2001) reported that contents of T3 and T4 in plasma had increased

Table 6. Effects of dietary BSB supplementation on endocrine response of Holstein dairy heifers in Experiment 2[1]

Item	Control	2% BSB	4% BSB	SEM	p-value
Before BSB offered					
Estrogen (pg/mL)	20.57	20.57	23.14	1.417	0.3101
Progesterone (ng/mL)	3.26	3.26	1.94	0.438	0.0917
T3 (ng/dL)	167.24	167.24	147.47	2.865	0.0163
T4 (μg/dL)	6.52	6.53	6.19	0.145	0.1531
Conception at 120 d					
Estrogen (pg/mL)	55.71	94.14	72.73	7.314	0.0764
Progesterone (ng/mL)	4.92	5.84	4.61	0.450	0.4479
T3 (ng/dL)	159.69	167.71	153.12	3.695	0.1843
T4 (μg/dL)	5.95	6.25	5.35	0.235	0.1957
Conception at 240 d					
Estrogen (pg/mL)	121.57	189.57	165.22	20.159	0.3871
Progesterone (ng/mL)	5.53	5.19	5.00	0.446	0.8902
T3 (ng/dL)[2]	160.38	167.29	156.14	2.419	0.0888
T4 (μg/dL)[3]	5.45	5.95	5.36	0.212	0.4149

BSB, brown seaweed by-products; SEM, standard error of the mean.

[1] Experimental animals were heifers and were conceived during the study.

[2] Triiodothyronine. [3] Thyroxine.

significantly compared with the control when I at the level of 0.05 mg and 0.075 mg per head a day was added in the basic feeds for goats. However, a pattern of increase in hormone synthesis in the thyroid glands was not exhibited per stage in the present study. In the case of T3, only a slight increase of 3.83% and 5.87% was observed when compared with the beginning of the study for a pregnancy of 120 days and 240 days, respectively.

Milk production and composition: Milk productions and milk composition 1 month after delivery of dairy cows are given in Table 7. DM intakes showed similar results among treatments with 21.05 kg, 20.88 kg, 20.52 kg for the control, 2% BSB, 4% BSB, respectively. Although the concentrations of estrogen and progesterone during pregnancy period, which tended to show higher values for 2% BSB compared with other treatments, were expected to influence duct development causing differences in milk production, no differences in milk production were brought about with resultant values of 27.14 kg, 28.77 kg, 27.65 kg corresponding to the control, 2% BSB and 4% BSB, respectively at 4th week of lactation. Contents of milk protein, milk fat, lactose, SNF were shown to be similar among treatments, indicating that addition of BSB during pregnancy period did not have an influence in the lactation period. Our results are somewhat different compared with previous reports. For example, Bendary et al. (2013) reported increased milk yield when dairy cows were supplemented with the mixture of seaweed and premix, and another study also has shown a similar result, an increased milk yield in high-yielding dairy cows (Hostens et al., 2011). The discrepancy in terms of milk yield between previous studies and our results remains to be determined. It is interesting to note, however, that in the report by Kellogg et al. (2006) where they concluded from a series of studies that supplementation of commercially available brown seaweed such as Tasco (Acadian Seaplants Ltd., Dartmouth, Nova Scotia, Canada) is beneficial for milk production in large dairy cows during moderate to severe heat stress, but not with cows at first-lactation where the body size is still relatively small compared with mature cows.

Table 7. Effects of dietary BSB supplementation on DMI, milk yield and composition of Holstein dairy cows in Experiment 2

Item	Treatments			SEM	p-value
	Control	2% BSB	4% BSB		
Number of cows	5	6	6		
DMI (kg/d)	21.05	20.88	20.52	0.525	0.8752
Milk yield (kg/d)	27.14	28.77	27.65	0.342	0.7963
Milk fat (%)	4.07	4.12	4.03	0.230	0.5364
Milk protein (%)	3.11	3.06	3.12	0.158	0.8897
Milk lactose (%)	4.71	4.81	4.73	0.112	0.7856
Solid-not-fat (%)	8.27	8.31	8.32	0.175	0.9982

DMI, dry matter intake; BSB, brown seaweed by-products; SEM, standard error of the mean.

IMPLICATIONS

The present study examined the influence of BSB on fermentation characteristics *in vitro* and also on animal performance, endocrine response and milk production in Holstein dairy cows. From the results of both *in vitro* (Experiment 1) and *in vivo* studies (Experiment 2), it is concluded that addition of BSB hardly changes pH, induces fermentation synchronization *in vitro* and has little influence on estrogen and progesterone, and no increase in milk production was observed up to 1 month after delivery. The results from our study may provide an insight in the use of by-products or waste materials in the diet of dairy cows from fisheries.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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