

EFFECT OF SUPPLEMENTATION OF BROWN SEAWEED MEAL ON SERUM ENZYMES AND MINERAL STATUS OF GOATS

KANTI RAJE*, A.K. VERMA, A. DAS, PUTAN SINGH, NIRMALA MUWEL,
Y. ROJITA DEVI, BRIJESH K. OJHA¹ and ALOK MISHRA

Centre of Advanced Faculty Training in Animal Nutrition,
ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, India

¹Department of Animal Nutrition, College of Veterinary Science & Animal Husbandry,
Rewa, NDVSU, Jabalpur-486001, India

Received: 26.06.2018; Accepted: 12.02.2019

ABSTRACT

The study was conducted to assess the effects of brown seaweed (BSW) supplementation on different blood metabolites in adult female goats. Fifteen adult female goats were randomly divided into 3 groups following randomized block design. Concentrate mixture in the Control group had no BSW, whereas in T₁ and T₂ group, BSW was added at 4% and 8% level, respectively. The goats were fed as per ICAR nutrient requirements (2013). Before start of experiment (day 0), on 30th and 60th day of experiment, blood samples were collected for harvesting serum. A linear increase ($P < 0.001$) in serum concentration of calcium as well as superoxide dismutase activity were observed in the BSW supplemented groups. Rest of the parameters were comparable ($P > 0.05$) among the groups. Thus, BSW shows a potential as a functional feed additive which can improve antioxidant status and Ca balance in the goats.

Keywords: Antioxidant, Brown seaweed, Goat, Mineral, Serum enzymes

Seaweed could be a potentially valuable resource for animal feeding and may contribute in solving the fodder scarcity problem in animals (Oliveira *et al.*, 2009). Seaweed consumption has been found to increase activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and sometimes catalase (CAT) (Matanjun *et al.*, 2010). They are rich in soluble dietary fibers, proteins, minerals, vitamins, antioxidants, phytochemicals and polyunsaturated fatty acids but are low in caloric value. Brown seaweed also contains a large amounts of both macro (Ca, Mg, Na, P and K) and trace elements (Zn, I and Mn) with higher contents of iodine (I) and calcium (Ca) compared with other feeds (Matanjun *et al.* 2009). Seaweeds are valuable alternative feeds for livestock, mostly as sources of valuable chelated micro-minerals, the availability of which is higher than that of inorganic ones; complex carbohydrates with prebiotic activities; pigments beneficial to consumer health (Evans and Critchley, 2014). Considering the positive findings regarding seaweeds obtained from several feeding experiments, our study was planned to evaluate the effects of brown seaweed (BSW) supplementation on blood metabolites of indigenous goats.

MATERIALS AND METHODS

The present study was carried out in the Animal Nutrition Division, Indian Veterinary Research Institute, Izatnagar, Bareilly.

Experimental design: Fifteen female goats (does) of

same age were randomly divided into 3 groups (T₀, T₁ and T₂) following randomized block design. The goats were randomly allocated into three dietary treatments; control and 2 treatment groups. Does in the control group (T₀) were fed a standard concentrate mixture having no BSW, whereas the concentrate mixture for goats in groups T₁ and T₂ contained 4% and 8% of BSW, respectively. The animals were housed in a well ventilated concrete-floored shed with facilities for individual feeding and watering. Proper ethical care and management procedures were adopted throughout the study period according to the guidelines for institutional animal ethics committee for experimentation. They all were treated for endo and ectoparasites before start of the experiment.

Feeds and feeding: All the goats were offered daily a weighted amount of respective concentrate mixture and *ad libitum* wheat straw in the morning at 10.00 AM to meet their nutrient requirements for maintenance as per ICAR (2013). Wheat straw refusals were weighted daily on the next morning to estimate wheat straw consumption per day and sampled at weekly intervals for subsequent analysis of dry matter (DM) to assess the average DM intake during the experiment. A small amount of green berseem fodder (400 g on DM basis) was given to all the goats to satisfy the part of their respective nutrient requirements. The animals were provided fresh and clean tap water twice daily. Duration of experimental feeding was 60 days. The ingredients composition of concentrate mixtures is given in Table 1.

Blood Profile: To correlate the results of nutritional value

*Corresponding author: drkantiraje@gmail.com

Table 1
Ingredients composition of concentrate mixtures

Ingredients (kg)	T0	T1	T2
Maize	40	40	40
Wheat bran	40	40	40
SBM	17	17	17
Mineral mixture	2.0	2.0	2.0
Common salt	1.0	1.0	1.0
Brown seaweed (%)	-	4	8
DCP(%)	13.72	13.78	13.85

of seaweeds, periodical monitoring of blood parameters was carried out. Blood samples were drawn before start of experiment (day 0), on day 30 and 60 of the experiment. About 6 ml of whole blood was collected and taken in well cleaned, dry, sterilized centrifuge tubes and allowed for clotting. After clotting, blood samples were centrifuged at 2500 rpm for 15 min at 4 °C and transparent straw colour portion were separated and stored in deep freeze (-20 °C) for further analysis.

Estimation of serum enzymes: The serum samples were analyzed for different serum antioxidant enzymes by using commercial assay kits which includes Catalase (CAT) by Catalase Assay Kit (ELISA) by Cayman Chemical Ann Arbor, MI; Superoxide dismutase (SOD) by SOD Assay kit by SIGMA-ALDRICH Co. LLC and Glutathione peroxidase (GSH-Px) by Glutathione Peroxidase Cellular Activity Assay Kit by SIGMA-ALDRICH Co. LLC. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) in serum was determined as per the method given by Reitman and Frankel (1957) and serum alkaline phosphatase (ALP) activity was estimated by the method of Kind and King (1954) using diagnostic kits manufactured by Span Diagnostics Limited, Surat, India.

Estimation of minerals in serum samples: Serum mineral extracts prepared by using one ml serum in a 50 ml digestion flask and digest with 15-20 ml triple acid (HNO₃, H₂SO₄, HClO₄; 4:2:1) mixture and after cooling make volume 50 ml by using distilled water. Calcium and phosphorus in serum sample were estimated using diagnostic kit from Span Diagnostics Limited, Surat, India. Concentration of trace minerals *viz.* zinc, copper,

iron and manganese was estimated by using atomic absorption spectrophotometer.

Statistical Analysis: The experimental data generated were analyzed using two-way ANOVA (statistical package SPSS 20.0) and Duncan's multiple range test was used to compare difference among the treatment groups. The P value less than 0.05 indicated significant difference among treatments by adopting standard statistical procedures (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Data pertaining to serum antioxidant enzymes i.e. CAT, SOD and GSH-Px activity are presented in Table 3. Activity of catalase and glutathione peroxidase enzymes were comparable ($P>0.05$) among treatments over different period of experiment. Supplementation of BSW at the level of 4% and 8% of concentrate mixture significantly improved ($P<0.001$) the serum concentration of SOD. Similar to our results Wresdiyati *et al.* (2008) observed that supplementation of 5% and 10% seaweed powder increased of Cu, Zn-SOD content in the liver tissues of rats compared to control group. BSW show various biological activities, including anticoagulant, antioxidant, antiviral, anticancer and immunomodulating activities (Zhang *et al.*, 2009; Wijesekara *et al.*, 2011). Zhang *et al.* (2004) reported that sulfated polysaccharides (BSW) play important roles as free-radical scavengers and antioxidants, which can prevent oxidative damage in living organisms. However, lambs fed brown seaweed Tasco (*Ascophyllum nodosum*) diets tended ($P=0.07$) to exhibit higher Red and white blood cell glutathione peroxidase across time when compared with controls. White blood cell SOD activity (mU/mg protein) also tended ($P=0.13$) to be greatest in heat induced oxidative stress lambs fed pre- or post-harvest Tasco groups (Saker *et al.*, 2004). Kang *et al.* (2005) observed antioxidant properties of red seaweed (*Callophyllis japonica*) *in vitro*. Chen *et al.* (2006) found that agaro-oligosaccharides derived from red seaweed can exert their *in vitro* and *in vivo* hepatoprotective effect through scavenging oxidative damage induce by reactive oxygen species. BSW is an excellent source of acid water-soluble polysaccharide of fucoidan that has shown anti-oxidation effect (Allen *et al.*, 2001).

Serum minerals status of supplemented goats is presented in Table 4. Supplementation of BSW at levels of 4% and 8%, significantly ($P<0.001$) increase the serum Ca and P level, respectively, as compared to control groups. The increased serum concentration of Ca and P might be due to their increased supply through BSW supplementation. The level of trace minerals Zn, Cu, Fe and Mn in serum were comparable ($P>0.05$) among different treatment

Table 2
Chemical Composition of Feeds and Fodder (% DM Basis)

	DM	CP	EE	NDF	ADF	OM	Ca	P
Straw	91.6	2.90	0.92	75.4	51.00	92.7	0.24	0.16
Berseem	17.2	18.16	1.52	49.75	89.63	86.6	2.32	0.36
Seaweed	90.86	7.05	2.01	21.10	20.45	54.46	1.16	0.31
Con. mix	88.9	26.82	1.60	17.72	8.46	92.12	0.89	0.42

Table 3
Effect of supplementation of BSW on serum antioxidant status in goats (n=5)

Group	Period			Treatment Mean ±SE	P value		
	Day 0	Day 30	Day 60		T	P	T*P
CAT (U/ml)							
T ₀	88.56±3.19	89.13±1.22	89.78±2.41	89.16±1.97	0.321	0.124	0.231
T ₁	89.15±4.38	90.32±1.27	91.59±5.16	90.35±1.97			
T ₂	88.98±2.62	88.17±3.72	89.52±4.41	88.89±1.97			
Period mean ±SE	88.90±3.40	89.21±2.07	90.30±3.99				
SOD (U/ml)							
T ₀	48.56 ^a ±1.74	50.88 ^a ±1.51	53.68 ^b ±2.78	51.37 ^A ±1.21	0.051	0.000	0.004
T ₁	49.18 ^a ±1.53	54.94 ^b ±1.23	56.32 ^{bc} ±1.36	53.15 ^B ±1.21			
T ₂	49.21 ^a ±3.42	55.88 ^{bc} ±1.28	58.72 ^c ±2.75	54.60 ^B ±1.21			
Period mean ±SE	48.98 ^a ±1.81	53.23 ^b ±1.81	56.91 ^{bc} ±1.81				
GSH-Px (U/ml)							
T ₀	19.87±0.91	21.20±0.55	22.13±0.94	21.07±0.54	0.234	0.089	0.107
T ₁	20.53±0.65	21.39±1.12	22.83±0.85	21.58±0.54			
T ₂	21.14±1.51	22.80±0.91	23.51±0.68	22.49±0.54			
Period mean ±SE	20.51±0.55	21.80±0.54	22.82±0.54				

Means with different superscripts (^{abc} & ^{AB}) within row and column, respectively differ significantly

Table 4
Effect of supplementation of BSW on serum ALP, AST and ALT in goats (n=5)

Group	Period			Treatment Mean ±SE	Pvalue		
	Day 0	Day 30	Day 60		G	P	G*P
ALP(IUL-1)							
CON	124.4±7.97	125.5±8.04	126.9±8.19	125.6±2.77	0.918	0.923	1.000
T ₁	120.4±1.17	120.7±1.48	122.9±0.96	121.4±2.77			
T ₂	121.9±1.81	122.8±1.44	123.1±1.12	122.4±2.77			
Period mean ±SE	124.2±2.77	123.1±2.77	124.3±2.77				
AST (IUL-1)							
CON	81.81±9.93	81.94±8.44	82.41±8.51	82.05±4.34	0.996	0.999	1.000
T ₁	80.69±6.44	81.06±8.37	80.81±6.76	80.85±4.34			
T ₂	79.19±4.91	79.98±5.53	80.44±7.30	79.87±4.34			
Period mean ±SE	80.56±4.34	80.99±4.34	81.22±4.34				
ALT (IUL-1)							
CON	20.57±2.85	22.69±3.11	22.18±2.44	21.81±0.99	0.140	0.190	0.260
T ₁	18.76±0.86	18.90±0.90	19.02±0.58	18.89±0.99			
T ₂	18.20±0.33	18.92±0.72	19.48±0.53	18.87±0.99			
Period mean ±SE	19.18±0.99	20.17±0.99	20.41±0.99				

groups.

The level of serum enzymes like ALP, ALT and AST (Table 5) did not differ significantly (P>0.05) among different groups on dietary supplementation of brown seaweed at level of 4% and 8% to the indigenous breed of adult female goats. In above study, these serum enzymes level have

within normal range so seaweed not showing any detrimental effect on liver function up to the level of 8% in diet of adult goats. Similar to our study Nogueira *et al.* (2017) have reported no significant (P>0.05) changes in serum AST and ALT level between ME (methanolic extract of *Padinasanctae-crucis*) treated mice and the control. Contrary to our result, the ethanol extract of *S. binderi*

Table 5
Effect of supplementation of BSW on serum mineral status in adult goats (n=5)

Group	Period			Treatment Mean±SE	P value		
	Day 0	Day 30	Day 60		G	P	G*P
Calcium (mgdl⁻¹)							
CON	9.17 ^a ±0.08	10.34 ^{ab} ±0.14	10.56 ^{ab} ±0.13	10.02 ^A ±0.10	0.01	0.021	0.010
T ₁	9.39 ^a ±0.06	10.68 ^{ab} ±0.14	11.71 ^b ±0.13	10.59 ^{AB} ±0.10			
T ₂	9.58 ^a ±0.17	11.45 ^b ±0.10	12.81 ^c ±0.35	11.28 ^B ±0.10			
Period Mean±SE	9.38 ^a ±0.10	10.82 ^{ab} ±0.10	11.69 ^b ±0.10				
Phosphorus (mgdl⁻¹)							
CON	2.98 ^a ±0.05	3.01 ^a ±0.04	3.14 ^a ±0.06	3.04 ^A ±0.05	0.001	0.001	0.001
T ₁	2.99 ^a ±0.08	3.36 ^b ±0.12	4.23 ^c ±0.06	3.53 ^B ±0.05			
T ₂	3.11 ^a ±0.02	4.08 ^b ±0.12	5.17 ^c ±0.08	4.12 ^C ±0.05			
Period Mean±SE	3.03 ^a ±0.05	3.48 ^b ±0.05	4.18 ^c ±0.05				
Zinc (ppm)							
CON	0.81±0.14	0.79±0.18	0.80±0.11	0.80±0.14	0.112	0.672	0.118
T ₁	0.82±0.24	0.80±0.13	0.81±0.16	0.81±0.18			
T ₂	0.80±0.17	0.78±0.15	0.82±0.23	0.80±0.18			
Period Mean±SE	0.81±0.18	0.79±0.15	0.81±0.17				
Copper (ppm)							
CON	0.63±0.23	0.65±0.25	0.68±0.22	0.65±0.23	0.115	0.654	0.214
T ₁	0.65±0.31	0.67±0.16	0.69±0.23	0.67±0.23			
T ₂	0.62±0.20	0.64±0.26	0.67±0.37	0.64±0.28			
Period Mean±SE	0.63±0.25	0.65±0.22	0.68±0.27				
Iron (ppm)							
CON	4.80±0.53	4.95±0.31	4.99±0.24	4.91±0.36	0.212	0.542	0.321
T ₁	4.73±0.22	4.85±0.21	4.88±0.27	4.82±0.23			
T ₂	4.84±0.35	5.06±0.33	5.30±0.33	5.07±0.34			
Period Mean±SE	4.79±0.37	4.95±0.28	5.06±0.28				
Manganese (ppm)							
CON	0.66±0.26	0.57±0.32	0.59±0.12	0.61±0.23	0.322	0.894	0.421
T ₁	0.65±0.21	0.66±0.27	0.62±0.14	0.64±0.21			
T ₂	0.63±0.25	0.63±0.12	0.61±0.11	0.62±0.16			
Period Mean±SE	0.65±0.24	0.62±0.24	0.61±0.12				

Means with different superscripts (abc&AB) within row and column, respectively differ significantly

slightly increased the concentration of liver enzymes (AST, ALT and ALP) level in serum when administered at the rate of 200 mg/kg to rats for 14 days, as compared with control (Hira *et al.*, 2017). Although seaweeds contains various biological activity but their use as dietary supplement needs toxicological study in future.

From the results of this, experiment it is evident that supplementation of BSW improved serum concentrations of Ca, P and SOD. Thus, BSW shows a potential as a functional feed additive that may increase antioxidant

status and utilization of minerals in goat.

REFERENCES

- Allen, V.G., Pond K.R., Saker, K.E., Fontenot, J.P., Bagley, C.P., Ivy, R.L., Evans, R.R., Brown, C.P., Miller, M.F., Montgomery, J.L., Dettle, T.M., and Wester, D.B. (2001). Tasco-Farage: III. Influence of a seaweed extract on performance, monocyte immune cell response, and carcass characteristics in feedlot-finished steers. *J. Anim. Sci.* **79**: 1032–1040.
- Chen, H., Yan, X., Zhu, P. and Lin, J. (2006). Antioxidant activity and hepatoprotective potential of agaro-oligosaccharides *in vitro* and *in vivo*. *Nur. J.* **5**: 31-42.

- Evans, F.D. and Critchley, A.T. (2014). Seaweeds for animal production use. *J. Appl. Phycol.* **26**(2): 891-899.
- Hira, K., Tariq, R.M., Sultana, V., Ara, J. and Ehteshamul-Haque, S. (2017). Effect of seaweeds occurring at Karachi coast on mosquito larvae and liver function in rats. *Pak. J. Pharm. Sci.* **30**(2): 387-391.
- ICAR (2013). Nutrient requirement for Goats. Indian Council for Agricultural Research, New Delhi.
- Kang, K.A., Bu, H.D., Park, D.S., Go, G.M., Jee, Y., Shin, T. and Hyun, J.W. (2005). Antioxidant activity of ethanol extract of *Callophyllis japonica*. *Phytother. Res.* **19**(6): 506-510.
- Kind, P.R.N. and King, E.J. (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J. Clin. Pathol.* **7**(4): 322-326.
- Matanjun, P., Mohamed, S., Kharidah, M. and Noordin, M. M. (2010). Comparison of cardiovascular protective effects of tropical seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*, on high-cholesterol/high-fat diet in rats. *J. Med. Food.* **13**: 792-800.
- Matanjun, P., Mohamed, S., Mustapha, N.M. and Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *J. Appl. Phycol.* **21**: 1-6.
- Nogueira, R.B., Tomaz, A.C.A., Pessoa, D.R., Xavier, A.L., Pita, J.C.L., Sobral, M.V., Pontes, M.L., Pessôa, H.L., Diniz, M.F., Miranda, G.E.C. and Vieira, M.A.R. (2017). Brown algae *Padina Sanctae-crucis* Børgesen: A Potential Nutraceutical. *Mar. Drugs.* **15**(10): 251.
- Oliveira, M.N., Freitas, A.L.P., Carvalho, A.F.F.U., Sampaio, T.M.T, Farias, D.F., Teixeira, D.I.A., Gouveia, S.T., Pereira, J.G. and Sena, M.M.C.C. (2009). Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. *Food Chem.* **115**: 254–256.
- Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **28**(1): 56-63.
- Saker, K.E., Fike, J.H., Veit, H. and Ward, D.L. (2004). Brown seaweed-(Tasco) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. *J. Anim. Physiol. Nutr. (Berl)*. **88**: 122-130.
- Snedecor, G.W. and Cochran, W.G. (1994). Statistical Methods, 8th edition. The Iowa State University Press, Ames, Iowa, USA.
- Wijesekara, I., Pangestuti, R. and Kim, S.K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydr. Polym.* **84**: 14–21.
- Wresdiyati, T., Hartanta, A.B. and Astawan M. (2008). The Effect of Seaweed *Eucheuma cottonii* on superoxide dismutase (SOD) liver of hypercholesterolemic rats. HAYATI. *J. Biosci.* **15**(3): 105-110.
- Zhang, Q., Li, N., Liu, X., Zhao, Z., Li, Z. and Xu, Z. (2004). The structure of a sulfated galactans from *Porphyra haitanensis* and its *in vivo* antioxidant activity. *Carbohydr. Res.* **339**: 105–111.
- Zhang, Z., Zhang, Q., Wang, J., Zhang, H., Niu, X. and Li, P. (2009). Preparation of the different derivatives of the low-molecular-weight porphyran from *Porphyra haitanensis* and their antioxidant activities *in vitro*. *Int. J. Biol. Macromol.* **45**: 22–26.