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Effect of Supplementation of Seaweed Formulations on Haematological, Blood Biochemical Parameters and Immune Response of Crossbred Calves

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ABSTRACT

Background: Seaweeds are rich in complex carbohydrates, proteins and low molecular weight nitrogenous compounds, lipids, organic minerals, vitamins and pigments. The aim of the study is to evaluate the effect of supplementation of seaweed formulations on haematological, blood biochemical parameters and immune response of crossbred calves.

Methods: A total of 18 male crossbred calves (130±7.56 kg live weight) were divided into 3 groups of 6 each based on complete randomized design. Calves in control (T_0) was fed on standard diet, while treatment groups: T_1 and T_2 were supplemented with AF-KWP or AFRD-5 at the rate of 4% of concentrate mixture with 1% calcium carbonate and 1% dicalcium phosphate replacing mineral mixture and common salt offered in T_0. The experiment was conducted for a period of 240 days. Blood was collected from each calf on 0, 100 and 200 days of experimental periods.

Result: Concentration of Hb, PCV and RBC count was significantly higher in T_1 and T_2. Supplementation of seaweed increased significantly (P<0.001) the lymphocytes to neutrophil ratio. Serum total protein, albumin (A), globulin (G) and A:G ratio were comparable. Humoral immune response was significantly higher (P<0.001) in T_1 and T_2. Study concluded that supplementation of seaweed formulations of T_1 and T_2 improved erythropoiesis, alleviate stress and increased humoral immune responses in crossbred calves and recommend as replacement to mineral mixture and common salt.

Key words: Crossbred calves, Haematology, Immunity, Seaweed formulations, Serum biochemical.

INTRODUCTION

Livestock sector contributes 4.11% GDP and 25.6% of total agriculture GDP in India and there is scope to improve the livestock sector from low production and poor productivity with alternative feed resources to bridge the gap in feed resources between requirement and availability. The deficit in feed resources accounted to 44% concentrate feeds, 35.6% green fodder and 10.95% dry crop residues (IGFRI, 2013). There are many potentially important feed resources having significant nutritional value which are available inexpensively in large quantity. Aquatic plant based non-conventional feed resources are one among them.

Marine macroalgae popularly known as seaweeds are renewable natural resources grow in large quantities along the coasts of India and the estimated length of coastalian in India is 7516.6 km. In European countries and USA, there were numerous reports of occasional or systematic use of seaweeds to feed the livestock in 19th and early 20th centuries (Hansen *et al*., 2003; Makkar *et al*., 2016). There are about 10,000 species of seaweeds (Guiry, 2014) but only a few of them are being used for animal feeding. Seaweed serves mainly as a source of minerals and, to an extent, of vitamins and energy. Therefore, it can be assumed that supplements of seaweed may improve the nutritive quality of diet and growth of small animals and birds in terms of body weight gain, fats and protein contents (Zahid Phool *et al*., 1995). There are 3 different groups of seaweed on the basis of thallus colour brown, red and green seaweed. They are also different in many ultra structural and bio-chemical features including photosynthetic pigments, storage compounds, composition of cell walls and presence or absence of flagella. Seaweeds are mostly rich in complex carbohydrates, proteins and low molecular weight nitrogenous compounds, lipids, organic minerals, vitamins, volatile compounds and pigments (Makkar *et al.,* 2016). Seaweeds contain 10 to 20 times more minerals as compared to land plants and thus, they are potential sources of minerals (Makkar *et al*. 2016). Seaweeds are rich source of sodium, potassium, magnesium, chlorine, sulfur, phosphorus, iodine, iron, zinc, copper, selenium and molybdenum (Okab *et al*., 2013). Moreover, they also contain heavy metals and some minerals

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are in toxic concentration that may interfere with availability of other minerals (Cabrita *et al*., 2016). Many species of macro-algae have anti-bacterial, anti-viral, anti-oxidant and anti-inflammatory properties that improve animal health and body functions (Bach *et al.,* 2008). Haematological and serum metabolites are used to monitor the metabolic and health status of the animals. An index neutrophil/lymphocyte (N: L) which is widely used to assess stress in mammals (Davis *et al.,* 2008; Stella *et al.,* 2013). Supplementation of *Kappaphycus alvarezii* based formulations improved cellular and humoral immunity in chicken (Qadri *et al*. 2018).

Red seaweeds *Kappaphycus alvarezii* and *Gracilaria salicornia* are mostly used as sources of carrageenan. The by-products after extraction of carrageenan can be used as animal feed. There is dearth of information about effect of feeding seaweeds in cattle. The present experiment was conducted to evaluate the effect of seaweed formulations on haematological, serum biochemical parameters and humoral immune response in crossbred calves.

MATERIALS AND METHODS

This study was conducted at experimental animal shed of Animal Nutrition Division, ICAR-IVRI, Izatnagar in Uttar Pradesh of India in the year 2019-2020. The experiment was approved by Institutional Animal Ethics Committee (IAEC) and conducted under guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Experimental design, animal management and diet

Eighteen male crossbred calves (130 ± 7.56 kg body weight) were divided into 3 groups of 6 each based on complete randomized design. The experimental calves were offered concentrate mixture, wheat straw and green fodder to meet out nutrient requirements as per ICAR (2013) feeding standard recommendation for a body weight gain of 500 g/ day. All the animals were provided fresh and clean drinking water twice *ad lib*. The feeding trial was conducted for 240 days. Concentrate mixture was formulated with crushed maize, de-oiled soybean meal, wheat bran, mineral mixture and common salt for feeding of calves in control (T_0) group. Two different concentrate mixtures were formulated with crushed maize, de-oiled soybean meal, wheat bran, calcium carbonate, dicalcium phosphate, seaweed based formulations for feeding the T_1 and T_2 groups. Treatment T_1 and T_2 were supplemented with AF-KWP or AFRD-5 at 4% with 1% calcium carbonate and 1% dicalcium phosphate replacing mineral mixture and common salt (Table 1). AF-KWP contains *Kapaphycus alvarezi* (thrashed weed), *Gracilaria salicornia* (washed weed) and *Kapaphycus* water extract at a ratio of 1:1:1 while AFRD-5 comprise of *Kapaphycus alvarezi* (thrashed weed), *Gracilaria salicornia* (washed weed) and *Turbinaria conoides* at a ratio of 2: 2: 1. Roughage to concentrate ratio in the present experiment was 60:40. Available green fodder was given to experimental calves to meet out the Vitamin A or carotene requirement.

Blood sample collection and analysis

Blood was collected on 0, 100 and 200 days of the experimental period from each calf by puncturing jugular vein in the morning (before watering and feeding) in the vaccutainer tubes. Blood samples were brought to laboratory immediately, centrifuged at 3000 rpm for 15 minutes to separate serum and stored at -20°C until further analysis. The haemoglobin (Hb) concentration in blood was estimated by cyanomethaemoglobin method. RBC and WBC were analyzed by using the haemocytometer. Lymphocyte: neutrophil ratio was calculated by dividing percent lymphocyte by percent neutrophil. Serum biochemical parameters were estimated by using commercial kits (Coral Clinical Systems - Tulip Diagnostics, India). At the end of the experiment humoral immune response was assessed by micro-haemagglutination assay. All the calves were injected with 1ml of 20% suspension of washed chicken red blood cells (CRBC) by intravenous route and blood samples were collected on day 0, 7, 14, 21 and 28.

Data processing and analysis

Data were analyzed by using SPSS computer package (SPSS version 20.0, SPSS Inc., Chicago, USA). One and two way ANOVA was used for comparison of means according to Duncans multiple range test at 5% level of significant.

RESULTS AND DISCUSSION

The chemical composition of concentrate mixture, wheat straw and maize green fodder was presented in Table 2. The crude protein (CP) content of $T_{0,}$ T_1 and T_2 was almost similar *i.e.*19.63, 19.55 and 19.60%, respectively. Total ash, calcium and phosphorus content of concentrate mixture containing calcium and phosphorus added seaweed formulations (AF-KWP and AFRD-5) powder were higher than control with mineral mixture due to higher content of OM (more than 50%). Our results are in agreement with Hong *et al.* (2015) who reported lower CP, EE, ADF and NDF in brown seaweed byproducts supplemented group than control. The chemical composition of maize fodder and wheat straw was within the normal range reported by

Table 1: Ingredient compositions of concentrate ration.

Ingredients	T_0	T_1	T_2
Crushed maize	40	‘40	40
Deoiled-soybean	22	22	22
Wheat bran	35	32	32
Mineral mixture	02	-	-
AF-KWP	-	04	-
AFRD-5	-	-	04
Calcium carbonate	-	01	01
Dicalcium phosphate	-	01	01
Common salt	01	-	-
Total	100	100	100

Ranjhan, 1988. The overall feed intake (g/d) was presented in Table 3. The overall daily intake of concentrate, maize fodder and wheat straw did not find significant difference among all the groups. Similar result reported by many studies (Franklin *et al.*, 1999; Munde, 2018). Cell walls of seaweeds composed of alginic acids which might attributed to form viscous gel that reduce the feed intake by decrease the palatability (Beresford *et al.,* 2000).

Results of haematological parameters in different groups of calves were presented in Table 4. A significant ($P<0.001$) effect was observed with in Hb concentration, PCV (%), count of WBC and RBC. Concentration of Hb, PCV (%), count of WBC and RBC were higher in T_1 and T_2 compared to T_0. According to Alwarswamy *et al*. (2016) AFRD-5 contained brown seaweed; *Turbinaria conoides* which is a good source of fucoidan that are stimulators of erythropoiesis, increased Hb concentration. Hence improvement in Hb in T_2 could be attributed to fucoidan present in the brown seaweed. However, there are reports those did not observed any significant effect on Hb concentration after feeding brown seaweed in crossbred calves (Venkateswaran, 2018). Seaweed supplementation had insignificant effect on the platelets count but significant ($P<0.05$) effect was observed on lymphocytes and neutrophils. Archor *et al.* (2008) also reported increased white blood cell and lymphocyte counts in seaweed supplemented group. Supplementation of seaweed formulations increased the relative abundance of lymphocytes with decrease in neutrophil resulting in their increased ($P<0.001$) ratio. Higher lymphocyte to neutrophil ratio was observed in T_2 (1.89) followed by T_1 (1.80) and T_0 (1.33). Lymphocyte to neutrophil ratio is widely used to determine stress in mammals (Davis *et al*., 2008; Stella *et al*. 2013) thus their higher ratio in T_1 and T_2 signified reduce stress in these crossbred calves

Table 5 shows the serum biochemical parameters in calves. Serum glucose is significantly high in T_1 and T_2 than T_0 which is in agreement with the finding of El-Banna *et al*. (2005) on rabbits fed green seaweed (*Ulva lactuca* or *Entromorpha intestinalis*). Serum total protein, albumin, globulin and A/G ratio were within the normal range reported by Dukes, (1984) therefore, all the experimental cossbred calves were in good health during the period of experimentation. Okab *et al*. (2013) also observed similar impact of feeding green seaweed (*U. lactuca*) to the male and female rabbits. Serum creatinine in different groups was lowering side but non - significant. Even feeding of brown algae *Padina sanctae-crucis* to mice at 2000 ppm also unaltered serum creatinine concentration. Mean BUN concentration was significantly ($P<0.001$) differ among treatment. Similarly El-Banna *et al*. (2005) reported that diets supplemented with green seaweed resulted in elevated BUN concentration in Baladi rabbits and contrary was reported by Okab *et al*. (2013). Urea and creatinine represent the two nitrogenous components that are eventually excreted by the kidney; therefore, changes in their levels in blood stream would reflect the insufficiency of kidney tubules or kidney malfunction (Miller, 1966). Serum values of uric acid, total cholesterol, HDL and LDL were comparable between groups but triglycerides were significant ($P=0.054$). The decreases in triglyceride in the present study particularly in animals fed on seaweed formulations indicate that selenium and other antioxidants content of the seaweed may play a major role in preventing lipid peroxidation.

Hira *et al*. (2017) reported that rat fed with 200 ppm of brown seaweed (*Sargassum binderi*) extract did not alter

Table 2: Chemical composition (%) of concentrate mixture, green fodder and wheat straw.

Attributes	T_0	T_1	T_2	Wheat straw	Maize fodder
Dry matter	88.74	88.76	88.77	93.55	19.00
Crude protein	19.63	19.55	19.60	3.65	9.85
Ether extract	2.23	2.16	2.20	1.79	1.07
Total ash	7.21	7.24	7.18	6.53	10.20
Organic matter	92.86	92.76	92.82	93.47	89.80
Acid insoluble ash	1.36	1.43	1.46	2.20	9.00
Crude fibre	5.80	5.68	5.77	38.12	26.40
Nitrogen free extract	65.13	65.37	65.25	49.91	52.48
Total CHO	70.93	71.05	71.02	88.03	78.88
Neutral detergent fibre	24.10	22.43	22.66	85.20	58.42
Acid detergent fibre	7.23	7.18	7.33	57.13	37.91

Table 3: Overall feed intake of crossbred calves in different groups.

Attributes	T_0	T_1	T_2	SEM	P value
Concentrate (g/d)	2525.13	2504.94	2481.98	113.75	0.989
Green (g/d)	1161.39	1145.93	1132.31	62.61	0.984
Wheat straw (g/d)	1905.00	1871.48	1865.65	48.17	0.944
Total (g/d)	5591.52	5522.35	5479.94	223.49	0.981

serum cholesterol concentration. In contrary to this result El-Banna *et al*. (2005) reported that diets supplemented with green seaweed (*Ulva lactuca* or *Entromorpha intestinalis*) resulted in elevated cholesterol concentration than control diet fed Baladi rabbits. Chung *et al*. (2008) and Hira *et al*. (2017) also reported that brown algae *Ecklonia stolonifera* and *Sargassum binderi* reduce serum triglyceride level in rats. In contrary to this result, El-Banna *et al*. (2005) reported that diets supplemented with green seaweed (*Ulva lactuca* or *Entromorpha intestinalis*) resulted in triglycerides concentration is comparable to control diet in Baladi rabbits.

Humoral immunity of calves in different dietary groups is presented in Table 6. The titre was significantly (P<0.001) lower in T_0 (2.78) as compared to T_1 (3.00) and T_2 (3.21). The mean value at 0 day against CRBC was significantly (P<0.001) lower than subsequent days. The titre value at 14 day (5.05) was significantly (P<0.001) higher as compared to day 0 (1.00), 7 (2.62), 21 (5.05) and 28 (2.63).

Table 4: Haematological parameters of crossbred calves in different groups.

Attributes	0d	100d	200d	Mean	SEM	T	P	TxP
Haemoglobin (g/dl)								
T_0	9.38	9.83	10.66	9.95^a	0.074	0.000	0.000	0.000
T_1	9.42	11.56	12.32	11.10^b				
T_2	9.44	11.64	12.43	11.17^c				
Mean	9.41^a	11.01^b	11.80^c					
PCV (%)								
T_0	32.13	33.54	34.36	33.34^a	0.065	0.003	0.000	0.909
T_1	32.62	33.81	34.54	33.66^b				
T_2	32.91	34.04	34.82	33.92^c				
Mean	32.55^a	33.80^b	34.57^c					
WBC (x10^3/µl)								
T_0	5.24	7.64	8.53	32.14^a	0.055	0.000	0.000	0.000
T_1	5.62	8.05	9.26	33.31^b				
T_2	5.84	8.85	9.69	33.79^c				
Mean	5.57^a	8.18^b	9.16^c					
RBC (x10^6/µl)								
T_0	5.59	6.65	8.44	6.89^a	0.05	0.007	0.000	0.117
T_1	5.70	6.84	8.25	6.93^a				
T_2	5.65	6.76	8.83	7.08^b				
Mean	5.65^a	6.75^b	8.51^c					
Platelets (x10^3/µl)								
T_0	354	356	369	359.67	5.22	0.861	0.436	1.000
T_1	357	360	373	363.33				
T_2	360	364	376	366.67				
Mean	357	360	372					
Lymphocytes (%)								
T_0	55.39	48.87	45.87	50.04^a	1.04	0.017	0.008	0.056
T_1	52.78	57.74	51.77	54.10^b				
T_2	55.80	59.23	57.93	57.65^c				
Mean	54.66^b	55.28^c	51.86^a					
Neutrophils (%)								
T_0	33.23	38.91	40.79	37.64^b	0.549	0.018	0.003	0.024
T_1	29.67	30.00	30.46	30.05^a				
T_2	33.66	27.65	30.07	30.46^a				
Mean	32.19^a	32.19^a	33.77^b					
L:N ratio								
T_0	1.67	1.26	1.12	1.33^a	0.024	0.000	0.004	0.001
T_1	1.77	1.93	1.69	1.80^b				
T_2	1.66	2.18	1.94	1.89^c				
Mean	1.70^a	1.89^c	1.74^b					

^{abc}Means in the same row and column bearing different superscripts differ significantly (P<0.001, P<0.05)

Table 5: Serum biochemical parameters of crossbred calves in different groups.

Treatment	Periods			Mean ±SE	SEM	P value		
	0d	100d	200d			T	P	T*P
Glucose (mg/dl)								
T_0	64.45	62.18	61.76	62.79[a]	0.088	0.001	0.000	0.024
T_1	64.26	63.24	61.19	62.90[a]				
T_2	64.72	63.53	62.46	63.57[b]				
Mean	64.47[c]	62.98[b]	61.80[a]					
Total protein (g/dl)								
T_0	6.46	6.38	6.37	6.40	0.059	0.429	0.990	0.980
T_1	6.42	6.48	6.45	6.45				
T_2	6.53	6.57	6.66	6.59				
Mean	6.47	6.48	6.49					
Albumin (g/dl)								
T_0	3.26	3.33	3.10	3.23	0.065	0.176	0.317	0.759
T_1	3.30	3.81	3.32	3.47				
T_2	3.29	3.46	3.37	3.37				
Mean	3.28	3.53	3.26					
Globulin (g/dl)								
T_0	2.58	3.16	3.05	2.93	0.088	0.372	0.309	0.858
T_1	3.11	3.25	3.28	3.21				
T_2	3.15	3.16	3.35	3.22				
Mean	2.95	3.19	3.23					
A:G								
T_0	1.08	1.19	0.96	1.08	0.05	0.361	0.133	0.643
T_1	1.07	1.54	1.13	1.25				
T_2	1.08	1.15	1.10	1.11				
Mean	1.08	1.29	1.06					
Creatinine (mg/dl)								
T_0	1.33	1.51	1.62	1.48	0.040	0.759	0.018	0.990
T_1	1.38	1.57	1.71	1.55				
T_2	1.42	1.51	1.68	1.54				
Mean	1.37[a]	1.53[ab]	1.67[b]					
BUN (mg/dl)								
T_0	18.53	19.02	21.11	19.56[a]	0.050	0.000	0.000	0.007
T_1	18.81	19.65	21.61	20.02[b]				
T_2	19.21	20.82	22.04	20.69[c]				
Mean	18.85[a]	19.83[b]	21.59[c]					
Uric acid (mg/dl)								
T_0	2.24	2.13	1.88	2.09	0.039	0.775	0.001	0.994
T_1	2.28	2.17	1.93	2.13				
T_2	2.35	2.21	1.90	2.15				
Mean	2.29[b]	2.17[b]	1.90[a]					
Total cholesterol (mg/dl)								
T_0	68.37	71.26	75.07	71.57	1.062	0.577	0.178	0.938
T_1	67.91	69.05	72.13	69.69				
T_2	68.78	66.45	71.43	68.89				
Mean	68.35	68.92	72.88					
Triglyceides (mg/dl)								
T_0	40.25	42.54	43.16	41.98[b]	0.549	0.070	0.054	0.974
T_1	37.74	39.60	40.16	39.17[a]				

Table 5: Continue....

Table 5: Continue....

T_2	36.65	40.63	40.52	39.27[a]				
Mean	38.21[a]	40.92[ab]	41.28[b]					
HDL (mg/dl)								
T_0	35.74	41.06	42.26	39.69	0.499	0.209	0.005	0.110
T_1	34.75	40.32	38.08	37.72				
T_2	37.56	36.47	39.52	37.85				
Mean	36.02[a]	39.28[b]	39.95[b]					
LDL (mg/dl)								
T_0	24.12	22.15	24.18	23.48	1.194	0.945	0.628	0.984
T_1	25.23	21.18	26.02	24.14				
T_2	23.09	22.65	23.81	23.19				
Mean	24.15	21.99	24.68					

[abc]Means in the same row and column bearing different superscripts differ significantly (P<0.001, P<0.05).

Table 6: Humoral immune response of crossbred calves measured as antibody response to chicken RBC.

Treatment	Periods					Mean	SEM	P value		
	0d	7d	14d	21d	28d			T	P	T*P
T_0	1.00	2.57	4.37	3.54	2.44	2.78[a]				
T_1	1.00	2.62	5.14	3.69	2.57	3.00[b]	0.025	0.000	0.000	0.000
T_2	1.00	2.68	5.64	3.83	2.88	3.21[c]				
Mean	1.00[a]	2.62[b]	5.05[d]	3.69[c]	2.63[b]					

*[abc]Means in the same row and column bearing different superscripts differ significantly (P<0.001, P<00.05.)

Read *et al*. (1996) and Islam *et al.* (2016) reported similar results in seaweed extract supplemented diet of cows and sows, respectively. Munde (2018) found that humoral immunity (P<0.05) is improved due to supplementation seaweed based formulations in calves. The strong hemagglutination activity of *Kappaphycus alvarezii* was linked to high lectin content which is 185-338 ìg/g dry algae (Hung *et al*., 2009). Kuznetsova *et al*. (2015) also observed that fucoidans are agonists for receptors of innate immunity and potent inducers of the cell-mediated and humoral immune response.

CONCLUSION

Supplementation of seaweed formulations improved haemoglobin, relative abundance of lymphocytes, lymphocyte to neutrophil ratio and humoral immune response of crossbred calves. The study recommend supplementation of growing crossbred calves with 1% each of calcium carbonate and dicalcium phosphate along with 4% of seaweed formulation AF-KWP or AFRD-5 as substitute to mineral mixture and common salt.

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