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Effect of Dietary Supplementation of Seaweed Formulations on Nutrient Utilization, Growth Performance, Serum Antioxidant Activity and Immunity in 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 was to evaluate the effect of seaweed formulations on nutrient utilization, growth performance, serum antioxidant activity and cell mediated immune response of crossbred calves.

Methods: Eighteen male crossbred calves (130±7.56 kg body weight) were divided into 3 groups of 6 each based on complete randomized design. 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, 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. Experiment was conducted for a period of 240 days. Blood was collected from each calve at 0, 100 and 200 days of experiment. At the end of the experimental feeding a metabolism trial was conducted and cell mediated immune response was asassed.

Result: There was no significant difference between groups regarding nutrient utilization, total dry matter intake, gain in body weight and feed conversion ratio. However, significant ($P<0.001$) differences were observed in serum antioxidants status. Moreover, the mean values for skin thickness differed significantly ($P<0.001$) among different groups and values were 14.03, 15.54 and 16.62 in T_0, T_1 and T_2 group, respectively. Hence seaweed formulations might improve antioxidant status and cell mediated immune response without affecting the nutrient utilization and growth performance of crossbred calves.

Key words: Antioxidants, Crossbred calves, Growth performance, Immunity, Nutrient utilization, Seaweed formulations.

INTRODUCTION

Seaweeds are rich in proteins, lipids, polysaccharides, mineral, vitamins and enzyme (Rimber 2007). In contrast to conventional mineral supplements, seaweed is unique in being of plant origin containing a wide range of naturally balanced chelated minerals, trace elements, amino acids and vitamins. Being totally natural and of vegetable origin seaweed is easily digested and is safely fed to animals of all ages (Sykes 2009). 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 rich source of Na, K, Mg, Cl, S, P, I, Fe, Zn, Cu, Se and Mo (Okab *et al*. 2013). The use of seaweeds in livestock feed increased growth rate and feed conversion efficiency in ruminants (Chowdhury *et al.* 1995). Many species of macro-algae have anti-bacterial, anti-viral, anti-oxidant and anti-inflammatory properties that improve animal health and function (Bach *et al.* 2008). Many researchers reported that phlorotannins which is derived from the marine brown algae have strong antioxidant properties (Heo *et al.,* 2005; Shibata *et al.,* 2008). Allen *et al.,* (2001) reported that supplementation of seaweed meal enhanced immunity in beef cattle.

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Limited information is available about feeding and evaluation of seaweed in crossbred calves. Therefore, present experiment was designed to assess the effect of seaweed formulations on nutrient utilization, growth performance, serum antioxidant activity and immunity of crossbred calves.

MATERIALS AND METHODS

Eighteen male crossbred (Holstein Friesian × Indigenous) calves (130±7.56 kg live weight) were divided into 3 groups of 6 each based on CRD. The 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. While the concentrate mixture of calves in groups T_1 and T_2 were supplemented with AF-KWP and AFRD-5 at 4% with 1% calcium carbonate and 1% dicalcium phosphate, respectively 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. The experimental calves were offered feed 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. Roughage to concentrate ratio was 50:50. Available green fodder was given to experimental calves to meet out the Vitamin A or carotene requirement.

A record of feed intake and live weight was maintained for assessing the growth performance of calves during the entire experimental periods. A metabolism trial of 9 days was conducted at the end of the experimental feeding including 3 days of adaptation and 6 days of collection. Faeces and urine voided in 24 hour by each animal were collected and processed for storage and analysis. A separate aliquot of faecal samples was preserved with dilute (25% v/v) sulphuric acid for nitrogen (N) estimation. Similarly, urine sample was collected under acidic (dilute sulphuric acid) condition and sampled for estimation of N. Feeds, faeces and seaweed samples were also analyzed for the proximate principles (AOAC 2012) and fibre fractions *viz*. NDF and ADF (Van Soest *et al.* 1991).

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). Blood samples were brought to laboratory and centrifuged at 3000 rpm for 15 minutes to separate serum and stored at -20°C until further analysis. SOD and Catalase concentration was determined by using Cayman's Superoxide Dismutase Biont Assay ELISA kits based on the biotin double antibody sandwich technology. Glutathione peroxidase was determined by cellular activity assay

The cell mediated immune response was estimated in crossbred calves through *in vivo* delayed type hypersensitivity (DTH) reaction against phytohaemagglutinin- P (PHA-P). All the calves were injected intra-dermally with 125 µl of either saline solution (8.5 g NaCl) as a negative control on one side and PHA-P (20 µg 125 µ1-1 PHA-P solution) on the other side of neck region. The thickness of the skin was subsequently measured at 0, 24, 48, 72 and 96 hrs.

The statistical analyses were performed by using SPSS computer package (SPSS version 20.0, SPSS Inc., Chicago, USA). The data were statistically analyzed by using ANOVA with Duncan's post hoc testing to compare experimental groups. For all statistical analyses, probability values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Seaweed formulations (AF-KWP and AFRD-5) were higher in total ash and insoluble ash content (Table 2). Total ash, which mainly represents mineral content, in the present experiment was higher than the values reported by Cabrita *et al. (*2016) with *Ulva* sp. and *G. vermiculophylla*. EE content was in agreement with many studies (Khairy and El-Shafay 2013; Mouritsen *et al.* 2013). The CP content of concentrate mixture was almost similar. The chemical composition of maize fodder and wheat straw was within the normal range reported by Ranjhan, 1988. Our results are also similar to Abdoun *et al.* (2013) who reported that NDF and ADF were less in seaweed (*Ulva lactuca*) supplemented group than control.

The intake and digestibility of DM, OM, CP, EE, total carbohydrate, ADF and NDF was comparable in all three groups (Table 3). Therefore it is evident that supplementation of seaweed formulations has no adverse impact on overall rumen microbial and digestive efficiency of the animals. This corroborates well with the findings of Berry and Turk (1944) who have reported no influence of brown seaweed supplementation on DMI. Our findings were in contrary to Antaya *et al*. (2016) who have reported increased digestibility of OM and NDF due to supplementation of *Ascophyllum nodosum*.

The results showed that all the experimental calves were in positive Ca, P and N balance (Table 4). The Ca intake (g/d) was significatly ($P<0.001$) higher in the seaweed supplemental group than control. However, P intake (g/d) was significatly lower ($P<0.001$) in the seaweed supplemental group than control. The N intake was similar in all 3 groups. The retention (% intake) of Ca and P was significant ($P<0.05$) among the different groups. Munde (2018) reported that intake (g/d), retention (% intake) of Ca and P were similar among the groups by feeding *Kappaphycus* and *Gracilaria*. Munde (2018) also reported that retention of N (% intake) did not differ significantly among the group by feeding *Kappaphycus* and *Gracilaria*. Similarly, Venketeswaran (2018) reported that daily intake and retention of N were statistically similar in 3 groups by feeding 2% and 4% *Turbinaria conoides*.

Table 1: Ingredient compositions of concentrate mixture.

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

Non-significant effect was observed in total gain in body weight, ADG, total DMI and FCR among the 3 treatment groups (Table 5). Our results were in agreement with Anderson *et al.* (2006) who did not observed any influence of *Ascophyllum nodosum* supplementation on growth performance of crossbred cattle. Our results are in contarary

Table 2: Chemical composition (%) of seaweed formulations, concentrate mixture and roughage.

Attributes	AF-KWP	AFRD-5	T_0	T_1	T_2	Wheat straw	Maize fodder
DM	93.10	91.33	88.74	88.76	88.77	93.55	19.00
OM	37.90	44.60	92.86	92.82	92.76	93.47	89.80
Total ash	62.10	55.40	7.21	7.24	7.18	6.53	10.20
CP	4.87	7.26	18.73	18.65	18.62	3.65	9.85
EE	0.90	1.20	2.30	2.16	2.15	1.79	1.07
AIA	7.67	8.35	1.36	1.44	1.46	2.20	9.00
NDF	10.17	20.52	24.10	22.43	22.66	85.20	58.42
ADF	9.04	19.11	7.33	7.18	7.23	57.13	37.91
ADL	1.40	3.52	1.38	1.35	1.31	7.53	6.96
Hemicellulose	1.13	1.41	16.87	15.25	15.33	28.07	20.51
Cellulose	7.64	15.59	5.85	5.83	6.02	48.77	30.95
Ca	2.70	1.95	0.73	1.31	1.18	0.25	1.30
P	0.078	0.090	0.82	0.78	0.79	0.28	0.50

Table 3: Intake and digestibility of nutrients in different groups.

Attributes	Dietary treatments			SEM	P- value
	T_0	T_1	T_2		
Feed and nutrient intake(g/d)					
DM	7040.96	7014.46	6997.38	11.92	0.345
OM	2863.18	2853.92	2849.87	2.36	0.150
CP	844.78	839.73	839.37	0.80	0.154
EE	128.56	125.97	126.90	0.32	0.232
Total CHO	5351.69	5338.18	5321.28	9.64	0.461
ADF	3704.27	3636.87	3634.48	11.03	0.105
NDF	2189.15	2177.58	2176.40	5.22	0.574
Digestibility (%)					
DM	64.18	67.46	67.31	0.67	0.069
OM	66.01	68.45	68.15	0.66	0.273
CP	56.12	58.61	57.35	0.55	0.266
EE	52.05	50.71	51.48	0.59	0.677
Total CHO	58.09	60.10	60.25	0.58	0.244
ADF	57.24	54.05	61.48	1.09	0.010
NDF	65.35	58.45	61.48	1.00	0.009

Table 4: Balances of calcium, phosphorus and nitrogen in different groups.

Attributes	Treatment			SEM	P value
	T_0	T_1	T_2		
Ca					
Intake (g/d)	58.50^a	61.95^c	61.50^b	1.83	0.042
Retention (% intake)	49.93^a	52.95^b	54.55^b	1.59	0.004
P					
Intake (% intake)	48.19^b	50.13^a	52.59^a	1.90	0.003
Retention (g/d)	19.15	19.31	20.3	0.46	0.162
N					
Intake (g/d)	132.40	131.17	134.49	2.43	0.659
Retention (% intake)	48.92	50.93	51.57	1.39	0.572

^{abc}Mean values bearing different superscripts in a row differ significantly ($p<0.001$). ($p<0.05$).

with many studies who observed that seaweed supplementation improved body weight gain (Al-Shorepy *et al.,* 2001; Turner *et al.,* 2002).

The level of different antioxidant parameters were presented in Table 6. In the present study, seaweed formulations supplementation increased the activity of SOD, catalase, MDA and GPx. Our findings were similar to Allen *et al.* (2001) and Saker *et al*. (2004) who reported that inclusion of *Ascophyllum nodosum* improved antioxidant status in cattle. The seaweed formulation AFRD-5 used in the present experiment contained a brown seaweed thrashed powder (*Turbinaria conoides*) which contain phlorotannin, has antioxidant activities. Moreove extracts of *Kappaphycus* sp. showed higher reducing power and-OH radical scavenging activity than other antioxidants (Holdt and Kraan 2011). Sulfated polysaccharide from *Gracilaria* sp. exhibited moderate effect in inhibits the formation of free radicals. *Kapaphycus alvarezii* contains β-carotene, vitamin C and vitamin E which have antioxidant activity (Mantajun *et al*. 2010). Our result is in contradiction with Abdoun *et al.* (2013) who reported that seaweed inclusion did not alter the blood antioxidant activity. Similarly, Venketeswaran (2018) reported non-significant effect in serum SOD and GPx concentrations by supplemementation of *Turbinaria conoides* in crossbred calves.

The mean values for skin thickness differed significantly ($P<0.001$) among different groups and significantly ($P<0.001$) maximum skin thickness was observed in group T_2 followed by T_1 and T_0 groups (Fig 1). Maximum average thickness was observed after 24 h and then gradually reduced. Our results are in agreement with Allen *et al*. (2001) who have observed that *Ascophyllum nodosum* improved immune response in mammals. Kuznetsova *et al*. (2015) also observed that fucoidans are agonists for receptors of innate immunity and potent inducers of the cell-mediated immune response. In the present experiment, increased GPx

Table 5: Growth performance of crossbred calves in different groups.

Attributes	Treatment			SEM	P value
	T_0	T_1	T_2		
Initial BW (Kg)	130.05	130.13	130.83	7.56	0.999
Final BW (Kg)	270.70	275.15	279.22	8.58	0.893
Total gain (Kg)	140.65	145.02	148.39	2.00	0.976
ADG (g)	586.04	604.25	618.29	8.35	0.129
Total DMI (kg)	1341.96	1325.36	1315.19	53.64	0.981
FCR	9.54	9.14	8.86	0.33	0.620

Table 6: Serum antioxidant status of crossbred calves in different groups.

Treatment	Period			Mean	SEM	P value		
	0d	100d	200d			T	P	TxP
SOD (U/ml)								
T_0	7.64	10.21	14.65	10.83^a	0.148	0.000	0.000	0.073
T_1	8.22	11.32	17.59	12.38^b				
T_2	9.37	11.93	18.20	13.17^c				
Mean	8.41^a	11.15^b	16.81^c					
Catalase (nmol/min/ml)								
T_0	9.29	10.33	12.11	10.58	0.219	0.148	0.000	0.516
T_1	9.32	12.52	12.72	11.52				
T_2	9.40	11.81	13.25	11.49				
Mean	9.34^a	11.55^b	12.69^c					
MDA(mmol//L)								
T_0	2.01	3.23	3.36	2.87^a	0.035	0.000	0.000	0.095
T_1	1.74	2.48	2.83	2.35^b				
T_2	1.58	2.29	2.51	2.13^c				
Mean	1.78^a	2.67^b	2.90^b					
GPx (nmol/min/ml)								
T_0	154.46	157.73	160.73	157.64^a	0.284	0.000	0.000	0.082
T_1	156.79	162.46	164.03	161.09^{ab}				
T_2	158.23	160.23	166.31	161.59^b				
Mean	156.49^a	160.14^b	163.69^c					

^{abc}Mean values bearing different superscripts in a row and column differ significantly ($p<0.001$).

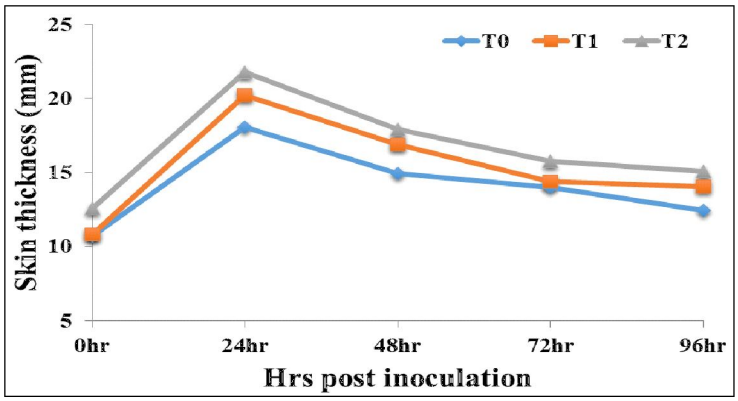

Fig 1: Absolute skin thickness (mm) after injection of PHA-p at different hours in different groups.

and SOD activity was observed which might contributed to increased cellular immunity. This is similar with the finding of Fike *et al*. (2001) who have reported increased SOD in ruminants with supplementation of brown seaweed. *Kappaphycus* and *Gracilaria* are rich in Se and vitamin E which were positively linked to cellular immunity (Fike *et al*. 2001). Our results are in agreement with Venketeswaran (2018) and Munde (2018) who reported that cell mediated immune response was significantly higher seaweed supplemented groups.

CONCLUSION

Present study concluded that calcium and phoshporus added seaweed formulations improves antioxidant status and cell mediated immune response without affecting growth performance and nutrient utilization in crossbred calves.

Conflict of interest: None.

REFERENCES

Abdoun, K.A., Okab, A.B., EL-Waziry, A.M., Samara, E.M. and AL-Haidary, A.A. (2013). Dietary supplementation of seaweed (*Ulva lactuca*) to alleviate the impact of heat stress in growing lambs. Pakistan Veterinary Journal. 34: 108-111.

Allen, V., Pond, K.E. and Saker. (2001). Effect of brown seaweed on antioxidants in forage and livestock. Journal of Animal Science. 79: 21-31.

Al-Shorepy, S.A., Alhadrami, G.A. and Jamali, I.A. (2001). Effect of feeding diets containing seaweed on weight gain and carcass characteristics of indigenous lambs in the United Arab Emirates. Small Ruminant Research. 41: 283-287.

Anderson, M.J., Blanton, J.R., Gleghorn, J., Kim, S.W. and Johnson, J.W. (2006). *Ascophyllum nodosum* supplementation strategies that improve overall carcass merit of implanted English crossbred cattle. Asian-Australia Journal of Animal Science. 19: 1514-1518.

Antaya, N.T. (2016). Kelp meal (*Ascophyllum nodosum*) supplementation to organic lactating dairy cows: Effects on milk production, milk composition, animal health and nutrient utilization during the non-grazing and grazing seasons in New Hampshire. Master's Thesis and Capstones. 887. University of new Hampshire, Durham, USA.

AOAC. (2012). Official Method of Analysis (19th ed.). Washington, DC: Association of Official Analytical Chemist.

Bach, S.J., Wang, Y. and McAllister, T.A. (2008). Effect of feeding sun-dried seaweed (*Ascophyllum nodosum*) on fecal shedding of *Escherichia coli* O157: H7 by feedlot cattle and on growth performance of lambs. Animal Feed Science and Technology. 142: 17-32.

Berry, M.H. and Turk, K.L. (1944). The value of kelp meal in rations for dairy cattle. Journal of Dairy Science. 27: 861-866.

Cabrita, A.R., Correia, A., Rodrigues, A.R., Cortez, P.P., Vilanova, M. and Fonseca, A.J. (2016). Assessing *in vivo* digestibility and effects on immune system of sheep fed alfalfa hay supplemented with a fixed amount of *Ulva rigida* and *Gracilaria vermiculophylla*. Journal of Applied Phycology. 1-11. DOI:10.1007/s10811-016-0999-9.

Chowdhury, S.A., Huque, K.S. and Khatun, M. (1995). Algae in animal production.

Fike, J.H., Allen, V.G., Schmidt, R.E., Zhang, X., Fontenot, J.P., Bagley, C.P., Ivy, R.L., Evans, R.R., Coelho, R.W., Wester, D.B. (2001). Tasco forage. I. Influence of a seaweed extract on antioxidant acivity in tall fescue and in ruminants. Journal of Animal Science. 79: 1011-1021.

Heo, S.J., Park, E.J., Lee, K.W. and Jeon, Y.J. (2005). Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresource Technology. 96: 1613-1623.

Holdt, S.L. and Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Phycology. 23: 543-597.

ICAR. (2013). Nutrient Requirements of Cattle and Buffalo. Indian Council of Agricultural Research, New Delhi.

Khairy, H.M. and El-Shafay, S.M. (2013). Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt. Oceanologia. 55: 435-452.

Kuznetsova, T.A., Besednova, N.N., Zaporozhets, T.S. and Somova, L.M. (2015). Prospects for the use of extracts and polysaccharides from marine algae to prevent and treat the diseases caused by *Helicobacter pylori*. Helicobacter. 20: 89-97.

Matanjun, P., Mohamed, S., Muhammad, K. and Mustapha, N.M. (2010). Comparison of cardiovascular protective effects of tropical seaweeds, *Kappaphycus alvarezii*, *Caulerpa lentillifera* and *Sargassum polycystum*, on high-cholesterol/ high-fat diet in rats. Journal of Medicinal Food. 13: 792-800.

Mouritsen, O.G., Dawczynski, C., Duelund, L., Jahreis, G., Vetter, W. and Schroder, M. (2013). On the human consumption of the red seaweed dulse (*Palmaria palmata*). Journal of Applied Phycology. 25: 1777-1791.

Munde, V.K., Das, A., Singh, P. and Verma, A.K. (2018). Effect of supplementation of seaweed by-products based formulations on haematological and serum metabolites profile in crossbred calves. Indian Journal of Animal Nutrition. 35: 271-281.

Munde, V.K. (2018). Evaluation of red seaweed meal as ruminant feed supplement. PhD Thesis. Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India.

Okab, A.B., Samara, E.M., Abdoun, K.A., Rafay, J., Ondruska, L., Parkanyi, V., Pivko, J., Ayoub, M.A., Al-Haidary, A.A., Aljumaah, R.S. and Peter, M. (2013). Effects of dietary seaweed (Ulva lactuca) supplementation on the reproductive performance of buck and doe rabbits. Journal of Applied Animal Research. 41: 347-355.

Ranjhan, S.K. (1988). Nutrient Requirements for Livestock and Poultry, 2nd ed. Indian Council for Agricultural Research, New Delhi, India.

Rimber, Ir Indy. (2007). Why is seaweed so important? M.Sc thesis, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Jln. Kampus Bahu, Manado 95115, Indonesia.

Saker, K.E., Fike, J.H., Veit, H. and Ward, D.L. (2004). Brown seaweed (TascoTM) treated conserved forage enhances antioxidant status and immune function in heat stressed wether lambs. Journal of Animal Physiology and Animal Nutrition. 88: 122-130.

Shibata, T., Ishimaru, K., Kawaguchi, S., Yoshikawa, H. and Hama, Y. (2008). Antioxidant activities of phlorotannins isolated from Japanese Laminariaceofae. Journal Applied Phycology. 20: 705.

Sykes, B. (2009). Seaweed and probiotics. Langcliffe Road, Settle, North Yorhshire.

Turner, J.L., Dritz, S.S., Higgins, J.J. and Minton, J.E. (2002). Effects of *Ascophyllum nodosum* extract on growth performance and immune function of young pigs challenged with *Salmonella typhimurium*. Journal of Animal Science. 80: 1947-1953.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991). Methods of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583-97.

Venketeswaran, K. (2018). Effect of replacement of mineral mixture by calcium enriched brown seaweed (*Turbinaria conoide*) on performance of crossbred calves. M.V.Sc. Thesis. Indian Veterinary Research Institute, Izatagar, Uttar Pradesh.