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ORIGINAL ARTICLE

Clinico-hemato-biochemical study of two commercial feed supplements for amelioration of rickets in growing male lambs

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Abstract The occurrence of rickets in young male lambs was investigated and amelioration was done with two commercial feed supplements (CFS-I and CFS-II) as a source of vitamins and minerals to see the beneficial effect in clinically affected lambs. Out of 113 male lambs, 18 lambs showed clinical symptoms of rickets such as bowing of legs, swelling of joints, stiff gait, and lameness. The ameliorative study was conducted for 45 days on 12 rickets affected lambs equally divided into two groups with commercial feed supplements as group I and group II. Six healthy lambs were served as control (group III). Clinical recovery after feed supplementation was assessed on the scale basis, and the hemato-biochemical alterations and serum mineral profile were evaluated on 0, 15, 30, and 45th days. The severity of clinical signs was found reduced in both the feed supplementation groups after 30th day in rickets affected animals and recovered completely on 45th day. The hematological values, serum total protein, globulin, calcium, phosphorus and magnesium levels revealed gradual improvement in rickets affected animals during the course of commercial feed supplementations and were comparable to the control animals at the end of experiment. The results of the study indicated that the feed supplementation with CFS-II was found to be more effective than CFS-I in the amelioration of rickets affected lambs.

Keywords Lamb · Rickets · Commercial feed supplement · Hemato-biochemical analysis

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Introduction

Rickets is a disease of young growing lamb with defective bone mineralization and calcification leading to bowing and swelling of extremities of long bones. It is a classic metabolic bone disorder of man and animals caused by a deficiency of either phosphorus or vitamin D or due to altered Ca-P ratio, but the disease may also be genetic in nature (Dittmer et al. 2009; O'Brien and Jackson 2012). Vitamin D is the most essential co-factor for normal calcium and phosphate homeostasis (DeLuca 2004). Mainly three types of rickets have been reported in animals. Hypophosphataemic rickets which is generally inherited forms of rickets, renal rickets which is thought to be vitamin D-dependent rickets type I (1 alpha-hydroxylase enzymes) and vitamin D-resistant rickets which is a hereditary disorder due to defect in the vitamin D receptors (Whyte 2002; Whyte and Thakker 2005). Rickets is characterized by stiffness of gait, recumbency, bowing of long bone, enlargement of costochondral junction called "rachitic rosary" in humans and reduced bone density which makes young ones prone to fractures (Radostits et al. 2000).

Rickets is uncommon in sheep, but generally, it is due to nutritional deficiency of vitamin D. In natural condition, the lamb grown on cereal crop feeding is much more prone for rickets due to ricketogenic effect of carotene. An outbreak has been reported in Scotland, which involved 50% of lambs aged 6 to 12 months grazing new grass and rape occurred during the early winter months and responded with vitamin D (Bonniwell et al. 1988). A similar study has been reported from South Island of New Zealand involving hoggets grazing green oats or green crop which are rich in rachitogenic carotenes in winter when solar irradiation is at the low level (Constable et al. 2016). An inherited form of rickets in Corriedale sheep has been described which is associated with

increased expression of the gene for 25-hydroxyvitamin D3-24-hydroxylase, the enzyme responsible for catabolism of vitamin D (Dittmer et al. 2011). Lameness as such poses a huge economic loss in a flock of sheep worldwide, but economics considering rickets as ailment has not been drawn till date.

In the 20th century, with the discovery of vitamin D, cod liver oil was used to treat rickets in human infants in the USA (Rajakumar 2003). In lambs, rickets has been treated successfully with injectable vitamin A and vitamin D (Van Saun 2004). With the advancement of therapeutics, it has been suggested with a balanced mineral and vitamin supplementation for management of rickets in animals such as calcium, phosphorus, copper, vitamin D, and vitamin A play important role in bone mineralization. Thus, the aim of present study was to evaluate two commercial feed supplements as a source of vitamins and mineral for amelioration of rickets in a flock of clinically affected lambs.

Material and methods

Animals and climatic conditions

The farm under study is situated at Avikanagar, Rajasthan, (26° 18′ 00″ N and 75° 25′ 39″ E). The maximum and minimum temperature during summer (April–July) and winter (November–February) varies from 38–45 °C and 2–10 °C, respectively. The average rainfall of area is about 400 mm and occurs mostly in July–August. There are occasional light showers in summer and winter with wind velocity of 14–16 km/h. About 3000 sheep of different breeds and 550 goats are maintained at separate units. All the animals are allowed nearly 8 h of pasture grazing during the pre-winter period and kept in partially covered corrals during night. Grazing is supplemented with balanced concentrate feed (approx. 150 g/day/animal for small breed and 300 g/day/animal for rest of the breeds) containing 2% mineral mixtures and 1% common salt throughout the year. Water is provided three times a day. At one sheep sector, out of 113, 18 (15.92%) male lambs of 4–6 months of age exhibited clinical manifestation of rickets in the month of September–October 2016 form the material of this study. The affected lambs were thoroughly examined for clinical symptoms such as bowing of legs, swelling of joints, stiffness of gait, lagging behind, and overall body score offered to each animal as per the criteria followed earlier (Van Saun 2004; Lovatt 2010). The improvement of the animals was judged on the basis of body score condition after the treatment with commercial feed supplements (Thompson and Meyer 1994).

Therapeutic groups

The randomly selected 12 diseased animals were divided into two equal groups for evaluation of effect of commercial feed supplements (CFS) in rickets. Group I received 25 ml of CFS-I (vitamin A, D3, E, H, calcium, and phosphorus) and group II was supplemented with 5 g of CFS-II (vitamin A, D3, E, nicotinamide, calcium, manganese, iron, copper, and cobalt) daily, orally for 45 days. The healthy lambs ($N = 6$) served as control (group III) and maintained without supplementation of CFS. All the animals were maintained up to 45 days with similar watering and feeding management and observed for clinical manifestations.

Hemato-biochemical analysis

Blood samples were collected in EDTA and non-EDTA vials (for serum) from jugular vein on 0th, 15th, 30th, and 45th day for monitoring hemato-biochemical and mineral analysis. Affected animals were observed clinically for changes in conformation of the bones and swelling of the joint, lameness, and body condition score. Hemoglobin (g %) was estimated in the whole blood by cyanmethemoglobin method (Vankampen and Zinglstra 1961). Packed cell volume (PCV %) was determined in whole blood by capillary microhaematocrit method (Coles 1980). Total erythrocyte count and total leucocyte count (TLC) were done manually by hemocytometer (Neubauer counting chamber) in whole EDTA blood. Differential leucocyte count was estimated in blood smear stained with Giemsa stain by routine method. Serum calcium, phosphorus, magnesium, alkaline phosphatase (ALP), total protein and albumin were estimated using commercial Accurex kit (Biomedical PVT. Ltd., Mumbai, India). Globulin was calculated by subtracting albumin concentration from total protein. AG ratio was derived by dividing albumin with globulin concentration.

Statistical analysis

The data obtained for hemato-biochemical and serum mineral profile was analyzed by using two-way ANOVA and Tuckey HSD using SPSS software version 22 for statistical significance at 5% level.

Results

Clinical signs observed in lambs were bowing and bending of long bones of limbs, swelling of epiphyses (mostly in radius ulna bone), stiffness in gait/lagging behind due to lameness, and poor body weight gain. The severity of clinical signs varied among the animals, but deformities of limbs were the common consistent feature. Clinical examination revealed no

evidence of increased rectal temperature, respiration, and heart rate, except the affected animals revealed slightly pale mucous membrane.

On 0th day, the severity of clinical signs on scale basis in group I and II, in relation to bow legs (3), swollen joints (2), and lameness (2), was found almost similar in between both the affected groups (Figs. 1 and 2). On 15th day, there was no much difference in severity of the clinical signs except the reduction in swollen joints (1) in both the groups and bow legs (2) and lameness (1) in the animals of group II. The body condition score for the animals of these groups on 0th and 15th day was found lower (2) than control animals. On 30th day, the severity of clinical signs was reduced in relation to bow legs (2), swollen joints (1), and lameness (1). The body condition score for group II animals was found comparable with the control animals; however, in group I animals, it was lower (3) than control. At the end of experiment (45th day), affected animals did not show any clinical symptoms of rickets and appeared to be normal as control animals (Figs. 3 and z Fig. 4). Scoring is depicted in Table 1.

Hematological changes

On 0th day of experiment, group I and group II lambs revealed significant ($P < 0.05$) decrease in level of Hb, PCV, and TEC as compared to healthy control group. After feed supplementation up to 45 days, the levels of Hb, PCV, and TEC were gradually increased on 15th and 30th day but were significantly lower ($P < 0.05$) than control. These values were found comparable with healthy control on 45th day. TLC and DLC changes were non-significant ($P > 0.05$) during the course of time. The data has been presented in Table 2.

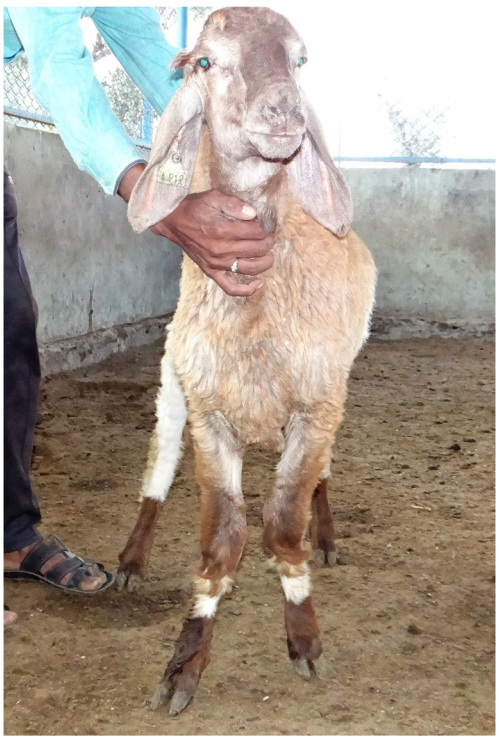

Fig. 1 Lamb of group I showing clinical signs of rickets on 0th day

Fig. 2 Lamb of group II showing clinical signs of rickets on 0th day

Serum calcium, phosphorus, magnesium, and Ca:P ratio

The level of serum calcium in group I and II found to be significantly ($P < 0.05$) low at 0th day as compared to healthy control (group III) but found non-significant ($P > 0.05$) at 15th, 30th, and 45th day of experiment as compared to healthy control. Serum phosphorus concentration was found significantly ($P < 0.05$) low at 0th and 15th day as compared to healthy control and found comparable with control animals on 30th and 45th day. Serum magnesium revealed significant ($P < 0.05$) low concentration on 0th day in group I and II. On 15th day, these values were low only in group II but found comparable on 30th and 45th day as compared to control group. Ca:P ratio was significantly ($P < 0.05$) high in group I and II at 0th and 15th day but found non-significant ($P > 0.05$) at 30th and 45th day as compared to healthy control. Data has been presented in Table 3.

Biochemical alterations

Alkaline phosphatase (ALP) was found to be significantly ($P < 0.05$) high in group I and II at 0th day and non-

Table 1 Observational clinical improvement scoring of rickets-affected lambs

Sl. no	Clinical signs	Group	0th day	15th day	30th day	45th day
1	[1]Bow legs (0–4)	I	3	3	2	1
		II	3	3	2	1
		III	0	0	0	0
2	[2]Swollen joints (0–3)	I	2	1	1	0
		II	2	1	1	0
		III	0	0	0	0
3	[3]Lameness (0–3)	I	2	2	1	0
		II	2	1	1	0
		III	0	0	0	0
4	[4]Body condition score(1–5)	I	2	2	3	4
		II	2	2	4	4
		III	4	4	4	4

An average of improvement of group was considered to draw the conclusion of scoring

[1] Bow legs scoring (0-no curvature; 1-minimum curvature, 2-mild curvature, 3-moderate curvature, 4-severe curvature)

[2] Swollen joint score was followed with of modification of Thompson et al. (1987) (0-no swelling, 1-mild swelling, 2-moderate swelling, and 3-severe swelling)

[3] Lameness scoring was done on 0–3 score following Angell et al. (2015)

[4] Body condition scoring was considered according to Thompson and Meyer (1994)

significant ($P > 0.05$) at 15th, 30th, and 45th day as compared to control. Total protein was found significantly ($P < 0.05$) low at 0th and 15th day, but found comparable to healthy control on 30th and 45th day. Serum albumin showed non-

Table 2 Hematological alterations

Parameter	Group	0th day	15th day	30th day	45th day
Hb (G/dl)	I	8.87 ± 0.23^{aA}	10.77 ± 0.32^{aB}	12.03 ± 0.32^{bC}	12.73 ± 0.20^{bC}
	II	8.33 ± 0.16^{aA}	10.33 ± 0.22^{aB}	11.53 ± 0.36^{aC}	11.83 ± 0.24^{aC}
	III	13.00 ± 0.32^{bA}	12.67 ± 0.32^{bA}	13.10 ± 0.38^{bA}	13.47 ± 0.22^{bA}
PCV (%)	I	25.07 ± 0.77^{aA}	27.32 ± 0.90^{aB}	32.17 ± 0.29^{aC}	35.23 ± 0.41^{aD}
	II	24.10 ± 0.61^{aA}	27.37 ± 0.64^{aB}	30.43 ± 0.72^{aC}	33.17 ± 0.66^{aD}
	III	37.00 ± 2.22^{bA}	36.90 ± 1.39^{bA}	38.80 ± 1.45^{bA}	39.10 ± 1.14^{bA}
TEC (106/μL)	I	4.18 ± 0.13^{aA}	4.51 ± 0.11^{aB}	5.36 ± 0.05^{aC}	5.87 ± 0.07^{aD}
	II	4.02 ± 0.10^{aA}	4.56 ± 0.11^{aB}	5.07 ± 0.12^{aC}	5.53 ± 0.11^{aD}
	III	6.50 ± 0.16^{bA}	6.33 ± 0.16^{bA}	6.55 ± 0.19^{bA}	6.73 ± 0.11^{bA}
TLC(103/μL)	I	10.57 ± 0.58^{aA}	11.72 ± 0.21^{aA}	10.64 ± 0.89^{aA}	9.37 ± 0.64^{aA}
	II	10.76 ± 0.76^{aA}	11.79 ± 0.54^{aA}	11.06 ± 1.32^{aA}	10.45 ± 1.32^{aA}
	III	10.68 ± 1.24^{aA}	11.94 ± 0.51^{aA}	10.70 ± 0.54^{aA}	10.51 ± 0.83^{aA}
Lymphocyte (%)	I	57.00 ± 1.59^{aA}	50.16 ± 1.46^{aA}	57.13 ± 3.98^{aA}	50.36 ± 1.88^{aA}
	II	57.96 ± 2.96^{aA}	55.51 ± 2.47^{abA}	57.07 ± 2.45^{aA}	48.79 ± 3.83^{aA}
	III	61.35 ± 2.84^{aA}	59.25 ± 1.57^{bA}	60.79 ± 3.16^{aA}	61.00 ± 2.47^{bA}
Neutrophils (%)	I	36.79 ± 1.67^{aA}	41.74 ± 1.96^{abA}	38.73 ± 3.43^{aA}	43.49 ± 2.17^{abA}
	II	36.57 ± 3.18^{aA}	35.01 ± 2.32^{aA}	38.15 ± 3.16^{aA}	51.55 ± 3.30^{aA}
	III	42.09 ± 2.82^{aA}	44.36 ± 1.50^{bA}	43.94 ± 3.24^{aA}	36.16 ± 2.54^{bA}
Eosinophils (%)	I	3.24 ± 0.76^{aA}	3.01 ± 0.98^{aA}	1.87 ± 0.52^{aA}	3.56 ± 0.83^{aA}
	II	2.72 ± 0.63^{aA}	2.57 ± 0.34^{aA}	1.99 ± 0.57^{aA}	2.08 ± 0.51^{aA}
	III	1.38 ± 0.18^{aA}	1.24 ± 0.09^{aA}	2.01 ± 0.18^{aA}	1.45 ± 0.34^{aA}
Basophils (%)	I	1.04 ± 0.57^{aA}	2.80 ± 0.81^{aA}	0.70 ± 0.22^{aA}	1.00 ± 0.49^{aA}
	II	1.27 ± 0.46^{aA}	2.60 ± 0.43^{aA}	1.48 ± 0.56^{aA}	0.30 ± 0.20^{aA}
	III	0.69 ± 0.25^{aA}	1.30 ± 0.09^{aA}	1.63 ± 0.23^{aA}	0.50 ± 0.21^{aA}
Monocytes (%)	I	1.77 ± 0.19^{aA}	2.33 ± 0.29^{aA}	1.66 ± 0.22^{aA}	1.85 ± 0.38^{aA}
	II	1.48 ± 0.33^{aA}	2.29 ± 0.43^{aA}	1.31 ± 0.25^{aA}	1.71 ± 0.46^{aA}
	III	1.36 ± 0.15^{aA}	1.08 ± 0.23^{bA}	1.08 ± 0.13^{aA}	0.89 ± 0.13^{aA}

Superscript a, b, c, and d depicting significant change ($P < 0.05$) between the groups

Superscript A, B, C, and D depicting significant change ($P < 0.05$) from 0th, 15th, 30th, and 45th day

Table 3 Serum calcium, phosphorus, magnesium, and Ca:P ratio

Parameter	Group	0th day	15th day	30th day	45th day
Calcium (mg/dl)	I	9.14 ± 0.41^{aA}	11.26 ± 0.66^{aB}	11.68 ± 0.14^{aB}	11.95 ± 0.14^{aB}
	II	9.44 ± 0.47^{aA}	11.92 ± 0.34^{aB}	12.31 ± 0.22^{aB}	12.72 ± 0.37^{aB}
	III	11.60 ± 0.32^{bA}	11.98 ± 0.37^{aA}	11.33 ± 0.42^{aA}	11.74 ± 0.45^{aA}
Phosphorus (mg/dl)	I	4.18 ± 0.27^{aA}	5.17 ± 0.20^{aB}	6.58 ± 0.11^{aC}	7.19 ± 0.29^{aC}
	II	4.19 ± 0.21^{aA}	5.46 ± 0.17^{aB}	6.50 ± 0.18^{aC}	7.16 ± 0.14^{aC}
	III	6.89 ± 0.09^{bA}	6.79 ± 0.12^{bA}	6.81 ± 0.13^{aA}	6.74 ± 0.11^{aA}
Magnesium (mg/dl)	I	1.94 ± 0.16^{aA}	2.22 ± 0.14^{abAB}	2.27 ± 0.18^{aAB}	2.54 ± 0.11^{aB}
	II	1.83 ± 0.15^{aA}	1.98 ± 0.10^{aA}	2.13 ± 0.09^{aAB}	2.53 ± 0.14^{aB}
	III	2.40 ± 0.05^{bA}	2.41 ± 0.04^{bA}	2.53 ± 0.06^{aA}	2.34 ± 0.09^{aA}
Ca:P ratio	I	2.27 ± 0.28^{aA}	2.56 ± 0.08^{aA}	1.78 ± 0.04^{abB}	1.68 ± 0.07^{aB}
	II	2.27 ± 0.11^{aA}	2.19 ± 0.09^{bA}	1.90 ± 0.05^{aB}	1.78 ± 0.07^{aB}
	III	1.69 ± 0.03^{bA}	1.77 ± 0.07^{cA}	1.66 ± 0.06^{bA}	1.74 ± 0.08^{aA}

Superscript a, b, c, and d depicting significant change ($P < 0.05$) between the groups

Superscript A, B, C, and D depicting significant change ($P < 0.05$) from 0th, 15th, 30th, and 45th day

significant ($P > 0.05$) alteration in the same group and as compared to healthy control group. Serum globulin was significantly ($P < 0.05$) low in group I and II at 0th day as compared to healthy control. In group I, the values of globulin found significantly ($P < 0.05$) lower from group II and control animals on 15th day but were comparable on 30th and 45th day to control. AG ratio was significantly ($P < 0.05$) high in group I and II at 0th day and became comparable to the control group on 15th, 30th, and 45th day. Data is depicted in Table 4.

Fig. 3 Recovery of lamb of group I after 45th day of commercial feed supplement (CFS-I)

Discussion

Rickets is a complex metabolic bone disease in young animals due to impaired mineralization of osteoid matrix during bone growth affecting epiphyseal growth plate (Menezes Filho et al. 2006). The disease has been reported in young ones of human, cattle, sheep, goats, and horse when deficient mineralization occurs at growth plates (Dittmer and Thompson 2011; Sahay and Sahay 2012). The etiology of this complex condition is still unexplained in animals. Many factors such as

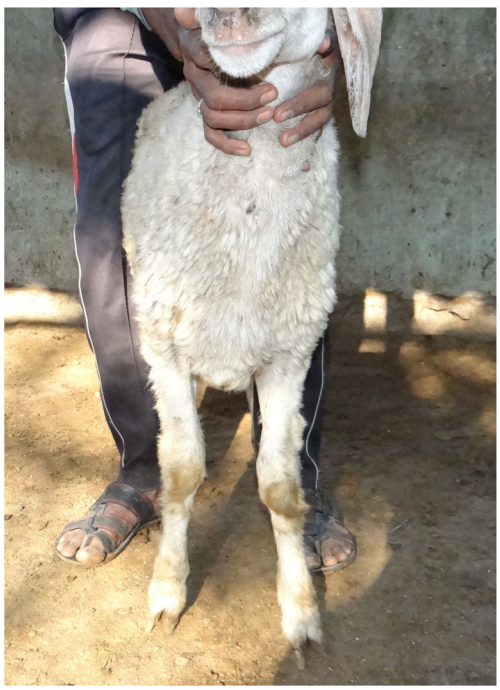

Fig. 4 Recovery of lamb of group II after 45th day of commercial feed supplement (CFS-II)

Table 4 Serum biochemical changes

Parameter	Group	0th day	15th day	30th day	45th day
ALP (IU/L)	I	458.10 ± 27.98^{aA}	194.16 ± 33.26^{aB}	129.73 ± 16.60^{aB}	170.09 ± 36.82^{aB}
	II	417.80 ± 44.71^{aA}	155.56 ± 16.86^{aB}	128.13 ± 10.25^{aB}	103.60 ± 9.47^{aB}
	III	150.33 ± 19.44^{bA}	141.33 ± 14.83^{aA}	149.17 ± 12.62^{aA}	140.67 ± 11.86^{aA}
Total protein (g/dl)	I	5.90 ± 0.30^{aA}	5.97 ± 0.20^{aA}	6.65 ± 0.17^{aAB}	7.12 ± 0.06^{aB}
	II	5.83 ± 0.18^{aA}	6.20 ± 0.13^{aA}	6.99 ± 0.06^{aB}	7.12 ± 0.07^{aB}
	III	7.09 ± 0.15^{bA}	6.93 ± 0.15^{bA}	6.98 ± 0.13^{aA}	7.05 ± 0.11^{aA}
Albumin (g/dl)	I	2.91 ± 0.07^{aA}	2.78 ± 0.09^{aA}	2.69 ± 0.12^{aA}	2.78 ± 0.09^{aA}
	II	2.85 ± 0.11^{aA}	2.77 ± 0.09^{aA}	2.99 ± 0.05^{bA}	2.69 ± 0.18^{aA}
	III	2.81 ± 0.12^{aA}	2.91 ± 0.06^{aA}	2.77 ± 0.05^{abA}	2.78 ± 0.09^{aA}
Globulin (g/dl)	I	2.99 ± 0.26^{aA}	3.19 ± 0.21^{aA}	3.97 ± 0.18^{aB}	4.33 ± 0.09^{aB}
	II	2.98 ± 0.13^{aA}	3.42 ± 0.16^{abA}	4.01 ± 0.09^{aB}	4.43 ± 0.15^{aB}
	III	4.28 ± 0.20^{bA}	4.02 ± 0.18^{bA}	4.21 ± 0.15^{aA}	4.27 ± 0.07^{aA}
A:G ratio	I	1.01 ± 0.08^{aC}	0.90 ± 0.07^{aBC}	0.69 ± 0.05^{aAB}	0.65 ± 0.03^{aA}
	II	0.97 ± 0.05^{aC}	0.82 ± 0.06^{aBC}	0.75 ± 0.03^{aAB}	0.62 ± 0.06^{aA}
	III	0.67 ± 0.05^{bA}	0.73 ± 0.04^{aA}	0.66 ± 0.03^{aA}	0.65 ± 0.02^{aA}

Superscript a, b, c, and d depicting significant change ($P < 0.05$) between the groups

Superscript A, B, C, and D depicting significant change ($P < 0.05$) from 0th, 15th, 30th, and 45th day

deficiency of vitamin D, phosphorus, calcium or altered Ca:P ratio in feed materials, and hereditary disorder has been implicated to rickets. In many reports, deficiency of copper and fluorosis have been correlated for similar manifestation in animals (Suttle and Angus 1978; Ranjan et al. 2008).

In the present study, cases of rickets were observed in approximately 16% of growing lambs born in the pre-winter season. The affected animals revealed clinical signs such as bowing and bending of long bones of limbs, swelling of epiphyses (mostly in radius ulna bone), stiffness in gait/lagging behind due to lameness, and poor body weight gain as observed in previous reports in sheep (Duckworth et al. 1961; Bonniwell et al. 1988; Van Saun 2004), goats (Dercksen and Berger 1992), and cattle (Theiler 1931; Thompson and Cook 1987). It was observed during the pre-winter season that there was ample availability of lush green pasture in the grazing area of the institute, and the animals under study were grazed nearly 8 h during this period. There are few reports from Scotland and New Zealand on rickets in sheep indicated that the sheep grazed on lush green pasture which is rich in carotenoid produces rachitogenic effect due to antagonism of vitamin D (Rohde and DeLuca 2005). For the therapeutic and prophylactic regime, the affected animals were divided into two groups and fed with CFS-I and II for 45 days. After supplementation of CFS, on the scale basis, there was no much difference in severity of the clinical signs except the reduction in swollen joints at 15th day. The severity of clinical signs was found reduced in these groups on 30th day of feed supplementation, and the body condition score was comparable with the control animals except group I animals, it was found slightly lower. On 45th day, the affected animals did not show any clinical signs of rickets and appeared healthy as control animals. The hematological (Hb, PCV, and TEC) values were significantly low in affected animals on 0th day; however, after supplementation of CFS-I and CFS-II, these values revealed gradual increase and found comparable with control animals at the end of experiment. Hematological changes in our study revealed that affected lambs were suffering from mild anemia which showed significant improvement in hematological values after supplementation of CFS-I and CFS-II. Sim et al. (2010) have shown correlation between vitamin D deficiency and anemia, but a letter to the editor by Ozsoylu et al. (1976) has reported that 25-hydroxycholecalciferol might be correlated with anemia, but anemia has no correlation with rickets in children. Fuhr and Steenbock (1943) reported that there was no significant reduction in hemoglobin observed in experimentally induced calcepenic or phosphopenic rickets in rats. In our study, anemia in rickets-affected lambs may be explained as reduced nutrient access due to lagging behind in pasture. Total leukocyte count was normal with no significant alteration in differential leukocyte count which differentiates the condition as rickets from other systemic inflammatory conditions affecting the bone or joint.

In the present study, the serum Ca, P, and Mg levels were found lower in rickets affected animals. The improvement in these levels along with Ca:P ratio was started after 15th day of CFS supplementation and was comparable to control animals after 30th day. Initially, in the affected animals, serum total protein and globulin level was significantly low and ALP was very high; however, these values were improved gradually

and found comparable to healthy control on 30th and 45th day. The animals under study were supplemented with equal unit of vitamin D, which is an essential pro-hormone for successful absorption and assimilation of calcium (Sahay and Sahay 2012). Vitamin D regulates the function of osteocalcin and osteopontin channels which are essential for regulation of calcium homeostasis in the body (Harter et al. 1995). The hypophosphatemia observed in rickets affected animals on serum analysis in our study could be considered as one of the etiologies of rickets because phosphorus homeostasis is maintained through intestinal absorption, renal tubular reabsorption, between bone pools and intracellular ion exchange process. In addition, parathyroid-calcitriol axis also plays a role in phosphorus regulation. Out of all, the kidney is a fast regulator of phosphorus balance through sodium-phosphate (Na-Pi) co-transport system (Menezes Filho et al. 2006). The low level of serum protein in rickets affected animals may be due to less feed accessibility and poor growth in lambs which showed remarkable improvement after 30th day of supplementation, and improvement was noted in both groups irrespective of change in conformation of the bones. It has been reported that rickets-suffered animals are hypoproteinaemic (Smith and Sherman 1994) which is in concurrence with our study. When the results of two treatments (CFS-I and CFS-II) were compared, the feed supplementation with CFS-II showed better improvement in rickets-affected lambs may be due to added advantage of essential micronutrients such as copper, cobalt, manganese, choline, and nicotinamide as compared to the CFS-I which lack essential micronutrient.

Conclusion

Rickets-affected lambs revealed low serum calcium, phosphorus, and high serum activity of alkaline phosphatase and high Ca:P ratio which can be a diagnostic in conjunction with clinical signs of bowing or bending of long bone grazing lush green pasture. Commercial feed supplementation may be used for amelioration of rickets as both are source of vitamin D_3 and calcium, but CFS-II in our study depicted added advantage of other minerals like copper, cobalt, iron, and manganese which are useful as micronutrients and fasten the recovery in affected lambs suffering from rickets.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical clearance All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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