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REGULAR ARTICLES

Response of anestrous heifers fed local grass or oak foliage-based diet with two different mineral mixtures

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Abstract

The effect of incorporation of two different mineral mixtures and/or oak leaves was studied on nutrient utilization and reproductive performance in anestrous heifers. Twenty-one anestrous heifers (18.2 ± 1.45 months; 229 ± 14.2 kg body weight) were randomly distributed into three similar groups. Heifers in control (T_1) and first treatment group (T_2) were fed concentrate mixture incorporated with Bureau of Indian Standards (BIS) specific mineral mixture and a customized mineral mixture developed specially for Kumaon hills (MMKH), respectively, along with local green grass (*Pennisetum orientale*). In the second treatment group (T_3), concentrate mixture was the same as that of T_2, while the source of roughage was local oak (*Quercus leucotricophora*) leaves containing 3.35% condensed tannin. A digestibility trial was conducted after 120 days of study. The feed intake was similar among the groups. Digestibility coefficient of crude protein (CP) was lower in T_3 than T_2 and comparable to T_1. Feeding oak leaves improved absorption of calcium as compared to grass-fed animals. Bioavailability of copper and zinc was higher ($P < 0.05$) in oak leaves and MMKH fed group (T_3) as compared to T_1, but similar to T_2. Conversely, absorption of iron had the reverse trend and was reflected in serum Fe concentration. Hematological, biochemical, enzyme and hormonal profiles were not influenced by any of the treatments. The relative occurrence of estrus cyclicity and conception rate was more in groups T_2 and T_3, respectively, than other groups. It was concluded that feeding oak foliage-based diet containing 1.87% tannin along with customized mineral mixture developed for Kumaon hills improved certain nutrient utilization and reproductive performance as compared to local green grass supplemented with BIS-specific mineral mixture or MMKH.

Keywords Blood biochemistry · Heifers · Minerals · Oak leaves · Reproduction · Tannin

Introduction

The livestock rearing in the Kumaon hilly regions in India is mostly dependent on tree leaves, which are generally not adequate in available nutrients to support optimum performance of livestock (Sahoo et al. 2016). Besides, it also adversely affects reproduction of farm animals starting from delayed puberty, anestrous, reduced ovulation, lower conception rate, infertility or even sterility (Shukla et al. 2010). This problem is further exacerbated by the fact that animals in the hilly areas are usually fed a meager quantity of homemade concentrate mixture. Oftentimes, supplementation of mineral mixture is also a very rare practice because of its cost-escalating factor and therefore, most of the animals suffer from chronic mineral deficiencies (Sharma and Joshi 2004). The Bureau of Indian Standards (BIS) has recommended a general mineral mixture specification for India. However, mineral deficiency differs from one region to another depending upon many factors arising from soil and water to plant-animal interrelationships, among others (Suttle 2010). In contrast, supplementation of area-specific mineral mixture in the diet could help in optimal utilization of nutrients, thereby improving the productive and reproductive performance of animals (Selvaraju et al. 2009; Mohanta and Garg 2014; Sahoo et al. 2017). Therefore, a

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specific mineral mixture was developed for the Kumaon Hill region (MMK) based on reviewing past works on composition of feeds and forages (Paswan and Sahoo 2012; Sahoo et al. 2014; Sahoo et al. 2016).

Tree foliage, especially oak (*Quercus leucotricophora*), forms the major green forage source for livestock in the Western Himalayan region (Paswan and Sahoo 2012). These evergreen oak trees, also known as 'Banjh' in Kumaon hills, dominate the major vegetation cover of the forests. Oak leaves contain polyphenols known as 'tannins', which can form stable complexes with proteins and mineral ions (Singh and Sahoo 2004; Patra and Saxena 2011; Naumann et al. 2017). Tannin exerts its effect by precipitating protein and also certain minerals through the polyphenolic groups (Pagan-Riestra et al. 2010; Minz et al. 2019). The effect of tannin with chelation of minerals is still not clearly established (Min et al. 2015; Naumann et al. 2017). Minerals, particularly calcium, phosphorus, copper and zinc, play a very important role in animal life including production and reproduction functions (Suttle 2010). Interference, positive or negative, on any of these minerals may influence animal production and economic sustainability of dairy farming.

Considering the aforementioned background, a comparative evaluation was attempted by comparing the effect of the specific mineral mixture developed for Kumaon Hills (MMKH) with BIS-specific mineral mixture on either local grass or oak forage-based diets in anestrous breedable heifers.

Materials and methods

Study location

The animal shed of Indian Veterinary Research Institute, Mukteshwar located at 28° 53′ 24″–31° 27′ 50″ N and 77° 34′ 27″–81° 02′ 22″ E at an altitude of 2250–2350 m above mean sea level was the location for the study.

Heifers and feeding management

Twenty-one heifers with a history of not showing any symptoms of estrous for a long time were chosen from the experimental cattle farm and randomly divided into three similar groups based on comparable body weight (229 ± 14.2 kg) and age (18.2 ± 1.45 months). The animals in control group (T_1) were fed a diet based on concentrate mixture (CM-I) with BIS mineral mixture and local green grass (*Pennisetum orientale*). In groups T_2 and T_3, a specific mineral mixture developed for the Kumaon Hill regions (MMKH) was incorporated in the concentrate mixture (CM-II). The roughage source for animals in T_2 and T_3 was local grass (*P. orientale*) and oak foliage (*Q. leucotricophora*), respectively. All animals enrolled in the study were housed in the well-ventilated shed receiving adequate sunshine, fed individually and managed uniformly throughout the experiment. The floors were disinfected periodically, thereby ensuring the hygienic surroundings. Clean and wholesome water was offered freely twice daily. The ingredient composition of mineral mixtures is mentioned in Table 1. The specific mineral mixture was formulated after evaluation of feed and forage resources of the region (Sahoo et al. 2014; Sahoo et al. 2016).

Sample collection and chemical analysis

The feeding experiment was conducted within four months of feeding before performing the digestibility trial for exhibiting the effect of the nutrients in different groups. A 6-day digestibility experiment was started on day 120 of the study. Quantity of forage and concentrate offered as well as residue left from all animals were daily weighed on-farm using spring balance and sampled at fortnightly intervals for dry matter (DM), to arrive at average DM intake (DMI) during the experimental period. Body weight (BW) of each animal was recorded at weekly interval prior to morning feeding and watering to estimate BW changes. Blood sample was collected in the morning before offering feed through jugular vein into test tubes, one containing sodium ethylenediaminetetraacetic acid (EDTA) and the other tube without EDTA. Serum was harvested at 0, 60 and 120 days of experiment and preserved at −20°C until analysed.

Daily DMI was recorded, and quantitative faecal output from all animals was noted. The samples of feed, orts and faeces collected during the digestibility trial were dried in hot air oven (60°C for 48 h), milled to pass through a one-millimeter sieve and analysed using the methods of AOAC (2000) to determine dry matter (DM), ether extract (EE), crude protein (CP), ash, calcium (Ca) and phosphorus (P). Analyses of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were done by the methods of Van Soest et al. (1991). The apparent total tract digestibility of nutrients was calculated from the total dietary intake and total faecal excretion

Table 1 Ingredient composition (kg/100 kg) of mineral mixtures

Ingredient	BIS (2003)	MMKH*
Di-calcium phosphate	65.0	60.0
Calcium carbonate	9.80	20.0
Magnesium sulphate	21.4	15.0
Ferrous sulphate	0.75	1.50
Potassium iodate	0.04	0.10
Copper sulphate	0.25	0.50
Zinc sulphate	2.25	2.50
Manganese sulphate	0.40	0.50
Cobalt sulphate	0.04	0.08
Sodium selenite	-	0.05

*Kumaon hill-specific mineral mixture

averaged for six days (Sharma et al. 2014). Based on intake and digestibility, nutritive value of diets was computed. Total phenol content in oak foliages was estimated using methods of Makkar (2000). The condensed tannin (CT) fraction was estimated by treating the extract with butanol–HCl in the presence of ferric ammonium sulphate, whereas non-tannin phenols were estimated after binding of tannin with polyvinyl–polypyrrolidone. Total tannin phenol and hydrolysable tannin (HT) values were calculated by difference.

The mineral extract was prepared following the wet digestion method in triple acid mixture, i.e. perchloric acid, sulphuric acid, and nitric acid mixture (1:2:4). Estimation of trace elements (Zn, Cu, Fe, Mn and Se) was done in the triple acid extracts using atomic absorption spectrophotometer (AAS, Model 4141, ECIL, Hyderabad) while selenium was estimated using hydride generator with AAS (Hershey et al. 1988).

The blood hemoglobin (Hb), serum activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) along with biochemical parameters in serum were analyzed using diagnostic kits (Span Diagnostic Limited, Surat, India). Total tri-iodothyronine (T_3) and total thyroxine (T_4), insulin, estrogen and progesterone in blood serum sample were analyzed through enzyme-linked immunosorbent assay kits from Labor Diagnostika Nord (Nordhorn, Niedersachsen, Germany).

Statistical analysis

The data generated were analyzed using SPSS 17.0 software (SPSS 2005). Data among the groups were compared using generalized linear model and Duncan's multiple range test (Snedecor and Cochran 1994) with the following model:

$Yij = \mu + Ti + \varepsilon ij$, where

Yij= response variable (e.g., intake, digestibility etc.)

μ= overall mean

Ti= effect of ith treatment (i=T_1, T_2 and T_3), and

εij=error component.

The significance among means of three groups was established when the *P* value for null hypothesis was ≤0.05.

Results

Chemical composition

The chemical composition of feed and forages is presented in Table 2. Proximate composition like OM, EE and CP content of oak foliages was comparatively higher than local grass (*P. orientale*). The NDF content of oak foliages was lower and ADF content was higher than *P. orientale*. Minerals like Ca, Mn and Se were higher and P was lower in oak foliages than grass *(P. orientale)*. The concentration (%) of CT and HT in oak foliages was 3.35 and 3.10, respectively.

Effect on nutrient intake and utilization

The DMI was similar among the treatment groups (Table 3). Feeding of oak foliages (T_3) reduced ($P < 0.05$) digestibility coefficient of CP as compared to grass-fed group (T_2). EE digestibility (%) was higher ($P < 0.05$) in group T_3 than T_2, but at par with T_1. Nutrient intake and nutritive value were similar among all the groups except DCP, which was higher ($P < 0.05$) in group T_2 as compared to T_3, but at par with that of group T_1.

Intake and outgo of Ca and P were similar in all the treatment groups (Table 4). Ca absorption was higher ($P < 0.05$) in oak foliages fed group (T_3) than T_1 and T_2 which were at par. However, absorption of P was higher ($P < 0.05$) in group T_2 than in T_3, but at par with T_1. Although intake and faecal outgo of Fe, Cu and Zn were similar, absorption of Cu and Zn was higher ($P < 0.05$) in group T_3 than T_1 but at par with T_2. However, absorption of Fe was lower ($P < 0.05$) in group T_3 than T_2 and T_1 which were at par. Mn intake and outgo were higher ($P < 0.01$) in group T_3 than T_1 and T_2 resulting to similar absorption in animals. Further, Se intake and faecal excretion were lower ($P < 0.05$) in group T_1 than T_3, but similar with that of group T_2.

Effect on serum mineral profile

Feeding oak foliages in place of grass did not influence the serum mineral level, except that of Fe, which was lower ($P < 0.05$) in oak foliages fed animals (group T_3) irrespective of mineral mixture types (Table 5). Serum level of Ca, P, Cu, Co and Mn progressively increased with the advance in experimental feeding.

Effect on blood biochemistry

Replacement of grass with oak foliages irrespective of type of supplemental mineral mixture increased ($P < 0.05$) blood Hb, PCV, albumin and ALP enzyme level. Most of the other blood biochemical and enzyme profiles were not influenced except cholesterol level, which was found to be lower ($P < 0.05$) in the oak foliages fed (T_3) animals (Table 6). Thyroid hormone and insulin levels were not influenced by any of the dietary treatments.

Effect on reproductive performance

Reproductive performance and estrus pattern (Fig. 1) showed that heifers in group T_3 exhibited a reduction in anestrus condition from 29 to 14%. The cyclicity was higher in T_2 (72%) as compared to T_1 (57%) and T_3 (57%). In addition, pregnancy rate was higher in T_3 (29%) as compared to T_1 (14%) and T_2 (14%).

Table 2 Chemical composition (DM basis) of feeds and forages

Nutrient	Concentrate mixture (T_1)	Concentrate mixture (T_2 and T_3)	Grass	Oak forages
Proximate composition (%)				
Organic matter	89.4	88.8	94.0	95.8
Crude protein	21.09	21.4	9.47	10.1
Ether extract	3.0	2.68	1.40	5.67
Total carbohydrates	65.2	64.7	83.1	80.0
Neutral detergent fiber	46.7	44.4	74.3	65.5
Acid detergent fiber	15.3	14.8	46.6	52.7
Mineral composition				
Calcium (%)	2.11	2.46	0.60	1.47
Phosphorus (%)	0.76	0.75	0.32	0.20
Cobalt (ppm)	3.19	3.47	1.92	1.78
Copper (ppm)	24	35	15	14
Iron (ppm)	224	250	213	193
Manganese (ppm)	84	89	149	351
Zinc (ppm)	75	82	42	39
Selenium (ppm)	0.35	0.70	0.28	0.40
Tannin (%)				
Total tannin	-	-	-	6.45
Hydrolyzable tannin	-	-	-	3.10
Condensed tannin	-	-	-	3.35

Discussion

Composition

Earlier reports indicate that the feed and forages of Kumaon hill region contain low levels of Cu and Zn, but high levels of Fe, Mn and Co (Sharma and Joshi 2004; Sahoo et al. 2016). The oak foliages available in this region had comparatively lower tannin content than those reported from other regions, which might be attributed to difference in the level of climatic stress, maturity of leaves, age of plant and soil type (Patra and Saxena 2011; Naumann et al. 2017). The variation in mineral composition between BIS and MMKH resulted in the difference in majority of mineral levels in the concentrate mixture offered to groups T_1 and T_2.

Intake and utilization of nutrients

A lack of effect on DMI signifies that neither forage source (grass vs. foliage) nor mineral (BIS or MMKH) had any influence on voluntary intake by heifers. Oak foliages are natural forage source for livestock in Himalayan hilly terrains, and are palatable (Paswan and Sahoo 2012; Sahoo et al. 2016; Naumann et al. 2017). Nonetheless, tannin may have caused some depressing effect on the appetite and DMI when fed at a level >3% of CT in the diet (Bengaly et al. 2007; Naumann et al. 2017). In the present study, digestibility of nutrients was not altered when fed 1.87% CT in the total diet as reported by earlier workers (Raju et al. 2018; Norris et al. 2020). Tannin mainly exerts its effects on protein, but it also affects other nutrients to varying degrees depending on its ability to form hydrogen bonds that are stable between ruminal pH 3.5 and 8 (Kumar and Singh 1984). The stable complexes dissociate at pH 2.5–3.0 in the abomasum or at pH>8 in the duodenum resulting in the improvement in bioavailability of nutrients. Low to moderate level of tannin containing <3% of CT in the total diet did not alter palatability, intake and utilization of nutrients, which supported the present findings (Raju et al. 2015; Minz et al. 2019; Norris et al. 2020).

Higher ($P < 0.05$) absorption of Ca in heifers of group T_3 might be attributed to the higher level of Ca in oak foliages than grass (*P. orientale*; Table 4). Similar Ca excretion pattern in all the treatment groups, irrespective of dietary treatments, is a clear indication of no effect of dietary treatment on Ca absorption in animals (Minz et al. 2019). In contrast to Ca, P absorption followed a negative trend that may be due to the higher level of Ca in MMKH-based diets. Feeding of tanniferous oak foliages and different mineral mixtures did not alter the intake and outgo of Cu and Zn, however, the absorption of these minerals as percent of intake was higher ($P < 0.05$) in oak foliages fed group (T_3) due to higher level of these minerals in oak foliages showing positive interaction of

Table 3 Nutrient digestibility and plane of nutrition among experimental heifers

Attribute	T_1	T_2	T_3	SEM	*P* value
Body weight (kg)	271	250	266	15.25	0.94
Dry matter intake (kg/d)					
Concentrate	3.22	2.91	3.20	0.29	0.90
Forage	4.00	3.38	4.05	0.33	0.68
Total dry matter	7.22	6.29	7.25	0.61	0.78
Organic matter	6.63	5.75	6.67	0.56	0.76
Concentrate: Forage	43:57	46:54	44:56		
Nutrient digestibility (%)					
Dry matter	60.4	62.6	59.7	1.81	0.79
Organic matter	62.1	64.1	60.5	1.79	0.72
Crude protein*	59.9[ab]	62.3[b]	57.4[a]	0.58	0.04
Ether extract*	63.3[a]	62.2[a]	69.5[b]	1.26	0.03
Neutral detergent fibre	60.0	62.3	57.1	1.75	0.50
Acid detergent fibre	58.0	56.3	53.1	1.62	0.45
Total carbohydrates	56.9	59.5	55.6	1.93	0.71
Nutritive value (%)					
Crude protein	14.7	14.8	15.1	0.12	0.39
Digestible crude protein*	8.8[a]	9.25[b]	8.7[a]	0.10	0.05
Total digestible nutrients	54.6	56.5	55.4	1.55	0.87

BIS, Bureau of Indian Standards; *SEM*, Standard error of mean; T_1, heifers given BIS mineral mixture based concentrate and green grass; T_2, heifers offered specific mineral mixture based concentrate and green grass; T_3, heifers offered specific mineral mixture based concentrate and oak leaves in their diet

[a,b] Means bearing different superscripts in a row differ significantly * $P < 0.05$

Table 4 Mineral intake and utilization among experimental heifers

Attribute	T_1	T_2	T_3	SEM	*P* value
Calcium (mg/d)					
Total intake	9.19	9.18	13.72	0.96	0.12
Outgo in faeces	6.64	6.58	9.27	0.65	0.19
Absorbed*	2.55[a]	2.60[a]	4.45[b]	0.32	0.04
Absorbed (% intake)*	27.74[a]	28.32[a]	32.43[b]	0.56	0.01
Phosphorus (mg/d)					
Total intake	3.73	3.26	3.19	0.31	0.75
Outgo in faeces	2.25	1.95	2.05	0.19	0.81
Absorbed	1.48	1.31	1.14	0.12	0.55
Absorbed (% intake)*	39.67[ab]	40.18[b]	35.74[a]	0.67	0.04
Zinc (mg/d)					
Total intake	410.9	381.4	415.3	35.94	0.92
Outgo in faeces	295.2	265.0	277.9	25.57	0.89
Absorbed	115.7	116.4	137.4	11.21	0.68
Absorbed (% intake)*	28.16[a]	30.52[ab]	33.08[b]	0.71	0.02
Copper (mg/d)					
Total intake	135.4	151.0	168.0	13.36	0.62
Outgo in faeces	101.6	109.7	117.8	9.90	0.80
Absorbed	33.8	41.3	50.2	3.67	0.22
Absorbed (% intake)**	24.57[a]	27.00[ab]	30.14[b]	0.62	0.01
Iron (mg/d)					
Total intake	1573.2	1446.9	1570.0	134.8	0.91
Outgo in faeces	1036.6	945.9	1111.1	87.24	0.74
Absorbed	536.6	501.0	458.9	49.70	0.81
Absorbed (% intake)*	34.10[b]	34.62[b]	29.23[a]	0.76	0.01
Manganese (mg/d)					
Total intake**	866.3[a]	762.0[a]	1691.0[b]	100.5	0.01
Outgo in faeces**	563.1[a]	499.4[a]	1052.6[b]	60.40	0.01
Absorbed**	303.2[a]	262.6[a]	638.4[b]	41.11	0.01
Absorbed (% intake)	34.99	34.46	37.75	0.93	0.13
Cobalt (mg/d)					
Total intake	18.00	16.71	18.26	1.56	0.90
Outgo in faeces	11.86	10.86	12.29	1.05	0.85
Absorbed	6.00	6.00	6.14	0.59	0.99
Absorbed (% intake)	33.59	35.76	32.80	0.84	0.35
Selenium (mg/d)					
Total intake	2.27[a]	3.00[ab]	3.83[b]	0.28	0.05
Outgo in faeces	1.10[a]	1.55[ab]	2.08[b]	1.13	0.01
Absorbed	1.17	1.45	1.75	1.57	0.33
Absorbed (% intake)	49.21	47.66	43.39	1.37	0.22

BIS, Bureau of Indian Standards; *SEM*, standard error of mean; T_1, heifers given BIS mineral mixture based concentrate and green grass; T_2, heifers offered Kumaon-specific mineral mixture-based concentrate and green grass; T_3, heifers offered Kumaon-specific mineral mixture-based concentrate and oak leaves in their diet.[a,b] Means bearing different superscripts in a row differ significantly * $P < 0.05$

Zn and Cu with tannin. It is possible that CT when fed at low to moderate level (1.87% in the diet) might chelate with Cu and Zn, enhance their absorption at post-ruminal tract and further utilization at the tissue level. Consumption of tannin containing feed did not affect the intake and excretion of Fe. However, absorption as percent of intake was lowest in group T_3 showing negative interaction between tannin and Fe. Polyphenolic compounds like tannins precipitate different nutrients such as proteins and minerals thereby lowering their bioavailability. Metallic ions with divalent properties form insoluble complexes with tannin through their carboxyl and hydroxyl groups, thus lowering their absorption (Khokhar and Apenten 2003). There are very few reports available on the interaction of minerals with tannin, most of them confine to in vitro studies, monogastric animals or human beings (Raju et al. 2018). The type, molecular size, conformational mobility and concentration of tannin play an important role in determining their chelating ability with minerals. Mineral intake and outgo followed similar trend, which was dependent upon feed intake. CT showed an adverse effect with Fe which might be due to high affinity and chelating ability of different functional groups of tannin with Fe, which lead to higher levels of Fe excretion through faeces

Table 5 Serum mineral profile among experimental heifers

Treatment	Day of experiment			Mean ± SEM	*P* value	
	0	60	120		T	P
Calcium (mg/dL)						
T_1	8.36	9.01	8.81	8.73 ± 0.26	0.13	0.05
T_2	8.45	8.54	8.93	8.64 ± 0.23		
T_3	8.69^{A}	8.80^{AB}	8.90^{B}	8.73 ± 0.23		
Phosphorus (mg/dL)						
T_1	4.24^{A}	5.45^{B}	4.77^{AB}	5.06 ± 0.31	0.68	0.03
T_2	4.15^{A}	5.98^{AB}	5.63^{B}	5.25 ± 0.27		
T_3	5.10	5.55	5.49	5.38 ± 0.17		
Zinc (ppm)						
T_1	1.05	1.07	1.08	1.07 ± 0.65	0.17	0.30
T_2	1.04	1.10	1.14	1.10 ± 0.40		
T_3	1.09	1.21	1.37	1.23 ± 0.77		
Copper (ppm)						
T_1	1.06	1.04	1.11	1.09 ± 0.18	0.68	0.03
T_2	1.04	1.14	1.16	1.11 ± 0.31		
T_3	1.06^{A}	1.17^{B}	1.21^{B}	1.15 ± 0.37		
Iron (ppm)						
T_1	11.78	13.16	12.40	12.45 ± 7.82^{b}	0.04	0.66
T_2	11.12^{A}	12.78^{AB}	11.57^{B}	11.82 ± 2.81^{ab}		
T_3	11.10	9.92	9.87	10.30 ± 5.80^{a}		
Manganese (ppm)						
T_1	5.63	8.69	8.60	7.64 ± 0.75	0.25	0.03
T_2	5.91	8.00	8.21	7.37 ± 0.53		
T_3	5.71^{A}	8.61^{B}	8.93^{B}	7.75 ± 0.45		
Cobalt (ppm)						
T_1	2.68	3.00	3.16	2.95 ± 0.11	0.10	0.03
T_2	2.35^{A}	3.14^{AB}	3.42^{B}	2.97 ± 0.20		
T_3	2.51^{A}	3.13^{AB}	3.22^{B}	2.95 ± 0.14		
Selenium (ppm)						
T_1	0.12	0.14	0.15	0.14 ± 0.65	0.19	0.23
T_2	0.13	0.17	0.19	0.16 ± 0.40		
T_3	0.14	0.19	0.21	0.18 ± 0.77		

BIS, Bureau of Indian Standards; *SEM*, standard error of mean; T_1, heifers given BIS mineral mixture based concentrate and green grass; T_2, heifers offered Kumaon-specific mineral mixture-based concentrate and green grass; T_3, heifers offered Kumaon-specific mineral mixture-based concentrate and oak leaves in their diet.

[a,b] Means bearing different superscripts in a column differ significantly $^{*}P < 0.05$

[A, B, AB] Means bearing different superscripts in a row differ significantly $^{*}P < 0.05$

and lower absorption of Fe as a percent of intake. The interaction/bonding of tannin or its derivatives with Cu and Zn may not be as strong as Fe so that the absorption of Cu and Zn was higher in the acidic pH of post-ruminal tract of animals that lead to higher absorption as a percent of intake in Cu and Zn showing their synergistic interaction (Naumann et al. 2017).

Serum biochemistry

Serum mineral level was not affected by supplementation of different mineral mixture with or without oak foliages with low to moderate CT concentration because of the homeostasis mechanism maintained by the animal. However, a progressive increase ($P < 0.05$) in concentration of Ca, P, Mn and Cu with advance in experimental feeding showed that goats were earlier deficient in minerals and mineral mixture supplementation helped to overcome the deficiency (Raju et al. 2018; Minz et al. 2019). Tanniferous oak foliages fed group had lower ($P < 0.05$) serum iron concentration, which might be due to its negative interaction with tannin, thereby reducing the bioavailability of Fe to host tissue. Similarly, the serum Fe level was lowered from 6.24 to 5.68 (mg/L) by increasing the level of CT through the feeding of oak foliages from 1 to 1.9% confirming the negative interaction of tannin with Fe at a higher level (Raju et al. 2015).

Blood profile of specific biomarkers reflects nutritional and health status of animals (Sharma et al. 2014). Blood biochemical and enzyme profiles were influenced by supplemental area-specific mineral mixture (Table 6). Chelating ability of CT (1.87% in diet) might have protected Fe loss in gastrointestinal tract of ruminants and improved the utilization efficiency at tissue level. A low serum urea concentration in oak foliages fed group (T_3) indicates that there is a reduction in rumen proteolysis due to interaction of CT with protein along with increased N utilization efficiency through bypass protein effect at post-ruminal tract and subsequently at tissue level resulting in enhanced production performance (McAllister et al. 1994).

However, feeding of oak foliages increased serum protein level in heifers (Paswan and Sahoo 2012) as compared to control group of animals fed grass hay. The effect on ingestion of the tannin depends on the physiological adaptations of mammals. Similar to our findings, a reduction in cholesterol level by tannin supplementation was also noted by previous workers through feeding different plants (Owens et al. 2012; Nishida et al. 2020). This property might be attributed to the binding of tannin with certain bile salts influencing glucose and cholesterol metabolism (Nishida et al. 2020).

Since animals were previously not adapted to oak foliages, its introduction might have increased the enzyme level, although it was within the normal range. Similar to our findings, Paswan and Sahoo (2012) noted no adverse effect of increasing the level of oak foliages (*Q. leucotricophora*) from 0 to 64% in diet of heifers on serum enzymatic activity. Furthermore, as expected, dietary treatments did not alter activities for thyroid and insulin hormones among heifers of all the three groups.

Reproductive performance

In our study, the bioavailability of calcium, phosphorus, copper and zinc was lower in T_1 when compared to the heifers in T_2 and T_3 groups, which might be the reason for lower reproductive performance in these heifers as minerals play an important role in neuro-endocrine system (Suttle 2010) influencing reproductive system. The better effect of Kumaon-specific mineral mixture than BIS mineral mixture might be due to the

Table 6 Blood biochemical profile among experimental heifers

Attributes	T_1	T_2	T_3	SEM	*P* value
Hemoglobin (g/dL)*	11.33[a]	11.53[a]	12.00[b]	0.15	0.02
Packed cell volume (%)*	40.00[a]	42.38[ab]	44.24[b]	0.75	0.03
Glucose (mg/dL)	42.62	43.95	40.62	1.35	0.21
Total protein (g/dL)	7.47	7.80	7.77	0.34	0.76
Albumin (g/dL)*	3.78[a]	4.03[ab]	4.26[b]	0.16	0.03
Globulin (g/dL)	3.69	3.77	3.51	0.33	0.85
Serum urea (mg/dL)	31.92	32.31	26.23	0.97	0.03
Cholesterol (mg/dL)	132[a]	142[b]	127[a]	10.45	0.04
Alkaline phosphatase (IU/L)*	119[a]	124[a]	150[b]	5.23	0.04
Aspartate aminotransferase (IU/L)	167	175	184	5.82	0.15
Alanine aminotransferase (IU/L)	17.78	18.94	19.60	0.41	0.45
Thyroxine (μg/dL)	10.54	12.69	11.60	0.78	0.15
Triiodothyronine (ng/mL)	2.98	2.39	2.50	0.17	0.21
Insulin (μIU/mL)	14.70	16.01	16.09	2.11	0.86

BIS, Bureau of Indian Standards; *SEM*, standard error of mean; T_1, heifers given BIS mineral mixture-based concentrate and green grass; T_2, heifers offered Kumaon-specific mineral mixture-based concentrate and green grass; T_3: heifers offered Kumaon-specific mineral mixture-based concentrate and oak leaves in their diet.

[a,b] Means bearing different superscripts in row differ significantly * $P < 0.05$

addition of more amount of essential minerals like Ca, Fe, Cu Se and Zn that regulate cellular metabolism through their role as many cofactors/co-enzymes. Higher mineral content in oak foliages could also be responsible for the additional positive effect on reproduction in the diet (Naumann et al. 2017). A positive correlation exists between serum progesterone-Cain cows and serum progesterone-Cu and estrogen-Cu in heifers throughout the estrus cycle (Rosenfeld et al. 2001).

Conclusion

Based on the present study, it may be recommended that oak foliages can be included safely in the overall diet as a sole forage source with about 1.87% condensed tannin in the diet for heifers. Development of customized mineral mixture, fed with either local grass or oak foliage, played a role in augmenting reproductive performance of anestrous heifers by improving the retention of Ca, Zn and Cu, and not causing any adverse effect on intake and digestibility of nutrients. Therefore, this study establishes that supplementing basal diets with MMKH should be advocated for optimum production and reproduction at field conditions.

Data and materials availability Available with the corresponding author

Code availability Not applicable

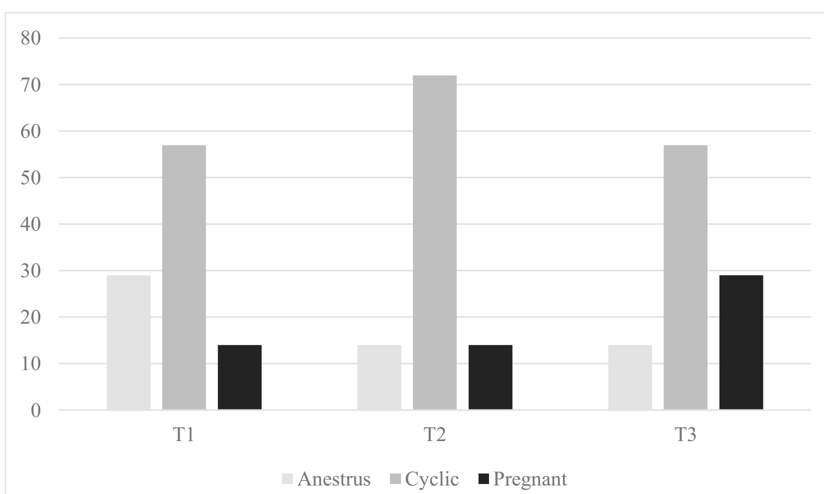

Fig. 1 Reproductive performance (%) among experimental heifers. BIS: Bureau of Indian Standards; T_1: heifers given BIS mineral mixture based concentrate and green grass; T_2: heifers offered Kumaon specific mineral mixture based concentrate and green grass; T_3: heifers offered Kumaon specific mineral mixture based concentrate and oak leaves in their diet

Declarations

Ethics approval and consent to participate The experiment was conducted after approval from Institute Animal Ethics Committee and experiment was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

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