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Effect of customised supplement on haemato-biochemical profile, serum minerals, metabolic hormones, antioxidant capacity and gene expression in crossbred calves

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ABSTRACT

Present experiment examined the supplementary effect of a tailor-made supplement to farmers'-based diet in crossbred calves. Male crossbred calves (15) were randomly allocated in 3 dietary treatments consisting of 5 calves in each. The dietary treatments were: Control- cereal straw-based diet with concentrate mixture as per the farmers' practices; CS (customised supplement)- control diet with additional customised supplement @ 0.25% of BW; SD- standard diet. Serum glucose was higher in SD than control, however, CS had an intermediate response. The serum macro (Ca and i-P) and trace (Zn, Cu, Fe and Mn) minerals were higher in SD and CS than control. The serum T_3 and T_4 hormones were significantly higher in SD and CS than control group. The serum growth hormone (GH) and insulin-like growth factor-1 (IGF-1) were significantly higher in SD than control groups, however, SD had an intermediate position. The total antioxidant capacity (TAOC) was significantly higher in SD and CS than control group. The relative mRNA expression of cytokines, viz. IL-2 and IL-4 was significantly higher in SD and CS than control group. The relative mRNA expression of leptin (LEP) was significantly higher and ghrelin (GHRL) was significantly lower in SD than control group, however, CS had a transitional position. Thus, it can be concluded that supplementation of the customised supplement (@ 0.25% BW) to farmers'-based diet significantly improved the serum glucose concentration, metabolic hormone profile, antioxidant capacity and relative mRNA expression of cytokines and genes involved in energy metabolism in crossbred calves.

Keywords: Antioxidant capacity, Customised supplement, Calves, Cytokines, Ghrelin, Leptin

Forage production and livestock farming complement each other. Fodder based economical feeding approaches are required to decrease the cost of quality livestock products as well as to sustain the optimum livestock production. There is a huge pressure of livestock on existing feeds and fodders, as land availability for fodder production has been decreasing gradually. As per Indian Grassland and Fodder Research Institute (IGFRI) vision 2030, presently, India is facing overall deficit of 64.21% green fodder and 24.81% dry crop residues. This shortage is forcing the livestock to feed mainly on low-quality crop residues and by-products, oil cakes and locally accessible green fodder which lacks balanced supply of fermentable energy, degradable protein, and mineral supplements. The unavailability of good quality fodder limits energy and protein availability to rumen thereby reduced rumen fermentation and microbial biomass production leading to compromised growth, immune response, and antioxidant defence of the animals (Uddin *et al.* 2015). Therefore, supplementing the animals' diet with customised supplement fortified with energy, protein and minerals may be an alternative strategy to overcome this problem (Wang *et al.* 2019). Keeping this brief idea in view, a customised supplement was formulated which is rich in fermentable energy, degradable protein as well as macro and trace minerals. In present study, the customised supplement was tested to ascertain its effects on haemato-biochemical parameters, serum minerals profile, metabolic hormones status, antioxidant capacity and relative mRNA expression of immune related cytokines and genes involved in energy metabolism in crossbred calves.

MATERIALS AND METHODS

The experiment was conducted at Animal Nutrition shed, ICAR-Indian Veterinary Research Institute, Izatnagar during September to October, 2018 and was approved by the Institute Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India.

Experimental animals and diets: Fifteen male crossbred calves (*Bos taurus* × *Bos indicus*) of about 18 months old, 166.53±4.93 kg mean BW were randomly divided into

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3 groups of 5 each in a completely randomized design and randomly allocated to Control, CS (customised supplement) and SD (standard diet) groups. The experimental calves in control group were fed on cereal straw-based diet with concentrate mixture (wheat bran: 85 and mustard cake: 15 kg) @ 20% of DM intake as per the farmers' practices (Ojha *et al.* 2017), whereas, the calves in CS group were fed according to control with additional customised supplement @ 0.25% of BW. The calves in SD group were fed as per Kearl (1982) to meet the nutrients requirement for maintenance and growth. The SD diet was composed of customised supplement and roughages.The experimental feeding trial period was of 30 days. All the calves were maintained under strict hygienic and uniform managemental conditions as described in Chaudhary *et al.* (2021). The chemical composition of feed offered to the calves is presented in Table 1.

Table 1. Chemical composition of feeds offered (% DM basis)

Attribute	Concentrate mixture		Wheat straw	Green maize
	Control	Customised supplement*		
DM	90.14	92.01	92.13	19.96
OM	93.67	93.65	93.55	87.33
CP	16.22	24.97	3.2	8.53
EE	4.30	3.03	1.15	1.25
TA	6.33	6.35	6.45	12.67
NDF	43.99	44.18	83.26	65.54
ADF	13.69	12.6	54.22	43.93

DM, Dry matter; OM, Organic matter; CP, Crude protein; EE, Ether extract; TA, Total ash; NDF, Neutral detergent fibre; ADF, Acid detergent fibre. *Patent application No. 202211032024 dated 03.05.2022.

Experimental procedures: All the experimental calves were individually offered a weighted amount of their respective concentrate mixture daily in the morning at 9.00 AM to meet their nutrient requirement for maintenance and growth. Wheat straw was provided *ad lib.* after ensuring complete consumption of the concentrate mixture. About 2 kg green maize was also given to all the experimental calves to satisfy the part of their respective nutrient requirements. The calves were provided with clean tap water *ad lib.* twice daily.

Chemical analysis: Samples of concentrate mixtures, wheat straw and green maize offered were milled to pass through a 1.0 mm sieve and then analysed for proximate composition as per AOAC (2012). NDF and ADF fractions were determined following the methods of Van Soest *et al.* (1991).

Collection of blood samples: Blood samples from all the calves were collected at the start and end of experimental feeding by jugular venepuncture with the help of clean sterilized needle early in the morning before feeding and watering. Out of 6 ml, 4 ml was dispensed into gel containing serum vacutainers and allowed to clot at room temperature for 45 min. The collected sera samples were stored in deep freeze (-20°C) for further analysis. Approximately 2 ml blood was dispensed into other vacutainers containing anticoagulant (EDTA) for estimation of haematological parameters.

Clinical chemistry: The blood Hb concentration was estimated by cyano-methaemoglobin method. The serum variables (glucose, NEFA, total protein, albumin, globulin, A:G ratio, urea, total-cholesterol, HDL-cholesterol, AST and ALT) and major minerals (calcium and inorganic phosphorus) were assessed spectrophotometrically (Multiskan™ FC Microplate Photometer, Thermo Scientific Ltd.) with the help of diagnostic kits manufactured by Coral Clinical Systems (Goa, India) following the standard protocol provided with the kits. The serum trace minerals (copper, zinc, iron and manganese) were assessed using atomic absorbance spectrophotometer (Model 4141, Electronic Corporation of India Ltd., Hyderabad, India) following standard procedure.

Metabolic hormone profile and total antioxidant capacity assay: The serum thyroid hormones (T_3 and T_4) were estimated using UBI MAGIWELTM (United Biotech Inc., USA) ELISA kits. The growth hormone (GH) and insulin-like growth factor-1 (IGF-1) assay in the serum samples were performed using Bovine GH and Bovine IGF-1 ELISA kits (GenAsia, China and RayBiotech, Georgia), respectively. The total antioxidant capacity (TAOC) assay was done using Total Antioxidant Capacity Assay Kit (Elabscience Biotechnology Inc., USA) following the standard protocol provided with the kit.

Gene expression study: The relative mRNA expression profile of cytokines, viz. IL-2, IL-4 and genes involved in energy metabolism, viz. LEP (leptin), GHRL (ghrelin) in peripheral blood mononuclear cells (PBMCs) were studied by Real-Time Quantitative Reverse Transcription PCR. About 4 ml blood was collected from all the calves at the start and end of the experimental trial period in a clean, autoclaved 15 ml Falcon tubes containing heparin (10-20 IU/ml blood) for PBMCs isolation. The PBMCs were isolated by density gradient centrifugation using Lymphocyte Separation Media (Histopaque 1077; MP Biomedicals). The total RNA was extracted from the PBMCs using Trizol reagent. The total RNA samples were quantified and the purity was tested using nanodrop spectrophotometer readings. The absorbance at 260 nm and 280 nm wavelength was recorded using 1 µl of re-suspended total RNA samples against nuclease free water as blank. The RNA samples showing the OD_{260}:OD_{280} value between 1.8-2.0 were expected to contain no protein and used in cDNA synthesis. Reverse transcription was carried out using QuantiTect Reverse Transcription Kit (Qiagen, Germany) in 20 µl reaction volume following the manufacturer's instruction.

Primers: Already published primers were standardized and used (Table 2). The PCR condition was optimized by amplifying the cDNA with the specific primers at different annealing temperatures (Tm). The qRT-PCR was performed after checking the specificity of each primer.

Table 2. Description of primers

Target gene	Primer sequence	Annealing temperature (°C)	Reference
β-actin	F 5'-CGC ACC ACT GGC ATT GTC AT-3' R 5'-TCC AAG GCG ACG TAG CAG AG-3'	55	Patra *et al.* 2013
IL-2	F 5'-TTT TAC GTG CCC AAG GTT AA-3' R 5'-CGT TTA CTG TTG CAT CAT CA-3'	57	
IL-4	F 5'-GTA CCA GTC ACT TCG TCC AT-3' R 5'-GCT CCT GTA GAT ACG CCT AA-3'	53	
LEP	F 5'-AGACCATAACAGCAGACAG-3' R 5'-TCCAGGCAATTCACTTCC-3'	53	Kumar *et al.* 2012
GHRL	F 5'-AGCTGTCAGGGGCTCAGTCC-3' R 5'-AGTGTCCCGGAAGCCAGGTGAG-3'	58	Gupta *et al.* 2014

IL-2, Interleukin-2; IL-4, Interleukin-4; LEP, Leptin; GHRL, Ghrelin.

Quantitative RT-PCR analysis: *q*RT-PCR reactions were run on RT-PCR system (Bio-Rad CFX Real-Time PCR System) and analysed by CFX Maestro Software. This was performed using Takyon No Rox SYBR MasterMix dTTP Blue (Eurogentec, Belgium). The reaction mixture was prepared comprising 10 µl SYBER green Master Mix (2×), 1.0 µl forward primer (10 pmole/µL), 1.0 µl reverse primer (10 pmole/µL), 2.0 µl template cDNA and nuclease free water up to 20 µl. The thermal cycling conditions used were: of one cycle of initial denaturation at 95°C for 3 min followed by 39 cycles of cyclic denaturation at 95°C for 15 sec, annealing for 1 min depending on primers Tm as specified in Table 2 and extension at 72°C for 30 sec. The results were expressed as cycle threshold (Ct) values. The $2^{-\Delta\Delta Ct}$ method, as elaborated earlier by Livak and Schmittgen (2001), was used to study the relative change in gene expression. Formula used to calculate the fold change in gene expression was

$$\text{Relative fold change} = 2^{-\Delta\Delta Ct}$$

Where, $\Delta\Delta Ct = (C_t$, target gene $- C_t$, reference gene) test $- (C_t$, target gene $- C_t$, reference gene) housekeeping.

Statistical analysis: The data recorded in the biological experiment were analysed using one-way analysis of variance (ANOVA). The means were compared by Tukey Test (SPSS Version 20.0) with level of significance ($P<0.05$) as per the procedures of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Haematological profile: The mean Hb and haematocrit levels were found to be comparable ($P>0.05$) irrespective of dietary treatments (Table 3) and were within the normal physiological range for cattle (Kaneko *et al.* 2008). The haematological profile (Hb and PCV) is considered to be an indicator of erythrocytic normalcy and overall welfare of the animals (Radostits *et al.* 1994). In agreement with our results, Lohakare *et al.* (2006) reported that crossbred calves fed different dietary levels of protein did not reveal any significant effect on Hb and haematocrit concentrations. Naqvi *et al.* (2011) also did not observe any significant change in Hb and PCV levels in ewes supplemented with concentrate mixture *vis-à-vis* non-supplemented control group. Findings of the present study suggest that supplementation did not exert any adverse effect on the haematological parameters and overall health of the calves persisted satisfactory during the experimental period.

Table 3. Haemato-biochemical profile of calves supplemented with customised supplement

Attribute	Control	CS	SD	SEM	P value
Hb, g/dl	11.56	12.00	11.64	0.18	0.536
Haematocrit, %	32.40	34.00	33.40	0.56	0.544
Glucose, mg/dl	62.78^{b}	67.90^{ab}	70.04^{a}	1.54	0.039
NEFA, ng/ml	7.55	7.35	7.06	0.14	0.331
Total protein, g/dl	7.44	7.35	7.26	0.15	0.902
Albumin, g/dl	3.80	3.82	3.82	0.05	0.977
Globulin, g/dl	3.64	3.53	3.44	0.14	0.148
A:G ratio	1.10	1.14	1.15	0.05	0.103
Urea, mg/dl	30.44	29.45	29.79	1.45	0.946
Total-cholesterol, mg/dl	104.19	108.42	103.44	1.84	0.534
HDL-cholesterol, mg/dl	42.38	43.31	41.63	1.23	0.87
AST, U/L	88.70	91.05	94.98	2.25	0.556
ALT, U/L	19.86	20.55	21.25	0.48	0.306

^{ab}Mean values with different superscript in a row differ significantly. CS, Customised supplement; SD, Standard diet; SEM, Standard error of mean; Hb, Haemoglobin; NEFA, Non-esterified fatty acids, AST, Aspartate transaminase; ALT, Alanine transaminase.

Serum biochemical profile: The data pertaining to the serum biochemical parameters of calves during the experimental trial is summarised in Table 3. Serum glucose concentration was significantly ($P<0.05$) higher in SD as compared to control groups, however, CS group occupied a transitional spot between SD and control groups. Serum glucose level is considered to be an indicator of the normal physiological condition and health of animals. Present results are in conformity with the findings of Lohakare *et al.* (2012), who reported that the serum glucose was significantly increased in the calves fed higher level of concentrate mixture (2 kg/d) relative to the calves offered lower level of concentrate mixture (1 kg/d). Similarly, Santra and Pathak (2000) also reported higher serum

glucose in calves fed high concentrate diet (60%) than low concentrate diet (30%). Naqvi *et al.* (2011) also reported significantly higher plasma glucose concentration in ewes supplemented with concentrate mixture *vis-à-vis* non-supplemented control group. The possible reason for the higher serum glucose in SD and CS groups might be attributed to the higher feed intake by calves as well as availability of easily fermentable energy source from the customised supplement (Chaudhary *et al.* 2021). The other biochemical parameters, viz. total protein, albumin, globulin, A:G ratio, urea, serum total-cholesterol, HDL-cholesterol and serum enzymes (AST and ALT) were not affected (P>0.05) by the dietary treatments and values were within the suggested normal physiological range for cattle (Kaneko *et al.* 2008). In association with the present findings, Lohakare *et al.* (2006) did not find any significant difference in the serum protein variables (total protein, albumin, globulin and serum urea) in crossbred calves fed different dietary protein levels. Naqvi *et al.* (2011) also did not find any significant change in total protein, albumin and globulin in ewes supplemented with concentrate mixture *vis-à-vis* non-supplemented control group. Serum proteins are an indicative of health status of animals and an increased serum albumin (the principal serum protein) indicates dehydration; however, decreased level indicates liver, kidney, GI disease or malnourishment (Kaneko *et al.* 2008, He *et al.* 2017) or thermal stress in goats (Dangi *et al.* 2012). In our study, the dietary supplementation of customised supplement in calves did not exert any adverse effect on serum total proteins and urea. The possible reason for non-significant effect on haemato-biochemical profile of crossbred calves following tailor-made supplementation may be attributed to the fact that the calves were kept on good plane of nutrition previously and shorter experimental duration.

Serum mineral profile: Serum Ca and i-P concentration were higher (P<0.01) in SD and CS than control group. The levels of serum trace minerals, viz. Zn, Cu and Fe were also significantly (P<0.01) higher in SD and CS groups than control group. The level of serum Mn was significantly (P<0.05) higher in SD relative to control, while CS had an intermediate position between SD and control group (Table 4). The present results are in conformity with the results of Yengkhom *et al.* (2018), who observed higher level of serum macro (Ca and i-P) and trace (Zn, Cu, Fe and Mn) minerals in kids supplemented with mineral mixture. Furthermore, higher levels of plasma trace minerals were also observed by Singh *et al.* (2010) in kids supplemented with mineral mixture. Gupta (2013) reported significantly higher serum Ca, Fe, Cu, Zn and Mn levels in heifers supplemented with different types of mineral mixture. Muralidharan *et al.* (2015) reported significantly higher concentration of serum Ca and i-P in lambs supplemented with concentrate mixture. The enhanced level of serum minerals may be associated with the fact that dietary inclusion of specialized supplement containing mineral mixture improved the mineral status of the calves which was reflected in the improved serum mineral concentration.

Table 4. Serum mineral profile of calves supplemented with customised supplement

Attribute	Control	CS	SD	SEM	P value
Calcium, mg/dl	9.14[b]	10.51[a]	10.48[a]	0.27	0.001
i-Phosphorus, mg/dl	5.66[b]	6.02[a]	6.10[a]	0.080	0.000
Zinc, µg/ml	0.77[b]	0.99[a]	1.03[a]	0.050	0.003
Copper, µg/ml	0.73[b]	0.98[a]	1.02[a]	0.060	0.008
Iron, µg/ml	3.09[b]	3.92[a]	4.48[a]	0.240	0.001
Manganese, µg/ml	0.21[b]	0.27[ab]	0.31[a]	0.020	0.016

[ab]Mean values with different superscript in a row differ significantly. CS, Customised supplement; SD, Standard diet; SEM, Standard error of mean.

Metabolic hormone profile

T_3 and T_4 hormones: The circulating concentration of T_3 (P<0.05) and T_4 (P<0.001) in calves was significantly higher in SD and CS than control group (Table 5). The thyroid hormone T_3 is an indicator of general resting metabolic rate (Cavallo *et al.* 1990, Rønning *et al.* 2009). The serum concentration of T_3 in calves is prerequisite since it increases the synthesis of uncoupling protein which is essential for brown adipose tissue thermogenesis (Carstens 1994). Wood *et al.* (2013) reported that the circulating levels of T_3 was improved in cows fed higher energy diet. The levels of T_3 are directly affected by level of feeding and reduce with lower feed intake in animals (Christopherson *et al.* 1979, Murphy and Loerch 1994). The proposed justification for the higher circulatory concentrations of T_3 and T_4 in SD and CS groups may be ascribed to the higher nutrient intake in SD and CS groups (Chaudhary *et al.* 2021).

GH and IGF-1 hormones: The circulating concentration of GH and IGF-1 was significantly (P<0.05) higher in SD relative to control group, however, CS had a transitional spot between SD and control groups (Table 5). GH and IGF-1 are the key regulators of postnatal growth in mammals (Nakae 2001). GH (somatotropin), binds to its receptors in liver; trigger the synthesis and release of IGF-1 into the circulation where it binds to specific IGF-binding proteins and circulates as its endocrine form (Lucy *et al.* 2001). IGF-1 (somatomedin C) is physiologically essential for growth (Yu *et al.* 2005, Junnila *et al.* 2013, Kumar and Laxmi 2015) and regulates the growth and differentiation of different mammalian cell types through cell cycle activation. Consequently, IGF-1 plays a crucial role in nutrient utilization and metabolism, postnatal growth, development of mammary gland, lactation as well as reproduction in dairy animals (Jiang and Lucy 2001, Renaville *et al.* 2002, Butler 2003, Lucy 2008, Kumar and Laxmi 2015). Velazquez *et al.* (2008) reviewed that circulating concentration of IGF-1 is closely associated with prepubertal growth of heifers which is highly influenced by nutritional regime. Studies in the laboratory animals demonstrated that the plane of nutrition directly influences the circulating level of IGF-1 (Schalch and Cree

1985) and IGF-1 along with insulin are reported to indicate the nutritional status of the animals (Ciccioli *et al.* 2003, Lents *et al.* 2005). Dietary supplementation of protein has been reported to enhance the circulating IGF-1 concentrations transiently (McLean *et al.* 2018). It has been well established that both dietary energy and protein are the principal nutritional determinant for basal circulating plasma concentration of IGF-1 and undernutrition can attenuate its response to GH as well as uncouple the regulation of IGF-1 generally attributed to GH (Elsasser *et al.* 1989).-Thus, the increased circulatory concentrations of GH and IGF-1 in SD and CS groups may also be attributed to the higher nutrient intake from customised supplement (Chaudhary *et al.* 2021).

Table 5. Metabolic hormone profile and total antioxidant capacity assay of calves

Attribute	Control	CS	SD	SEM	P value
T_3, ng/ml	2.69^{b}	3.12^{a}	3.10^{a}	0.09	0.032
T_4, ng/ml	47.91^{b}	57.37^{a}	67.43^{a}	3.08	0.000
GH, ng/ml	8.42^{b}	8.64^{ab}	9.35^{a}	0.280	0.017
IGF-1, ng/ml	70.90^{b}	75.55^{ab}	78.20^{a}	1.410	0.018
TAOC, U/mL	2.75^{b}	3.66^{a}	4.29^{a}	0.250	0.001

^{ab}Mean values with different superscript in a row differ significantly. CS, Customised supplement; SD, Standard diet; SEM, Standard error of mean; GH, Growth hormone; IGF-1, Insulin-like growth factor-1; TAOC, Total antioxidant capacity.

Total antioxidant capacity assay: The mean values for total antioxidant capacity (TAOC) assay were significantly (P<0.01) higher in CS and SD than control (Table 5). In agreement with our study, Singh *et al.* (2011) reported that long-term energy malnutrition in growing lambs fed on wheat straw-based diet reduces the erythrocytic antioxidant defence. Trace minerals are the important cofactors for various enzymatic antioxidants and deficiency on one or other leads to compromised antioxidant defence system of the body (McDonald *et al.* 2008). The enhanced TAOC in the CS and SD groups as evident in the current study may be attributed to the improved nutrients and trace minerals availability from the customised supplement.

Relative mRNA expression of immune related cytokines and genes involved in energy metabolism

Immune related cytokines: The relative mRNA expression of cytokines, viz. IL-2 and IL-4 and genes involved in energy metabolism, viz. LEP & GHRL in calves is presented in Figs 1 and 2. The fold expression of IL-2 and IL-4 genes varied significantly (P<0.001) among different treatment groups and was higher for SD and CS than control (Fig. 1). In agreement with the present results, trace minerals have been reported to regulate the response of pro-inflammatory cytokines such as IL-2, IL-6 and TNFα (Rink and Kirchner 2000). Chandra (2011) also reported significantly higher concentration of plasma IL-2 in cows following Zn supplementation. In present study, an increased relative mRNA expression of cytokines, viz. IL-2 and IL-4 reflects improved immune response of calves fed

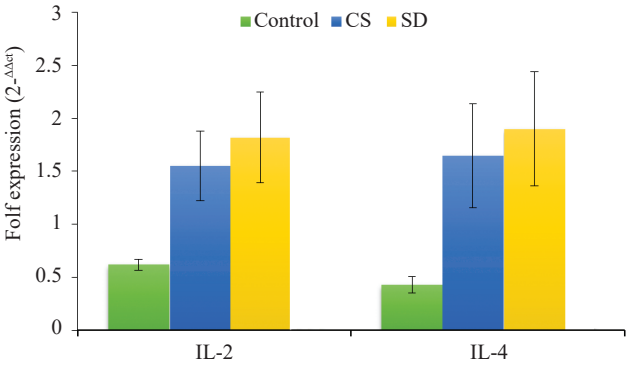

Fig. 1. Fold expression of immune related cytokines of calves supplemented with customised supplement. CS, Customised supplement; SD, Standard diet; IL-2, Interleukin-2; IL-4, Interleukin-4.

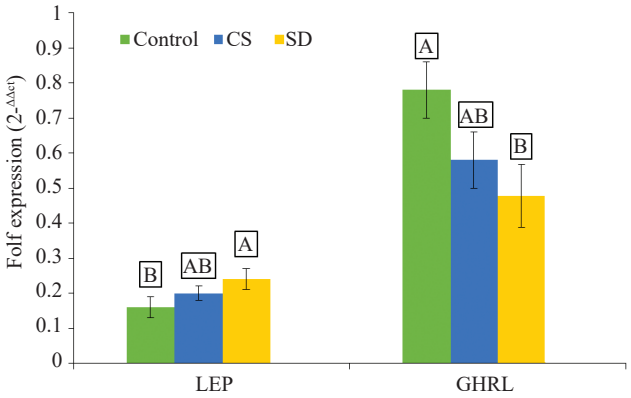

Fig. 2. Fold expression of genes involved in energy metabolism of calves supplemented with customised supplement. CS, Customised supplement; SD, Standard diet; LEP, Leptin; GHRL, Ghrelin.

customised supplement fortified with energy, protein and minerals.

LEP and GHRL: The relative mRNA expression of LEP was significantly (P<0.05) higher in SD as compared to control group, however, CS had an intermediate position between SD and control groups. Further, the fold expression of GHRL was significantly (P<0.01) lower in SD as compared to control group, whereas, CS had an intermediate position between SD and control groups (Fig. 2). The effect of nutrition on leptin dynamics was studied and reported that overfeeding increases and under or suboptimal feeding decreases the circulating concentration of leptin (Blache *et al.* 2000). It had been reported that, the plasma concentration of leptin positively correlates with the plasma concentrations of insulin and glucose, whereas, negatively correlates with the plasma concentration of NEFA (Block *et al.* 2001, Chelikani *et al.* 2008). Supplementation of trace minerals also positively affects the circulating concentration of leptin and reported significantly higher concentration of plasma leptin in cows following Zn supplementation (Chandra 2011). It had been reported that the plasma concentration of ghrelin showed positive correlation with the serum BHB and NEFA, however, negatively correlates

with leptin, T_3 and T_4 (Nowroozi-Asi *et al.* 2016). In agreement with our results, Wertz-Lutz *et al.* (2006) reported significantly higher concentration of plasma ghrelin in fasted cows as compared to well fed cows. In the present study, the increase in the relative mRNA expression of LEP gene and decrease in the relative mRNA expression of GHRL gene in SD and CS groups demonstrate the increased DM intake (Chaudhary *et al.* 2021) and serum glucose level in calves.

Based on the findings, it may be concluded that fortification of farmer's diet with customised supplement (0.25% of BW) had considerably enhanced the serum glucose concentration, metabolic hormone profile, total antioxidant capacity, relative mRNA expression of immune related cytokines and genes involved in energy metabolism in crossbred calves.

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