Original Research

Effect of Feed Restriction on Physio-Biochemical Changes, Milk Yield and Expression Profile of Leptin in Crossbred Cow

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Abstract

The present study investigated the effect of dietary feed restriction on physio-biochemical, milk yield, hormonal and leptin mRNA expression in crossbred cows. Fourteen lactating crossbred cows of uniform age were divided into two groups viz. Group I (n=7; optimal) and Group II (n=7; sub-optimal, 70% intake of the optimal group i.e., 30% feed restriction than the optimal group). The study was conducted for the period of 42 days. Blood and milk samples were collected at the interval of 7 days and individual milk production was recorded daily. There was a significant (p<0.05) reduction in body weight, body condition score and physiological responses like rectal temperature, respiration rate and pulse rate in GII cows. Glucose, albumin, creatinine, total protein and cholesterol were significantly (p<0.05) affected. Tri iodothyronine (T3), thyroxine (T4) and leptin concentration were found to be decreased (p<0.05), however, cortisol concentration was increased (p<0.05) in GII cows. In addition, milk yield was decreased (p<0.05) and also leptin gene expression was decreased significantly (p<0.05) in GII cows. The present findings indicates that 30% feed restriction adversely affect the physio-biochemical responses, hormonal profile, milk yield and leptin mRNA expression of crossbred cows.

Key words: Crossbred Cows, Cortisol, Feed Restriction, Leptin

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Introduction

India has the world's largest livestock population and managed to attain first position in milk production but with rapidly shrinking land and natural resources, the availability and quality of feed and fodder is becoming a challenge day by day. In the current scenario of feed shortage, the sustainable animal production relies upon the intelligent feeding strategies which exploit the physiological mechanisms by which animal become adapt to feed shortage. The productivity of cattle is fostered by the efficient utilization of nutrients

which is possible with an adequate supply of energy. Energy requirements are affected by age, body size, physiological state, environmental factor, muscular activity and relationship with other nutrients (Magdub *et al.*, 1983). Weather conditions such as ambient temperature, relative humidity, and wind velocity may modulate energy needs depending on the different geographic regions.

Nutritional stress and feed scarcity are one of the predisposing factors for the low productivity in cattle. In fed ruminants, substrate (fiber carbohydrate) supply is the major regulator of gluconeogenesis (Brockman *et al.*, 1979a). During energy restriction, when substrate availability is limited, hormonal interactions become major regulators of substrate supply through mobilization of body energy stores and control of hepatic gluconeogenesis (Brockman *et al.*, 1979b). Alteration in several body parameters like body weight, body condition score, pulse rate, respiration rate and rectal temperature during feed restriction help the animals to adapt to the various type of stressors (Bines *et al.*, 1983). Changes in biochemical profile such as glucose, protein, cholesterol, albumin, and creatinine occurs at cellular and sub-cellular level which tends to alleviate the nutritional stress within certain limits (Chilliard *et al.*, 2001). Adverse weather conditions that reduce pasture growth and quality may put lactating dairy cow under the period of transitory nutritional stress that leads to the reduction in milk yield. However, its effect depends on the intensity and duration of restriction and also the stage of lactation at which the feed restriction was imposed (Jabbar *et al.*, 2013). Leptin is a polypeptide with a molecular weight of approx 16kDa, which is the product of *ob* gene, could play a central role in the regulation of body energy homeostasis. In ruminants, leptin gene was shown to be expressed in adipose tissue (Chilliard *et al.*, 2001). It is thought as a hormone that helps the organism to survive in energy deficiency (Chilliard *et al.*, 2001). It has an important role in the regulation of neuroendocrine function and also has an important role in controlling body weight, metabolism and reproduction in different species (Rosenbaum *et al.*, 1999).

Previous studies conducted on species like sheep (Sejian *et al.*, 2010), goat (Hyder *et al.*, 2013) to study the various metabolic responses to feed restriction and adaptability of animals. Although the effect of dietary feed restriction on body physiological status has gained tremendous attention in recent years still the detail mechanism on adaption of crossbred cows during feed restriction has not been elucidated. Further, there is a dearth of information on the interaction between cortisol, thyroxine and leptin with specific fuel substrates during adaptation to energy restriction in crossbred cows. Hence, this study was designed to investigate the effect of feed restriction on physio-biochemical parameters, milk yield and expression profile of leptin in crossbred cows.

Materials and Methods

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Study Site and Experimental Animals

The present study was conducted at the Division of Physiology and Climatology, ICAR-Indian Veterinary Research Institute, Izatnagar. It is located at 170 m above sea level (28 °22'N and 79°24'E) in the northern upper Gangetic plain, having an annual rainfall of 90-120 cm. The mean temperature and relative humidity during the study were 15.5°C-24°C and 62% -75% respectively. Fourteen healthy non-pregnant lactating crossbred cows of uniform age group, weighing 340 to 450 kg of 3rd to 4th parity were selected from the experimental shed (University farm) and were used in the present study for a period of 42 days. The animals had *ad libitum* access to feed and good quality drinking water for a period of 30 days prior to the start of the study.

Experimental Procedure and Feeding Requirement

The animals were allocated into two groups of seven animals each viz. optimal (GI) and sub- optimal group (GII). The optimal group of the cow was fed concentrate @ 1 kg/2.5 litre of milk yield while green grass and wheat straw were fed according to their body weight and dry matter requirement. The suboptimal group animals were fed 70% intake of the optimal group (i.e., 30% feed restriction). The study was conducted after obtaining approval from the institute animal ethics committee. The details of the feeding are enlisted in Table 1.

Table 1: Details of the feeding for group I and II

Item	GI	GII	P value
DM intake, kg/d	9.43±0.58	7.99±0.36	0.057
DM intake, g/kgW$^{0.75}$	116.90±2.44	98.99±1.16	<0.001
ME intake, Mcal/d	21.68±0.35	14.23±0.32	<0.001
DP intake, g/d	762.41±11.31	484.73±10.97	<0.001
ME value, Mcal/kg DM	2.33±0.10	1.80±0.06	0.001
DP value, g/kg DM	82.38±4.37	61.30±2.65	0.001

DM: Dry Matter, ME: Metabolizable Energy, DP: Digestible Protein

Blood and Milk Sampling

Blood samples were collected from the jugular vein at an interval of 7 days for mRNA expression studies and for biochemical studies. Milk production was recorded daily.

Physio-Biochemical and Hormone Assay

Physiological parameter studied were body weight (BW), body condition score (BCS), rectal temperature (RT), respiration rate (RR), pulse rate (PR), milk yield (MY). The body weight (BW) and body condition scoring (BCS) were recorded at the start and end of the experiment whereas rectal temperature, respiration rate and pulse rate were recorded daily in the morning as well as in the evening. Serum biochemical parameters such as glucose, albumin, creatinine, total protein and cholesterol were estimated using

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commercial kits (Span diagnostic kits, India) with the standard method using double beam UV-Visible Spectrophotometer (Electronics Corporation of India Ltd, India). Tri-iodothyronine (T3), thyroxine (T4), and cortisol (Beckman coulter, Immunotech, Czech Republic) were estimated using radio immuno assay (RIA). Leptin (Boster Biological Technology Ltd., China) was estimated using commercial enzyme linked immunosorvent assay test (ELISA) kits as per manufacturer's recommendations. The analytical sensitivity of T3, T4, and cortisol were 0.26 nmol/L, 10.63 nmol/L, and 5 nM respectively while that of leptin was found to be 10 pg/ml.

Total RNA Extraction and cDNA Synthesis

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation method using Histopaque 1077 (Cat No#10771, Sigma-Aldrich, USA). Total RNA was isolated from PBMCs by using TRIzol reagent (Cat No#15596-026, Invitrogen, USA) by standard protocol. The concentration and purity of RNA were checked in Nanodrop Spectrophotometer (Thermo Scientific, USA). Samples with A260/280 values in between 1.8-2.0 were used for cDNA synthesis. Reverse Transcription was carried out using iScript cDNA Synthesis Kit (BioRad Laboratories, California, USA) in a total 20 µl reaction volume following the manufacturer's instruction. 1 µg of total RNA was used in the Reverse Transcription as a template. All components of the kit were mixed after thawing, briefly centrifuged and kept on ice. All the components were added as follows: 5X iScript Reaction mix- 4 µl, iScript Reverse Transcriptase- 1 µl, Nuclease-free water- variable, Template (RNA)- variable, Total volume-20 µl into a sterile, nuclease-free thin walled pre chilled micro centrifuge tube (0.5 ml). Reactions were carried out in three steps of incubation: 25°C for 5 minutes, followed by 42°C for 30 minutes, and finally 85°C for 5 minutes.

Quantitative Real Time PCR (qPCR) Analysis

The qPCR was performed in duplicate using KAPA SYBR^{R} FAST qPCR kit (KAPA Biosystem, USA) in Stratagene MX3005P qPCR System (Agilent technologies, USA). Primers for Leptin and Ribosomal protein S15A (RPS-15A, internal control) were designed by the Integrated DNA Technology (IDT) using Beacon software and depicted in Table 2.

Table 2: Target genes, primer sequences (5'→3') and amplicon length for qRT-PCR used in this study

Target	Primer Sequence (5'→3')	Product Length	Efficiency (%)	EMBL accession No/ Reference
Leptin	For: AGACCATAACAGCAGACAG	192	93.3	NM_173928.2
	Rev: TCCAGGCAATTCACTTCC			
RPS 15A	For: AGGGCTGGGAAAATTGTTGTGAA	125	104.8	Mishra *et al.* (2016)
	Rev:TGAGGGGATGGGAGCAGGTTAT			

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The master mix consisted of 10 µl of SYBR green, 8 µl nuclease free water (NFW), 1 µl of cDNA, 0.5 µl of each forward and reverse primer. The real-time reaction was performed with following cycling conditions: denaturation at 95°C for 7 min, 40 cycles of denaturation at 95°C for 15 sec, annealing at the primer specific annealing temperature for 15 sec and extension at 72°C for 10 sec. The cycle threshold (Ct) values and amplification plot were acquired by using the "KAPA SYBR (with dissociation curve)" method in Stratagene MX3005P qPCR System. No template control (NTC) was placed with each reaction set up to check any contamination in reaction. Relative mRNA expression was determined by the equation suggested by Pfaffl, 2001.

Statistical Analysis

ANOVA was used for analysing the data obtained in the experiment. The multiple comparisons between the group, week and interaction for different physio-biochemical parameters at different time period done by using Tukey's test at 5% level of significance. The analysis was done by JMP 9.0 software.

Results

Physiological Responses

The result for physiological responses is presented in Table 3. All the parameters were found to be significantly ($p<0.05$) decreased in GII as compared to GI cows.

Table 3: Effect of feed restriction on physiological parameters and milk yield of crossbred cows. Mean and standard error means (SEM) within a column having different superscripts differ significantly. The results represented are the combined means of all seven collections.

Item	BW*	BCS*	RT*	RR*	PR*	MY*
GI	351.07±0.56^a	3.42±0.01^a	100.34±0.13^a	25.79±0.49^a	54.36±0.41^a	9.41±0.24^a
GII	344.64±0.56^b	3.25±0.01^b	99.64±0.13^b	22.73±0.49^b	52.16±0.41^b	7.91±0.24^b

**p<0.05; BW: Body weight, BCS: Body Condition Score, RT: Rectal temperature, RR: Respiration rate, PR: Pulse rate, MY: Milk yield*

Biochemical Parameters

The result for biochemical parameters are presented in Table 4. Mean plasma glucose and albumin level was significantly ($p<0.05$) lower in GII compared to GI cows. However, serum creatinine level did not differ significantly between GI and GII. Mean plasma total protein was significantly ($p<0.05$) reduced in GII compared to GI cows. Furthermore, the total plasma cholesterol level was significantly ($p<0.05$) greater in GII compared to GI cows.

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Table 4: Effect of feed restriction on biochemical parameters of crossbred cow. Means and standard error means (SEM) within a column having different superscripts differ significantly. The results represented are the combined means of all seven collections.

Item	Glucose*	Albumin*	Creatinine*	Total Protein*	Cholesterol*
Average value of different parameters in GI and GII					
GI	70.35±2.74^{a}	3.46±0.08^{a}	61.88±3.01^{a}	8.50±0.22^{a}	152.90±8.43^{a}
GII	64.55±2.74^{b}	3.34±0.08^{b}	59.31± 2.92^{a}	7.45±0.22^{b}	164.23±8.43^{b}
Week wise value of different parameters in GII					
0	69.14±3.78^{b}	3.51± 0.07^{a}	59.37± 4.07	8.46±0.26^{a}	152.61± 6.84^{a}
1	70.25±3.78^{b}	3.50± 0.07^{a}	59.14± 4.07	8.24±0.26^{a}	147.08± 6.84^{a}
2	68.27±3.78^{b}	3.49±0.08^{a}	59.79± 4.07	8.14±0.26^{a}	158.83± 6.84^{b}
3	66.70±3.78^{a}	3.42± 0.07^{a}	56.47± 4.07	8.00±0.26^{b}	159.60± 6.84^{b}
4	66.19±3.78^{a}	3.37± 0.07^{b}	59.03± 4.07	7.86±0.26^{b}	160.10± 6.84^{b}
5	65.99±3.78^{a}	3.37± 0.07^{b}	60.58± 4.26	7.76±0.26^{b}	164.90± 6.84^{b}
6	65.62±3.78^{a}	3.12±0.08^{b}	60.31± 4.26	7.37±0.26^{b}	166.85± 6.84^{b}

$p<0.05$

Hormone

Plasma T_3 and T_4 level showed significant lower (Fig. 1 & 2) in the GII compared to GI. Serum cortisol level (Fig. 3) was significantly ($P<0.05$) higher in GII compared to GI cows. However, serum leptin (Fig. 4) showed significantly ($p<0.05$) lower level in GII cows.

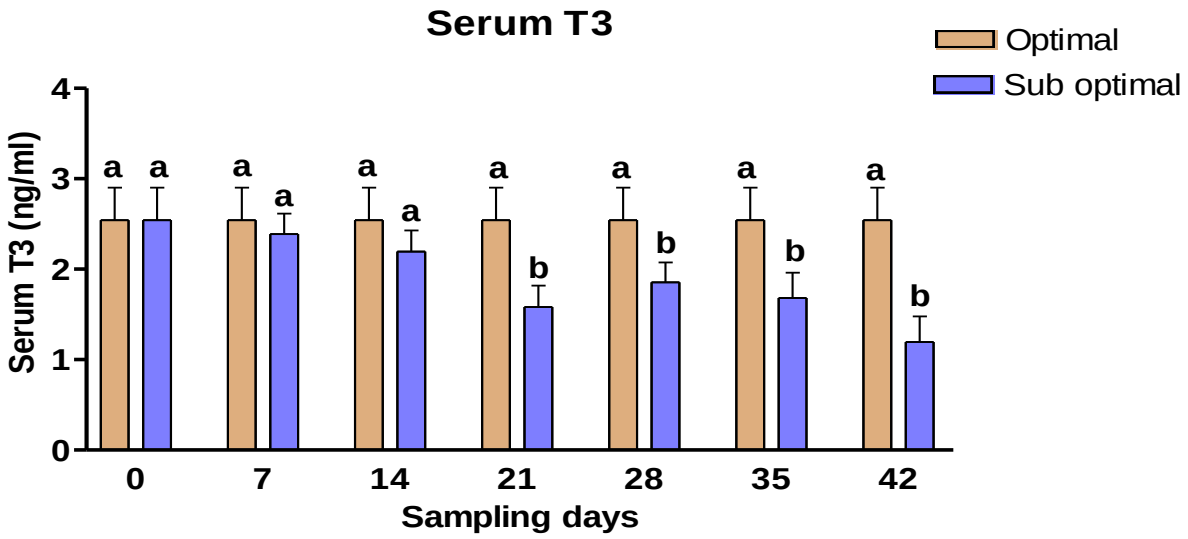

Fig. 1: Mean serum T3 concentration in GI and GII on different days. All values indicate Mean ± SEM. Values with different superscript within the same group are significantly different.

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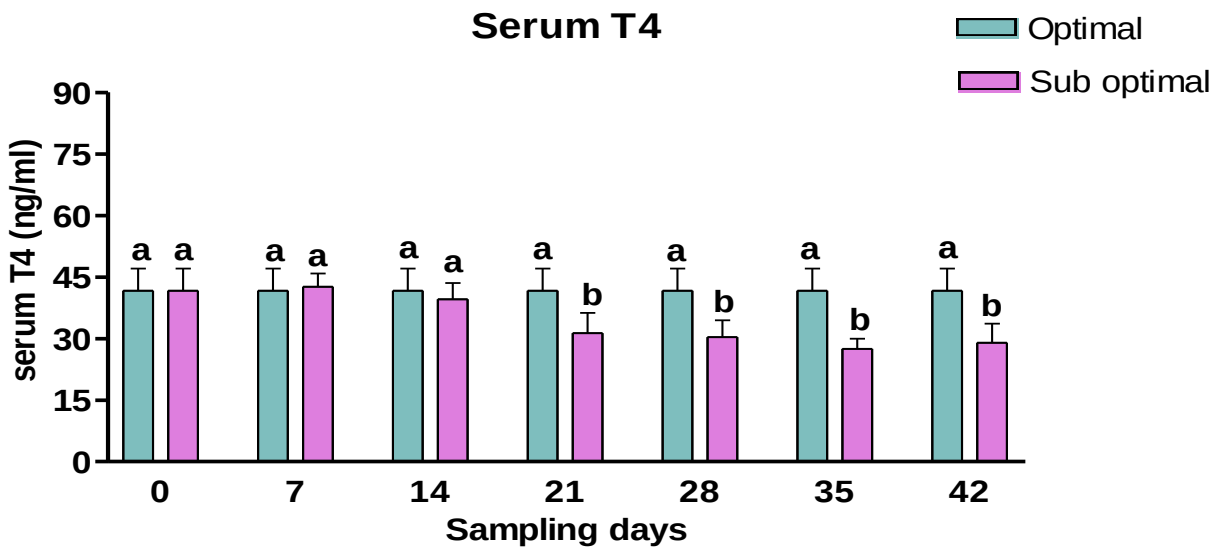

Fig. 2: Mean serum T4 concentration in GI and GII on different days. All values indicate Mean ± SEM. Values with different superscript within the same group are significantly different.

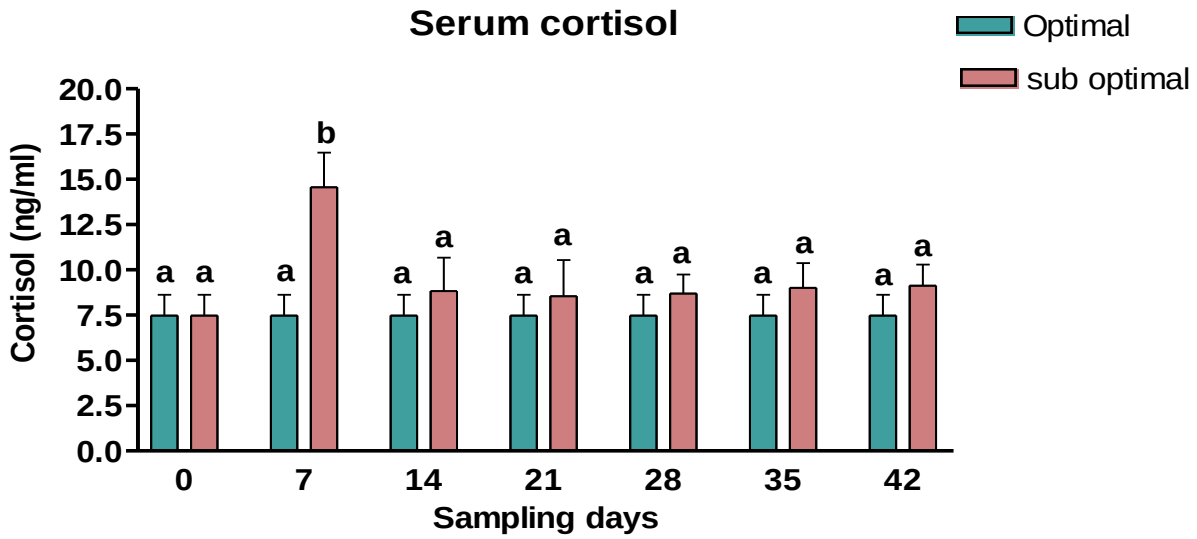

Fig. 3: Mean serum cortisol concentration in GI and GII on different days. All values indicate Mean ± SEM. Values with different superscript within the same group are significantly different.

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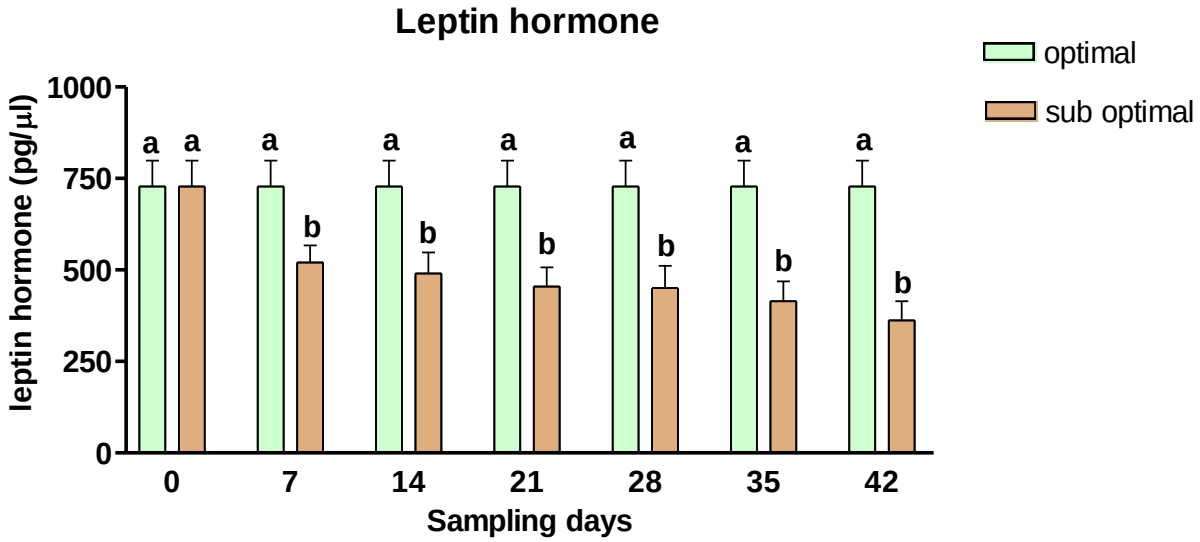

Fig. 4: Mean serum leptin concentration in GI and GII on different days. All values indicate Mean ± SEM. Values with different superscript within the same group are significantly different.

Leptin mRNA Expression

The leptin mRNA expression (Fig. 5) was significantly ($p < 0.05$) up-regulated on 7th day in GII compared to GI. Thereafter, the level of leptin transcript was decreased in GII compared to GI.

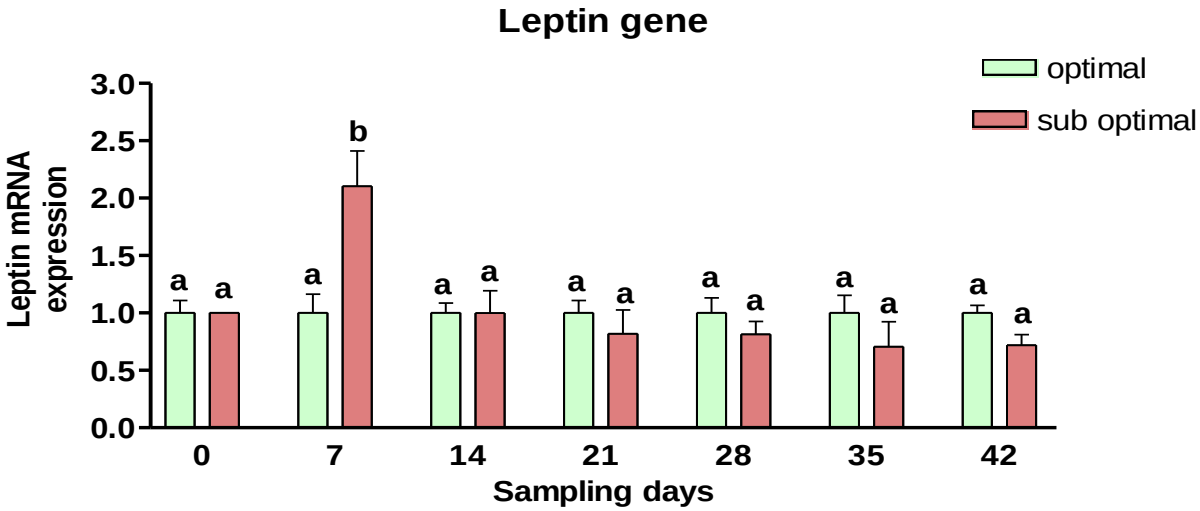

Fig. 5: Relative mRNA expression of leptin gene in GI and GII on different days. All values indicate Mean ± SEM. Values with different superscript within the same group are significantly different.

Discussion

The present study unravels the effect of different physio-biochemical, endocrine and gene expression factors which make the animal adapt during nutritional stress and the effect of nutritional stress on milk

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yield. Normally, the feed restriction results in the reduction in metabolic body weight which could be due to the reduction in mass of visceral tissue during feed restriction (Sainz *et al.,* 1997).

BCS is considered as the best indicator of the mechanism of body reserves management (Croston *et al.,* 1994). BCS was found to be decreased during lactation which was paralleled by decreased back fat thickness and muscle mass (Maurya *et al.,* 2010). Similarly, we observed lower BCS in feed restricted animals which may be due to fat mobilization during nutritional stress in feed restricted animals. Reduced metabolic state during feed restriction involves decrease in body temperature which further reduces metabolic rate as per Vant Hoff's rule. Additionally, animals further reduce their metabolic requirements to minimize heat loss to the environment (Martin *et al.,* 2008). Decrease in respiration rate as observed in the present study might be due to reduced metabolic requirements and decreased oxygen consumption. Likewise, pulse rate is an indicator of general metabolic status and lowered pulse rate the present study could be due to decrease in metabolic rate as a result of restricted feeding in goat (Hyder *et al.,* 2013). We found a significant decrease in milk yield in feed restricted animal's which corroborates with (Jabbar *et al.,* 2013). This may be due to reduced availability of glucose (required for lactose synthesis) in feed restricted animal's which could have decreased milk synthesis thereby affected the milk yield.

In the fed state, propionate provides a major proportion of gluconeogenic precursors in ruminants (Young *et al.,* 1997). During dietary energy restriction, homeostatic control of plasma glucose requires greater endogenous utilization of glycerol, lactate and amino acids for gluconeogenesis, suggesting glucose concentration began to increase initially but as substrate get depleted day by day the concentration of glucose in plasma goes down. In our study, the albumin level was reduced in the feed restricted animals. This could be due to the depletion of the amino acid pool in the skeletal muscle (Moorby *et al.,* 2002). In the present study, the total protein followed a similar trend as albumin. The reduction in total protein in feed restricted animals could be attributed to the decrease peripheral uptake of amino acids and increased catabolism of reserve protein thereby making available more amino acids for urea synthesis (Lyle *et al.,* 1984b). Our finding on creatinine level is consistent with (Keenan *et al.,* 1986) which could be due to enhanced nitrogen recycling, minimum proteolysis and might be due to variations in metabolic clearance of creatinine during feed restricted animals. Elevated levels of cholesterol in the present study could be due to decrease in lipoprotein lipase (LPL) activity which is required for selective uptake of LDL cholesterol in heart and skeletal muscle (Merkel *et al.,* 2002). Similarly, LPL activity was decreased by 37% during fasting in cattle (DiMarco *et al.,* 1981).

In the present study, the decrease in serum T3 and T4 concentration is in consonance with the earlier findings in goat (Todini *et al.,* 2007) and cattle (Murphy *et al.,* 1994). This could be attributed to down-regulation in liver type I deiodinase activity responsible for the peripheral conversion of T4 to T3 (Bianco *et al.,* 2002) in feed restricted animals while the reduction in T4 level could be due to variation in peripheral

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deiodinase activity. The greater cortisol level on day 7 may be due to lower blood glucose level during feed restriction which could have stimulated hypothalamic pituitary adrenal axis culminated in an acute rise in cortisol level. It has also been demonstrated that nutritional stress decreases leptin, which up-regulates cortisol, a metabolic adaptation of under nutrition (Bornstein *et al.,* 1997). In addition, serum leptin concentration was decreased with feed restriction which was in agreement with the previous finding (Chilliard *et al.*, 2001). The rapid decrease in plasma leptin in underfed animals was suspected as an acute signal to stimulate refeeding behavior and glucocorticoid secretion thereby decrease thyroid activity and energy expenditure (Heiman *et al.,* 1999).

Leptin mRNA expression was noticed to be greater during initial phase in feed restricted group animals which could be attributed to increase in circulating glucocorticoids and might be a physiological adaptation mechanism to compensate the reduced leptin level. Furthermore, *In vitro* and *In vivo* study of adipocyte depicted that glucocorticoids stimulate leptin gene expression (Slieker *et al.,* 1996). The subsequent fall in leptin mRNA expression in feed restricted group could be due to decreased level of leptin due to depleted fat reserves.

Conclusion

It may be concluded from our study that restricted feeding have an adverse effect on physio-biochemical responses, hormonal profile and leptin mRNA expression which could have tremendous impact on the physiological functions necessary to maintain homeostasis in crossbred cows reared under the tropical region of India.

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References

1. Bianco AC, Salvatore D, Gereben B, Berry MJ and Larsen PR. 2002. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocrine Reviews. 23: 38-89.
2. Bines JA and Morant SV. 1983. The effect of body condition on metabolic changes associated with intake of food by the cow. *British Journal of Nutrition*. 50: 81-89.
3. Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M and Scherbaum WA. 1997. Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland. Diabetes. 46: 1235-1238.
4. Brockman RP. 1979a. Effect of somatostatin suppression of glucagon secretion on glucose production in sheep. *Canadian Journal of Physiology and Pharmacology*. 57: 848-852
5. Brockman RP. 1979b. Roles for insulin and glucagon in the development of ruminant ketosis - Review. *Canadian Veterinary Journal*. 20: 121-126.

DOI 10.5455/ijlr.20180521073544

6. Chilliard Y, Bonnet M, Delavaud C, Faulconnier Y, Leroux C and Djiane J. 2001. Leptin in ruminants. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. Domestic Animal Endocrinology. 21: 271-295.
7. Croston D and Pollot G. 1994. Planned sheep production, second ed. Blackwell Scientific Publication, Oxford.
8. DiMarco NM, Beitz DC and Whitehurst GB. 1981. Effect of fasting on free fatty acid, glycerol and cholesterol concentrations in blood plasma and lipoprotein lipase activity in adipose tissue of cattle. *Journal of Animal Sciences.* 52: 75-82.
9. Heiman ML, Slopp KW, Chen Y and Caro JF. 1999. Extension of neuroendocrine axes to include leptin. *Journal of Animal Sciences.* 77: 33-42.
10. Hyder I, Ramesh K, Sharma S, Uniyal S, Yadav VP, Panda RP, Maurya VP, Singh G and Sarkar M. 2013. Effect of different dietary energy levels on physio-biochemical changes and mRNA expression profile of leptin in goat (Capra hircus). *Livestock Science.* 152: 63-73.
11. Jabbar MA, Fiaz M, Iqball T, Abdullah M and Marghazani IB. 2013. Effect of different dietary energy levels on milk production in lactating nili-ravi buffaloes. *Journal of Animal and Plant Sciences.* 23: 13-16.
12. Keenan DM and Allardyce CJ. 1986. Changes in plasma creatinine levels of sheep during sub maintenance feeding. *Australian Veterinary Journal*. 63: 29-30.
13. Lyle RR, de Boer G, Harrison RO and Young JW. 1984b. Plasma and liver metabolites and glucose kinetics as affected by prolonged ketonemia-glucosuria and fasting in steers. *Journal of Dairy Science.* 67: 2274-2282.
14. Magdub A, Johnson HD and Belvea RL. 1983. Effect of environmental heat and dietary fiber on thyroid physiology of lactating cows. *Journal of Dairy Science.* 65: 23-26.
15. Martin B, Golden E, Carlson OD, Egan JM, Mattson MP and Maudsley S. 2008. Caloric restriction: impact upon pituitary function and reproduction. *Ageing Research Reviews*. 7: 209-224.
16. Maurya VP, Sejian V, Kumar D and Naqvi SMK. 2010. Effect of induced body condition score differences on sexual behavior, scrotal measurements, semen attributes and endocrine responses in Malpura rams under hot semi-arid environment. *Journal of Animal Physiology and Animal Nutrition*. 96: 308-317.
17. Merkel M, Heeren J, Dudeck W, Rinninger F, Radner H, Breslow JL, Goldberg IJ, Zechner R and Greten H. 2002. Inactive lipoprotein lipase (LPL) alone increases selective cholesterol ester uptake in vivo, whereas in the presence of active LPL it also increases triglyceride hydrolysis and whole particle lipoprotein uptake. *Journal of Biological Chemistry*. 277: 7405-7411.
18. Mishra SR, Thakur N, Somal A, Parmar MS, Yadav VP, Bharati J, Bharti MK, Paul A, Verma MR, Chouhan VS, Sharma GT, Singh G, Gonzalez LA, D'Ochhio MJ and Sarkar M. 2016. Expression and localization of angiopoietin family in buffalo ovarian follicles during different stages of development and modulatory role of angiopoietins on steroidogenesis and survival of cultured buffalo granulosa cells. Theriogenology. 86: 1818-1833.
19. Moorby JM, Dewhurst RJ, Evans RT and Fishert WJ. 2002. Effects of level of concentrate feeding during the second gestation of Holstein-Friesian dairy cows. 2. Nitrogen balance and plasma metabolites. *Journal of Dairy Science*. 85: 178-189.
20. Murphy TA and Loerch SC. 1994. Effects of restricted feeding of growing steers on performance, carcass characteristics and composition. *Journal of Animal Sciences.* 72: 2497-2501.
21. Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research.* 29: 2002-2007.
22. Rosenbaum M and Libel RL. 1999. The role of leptin in human physiology. *The New England Journal of Medicine.* 341: 913-915.
23. Sainz RD and Bentley BE. 1997. Visceral organ mass and cellularity in growth-restricted and refed beef steers. *Journal of Animal Sciences.* 75: 1229-1236.

DOI 10.5455/ijlr.20180521073544

24. Sejian V, Maurya VP, Naqvi SMK, Kumar D and Joshi A. 2010. Effect of induced body condition score differences on physiological response, productive and reproductive performance of Malpura ewes kept in a hot, semi-arid environment. *Journal of Animal Physiology and Animal Nutrition*. 94: 154-161.
25. Slieker LJ, Sloop KW, Surface PL, Kriauciunas A, LaQuier F, Manetta J, Blue-Valleskey J and Stephens TW. 1996. Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. *Journal of Biological Chemistry. 271*: 5301-5304.
26. Todini L, Malfatti A, Valbonesi A, Trabalza-Marinucci M and Debenedetti A. 2007. Plasma total T3 and T4 concentrations in goats at different physiological stages, as affected by the energy intake. *Small Ruminant Research.* 68: 285-290.
27. Young J W. 1977. Gluconeogenesis in cattle: Significance and methodology. *Journal of Dairy Science.* 60: 1-15.

DOI 10.5455/ijlr.20180521073544