



Energy and Protein levels on Biochemical Parameters of Murrah Buffalo

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Studies on Growing Murrah Buffalo Fed on Diverse Energy and Protein Ration and their Effect on Biochemical Parameters

Sonali Prusty¹, S. S. Kundu¹, V. K. Sharma^{2*} and G. Mondal¹

Animal Nutrition Division, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India

¹ Assistant Professor, College of Veterinary Science and Animal Husbandry, Anjora, DSVCKV (Durg), C.G., India.

² Subject Matter Specialist (Animal Sciences), KVK Samba, SKUAST-Jammu, J & K, India.

*Correspondence: vjsharmandri@gmail.com

ABSTRACT

This study was undertaken to evaluate the hormonal profile of the Murrah buffalo when fed with varying levels of the energy and protein in the ration. Thirty growing male Murrah buffalo calves (202.5±6.75 kg BW) were randomly allocated into six groups based on their body weight (BW). Six total mixed rations (TMRs) with two energy levels and three protein levels were fed to the animals for duration of 150 days in a 2x3 factorial design. The energy levels were 10% higher and lower than the ICAR (2013) recommendation for 200kg BW bovines with expected average daily gain (ADG) of 600g. The protein levels were adequate to, 10% higher and 10% lower than the ICAR (2013) recommendations. Energy levels were 2.2 and 2.41 Mcal metabolizable energy (ME)/kg DM whereas protein levels were 12.3%, 13.6% and 15.0% of crude protein (CP). There was significant (P<0.05) increase in plasma urea concentration with increased CP and decreased ME in diet and also the increase was less apparent with the increased energy:protein ratio of diet. Total protein, urea and Insulin – like growth Factor 1 (IGF-1) levels were positively correlated to dietary energy and protein concentrations whereas, plasma growth hormone was negatively correlated. Plasma immunoglobulin status was unaltered by dietary energy and protein levels. Plasma biochemical parameters were within normal range in all the groups. It can be inferred that the ration with 10% lower or higher ME and CP than the requirement did not alter the biochemical and immune status of growing buffaloes.

Key words: Energy, IGF-1, Immunoglobins, Murrah buffalo, Protein

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INTRODUCTION

Imbalance or insufficiency in energy and protein nutrition in bovine lead to meager performance in terms of growth and production (Sharma et al., 2012; Sharma et al., 2013, Barbizan et al., 2020). This maybe due to the altered biochemical status of certain plasma metabolites and hormones. Alternatively, adequate dietary protein and energy maintains normal biochemical status of the animal. In growing ruminants, blood biochemical status along the growth data would be helpful to analyze the synchronized effects of energy and protein on growth precisely (Sharma et al., 2016). Plasma urea nitrogen is highly correlated with rumen ammonia concentration and thus varies with dietary protein and energy status within a short span (Whitelaw et

al., 1991). At constant energy intake, increased dietary protein may increase blood urea N concentration. Instead, if dietary protein intake is high, increased dietary energy would be expected to decrease BUN. Plasma protein has role in maintaining blood volume and tissue fluid content, shows an altered concentration with varying dietary composition (Raggio et al., 2007; Prusty et al., 2017). Adequate nitrogen balance depending upon the stage of growth is necessary to maintain the normal plasma immunoglobulin concentration. In bovines, interaction between growth hormone (GH), insulin like growth factor-1 (IGF-1) and their receptors have important role to play during growth phase. Serum IGF-1 concentration is regulated by GH status whereas tissue sensitivity to both GH and IGF-1 is

affected by nutritional status like energy and protein availability (Breier, 1999). There is altered/ reduced response of IGF-1 to GH if energy and protein in diet are inadequate. In farm animals a positive correlation of serum IGF-1 as well as liver IGF-1 mRNA concentration with body weight gain, feed efficiency and body protein synthesis has been observed (Hayden et al., 1993; Stick et al., 1998; Sharma et al., 2016). In the present study, plasma concentrations of different metabolites like plasma urea, protein, immunoglobulin, GH and IGF-1 in Murrah buffalo calves fed on varying levels energy and protein in diet are investigated.

MATERIALS AND METHODS

Ethical compliance

The use of the animals and the experimental procedure were approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) rules, laid down by the Government of India (386/01/ab/CPCSEA). All the experiment procedures including animals were approved by Institutional Animal Ethics Committee of ICAR-National Dairy Research Institute.

Animals, management and experimental design

Thirty growing male Murrah buffalo calves were selected from the Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal (India) and enrolled for this study by dividing into six similar groups of five each based on comparable body weight (202.5 ± 6.75 kg BW). Six total mixed rations (TMRS) with 2 levels of ME and 3 levels of CP (2x3 factorial design) were prepared. The proportions of green fodder, wheat straw and concentrate in the 6 groups were as follows, R1- 30:35:35, R2- 21:44:35, R3- 35:29:36, R4- 23:32:45, R5- 25:30:45 and R6- 28:28:44, respectively. Concentrate mixture was prepared using sorghum grain (red), deoiled mustard cake, cotton seed cake, wheat bran and rice bran at varying levels with 2% mineral mixture and 1% salt in each. The ME levels

were 10% below and above that of ICAR (2013) recommendation for 200kg BW male calves with an intended average daily gain (ADG) of 600g per day and similarly the CP levels were equal, 10% above and below that of ICAR (2013) recommendations (Table 1). The animals were fed these diets for 150 days duration.

Blood sampling and analysis

About 8 ml of blood sample from jugular vein of each buffalo calves was drawn at monthly interval in morning prior to feeding and watering. Immediately after collection into sterile heparinized vacutainers (Vacuette, Greiner bio-one, GmbH, Austria), these were placed in the icebox and transported to laboratory. Samples were centrifuged (Remi, Mumbai, India) at $1500 \times g$ at $4^\circ C$ for 10 minutes, recovered the plasma and frozen at $-20^\circ C$ until analyzed. Before analysis the sample were thawed properly to atmospheric temperature and then processed as per routine procedure. The samples were kept in replicate to avoid the unnecessary repeat thawing and freezing of the sample and thus caused minimum damage to the sample. Plasma concentration of urea was estimated by spectrophotometry by DAM (diacetyl monoxime) method using diagnostic reagent kit (Span diagnostic, Surat, India). The optical density was measured at 525 nm. Total plasma protein was estimated by modified biuret method using total protein test kit (Span diagnostic, Surat, India) at an optical wavelength of 578 nm. Immunoglobulin in the plasma sample was estimated by Zinc turbidity method (Mc Ewan et al., 1970) and the OD was taken at 460 nm. Bovine growth hormone (bGH) test kit (Endocrine Technology, Newark, USA) was based on the principle of solid phase enzyme-linked immune sorbent assay (ELISA) whereas bovine insulin like growth factor-1 (bovine/space IGF-1) test kit (Genxbio, New Delhi, India) was based on the principle of competitive enzyme immunoassay technique. OD for both the hormones was measured at 450 nm with a micro titer well reader.

RESULTS AND DISCUSSION

Performance and efficiency measures

The intake as well as average daily gain are provided in Table 1. No considerable variation in dry matter intake (DMI) among the treatment groups was recorded. Daily DMI ranged between 5.25 to 5.86 kg that is equivalent to 2.21 to 2.39% of BW. There was non-significant effect of dietary protein levels on the average daily gain (ADG). But ADG was affected ($P<0.05$) by varying energy levels and superior ADG was observed in groups fed on high energy. Similar, results were observed in female buffalo calves (Singh et al., 2009), Thai swamp buffaloes (Tatsapong, 2009) and male buffalo calves with significant ($P<0.05$) variation in ADG in response to different energy levels. Similarly, Singh et al. (2009) reported that weight gain in Bhadawari buffaloes was significantly ($P<0.05$) more when they were fed 20 percent more energy as compared to NRC (2001) recommendation and 20% lower than

recommendations. In this study ME intake was higher (21.61-22.90 MJ/100 Kg BW) in high energy groups, whereas DM intake was similar in all groups. Likewise, in Nili-ravi buffalo calves, fed on three levels of CP and ME (80, 100 and 120% of NRC (2001), ME intake increased with increased ME levels in the diet and protein intake was affected by both energy and protein levels (Basra et al., 2003a; 2003b). It describes the effect of varying energy levels on protein utilization when protein level was sufficient. It was observed that buffaloes on high protein and energy diet had higher CP intake compared to low protein and energy diet (Tatsapong, 2009; Basra et al. (2003b). Positive relationship between N intake and dietary CP level was observed in lactating cattle (Olmos Colmenero and Broderick, 2006), growing heifers (Devant et al., 2000) and dairy cows (Ipharraguerre and Clark, 2005), respectively. But no effect of dietary CP on BW gain was observed in crossbred calves (Lohakare et al., 2006).

Table 1. Effect of energy and protein levels on dry matter intake (DMI) and body weight (BW) gain of buffalo calves

Energy level	Low ME			High ME		
Protein level	Low CP	Medium CP	High CP	Low CP	Medium CP	High CP
Ration ME (Mcal/Kg)	2.19	2.19	2.21	2.41	2.43	2.41
Ration CP%	12.2	13.61	14.9	12.2	13.6	15.0
DMI (kg/day)	5.25±0.39	5.53±0.19	5.41±0.23	5.61±0.21	5.73±0.14	5.86±0.45
BW gain (g/day)	594±27.45 ^B	564±20.830 ^B	585±12.25 ^B	612±15.50 ^A	641±13.9 ^A	664±14.30 ^A

Effect of energy and protein levels on plasma biochemical status

The plasma urea (PU) concentration of buffalo calves fed on different energy and protein levels are presented in Table 2. Significant ($P<0.05$) effect of both dietary energy and protein levels on the plasma urea (mg%) concentration of the buffalo calves was observed. Plasma Urea content was lower ($P<0.05$) in high energy-low protein group compared to low energy-high protein group. Excess nitrogen relative to available energy in the rumen might result in increased rumen ammonia concentration that further

entered the portal blood and converted to urea in liver and appeared in the blood stream in the low energy and high protein group. Normal observed level of serum urea in calves is 200-300 mg/L (Kaneko et al., 1997), While in the present study the concentration was marginally higher (25.9 to 37.9 mg%). Hussain et al. (2001) also reported positive relationship between dietary protein and plasma urea nitrogen concentration post feeding in buffalo calves. With increasing CP concentration (11.9%, 16.7%, 18.1%, and 20.1%) PU nitrogen concentration was increased in Holstein heifers

(Gabler and Heinrichs, 2003). Similarly, direct relationship between blood urea-nitrogen and dietary CP in lactating dairy cows (Promkot and Wanapat, 2005) was recorded. Diet containing 12.5 and 13.7% CP showed normal BUN and MUN concentration in lactating cows whereas at 14.4% CP the values were higher (Promkot and Wanapat, 2005). When three iso-caloric diets at different CP levels (75%, 100% and 125% of Kearnl, 1982 CP recommendations) were fed to crossbred calves no significant effect of protein levels on serum urea concentration was observed and serum levels of urea was within normal range (Lohakare et al., 2006). Further, no significant effect of protein levels on growth rate of the calves was observed. A recommendation of 12.6% CP in diet was observed to be ideal to provide adequate digestible protein to the intestine for optimum growth beyond which no further improvement in growth rate was there. Different energy sources did not significantly affect plasma urea levels or rumen NH₃-N concentration in buffaloes (Wanapat et al., 2009). Protein at different levels (16 and 20% CP) had no significant effect on either plasma urea N or ADG and both the diets provided adequate amounts of metabolizable protein for growth (Richardel, 2004).

Total plasma protein level reflects availability of protein and their concentration decline in the case of dietary protein deficiency (Sahoo et al., 2009). Normal value of total proteins in crossbred cattle

was reported to be 6.75±7.82 g/100 ml (Gupta et al., 1988). Plasma protein concentration in growing female buffalo calves was 7.02 to 7.8 g/dl (Varma et al., 2012). In the present study total plasma protein concentration varied from 6.84-7.98 g/dl and was not significantly different among the groups except the reading taken on the 1st 30 days of experiment. This suggests CP as low as 12.3% was adequate for maintaining normal protein concentration in plasma. Similar studies reported protein levels at 16.8%, 20.5% and 25.3% in diet had no significant effect on serum total protein concentrations in crossbred calves (Lohakare et al., 2006). Similarly, no effect of different CP levels (100, 90 and 80% of Kearnl (1982) recommendations) was observed on blood protein concentration (5.62 to 6.49 mg/dl) in Murrah males (Verma et al., 2009; Sharma et al., 2018). Whilst, CP level in diet was adequate but reduced energy level lowered serum glucose and total protein concentration in lambs (Singh et al., 2013). Shukla et al. (1994) also observed significant (P<0.05) effect of dietary energy levels on the total plasma protein status in crossbred calves. The total plasma protein concentration was 6.88 g/dl at 100% and 6.50 g/dl at 75% energy level. Hence, role of energy in efficient utilization and availability of protein to ruminants is established from these studies. In light of these observations, in the present study the effectiveness of low dietary energy and protein concentration in maintaining plasma protein concentration may be inferred.

Table 2. Effect of energy and protein levels on plasma urea (mg%) concentration of male buffalo calves

Day	ME level						ME	CP	ME*CP
	Low			High					
	Low	Medium	High	Low	Medium	High			
30	28.2±0.58 ^{Ac}	33.6±1.84 ^{Ab}	37.5±0.84 ^{Aa}	23.0±1.16 ^{Bc}	28.0±1.34 ^{Bb}	32.8±0.73 ^{Ba}	<0.0001	<0.0001	NS
60	28.5±1.22 ^{Ac}	34.3±1.43 ^{Ab}	37.4±1.49 ^{Aa}	24.7±1.28 ^{Bc}	28.7±1.99 ^{Bb}	33.4±1.08 ^{Ba}	0.0009	<0.0001	NS
90	28.8±1.43 ^{Ac}	35.2±0.69 ^{Ab}	38.1±0.80 ^{Aa}	25.7±0.97 ^{Bc}	29.4±1.21 ^{Bb}	34.4±0.98 ^{Ba}	<0.0001	<0.0001	NS
150	29.6±1.96 ^{Ac}	35.0±0.74 ^{Ab}	38.7±0.92 ^{Aa}	25.1±1.03 ^{Bc}	29.0±1.38 ^{Bb}	33.2±1.50 ^{Ba}	<0.0001	<0.0001	NS

* Mean values bearing different superscript (A-B capital) in the same row differ significantly (P<0.05) for ME levels.

* Mean values bearing different superscripts (a,b,c small) in the same row within same ME level differ significantly (P<0.05) for CP levels.

Table 3. Effect of energy and protein levels on plasma protein (g/dl) concentration of growing male buffaloes

Day	ME level					
	Low			High		
	CP level					
	Low	Medium	High	Low	Medium	High
30	6.84±0.13 ^B	7.93±0.28 ^B	7.91±0.28 ^B	7.02±0.21 ^A	6.72±0.18 ^A	6.96±0.27 ^A
60	7.64±0.34	7.86±0.14	7.68±0.36	7.25±0.40	7.50±0.16	7.53±0.37
90	7.53±0.28	7.52±0.26	7.43±0.31	7.63±0.56	7.98±0.34	7.69±0.31
150	7.64±0.21	7.63±0.38	7.65±0.41	7.27±0.19	7.33±0.31	7.29±0.30

* Mean values bearing different superscript (A-B capital) in same row differ significantly (P<0.05) for ME levels.

Further, there was no significant variation in the Ig concentrations with dietary energy and protein at different intervals. In a study by Santra and Pathak (2000) no significant change of plasma globulin concentration was observed in crossbred calves fed on rations with varying proportion of concentrate and roughage. Ig concentration of 25.40 mg/ml was reported in Hariana heifers (Gupta et al., 2014). Globulin concentration ranged from 3.04 to 4.03 mg/dl on supplementing diet with different levels of protein in buffaloes (Verma et al., 2009)

Effect of energy and protein levels on plasma hormone status

A significant (P<0.05) effect of energy and protein levels on the growth hormone (GH) and Insulin-like growth Factor 1 (IGF-1) hormone status of buffalo calves was observed. The GH concentration (ng/ml) increased (P<0.05) with increased CP but decreased ME level of diet (Table 5). Indirect relationship between dietary energy and GH concentration has been reported in sheep (Waghorn et al., 1987), beef heifers (Houseknecht et al., 1988) and Holstein heifers (Dong et al., 2016). It became evident that when energy was sufficient, protein level in diet affected GH concentration and increased protein level facilitated growth in response to GH (Chevenne and Porquet, 1995). Protein level in diet may be the primary determinant of IGF-1 concentration, but response of IGF-I to CP is also affected by availability of ME (Elsasser et al., 1989). The growth hormone (ng/ml) concentration in this study was 7.53 (low CP, low ME), 9.01 (medium

CP, low ME), 9.54 (high CP, high ME), 7.33 (low CP, high ME), 8.59 (medium CP, high ME) and 9.13 (high CP, high ME). In buffalo heifers, GH concentration of 5.6-6.3 ng/ml of plasma was reported (Campanile et al., 2010; Sharma et al., 2016). There was no significant difference in GH concentration due to different levels of energy in the rations on 30th and 60th day of experiment. Subsequent samples showed a decrease in GH concentration with increase in energy levels. Similar trend in GH response to different energy levels in diet have been reported in yak (Yang et al., 2019 missing in reference section) and reported higher expression of GH receptors in liver of yak on low energy diet compared to medium and high energy diets. In the present study IGF-1 levels were significantly (P<0.05) different for different energy as well as protein treatments. The IGF-1 concentration varied from 169 to 186 ng/ml within low energy groups and 187 to 221 ng/ml within high energy groups (Table 5). A study by Lee et al. (2006) indicated a significant variation in IGF-1 level with variation in dietary energy and protein status. A direct relationship of IGF-1 with dietary CP:ME ratio was observed in Holstein heifers (Dong et al., 2016). But no significant difference in plasma IGF-1 concentrations with relation to low or high energy diets was observed in buffalo heifers and the concentration varied from 79.1 to 95.5 ng/ml (Campanile et al., 2010). Comparable response of IGF-1 to different energy levels were explained by the potential of buffalo heifers to reduce their requirement by metabolic adjustment when

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dietary energy was low. No effect of different protein levels (16% and 20% CP) in diet were observed on plasma levels of GH and IGF-1 in steers (Richardel, 2004). They calculated comparable rumen undegradable protein (RUP) value on either diets which was supposed to be

the cause for non-significant response in the high CP diet unlike other reported studies. In lambs, with increased CP (9, 12, 15%) in the diet, significantly (P<0.05) higher serum IGF-1 and hepatic IGF-1 mRNA concentrations were demonstrated (Davenport et al., 1995).

Table 4. Effect of energy and protein levels on plasma immunoglobulin (mg/ml) status of growing male buffaloes

Day	ME level					
	Low			High		
	CP level					
	Low	Medium	High	Low	Medium	High
30	30.75±2.61	31.00±0.93	30.81±2.33	30.47±3.83	31.54±1.82	30.40±1.50
60	30.54±1.15	31.08±2.28	31.25±2.00	30.98±1.44	31.50±1.67	31.00±0.53
90	32.01±1.07	32.05±2.77	31.76±1.58	33.89±2.43	31.98±1.97	31.74±0.34
150	29.46±1.78	30.62±1.67	30.10±0.97	29.90±0.58	30.90±0.67	30.30±1.09

* Mean values bearing different superscript (A-B capital) in same row differ significantly (P<0.05) for ME levels.

Table 5. Plasma GH (ng/ ml) status of growing male buffaloes fed on varying levels of energy and protein

Day	ME level								
	Low			High			Effect		
	CP level								
	Low	Medium	High	Low	Medium	High	ME	CP	ME*CP
30	7.68±0.13 ^b	9.62±0.24 ^a	9.41±0.16 ^a	7.50±0.07 ^b	9.66±0.07 ^a	9.29±0.33 ^a	NS	<0.0001	NS
60	7.35±0.12 ^b	8.97±0.25 ^a	9.52±0.18 ^a	7.44±0.12 ^b	8.87±0.07 ^a	9.06±0.23 ^a	NS	0.0001	NS
90	7.67±0.28 ^{Ab}	8.82±0.26 ^{Aa}	9.60±0.12 ^{Aa}	7.35±0.11 ^{Bb}	7.96±0.03 ^{Bb}	9.15±0.18 ^{Ba}	0.004	<0.0001	NS
150	7.47±0.35 ^{Ab}	8.63±0.17 ^{Ab}	9.49±0.18 ^{Aa}	7.04±0.14 ^{Bb}	7.88±0.07 ^{Bb}	9.01±0.22 ^{Ba}	0.008	0.0002	NS

* Mean values bearing different superscript (A-B capital) in the same row differ significantly (P<0.05) for ME levels.

* Mean values bearing different (a,b,c small) in the same row within same ME level differ significantly (P<0.05) for CP levels.

Table 6. Plasma IGF-1 (ng/ ml) status of growing male buffaloes fed on varying levels of energy and protein

Day	ME level								
	Low			High			Effect		
	CP level								
	Low	Medium	High	Low	Medium	High	ME	CP	ME*CP
30	162±10.72 ^B	156±9.82 ^B	167±14.07 ^B	171±16.53 ^A	194±3.95 ^A	202±25.68 ^A	0.003	NS	NS
60	167±11.46 ^B	169±16.05 ^B	188±12.17 ^B	190±17.54 ^A	214±32.10 ^A	226±26.53 ^A	0.001	NS	NS
90	164±4.86 ^{Bc}	176±12.83 ^{Bb}	197±11.05 ^{Bb}	187±9.85 ^{Ac}	224±17.95 ^{Ab}	220±20.78 ^{Aa}	0.001	0.03	NS
150	182±15.28 ^{Bb}	186±8.19 ^{Ba}	193±3.41 ^{Ba}	198±5.29 ^{Ab}	226±10.95 ^{Aa}	234±12.04 ^{Aa}	0.001	0.02	NS

* Mean values bearing different superscript (A-B) in the same row differ significantly (P<0.05) for ME levels

* Mean values bearing different superscripts (a,b,c) in the same row within same ME level differ significantly (P<0.05) for CP levels

Table 7. Correlation among physiological and intake parameters in growing Murrah buffaloes

Parameter	CP intake		DCP intake		MP intake		ME intake	
	R	P	R	P	R	P	R	P
Plasma Ig	0.07	NS	0.057	Ns	0.067	Ns	0.078	Ns
Total plasma protein		0.276	<0.01	0.245	<0.01	0.279	<0.01	0.236
Plasma urea	0.413	<0.001	0.447	<0.001	0.476	<0.001	0.204	<0.05
Plasma GH	-0.252	<0.01	-0.165	NS	-0.183	<0.05	-0.426	<0.001
Plasma IGF-1	0.307	<0.001	0.255	<0.01	0.332	<0.001	0.359	<0.001

* Mean values bearing different superscript (A-B) in the same row differ significantly ($P < 0.05$) for ME levels

* Mean values bearing different superscripts (a,b,c) in the same row within same ME level differ significantly ($P < 0.05$) for CP levels

Correlation between biochemical parameters and nutrient intake

Total protein is a reflection of the availability of protein and their concentration decline in the face of protein deficiency. Sahoo et al. (2009) reported lower levels of serum total protein on protein deficient diet, which as a matter of fact, is the condition in the present study as well. There was positive correlation between total plasma protein and CP (+0.276), DCP (+0.245), MP (+0.279) and ME(+0.236) intake. Total plasma protein levels were affected by dietary treatments and restricting the protein intake than optimum requirement resulted in lower plasma protein concentration in beef calves (Chang et al., 2013). Contrary to the present findings, no effect of dietary energy variation on serum total protein was observed by Ivey et al. (2000). In the present study, plasma Ig status was not significantly correlated to energy and protein intakes, that suggest the adequacy of even the lowest dietary energy (2.2Mcal/kg) and protein (12.3%) levels in maintaining desired growth and immunity in buffalo calves. Protein intake in relation to requirement, as well as the interaction between dietary energy and protein is the two main factors determining the plasma urea concentrations. Plasma urea showed high positive correlation with protein (CP, DCP and MP) intake. Qureshi et al. (2002) reported positive correlation of serum urea level with CP intake in dairy buffaloes. Similar relationship has been observed in 12-18 months old growing male swamp

buffaloes (Tatsapong et al., 2010). With increase in protein intake in sheep, rumen ammonia nitrogen and subsequently BUN was increased (Viswanathan and Fontenot, 2009). A couple of other studies also reported high correlation between BUN and dietary protein intake (Paengkoum and Tatsapong, 2009; Chantiratikul et al., 2009). In the present study plasma growth hormone showed negative correlation, whereas plasma IGF-1 showed positive correlation with the energy and protein intake. Serum IGF-I decreased ($P < 0.05$) whereas GH increased ($P < 0.05$) with restricted metabolizable protein in diet of lambs (Wester et al., 1995). Plasma IGF-1 concentration was about two times more in the high energy level group compared to low energy level group for young goats (Shen et al., 2004).

CONCLUSION

Different blood constituents were within normal range, even on the low ME and low CP diet. Plasma urea concentration increased significantly ($P < 0.05$) with increasing protein and energy levels. Total plasma protein and plasma urea and plasma IGF-1 levels were positively correlated to dietary energy and protein concentrations whereas plasma GH was negatively correlated. Plasma immunoglobulin status was unaltered by dietary energy and protein levels. Ration with 10% lower ME (2.2 Mcal/kg) and CP (12.3%) than ICAR (2013) recommendations was sufficient for growing buffalo (200 kg BW) for sustaining the normal physiological and immunity status

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