



Influence of metabolizable energy and protein levels on age and weight at puberty in male buffalo

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ABSTRACT

The objective of the present study was to determine the effects of different levels of metabolizable protein (MP) and metabolizable energy (ME) on nutrients utilization, growth performance, age and weight at puberty of Murrah male buffalo calves. Sixteen growing male buffalo calves weighing around 310 ± 15 kg were divided into four groups of 4 animals each. The animals were offered four different total mixed rations containing different ME and MP levels, viz. 100,100; 115,100; 100,115; 115,115% as per ICAR (2013) standard in groups T₁, T₂, T₃ and T₄, respectively. The animals were fed individually *ad lib.* from around 13 months of age to age at puberty and at the 18 months a metabolic trial was conducted. Dry matter intake in calves fed all the treatment diets remained similar. Feed conversion ratio (FCR), crude protein conversion ratio (CPCR) and metabolizable energy conversion ratio (MECR), live weight gain were also similar among the groups. Nutrient intake, digestibility and N balance were similar in all the groups. Age at puberty was comparatively lower (around 20 months) in T₄ than other groups (20–24 months), while body weight at that time was similar in all the groups. In conclusion, there were no significant improvement in body weight, ADG, nitrogen balance, age and weight at puberty in male buffalo calves with 15% increase in MP and ME in diet.

Keywords: Age at puberty, Buffalo, Metabolizable protein and energy, Performance

Buffalo husbandry is going through a phase of poor growth performance of male calves and delayed puberty, mainly due to inadequate and improper feeding, mechanization in agriculture and awareness of farmers on artificial insemination instead of natural service (Kumar *et al.* 2019). Inadequate weight gain or morbidity in growing male buffalo calves usually leads to poor growth rate and late age at puberty and sexual maturity in buffaloes (Sahoo *et al.* 2004) perhaps due to poor or imbalanced nutrition (Wynn *et al.* 2009). Inadequate or excess supply of either of these primary and major nutrients leads to inefficient utilization of nutrients and thereby compromised growth, puberty and lifetime production. Sufficient scientific literature regarding MP and ME requirements of growing exotic calves is available (NRC 2001), however, MP and ME requirements of exotic cattle established in temperate countries cannot be applied directly to buffalo reared in tropical countries because of differences in their physiology and quality of feedstuffs, though Shahzad *et al.* (2011)

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observed the effect of energy and protein levels in growing Nili Ravi buffalo and compared with NRC (2001) requirements and found less ME is required for growth than standard. Paul and Patil (2007) and Paul *et al.* (2003) determined the requirement in growing and lactating Nili Ravi buffalo, respectively, under tropical condition. Niranjana *et al.* (2017) observed that adding 15% extra ME and/or MP in the growing Murrah buffalo did not influence growth performance of males while Prusty *et al.* (2016) observed improved performance in growing heifers with increasing ME and MP in their ration. There are variation in results and recommendation for feeding the buffalo for attaining puberty or sexual maturity and under field condition providing higher feeds/ fodders make the enterprise less profitable. Keeping in view the limited and scanty scientific information regarding the MP and ME level of male buffalo calves, a study was designed to examine the influence of increasing MP and ME concentrations on nutrient utilization, nitrogen metabolism, growth, blood biochemical profile and age and weight at puberty of growing Murrah male calves.

MATERIALS AND METHODS

Animal management and feeding: Growing male buffalo calves (16) of similar weight (310 ± 15 kg) at Livestock

Research Centre, ICAR-National Dairy Research Institute, Karnal, divided into four groups of four animals each. The animals were offered four different total mixed rations containing different ME and MP levels, viz. 100,100; 115,100; 100, 115; 115, 115% of ICAR (2013) standard in groups T₁, T₂, T₃ and T₄, respectively. The ingredients, chemical composition of experimental diets and composition of TMRs are presented in Table 1. Two concentrate mixture were prepared with similar ingredients with varied quantity. Diets were offered total mixed ration twice daily at 9.30 am and 4.30 pm and feed intake was recorded daily and their representative samples were used for chemical analysis. The calves were weighed initially and fortnightly thereafter for two consecutive days. Calves were de-wormed and vaccinated against foot and mouth disease (FMD), black quarter and haemorrhagic septicaemia (HS) as per farm schedule at the Animal Health Complex, NDRI, Karnal, Haryana, India. The whole experiment lasted for 13 months of age to attaining puberty of animals.

Metabolic trial and chemical analysis: Metabolic trial was carried out to observe nutrient digestion, intake and N balance for a six days collection period. Total mixed ration (TMR) offered to the animals, residue left in the manger next day was collected for DM and other nutrients during the trial. Complete urine and faeces were also collected. At the end of each collection period, dried faecal samples were composited and 10% of the composited samples were analyzed for DM, OM, EE, CP and total ash (AOAC 2005), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (Van Soest *et al.* 1991). The equations of NRC (2001) were used to calculate ME, rumen degraded protein (RDP), rumen undegraded protein (RUP) and equations of AFRC (1993) were used for digestible microbial true protein (DMTP), digestible undegraded

protein (DUP) and MP content of feed. FCR (DMI/ kg gain), CPCRC (CPI, g/kg gain) and MECR (MJ/kg gain) in all four groups were also calculated.

Semen collection, analysis and blood biochemical: Observing the mounting activity at around 17 months of age, the animals were trained and semen was collected through hand massage in the artificial vagina from individual animals. Concentration was around 50 millions/ml and 10% mortality was the criteria for reaching puberty age to that particular animal. Blood was collected in heparinised test tubes once at the end last day of the experiment and plasma separated and preserved in -20°C until analysed for glucose, blood urea nitrogen (BUN), serum aspartate aminotransferase (AST) and alanine amino transferase (ALT).

Data were subjected to ANOVA using the difference within treatment means (error term). Differences among means were tested using Duncan's Multiple Range test at 5% level of significance.

RESULTS AND DISCUSSION

Performance of calves fed different levels of ME and MP: Experimental calves gained more than 55 kg during the experiment with average daily gain more than 510 g (Table 2). Daily DMI (kg/d) in various groups were similar and percent DMI (kg/100 kg BW) were also similar, varied from 2.64 to 2.97 kg in respective groups. Neither of the parameters were different (P>0.05). FCR in T₁, T₂, T₃, and T₄ was similar, though, trend (P=0.19) indicated that increasing energy and protein may improve FCR. CPCRC and MECR was also similar (P>0.05) in all the groups. Singh *et al.* (2009) observed similar results in Bhadwari buffalo; Tauqir *et al.* (2010) and Shahzad *et al.* (2011) reported similar results in *Nili Ravi* buffalo; Niranjan *et al.*

Table 1. Chemical composition of experimental diet (% DM)

Attribute	Concentrate mixture*		Maize green	Wheat straw	
DM	89.15±0.12		17.43±0.17	91.11±0.12	
CP	19.69±0.09		8.35±0.23	2.65±0.23	
EE	4.53±0.14		1.64±0.17	1.35±0.17	
Total ash	9.88±0.02		8.74±0.11	11.13±0.06	
OM	90.12±0.02		91.26±0.11	88.87±0.06	
NDF	28.71±0.11		55.08±0.23	77.14±0.25	
ADF	18.26±0.06		33.54±0.23	55.81±0.28	
Hemicellulose	10.42±0.21		20.54±0.13	21.33±0.24	
ADL	2.67±0.42		4.38±0.26	8.29±0.15	
TDN	72.04±0.08		56.55±0.12	43.79±0.06	
DE (MJ/kg)	13.28±0.15		10.23±0.19	8.07±0.14	
ME (MJ/kg)	10.89±0.08		8.39±0.07	6.62±0.08	
MP	11.88±0.04		4.87±0.02	1.16±0.03	
Composition of TRMs (ME : MP)	T ₁ (100:100)	T ₂ (115:100)		T ₃ (100:115)	T ₄ (115:115)
Roughage: Concentrate	74:26:00	75:25:00		79:21:00	72:28:00
ME (MJ/ kg)	8.32	8.94		8.32	8.96
MP (g/kg)	346	345		398	397

*Concentrate mixture, Maize-28, Bajra-5, Groundnut cake-10, Soybean meal-15, Mustard oilcake-13, Wheat bran-15, Rice polish-10, Mineral mixture-2, Salt-1 (% DM).

Table 2. Performance of male calves fed on different MP and ME levels in feed

Body weight	T ₁	T ₂	T ₃	T ₄
Initial weight, kg	311.80±15.9	310.00±17.0	312.75±11.9	309.00±15.7
ADG, g	0.51±0.04	0.51±0.03	0.52±0.03	0.54±0.03
DMI, kg/d	8.63±1.36	8.82±0.39	8.76±1.37	8.82±1.58
DMI, % BW	2.51±0.10	2.56±0.10	2.51±0.06	2.54±0.12
FCR (DMI kg/kg wt gain)	19.86±0.54	17.37±0.48	17.06±0.62	16.47±0.43
CPCR (CPI(kg)/kg wt gain)	2.57±0.29	2.30±0.32	2.24±0.33	2.21±0.31
MECR (MEI [MJ]/kg)	189.37±21.71	170.36±24.3	163.66±24.3	161.41±24.3
BW at puberty, kg	375.8±26.5	365.7±29.6	370.4±25.1	381.4±23.6
Age at puberty, months	24.88±2.88	23.80±1.98	20.88±1.28	20.15±1.78

(2017) in Murrah buffalo while Prusty *et al.* (2016) observed improved FCR and weight gain in buffalo heifers. DMI and ADG or FCR depends on many factors and in this experiment slight change in the protein and energy level did not influence these parameters. Singh *et al.* (2015) did not find influence of additional CP on growth performance above the requirement in Bhadwari buffalo growing @ around 300–400 g/d while FCR was in accordance with the present study though CPCR and MECR was higher than the present study. Higher protein degradation on additional CP in the diet may lead to N loss as ammonia from the rumen if carbohydrate fermentation not synchronized and in contrary, carbohydrate fermentation exceeds the N degradation, microbial protein synthesis may hamper (Nocek and Russel 1998).

Body weight at puberty among the experimental buffaloes were statistically similar ($P>0.05$). Age at puberty of male calves were comparatively ($P>0.05$) lower in T₄ groups (20 months) than T₁, T₂ and T₃ groups (around 24 months) which indicates that there was benefit of offering more ME and MP to the animals. Consequently, many factors like species, genetic potentiality or breed, plane of nutrition, growth, body weight, role of different hormones, health and other managerial conditions are responsible for the growth, puberty and sexual maturity in animals. Buffalo usually attain puberty when they reach about 60% of their adult body weight (250 to 400 kg), but the age at which they attain puberty can be highly variable, ranging

from 18 to 46 months (Jainudeen and Hafez 2013) which is similar to our findings.

Digestibility and intake of nutrients: Daily feed consumed by buffalo calves during metabolic trial remained similar ($P>0.05$) across all groups (Table 3). MP intake, ME intake was also similar ($P>0.05$) in all the groups but T₄ group showed comparatively higher ME intake. Calves fed diets containing high ME consumed higher than those fed diets containing low ME. The results are in accordance with the findings of Singh *et al.* (2009) in Bhadawari buffalo calves and Niranjana *et al.* (2017) in Murrah buffalo calves fed on rations with different energy and protein concentrations. Shahzad *et al.* (2011) observed linear ($P<0.05$) and quadratic ($P<0.05$) response of digestible NDF intake with respect to energy levels. With increasing the level of both MP and ME level in the diet digestibility of DM, OM and NDF remained similar. There was almost 7% increase in digestibility of CP and 4% increase in digestibility of NDF due to addition of both MP and ME level in the diet. The nutrients digestion and their intake increased as the forage to concentrate ratio decreased in the TMR, though it did not resulted in growth performance. Similar to present findings, higher apparent DM and NDF (Shahzad *et al.* 2011) and OM digestibility (Hussain *et al.* 2001) in buffalo calves was observed on high energy diets compared to low energy diets whereas no effect of protein levels on their digestibility was observed. Whereas, Arieli *et al.* (2004) reported increased CP digestibility when

Table 3. Digestibility of nutrients in experimental groups

Nutrient	T ₁	T ₂	T ₃	T ₄
DM	62.31±3.26	62.02±2.64	61.26±2.49	63.02±3.27
OM	64.18±2.51	63.88±2.87	63.09±3.25	64.91±2.19
CP	58.15±1.81	60.82±2.03	62.46±2.19	62.23±1.67
NDF	50.83±2.19	50.07±1.64	51.82±2.08	52.87±2.41
ADF	39.51±1.65	40.12±1.38	40.34±2.61	39.57±2.16
<i>Nutrient intake</i>				
DM (kg/day)	9.19±1.47	9.55±1.74	9.33±1.95	9.63±2.07
OM (Kg/day)	8.33±1.33	8.66±1.58	8.46±1.77	8.73±1.87
CP (kg/day)	1.11±0.18	1.17±0.22	1.15±0.24	1.19±0.25
MP (g/day)	0.61±0.10	0.67±0.12	0.64±0.13	0.66±0.14
ME (MJ/day)	788.09±33.02	789.65±26.37	803.39±43.62	810.35±54.22

Table 4. Nitrogen dynamics in experimental groups during metabolism trial

Parameter	T ₁	T ₂	T ₃	T ₄
N intake/d (g)	177.96±7.93	187.07±10.39	184.36±10.08	190.07±15.20
N outgo in urine/day (g)	103.65±4.83	110.25±6.35	105.36±7.22	110.54±9.60
N outgo in faeces/day (g)	54.26±4.42	55.39±6.58	51.64±4.51	51.54±5.91
Total N outgo/day (g)	157.91±6.65	165.54±11.05	157.00±10.59	162.08±14.77
N balance (g/d)	20.05±1.29	21.43±1.84	23.51±1.54	24.29±0.83

Table 5. Blood biochemical parameters of buffalo calves fed on different MP and ME levels in feed

Parameter	T ₁	T ₂	T ₃	T ₄
Glucose (mg/dl)	52.74±1.96	53.30±2.84	52.72±1.80	53.09±1.37
BUN (mg/dl)	19.92±0.68	19.79±0.74	19.65±0.74	17.48±0.77
ALT (IU/l)	24.47±0.67	24.54±0.68	25.03±0.68	23.42±0.47
AST (IU/l)	106.67±0.46	107.48±0.40	106.71±0.39	106.59±0.41

dietary CP level was increased from 15% to 17%. Singh *et al.* (2009) observed similar DM, OM and CP digestibility in Bhadawari buffalo calves fed on varying levels of dietary energy, in Murrah heifers (Prusty *et al.* 2016) with altered by dietary CP levels. Mondal and Kakati (2013) observed the difference in effects of dietary protein on nutrient digestibility might attributed to several factors such as like breed, sex, basal diet, balance of supplemental protein and soluble carbohydrate and their quality.

There was similar effect of dietary MP and ME levels on the nitrogen dynamics in various groups (Table 4). Nitrogen balance was similar ($P>0.05$) among all the groups, however the absorbed nitrogen was comparatively increased in T₃ and T₄ groups fed increased level of dietary MP. This might be due to the higher intake of MP and slightly higher digestibility of protein (7%). Higher CP intake, higher than optimum or imbalance between energy and protein intake or rapid protein degradation than microbial synthesis lead to more ammonia production in rumen and the ammonia absorbed into the blood carried to the liver and converted to urea and excreted via urine. This is in accordance with the study of Prusty *et al.* (2016) and Niranjana *et al.* (2017). Singh *et al.* (2015) observed nitrogen retention and urinary nitrogen loss was higher ($P<0.05$) in high protein group where urea was added to increase the CP level while N absorption (% of total N) was decreased with increasing the N level in the diet.

The overall mean values of blood glucose, BUN across the groups were similar ($P>0.05$) (Table 5). Prusty *et al.* (2016) and Niranjana *et al.* (2017) reported blood glucose and ammonia-N did not differ significantly among the treatments varying level of energy and protein which support present findings. Kumar *et al.* (2013) also observed that the dietary protein levels did not have any significant effect on the blood glucose and BUN level in growing Sahiwal calves perhaps due to better synchronization and utilization in rumen and lower gut of the animals. The overall mean values of ALT and AST activity among the experimental groups were similar ($P>0.05$). The results

indicated that the animals given varying in MP and ME content did not influence plasma ALT and AST activity in buffalo calves.

It was concluded that 15% increase in MP and ME levels in the growing buffalo calves did not produce any significant improvement in body weight, ADG, nitrogen balance, age and weight at puberty.

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