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Microbial protein estimation in Murrah buffalo calves fed on diets with varying levels of energy and protein

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ABSTRACT

A study was conducted to predict the effect of energy and protein levels in diet on rumen microbial protein production in Murrah buffalo calves. Microbial protein was estimated from urinary excretion of purine derivatives. Six diets were formulated to provide 90% (12.30% CP), 100% (13.70% CP), and 110% (15.15% CP) protein level, and 90% (2.20 Mcal ME/kg DM) and 110% (2.42 Mcal ME/kg DM) energy level of ICAR 2013 recommendations for buffalo calves. Thirty calves (body weight; 254±7.4 kg) were divided into 6 groups and fed in 2×3 factorial designs. Dry matter intake was recorded by feeding animals for experimental period of 150 days. At the end of feeding trial, urine samples were collected. Collection, preservation, analysis and calculation of urinary purine derivatives were performed by methods described by IAEA (1997). Allantoin constituted the principal PD in the urine. Allantoin and uric acid ranged from 19.93 to 21.37 mmol/day and 2.32 to 3.49 mmol/day, respectively. Total PD varied from 23.01 to 24.28 mmol/day, whereas PD per kg $BW^{0.75}$ was within ranges of 362.77 to 384.23 mmol. The microbial N per kg DOMI or digestible OM retention (DOMR) was significantly higher in lower dietary energy group compared to higher ones. There was no significant effect of the energy and protein levels on allantoin, uric acid, creatinine, total purine derivatives and microbial N production per metabolic body weight (kg) in buffaloes. But microbial N production per digestible organic matter intake increased with decreased energy levels, whereas there was no significant effect of protein levels on it. Thus there was efficient conversion of feed N to microbial protein at CP of 12.3% and ME of 2.20 Mcal/kg DM in 250 kg BW Murrah buffaloes.

Key words: Creatinine, Crude protein, Metabolizable energy, Microbial protein, Murrah buffalo, Purine derivative

Microbial protein synthesis in ruminants is chiefly dependent upon synchronization of available dietary energy and protein. Maximum microbial protein could be synthesized by matching the organic matter and protein degradation rate. The efficiency of microbial protein synthesis varies in animals according to the type of diet. When protein intake is not limiting, its utilization is affected by energy intake. Most amino acids absorbed by ruminants come from microbial protein synthesized in the rumen. Thus efficient feeding strategy aims at optimizing flow of microbial protein to small intestine. Quantification of microbial protein synthesized is useful in determining the factors affecting its production. Purines of dietary origin undergo extensive degradation in rumen. So, the purines leaving the rumen and absorbed at small intestine are of microbial origin. Absorbed purines are degraded and excreted in the urine as their derivatives-hypoxanthine, xanthine, uric acid and allantoin. Excretion rates of purine derivatives (PD) in the urine reflect the duodenal absorption of purine bases (PB) and thus predict the microbial N yield from the rumen.

Several works revealed a direct relationship between level of DM intake and microbial N production (Oldick *et al.* 1999, Dipu *et al.* 2006). But report on effect of energy and protein synchrony in diet on microbial N production is scarce. So, in the present study, microbial protein synthesis from growing Murrah calves was estimated subsequent to feeding varying levels of energy and protein diets.

MATERIALS AND METHODS

Murrah male buffalo calves (30; body weight 254±7.4 kg) were selected from cattle yard herd of ICAR-NDRI, Karnal and divided in to 6 groups of 5 animals each. All the experimental procedures including animals were approved by Institutional Animal Ethics Committee of ICAR-NDRI (IAEC/20/14). Six concentrate mixtures with 2 levels of ME and 3 levels of CP were formulated and mixed with berseem fodder and wheat straw to formulate total mixed rations (Table 1). Feeding of animals was conducted in a 2×3 factorial design. The ME levels were 10% below and above that of ICAR (2013) recommendation for 250 kg BW male calves intended for an average daily

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Table 1. Ingredients and chemical composition of the rations fed to buffalo calves

	TMR1	TMR2	TMR3	TMR4	TMR5	TMR6
Energy	Low			High		
Protein	Low	Medium	High	Low	Medium	High
Berseem	30	21	35	23	25	28
WS	35	44	29	32	30	28
Sorghum red	12.5	14.5	8	22.5	18.5	20
DOMC	16.5	17	23	9	10	14.5
CSC	12.5	-	-	5.5	5.5	5.5
Wheat bran		17	17.5		-	-
Rice bran	7	-	-	11.5	14.5	9
Mineral mixture	1	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition (% DM)						
OM	96.99	96.50	96.52	95.90	95.96	96.09
CP	12.28	13.61	14.98	12.29	13.66	15.05
EE	3.03	1.76	1.70	3.80	3.72	3.30
NDF	51.59	49.68	48.86	43.67	42.72	42.91
ADF	34.56	31.59	31.09	28.88	28.47	28.71
Lignin	7.03	6.66	6.74	5.70	5.91	6.43
NDICP	2.87	2.58	2.91	2.97	3.20	3.14
ADICP	1.44	1.28	1.38	1.48	1.53	1.47
NFC	24.62	26.39	25.92	31.61	31.41	30.47
TDN	58.49	58.05	58.19	63.63	63.74	62.81
ME, Mcal/kg	2.19	2.19	2.21	2.41	2.43	2.41

gain (ADG) of 600 g. Alike the CP levels were equal to and 10% above and below that of ICAR (2013) recommendations. ME levels were 2.20 and 2.42 Mcal/kg DM, and CP levels were 12.30, 13.70 and 15.1% of DM in feed. Quantity of feed offered and residuals left were recorded daily throughout the experimental period of 150 days for dry matter intake (DMI). Samples of feed offered, refusals and faeces were pooled daily and dried for further chemical analysis. Spot urine samples were collected manually during morning and evening time for 5 consecutive days. To prevent microbial degradation of purines, urine was acidified by 10% H_2SO_4 to a pH of 2–3. Urine was diluted by distilled water to prevent the precipitation of purine derivatives during the storage period. A sub sample of 40 ml was taken and stored at –20°c for further analysis. In order to measure the uric acid and allantoin in the urine sample, the stored urine samples were diluted further in a way that the concentrations in the final samples were within the range of the standards used in the assay and readable for the spectrophotometer. Stock solutions of allantoin (Himedia) within 10 to 60 mg/l and uric acid (Himedia) within 5 to 40 mg/l concentration were prepared. The optical density was measured at 522 nm and 293 nm for allantoin and uric acid, respectively. The concentration readings in mg/l were changed into mg/day and then altered to mmol/day by conversion. Then the amounts of uric acid and allantoin in each sample were added to obtain total purine derivatives (mmol/day). The total purine derivatives (PD) excreted in urine (Y, mmol/day) was set in the equation $Y = 0.12X + 0.20\ kgW^{0.75}$ (Liang *et al.* 2004) to estimate the absorbed microbial purine (X, mmol/d). Then absorbed microbial purine was multiplied by 6.25 to find out microbial protein synthesized. Creatinine was estimated by creatinine test kit with modified Jaffe's reaction and the optical density was measured at 505 nm by auto analyzer. PDC index was calculated from the formula $(PD/creatinine)*W^{0.75}$.

Data were analyzed in 2 × 3 factorial arrangements using the general linear model (GLM) procedure of the Statistical Analysis System (SAS 1996) version 9.3. The model contained effects of protein concentration, energy concentration and the interaction between these factors. High significance was declared at $P<0.01$ whereas significance was declared at $P<0.05$ and non-significance was declared at $P>0.05$. The model in this experiment was

$$Y_{ijk} = \mu + P_j + E_k + (PE)_{jk} + \sigma_{ijk}$$

where, Y_{ijk}, response of buffalo to protein level j and energy level k; μ, overall sample mean; P_j, effect of protein level; E_k, effect of energy level; $(PE)_{jk}$, interaction of jth protein level and kth energy level; σ_{ijk}, protein j and energy k error (error term).

RESULTS AND DISCUSSION

Daily nutrient intake: Dry matter and organic matter intake per 100 kg BW were comparable among the groups (Table 2). Increased dietary CP, increased CP intake; whereas no effect of energy levels or interaction between dietary protein and energy levels was observed on its intake. Dietary CP had strong ($P<0.001$) influence on the percentage of CP intake. Similarly, dietary ME significantly affected ($P<0.01$) the gross ME as well as percentage of ME intake. There was significant effect ($P<0.01$) of interaction between protein and energy ($P<0.05$) on ME intake/100kg BW.

Similar to present study, Singh *et al.* (2009) observed no significant effect of ME levels (as per NRC, 2001 and 20% higher or lower to that) on DMI of female Bhadawari buffalo calves. Similar results were also obtained by Basra *et al.* (2003) in Nilli-Ravi buffalo calves. In contrast to present findings, energy concentrations affected DMI in buffalo calves as reported by Nair *et al.* (2004), Puri *et al.* (2004) and Mahmoudzadeh *et al.* (2007). The lower intake in low energy groups might be due to reduced palatability for fibrous nature of diet. Similar results on DMI were also reported by Singh *et al.* (2009) in Bhadawari buffalo calves. Tauqir *et al.* (2011) also observed higher feed consumption by buffalo calves fed on high energy (2.23 Mcal/kg) than those fed low energy (1.86 Mcal/kg) diet. Tatsapong *et al.* (2010) and Mahmoudzadeh *et al.* (2007) observed no significant effect of dietary CP on DM intake of swamp buffalo calves. Similar effect of dietary CP was demonstrated in male crossbred calves (Lohakare *et al.* 2006). Tauqir *et al.* (2011) observed that feed consumption was unaltered in calves fed diet with 11.85% and 14.2% CP. However, intake was reduced on higher CP (16.5%) diet. Shahzad *et al.* (2011) observed significantly ($P< 0.05$)

Table 2. Nutrient intake in buffalo calves fed on different levels of energy and protein

Parameter	Low ME			High ME			Effect		
	Low CP	Medium CP	High CP	Low CP	Medium CP	High CP	E	P	E*P
BW, kg	249±27.49	248±16.71	250±19.00	251±14.29	252±8.50	255±26.65	ns	ns	ns
DM intake, kg	6.15±0.54	6.23±0.41	6.32±0.42	6.40±0.23	6.50±0.14	6.64±0.61	ns	ns	ns
DM/100kg BW	2.51±0.09	2.52±0.03	2.54±0.03	2.56±0.06	2.58±0.04	2.62±0.05	ns	ns	ns
OM intake, kg	5.71±0.50	5.81±0.39	5.88±0.38	5.90±0.22	6.00±0.15	6.16±0.57	ns	ns	ns
OM/100kg BW	2.33±0.09	2.35±0.03	2.36±0.04	2.36±0.06	2.38±0.04	2.42±0.04	ns	ns	ns
CP intake, kg	0.91^{b}±0.06	0.95^{ab}±0.07	1.02^{a}±0.05	0.86^{b}±0.03	1.06^{ab}±0.02	1.09^{a}±0.10	ns	*	ns
CP/100kg BW	0.37^{b}±0.02	0.38^{a}±0.01	0.41^{a}±0.01	0.35^{b}±0.01	0.42^{a}±0.02	0.43^{a}±0.01	ns	***	ns
MEI, Mcal	14.13^{B}±1.53	12.84^{B}±1.10	12.31^{B}±1.04	14.71^{A}±0.93	15.60^{A}±0.48	16.29^{A}±1.77	*	ns	ns
ME/100kg BW	5.79^{B}±0.22	5.24^{B}±0.17	5.01^{B}±0.14	5.87^{A}±0.29	6.14^{A}±0.10	6.32^{A}±0.08	***	ns	**

ns, nonsignificant; *, P<0.05; **, P<0.01, ***, P<0.001. Capital letters in the same row (|A-B), differ at P<0.05 by least squares means for ME levels effect. Lowercase letters in the same row (a-c), within same ME level, differ at P<0.05 by least squares means for CP levels effect.

Table 3. Effect of dietary protein and energy concentration on urinary purine derivatives, creatinine and microbial N production in male buffalo calves

Parameter	Low ME			High ME		
	Low CP	Medium CP	High CP	Low CP	Medium CP	High CP
Allantoin (mmol/d)	21.37±1.80	20.92±0.70	20.85±1.14	20.28±0.66	19.93±1.06	21.25±1.66
Allantoin (µmol/$BW^{0.75}$)	342.88±5.97	336.89±6.51	333.43±9.86	322.52±5.67	314.10±9.09	334.90±9.96
Uric acid (mmol/d)	2.32±0.46	2.97±0.27	3.02±0.64	3.49±0.34	3.08±0.15	3.03±0.54
Uric acid (µmol/$BW^{0.75}$)	35.98±5.17	47.34±2.56	46.77±8.44	54.86±3.27	48.67±1.73	46.09±5.48
Creatinine (mmol/d)	17.88±1.58	20.23±1.13	21.82±1.20	21.69±0.88	18.78±0.55	18.34±1.29
Creatinine (µmol/$BW^{0.75}$)	286.77±3.80	324.77±8.85	349.28±13.66	345.83±15.70	297.73±13.11	289.22±4.34
Total PD (mmol/d)	23.81±2.02	23.89±0.97	23.87±1.62	23.77±0.95	23.01±1.19	24.28±2.19
Total PD (µmol/$BW^{0.75}$)	382.30±7.16	384.23±6.35	380.20±8.93	377.37±3.73	362.77±10.15	381.00±11.24
Microbial N, g	68.73±6.13	69.23±2.56	68.70±5.88	67.63±2.66	62.74±5.37	70.03±7.73
MN/kg DOMI (g)	26.66^{A}±1.17	28.81^{A}±2.36	28.72^{A}±1.23	25.23^{B}±1.21	21.97^{B}±1.52	23.63^{B}±1.62
MN/kg DOMR (g)	29.82^{A}±1.31	32.22^{A}±2.64	32.13^{A}±1.38	28.22^{B}±1.36	24.57^{B}±1.70	26.43^{B}±1.81
PD:creatinine	1.34±0.04	1.19±0.04	1.09±0.04	1.10±0.04	1.23±0.08	1.32±0.03
PDC index (kg)	83.15±7.22	73.83±3.90	68.87±5.75	69.53±5.15	78.44±7.02	84.27±8.16

Capital letters in the same row (A-B), differ at P<0.05 by least squares means for ME levels effect.

higher intake of nutrients (DM, CP and NDF) in buffalo calves on medium CP (12.20%) and low ME (1.73 Mcal/kg) rations. Hussain *et al.* (2001) observed lower fibre intake (P<0.01) for high energy diets because of its low structural carbohydrates content. Increased CP intake with increased dietary CP concentration was observed in Nili ravi buffalo (Basra *et al.* 2003) and male crossbred calves (Lohakare *et al.* 2006).

Purine derivatives and creatinine excretion: The data on urinary purine derivatives (PD) and creatinine excretion in growing male buffaloes is provided in Table 3. There was no significant effect of varying energy and protein levels on allantoin, uric acid, creatinine, total purine derivatives and microbial N production. Allantoin constituted the principal PD in urine. Allantoin and uric acid ranged from 19.93 to 21.37 mmol/day and 2.32 to 3.49 mmol/ day, respectively. Total PD concentration varied from 23.01 to 24.28 mmol/day, whereas PD per kg metabolic BW was within 362.77 to 384.23 mmol ranges. Creatinine excretion was similar among all the groups and ranged from 17.88 to 21.82 mmol/day. When expressed per kg metabolic BW, creatinine excretion ranged from 287 µmol to 349 µmol. Since creatinine excretion was relatively constant, PD:creatinine ratio correlated linearly with daily PD excretion of individual animal. The linear relationship can be applied to all the animals by deriving PDC index. The relationship of PDC index (Y_1) with DOMI (kg/d, X) was described by the linear prediction equation: Y_1= 14.516 + 16.89*X (R^2= 0.58, P<0.001). Similarly, urinary excretion of total purine derivatives (mmol/day, Y_2) is dependent on total DOMI (kg, X), explained by the relationship Y_2= 9.89+3.79*X (R^2= 0.58, P<0.001). The above linear relationships are depicted in graph (Fig. 1). Pimpa *et al.* (2007) observed lower plasma PD excretion rate via the renal route whereas, a significant proportion (22%) was lost via saliva. That might be the reason for lower urinary

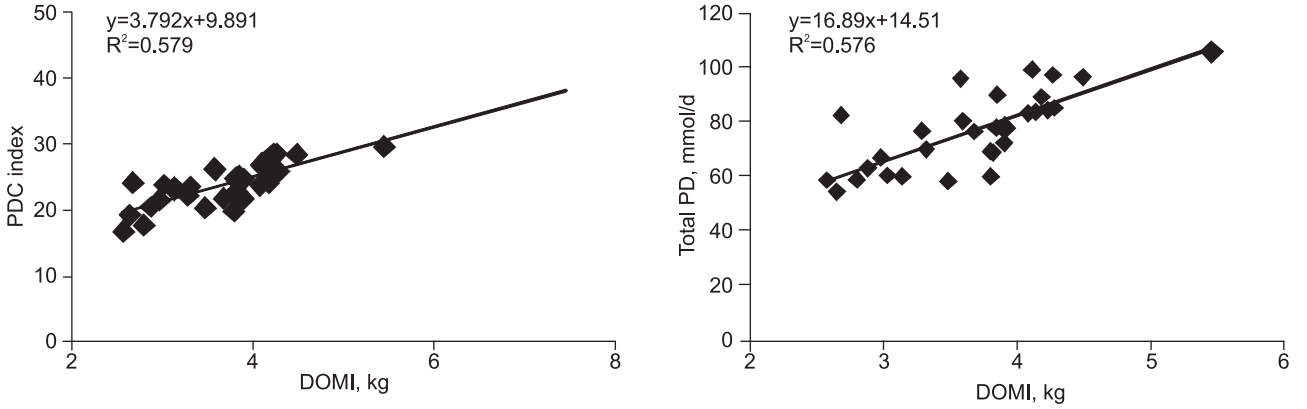

Fig. 1. Relationship of PDC index (kg) and total purine derivatives (mmol/day) with digestible organic matter intake (DOMI, kg/day).

purine derivatives and creatinine excretion from the buffalo calves in the present study compared to crossbred calves (Singh *et al*. 2007). Jetana *et al*. (2009) also reported significantly lower (P<0.01) urinary purine derivatives (PD) and creatinine (Cr) excretion by swamp buffaloes than Brahman cattle. The urinary allantoin excretion by swamp buffaloes was reported to fall between 28.1 to 45.9 mmol/day (Khampa and Wanapat 2006). Total purine derivatives excretion ranged from 14.54 to 24.55 mmol/day from Iranian buffaloes (140 kg BW), on varying levels of concentrate (Khorshidi *et al.* 2012) feeds. The rate of allantoin and total PD excretion were positively correlated with digestible organic matter intake (DOMI) in buffaloes (Dipu *et al.* 2006) and crossbred bulls (George *et al.* 2006). Singh *et al.* (2007) reported no difference (P>0.05) in the proportional contribution of allantoin and uric acid to total urinary purine derivatives (PD) at different levels of feed intake. Similar findings were recorded in crossbred bulls (George *et al.* 2006) and Murrah buffaloes (Dipu *et al.* 2006). Urinary excretion of creatinine was significantly different (P<0.05) in animals fed at different levels as observed by earlier workers (Dipu *et al.* 2006, George *et al.* 2006).

Microbial N production: The microbial N production in the low ME groups were 68.73 (low CP), 69.23 (medium CP) and 68.70 g (high CP), and high ME groups were 67.63 (low CP), 62.74 (medium CP) and 70.03 g (high CP) per day. The microbial N per kg DOMI or digestible OM retention (DOMR) was significantly higher in lower dietary energy group compared to higher energy group. The ratio of purine derivative to creatinine and PDC index was similar at all the levels of dietary energy and protein. The lower microbial N production in high energy group might be due to decreased efficiency of utilization in excess of energy. Thus the lower energy level that is 2.20 Mcal/kg DM was sufficient for efficient nutrient utilization in buffaloes of around 250 kg BW. Oldick *et al.* (1999) observed high positive association of DMI with microbial protein synthesis. The *in vitro* ruminal microbial N synthesis per kg truly digestible OM was reported to be 26.3 - 30.5g (Blummel and Lebzien 2001). Khampa and Wanapat (2006) reported variation in efficiency of microbial protein synthesis with changes in sources of nitrogen and carbohydrates. Disparity in harmonization between available fermentable energy and degradable nitrogen might be the reason for the variation. The variation was wide (4.3 –10.7 g/kg truly digestible organic matter) and was much lower compared to present study, might be due to diverse equation used for microbial purines absorbed (Chen and Gomes 1995). Singh *et al.* (2007) reported similar microbial N per kg DOMI irrespective of level of intake. Alike present study, Kang *et al.* (2012) reported higher efficiency of microbial protein synthesis (28.0 to 32.0 g N/kg DOMI) in higher energy group.

The microbial N production increased with decreased dietary energy concentrations. There was no significant effect of dietary protein concentration on microbial N production. It indicated that protein levels in the present study were well above the limiting concentration. At sufficient levels of N intake, microbial protein synthesis is dependent on energy level. There was efficient conversion of feed N to microbial protein at CP of 12.3% and ME of 2.20 Mcal/kg DM in 250 kg BW Murrah buffaloes.

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