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In Vitro Metabolizable Protein and Utilizable Amino Acid Estimation of Ruminant Feed Ingredients

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ABSTRACT

Present study was conducted to estimate *in vitro* metabolizable protein (IVMP) and utilizable amino acid (uAA) content of ruminant feed ingredients like grains *viz.* maize, red sorghum, pearl millet and barley; agro-industrial byproducts *viz.* wheat bran, rice bran, gram churi and maize gluten meal (MGM); oilseed cakes *viz.* groundnut cake-deoiled (DGNC), mustard cake-deoiled (DOMC) and cotton seed cake (CSC) and fodders *viz.* maize green, sorghum green, pearl millet green, berseem fodder, and wheat straw. The samples were incubated in nine replicates. Three replicates followed filtration of incubation mixture and N estimation in the filtrate and residue and ammonia N estimation in the filtrate. Ammonia N was subtracted from total N in filtrate and residue to obtain utilizable crude protein (uCP). Rest of the incubated replicates were freeze dried to obtain residue with CP concentration equivalent to that of uCP, of which half of samples were subjected to amino acid analysis. From uCP, methionine and lysine availability among grains ranged from 1.21 to 2.01% and 2.28 to 4.52%, respectively. The utilizable methionine (% CP) and lysine (% CP) from agro-industrial byproducts was highest from maize gluten meal (MGM; 1.82) and rice bran (5.27), respectively. Methionine (% CP) availability from groundnut cake-deoiled (DGNC; 0.74) and mustard cake-deoiled (DOMC; 0.89) were lower than that of cottonseed cake (CSC; 2.40) and soybean meal (SBM; 2.85). Rest of the sample residues were subjected to hydrolysis with pepsin and pancreatin for estimation of intestinal digestibility of uCP. Intestinal digestibility was multiplied with uCP to obtain *in vitro* metabolizable protein (IVMP). The regression equation developed by regressing estimated MP (IVMP) from calculated MP derived from protein fractions as per CNCP system was IVMP = 0.915× calculated MP + 1.489 (R^2 = 0.934 at $P<0.0001$, n=39).

Key words: Amino acid, CNCPS protein fractions, Metabolizable protein, Utilizable crude protein

INTRODUCTION

In ruminants, protein is digested by microbes in rumen followed by post ruminal digestion by intestinal and pancreatic enzymes. The absorbed protein at duodenum otherwise, metabolizable protein consists of intestinally digestible microbial protein (DMP) and digestible rumen undegradable protein (DUP). Metabolizable protein (MP) is available to animal for functional purposes after absorption from intestine. Thus, its estimation gives most reliable estimate of protein quality in ruminants. Various MP determination systems (NRC, 2000; 2001, AFRC, 1992) have been proposed. In above systems separate estimation of DMP and DUP was undertaken. To test the usefulness of absorbed protein for specific functions such as growth, lactation, knowledge about amino acid composition of absorbed protein is critical. *In vivo* MP estimation required duodenal canulated animal. Duodenal amino acids flow was estimated in canulated ruminants by Cecava *et al.,* 1988, McCuistion *et al.,* 2004. However, use of duodenal canulated animal is often not feasible due to stringent animal ethic laws, labour and cost on animal experiment. *In vitro* duodenal available amino acid (utilizable amino acid) could be effective alternative to determine utility of protein source to ruminants. An alternative method proposed by Zhao and Lebzien (2000) applying *in vitro* fermentation technique for estimation of utilizable crude protein (uCP) appears more precise. Treatment of uCP residue with pepsin and pancreatin is likely to yield quantity of metabolizable protein more accurately. Considering the paucity of data on IVMP and uCP under Indian context, feedstuffs commonly used in ruminant feeding were quantitatively estimated for *in vitro* metabolizable protein (IVMP) and utilizable amino

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acid composition.

MATERIALS AND METHODS

Dried ground samples of grains *viz.* maize, red sorghum, pearl millet and barley; agro-industrial byproducts *viz.* wheat bran, rice bran, gram churi and maize gluten meal (MGM); oilseed cakes *viz.* groundnut cake-deoiled (DGNC), mustard cake-deoiled (DOMC) and cotton seed cake (CSC) and fodders *viz.* maize green, sorghum green, pearl millet green, berseem fodder, and wheat straw were analyzed for chemical composition. Samples were incubated in nine replicates along with blank. Each group of feed ingredients was incubated in different runs. Each sample in triplicate was processed for utilizable crude protein (uCP) estimation, whereas, remaining six replicates were freeze dried after incubation. From the freeze dried samples, three replicates were analyzed for their amino acid composition to determine the utilizable amino acid (uAA) content in the feed ingredients by using HPLC (AOAC, 2005). Last three replicates were digested further by pepsin and pancreatin (Calsamiglia and Stern,1995) to determine the intestinal digestibility of uCP.

Proximate compositions (AOAC, 2005) and fibre fractions (Van Soest *et al.,* 1991) of the feedstuffs were estimated. NDF was analyzed by using heat stable β-amylase (Number A3306, Sigma Chemical Co.). Acid detergent and neutral detergent insoluble nitrogen (ADIN and NDIN) were estimated as per Licitra *et al.* (1996). Protein fractions *viz.* PA (instantly soluble), PB_1 (instantly degradable), PB_2 (readily degradable), PB_3 (available cell wall protein) and PC (unavailable cell wall protein) were determined as per Cornell net carbohydrate and protein system (Sniffen *et al.,* 1992).

In vitro incubation procedure of Zhao and Lebzien (2000) was adopted to determine uCP content of the feedstuffs. The rumen liquor (RL) from the rumen of fistulated buffalo was collected into thermos flask whose internal temperature was maintained at 39°C before collection. Collection of RL was performed early morning from fistulated animal fed on standard (NDRI) concentrate mixture along with maize green *ad lib* and had free access to water. Collected RL was filtered through 4 layers of surgical gauze and put into thermos and used at earliest for incubation. The following preparations were ready before collection of RL. Buffer I was prepared by dissolving Na_2HPO_4. $12H_2O$ (23.5 g), $NaHCO_3$ (15 g) and NH_4HCO_3 (9.5 g) in 400 ml distilled water. Buffer II was prepared by dissolving NaCl (23.5 g), KCl (28.5 g), $MgCl_2$. $6H_2O$ (6 g) and $CaCl_2$. $2H_2O$ (2.63 g) in 1000 ml distilled water. To 50 ml of buffer II, 400 ml of buffer I was added and the final volume was made to 500 ml. Then, 250 ml of the mixed buffer was diluted with 1000 ml of distilled water and kept at 39°C to maintain the temperature. Feed sample weighing 0.5 gm was taken in 100 ml glass bottle and put in incubator at 39°C before incubation.

For final incubation, 312.5 ml rumen fluid was added to the diluted mixed buffer in a laboratory flask and continuously gassed with CO_2. Then to feed sample, 50 ml of buffered rumen liquor was added and the bottles were sealed with rubber stopper and incubated in water bath maintained at 39°C for 24 h. Blanks without any substrate were incubated along with the samples. After incubation, all the bottles were taken out of the water bath and the pH was measured immediately. Six replicates from each sample and blank were freeze dried. Three replicates of each sample along with blank were filtered through ashless filter paper (no. 42) and washed with distilled water to obtain separate liquid filtrate and solid residue, which was dried in hot air oven.

Utilizable CP sample was freeze dried after 24 h of incubation. Amino acid profile was determined by using High Performance Liquid Chromatography (HPLC) after acid hydrolysis of sample with 6 M HCl at 110°C for 24 hr and further processing as described in AOAC (2005).

From the filtrate 15 ml was taken in glass tube for ammonia N estimation by Kjeldahl distillation method (Melesse *et al.,* 2013). To it, 15 ml of 0.25M phosphate buffer (90 g of $Na_2HPO_4/12.H_2O$/l of distilled water; pH = 11.0 adjusted with NaOH) was added. The pH was adjusted above 10.0 by adding NaOH, since NH_3 release was facilitated at alkaline pH. The liquid sample was then distilled and released ammonia was collected in 3% (w/v) boric acid solution and titrated against 0.05 M hydrochloric acid solution.Nitrogen content of the solid residue, liquid filtrate and blank were estimated by Kjeldahl distillation method. Residue along with filter paper was collected into a Kjeldahl tube for nitrogen determination. For filtrate, 25 ml of liquid were sampled for determination of N.

Calculation: The utilizable crude protein (uCP) was calculated as

$$uCP\ (\%) = \frac{[NH_3\text{-}N_{blank\ filtrate}(g) + N_{residue}(g) + N_{filtrate}(g) - NH_3\text{-}N_{sample\ filtrate}(g)] \times 6.25 \times 100}{\text{Sample weight (g)}}$$

After 24 h of incubation the bottle content was freeze dried which has equivalent CP content as that of uCP. The dried sample containing about 15 mg residual N was digested with pepsin followed by pancreatin (Calsamiglia and Stern, 1995) to obtain intestinal digestibility. Utilizable crude protein was multiplied by intestinal digestibility to obtain *in vitro* metabolizable protein (IVMP)

The attributes were compared by analysis of variance using the one-way analysis procedure of SAS System, version 9.3.

RESULTS AND DISCUSSION

Utilizable crude protein (uCP), intestinal digestibility of uCP and *in vitro* metabolizable crude (IVMP) protein content of feed ingredients (% DM) are presented in table 1.

Among grains, uCP (% DM) and MP (% DM) were similar in maize, sorghum and barley. Intestinal digestibility of uCP from grains was variable from 78.63% (barley) to 93.20% (sorghum). Among

Table 1. Crude protein (CP), fibre bound CP, Utilizable CP (uCP), intestinal digestibility of uCP and metabolizable protein (MP) content of common feed ingredients

Feed	CP (%DM)	NDICP (%DM)	ADICP (%DM)	uCP (% DM)	Intestinal digestibility of uCP	MP (%DM)
Grains						
Maize	$9.75^{ab}\pm0.20$	$1.22^{c}\pm0.12$	$0.56^{c}\pm0.05$	$9.12^{a}\pm1.02$	$86.27^{b}\pm0.54$	$7.88^{a}\pm0.93$
Sorghum red	$10.25^{ab}\pm0.25$	$1.26^{c}\pm0.06$	$0.78^{bc}\pm0.11$	$7.92^{a}\pm0.60$	$93.20^{a}\pm0.21$	$7.38^{a}\pm0.57$
Pearl millet	$10.34^{a}\pm0.18$	$1.66^{bc}\pm0.03$	$1.55^{a}\pm0.01$	$5.11^{b}\pm0.28$	$85.26^{b}\pm0.64$	$6.36^{b}\pm0.27$
Barley	$9.49^{b}\pm0.06$	$2.22^{a}\pm0.11$	$0.46^{c}\pm0.05$	$9.89^{a}\pm0.03$	$78.63^{c}\pm1.04$	$8.43^{a}\pm0.08$
Agro-industrial byproducts						
Wheat bran	$14.75^{b}\pm0.19$	$2.92^{a}\pm0.12$	$1.26^{b}\pm0.06$	$12.41^{b}\pm0.50$	$80.60^{c}\pm0.54$	$10.00^{b}\pm0.42$
Rice bran	$14.72^{b}\pm1.11$	$2.10^{b}\pm0.24$	$1.12^{b}\pm0.11$	$11.25^{b}\pm1.52$	$94.37^{a}\pm0.48$	$10.61^{b}\pm1.40$
Gram churi	$16.24^{b}\pm0.17$	$1.63^{b}\pm0.19$	$1.00^{b}\pm0.15$	$12.51^{b}\pm1.16$	$76.43^{d}\pm0.38$	$9.57^{b}\pm0.93$
Maize gluten meal	$58.39^{a}\pm0.37$	$3.59^{a}\pm0.02$	$2.22^{a}\pm0.10$	$21.33^{a}\pm0.14$	$86.06^{b}\pm1.51$	$18.36^{a}\pm0.20$
Oilseed cake						
DGNC	$41.76^{b}\pm0.40$	$4.21^{a}\pm0.42$	$0.74^{b}\pm0.05$	$33.25^{a}\pm0.59$	$84.85^{a}\pm0.54$	$28.21^{a}\pm0.33$
DOMC	$35.40^{c}\pm0.57$	$1.44^{bc}\pm0.02$	$0.30^{c}\pm0.08$	$29.11^{b}\pm0.80$	$75.75^{b}\pm1.42$	$22.07^{b}\pm1.01$
CSC	$23.90^{d}\pm0.52$	$1.02^{c}\pm0.04$	$0.37^{bc}\pm0.12$	$19.59^{c}\pm0.36$	$75.24^{b}\pm1.18$	$14.74^{c}\pm0.34$
SBM	$44.38^{a}\pm0.60$	$2.14^{b}\pm0.04$	$1.16^{a}\pm0.08$	$30.61^{ab}\pm1.03$	$83.70^{a}\pm0.67$	$25.62^{a}\pm0.86$
Fodder						
Maize	$11.33^{c}\pm0.12$	$6.05^{b}\pm0.19$	$1.72^{c}\pm0.11$	$9.53^{b}\pm0.73$	$75.38^{c}\pm0.38$	$7.18^{c}\pm0.55$
Sorghum	$9.53^{d}\pm0.18$	$3.23^{c}\pm0.07$	$0.82^{e}\pm0.03$	$7.37^{c}\pm0.22$	$81.26^{b}\pm0.96$	$5.99^{c}\pm0.24$
Pearl millet	$9.58^{d}\pm0.13$	$5.97^{b}\pm0.01$	$1.17^{de}\pm0.09$	$7.47^{bc}\pm0.03$	$76.08^{c}\pm0.40$	$5.69^{c}\pm0.03$
Berseem	$16.89^{a}\pm0.06$	$6.92^{a}\pm0.05$	$2.97^{b}\pm0.12$	$13.70^{a}\pm0.62$	$87.70^{a}\pm0.86$	$12.03^{a}\pm0.66$
Wheat straw	$3.31^{e}\pm0.28$	$1.95^{d}\pm0.02$	$1.32^{cd}\pm0.02$	$2.14^{d}\pm0.07$	$39.30^{e}\pm1.40$	$0.84^{d}\pm0.05$

agro-industrial byproducts, uCP ranged from 11.25 (rice bran) to 21.33% (maize gluten meal) and intestinal digestibility of uCP varied from 76.43 (gram churi) to 94.37% (rice bran), resulting IVMP ranged from 9.57 (Gram churi) to 18.36% (maize gluten meal). Among oil cakes uCP and IVMP were lowest in CSC (19.59 and 14.74%) and highest in DGNC (33.25 and 28.21%). Intestinal digestibility of uCP of DGNC (84.85%) and SBM (83.70%) was similar and higher compared to those of DOMC (75.75%) and CSC (75.24%). Among the fodders, highest uCP (16.89%) as well as intestinal digestibility (87.70%) was of berseem, thus resulting highest MP (12.03%) from berseem among fodders. Lowest uCP (2.14%) and MP (0.84%) was of wheat straw.

Essential and non-essential amino acid contents of the feed ingredients were expressed as percentage of CP (Table 2). Among grains, pearl millet had highest histidine (3.91%) and phenylalanine (6.81%), whereas, barley had highest arginine (5.35%) content. Red sorghum had the highest valine (4.90%) and leucine (12.87%) content. All the grains had >7.5% leucine and >4.5% of phenylalanine. Maize had the highest methionine (2.06%), cysteine (2.47%) and isoleucine (4.79%) among the grains, whereas, the lysine content was highest in pearl millet (4.11%). Among agro-industrial byproducts, wheat bran had highest % of histidine (5.82%), valine (4.92%), cysteine (2.12%), isoleucine (2.72%) and phenylalanine (8.83%). Phenylalanine was >5% in agro-industrial byproducts. Methionine ranged from 1.37 to 1.69% among the agro-industrial byproducts. Maize gluten meal was richest source of leucine and was least in lysine content (2.27%). In oil cakes, lysine content ranged from 3.13 to 6.77%, arginine ranged from 5.59 - 6.19% except DGNC (1.48%). CSC was richest source of arginine (7.37%) and cysteine (2.29%) among cakes. GNC was a poor source of methionine (0.72%), cysteine (0.22%) and was richest in histidine (4.98%). SBM was highest in leucine (7.98%) and methionine (2.29%). Among the amino acids (% CP), histidine (7.07%), phenylalanine (6.39%) and cysteine (1.99%) were analyzed to be highest in pearl millet fodder. Among fodders methionine was higher in pearl millet (2.17%) and

Table 2. Amino acid content of the feeds (% CP)

Feed	HIS	ARG	THR	VAL	MET	CYS	ILE	LEU	PHE	LYS	ASP	GLU	SER	GLY	ALA	PRO	TYR
Grains																	
Maize	2.42	4.63	4.24	3.31	2.06	2.47	4.79	11.01	5.44	2.06	2.70	7.33	2.47	2.01	0.21	2.31	1.21
Sorghum red	2.32	3.97	3.68	4.90	1.79	1.91	4.06	12.87	4.88	2.44	6.13	6.48	5.22	4.71	4.90	6.06	3.47
Pearl millet	3.91	4.11	1.51	3.31	1.41	0.81	2.51	4.11	6.81	4.11	4.11	6.81	9.31	9.81	2.71	6.91	2.91
Barley	2.91	5.35	2.90	4.45	1.57	2.30	3.40	7.51	4.68	3.66	6.02	7.21	10.01	6.79	6.57	9.21	6.01
Agroindustrial byproducts																	
Wheat bran	5.82	3.12	1.32	4.92	1.52	2.12	2.72	6.02	8.83	3.93	5.73	6.62	6.82	1.82	1.02	4.32	4.82
Rice bran	3.29	2.19	2.09	2.69	1.43	0.63	2.13	2.89	7.49	3.29	4.69	3.29	4.09	1.59	1.69	13.99	2.69
Gram churi	3.47	2.17	1.97	2.37	1.37	0.59	1.99	3.19	5.19	4.49	4.97	3.77	3.87	1.57	1.97	11.79	2.19
Maize gluten meal	3.49	3.89	0.99	1.99	1.69	0.37	2.27	19.17	8.17	2.27	4.59	3.99	9.13	2.13	4.03	4.02	3.02
Oil cakes																	
DOGNC	4.98	1.48	2.08	1.78	0.72	0.22	1.82	2.02	7.63	3.13	6.23	5.12	5.72	1.62	1.82	7.92	1.82
DOMC	1.99	6.19	4.39	4.29	0.63	1.63	4.23	5.19	5.99	4.68	4.48	12.08	2.58	2.99	0.89	2.79	1.79
CSC	2.57	7.37	2.37	4.17	1.37	2.29	3.79	2.29	7.49	5.29	4.27	10.07	2.37	2.27	0.07	1.69	3.29
SBM	2.49	5.59	2.69	4.19	2.29	1.47	3.47	7.98	4.58	6.77	6.79	10.59	2.73	2.33	1.03	2.32	1.32
Fodder																	
Maize	1.58	4.38	3.08	4.88	1.42	0.72	3.42	6.72	3.93	2.73	7.23	8.32	3.42	4.22	1.32	1.02	1.52
Sorghum red	3.49	2.59	1.39	4.29	2.13	1.13	4.23	4.69	3.79	3.68	9.48	7.38	4.28	4.19	1.29	4.99	1.99
Pearl millet	7.07	8.27	1.77	3.87	2.17	1.99	3.09	6.49	6.39	3.49	5.87	6.27	4.87	2.27	2.47	4.39	3.69
Berseem	1.39	5.59	4.89	4.79	1.49	1.47	4.37	6.88	4.28	5.37	8.49	7.99	2.03	7.03	1.13	3.12	1.82
Wheat straw	1.51	1.06	2.55	2.75	1.05	0.99	1.68	3.40	2.17	3.17	10.19	6.79	1.13	1.33	2.53	3.92	1.23

sorghum (2.13%) whereas highest lysine content was of berseem (5.37%). CP in berseem had highest concentration of threonine (4.89%) and valine (4.79%) among fodders.

The utilizable amino acid (uAA) was expressed as percentage of CP (Table 3). Among grains, the availability of arginine, valine and phenylalanine from uCP were 4.40 to 4.52%, 3.75 to 4.93% and 4.02 to 5.15%, respectively. The methionine and lysine availability from grains ranged from 1.21 to 2.01% and 2.28 to 4.52%, respectively and that of cysteine ranged from 1.11 to 2.33%. The utilizable arginine from agro-industrial byproducts was highest in rice bran (4.41%) followed by maize gluten meal (3.82%), wheat bran (3.67%) and gram churi (2.09%). The methionine and cysteine availability were highest from maize gluten meal (1.82%) and wheat bran (2.69%), respectively. The lysine availability from byproducts varied from 2.63% (maize gluten meal) to 5.27% (rice bran). Among oil cakes DGNC had highest histidine (5.26%) and lowest arginine (1.80%) availability at the duodenum. In other cakes the arginine availability at duodenum was much higher and ranged from 5.52% (SBM) to 6.03% (CSC). Methionine availability from DGNC (0.74%) and DOMC (0.89%) were lower than that of CSC (2.40%) and SBM (2.85%). But S containing amino acid cysteine was highest utilizable from DOMC (2.05%). The lysine and phenylalanine availability from the cakes ranged from 3.70% (DGNC) to 6.42% (SBM) and 4.60% (SBM) to 7.12% (DGNC), respectively. The utilizable histidine was highest from pearl millet (5.46%) followed by sorghum (4.51%) fodder, whereas that of valine and threonine was highest from berseem. Methionine availability at duodenum was highest from sorghum fodder (2.01%) followed by wheat straw (1.79%) whereas that of cysteine was highest from wheat straw (1.72%) and lowest from maize fodder (0.37%). The utilizable lysine from fodders varied from 2.58 to 5.59%.

The uCP of feeds analyzed by Zhao and Lebzien (2002) are in corroboration with present findings. The uCP (%DM) of maize, barley, MGM, rapeseed meal, CSC and SBM estimated from *in vitro* incubation with rumen liquor of cattle was 15.4, 16.4, 21, 23.3 and 28.9 whereas that estimated with rumen liquor of sheep was 14.4, 14.2, 17.2, 25.2 and 24.2, respectively (Zhao and Lebzien, 2002). Higher RDP and RUP was reported in MGM (Prusty *et al.,* 2013a), which might be the possible cause of higher uCP in MGM. The small intestinal digestibility of feed ingredients *viz.* cotton seed meal, rapeseed meal, SBM, maize gluten feed analyzed by *in vivo* methods ranged from 73.7-90.4%, 64.6-76.5%, 96.6-98.4% and 55-72.1% (Moloney *et al.,* 2001), are in corroboration with present findings. Similar MP of maize grain, CSC and wheat bran has been reported by Das *et al.* (2014) using nylon bag technique (AFRC, 1992). Taghizadeh *et al.* (2008) reported lower MP of maize (3.51%) and barley (4.85%) and higher MP of cottonseed meal (23.22%) compared to present findings by using *in situ* degradability method to estimate MP. Calsamiglia and Stern (1995) reported higher (85-90%) post ruminal digestion of SBM and maize gluten meal. The variations might be due to variation in processing methods for oil cakes or difference in rumen microbial population. In agreement to present findings, Promkot and Wanapat (2003) found higher intestinal digestibility of rumen residual CP of SBM compared to CSC. The uCP (%DM) of wheat straw analyzed from *in vitro* incubation with rumen liquor of cattle and sheep was 6.3 and 6.7%, respectively (Zhao and Lebzien, 2002). MP of fodders *viz.* maize, sorghum and berseem analyzed by Das *et al.* (2014) by *in sacco* incubation technique corroborated with present report.

In Cornell net carbohydrate and protein system (CNCPS), CP of feeds is classified according to rate of degradability (Prusty *et al.,* 2013b; Mondal *et al.,* 2008) into different fractions. Findings of the present study were validated from regression of IVMP from calculated MP derived from CNCPS protein fractions (Table 1). Rumen degradable protein (RDP) and rumen undegradable protein (RUP) based on the protein fractions were calculated (NRC, 2000). The MCP availability was obtained by multiplying 0.85 with the RDP. MCP contribution towards metabolizable protein (digestible microbial protein, DMP) was 64 percent

(NRC, 2001). Intestinal digestibility of RUP (NRC, 2001; Das *et al.,* 2014; De Boer *et al.,* 1987) was applied to RUP to obtain digestible undegradad protein (DUP). Thus, calculated MP was estimated by adding up DMP and DUP. Significant relationship between calculated MP and IVMP was established (Fig. 1). The developed regression equation was IVMP = 0.915× calculated MP + 1.489. The correlation coefficient between the calculated and *in vitro* analyzed MP was 0.97 with R^2 value of 0.93 at P< 0.0001 level of significance. Thus IVMP could be effectively estimated by pepsin and pancreatin hydrolysis of *in vitro* incubated sample. In case of unavailability of required set up for conducting *in vitro* incubation procedure, CNCP fractions also could give an idea of the availability of protein from a feed. Significant multiple regression relationship between the uCP and the CNCPS crude protein fractions, i.e. PB1, PB2, PB3 and PC of feeds was observed by Zhao and Cao (2004) which supported current findings.

Unlike present findings, NRC (1984) suggested maize to be a relatively good source of sulfur AA and a

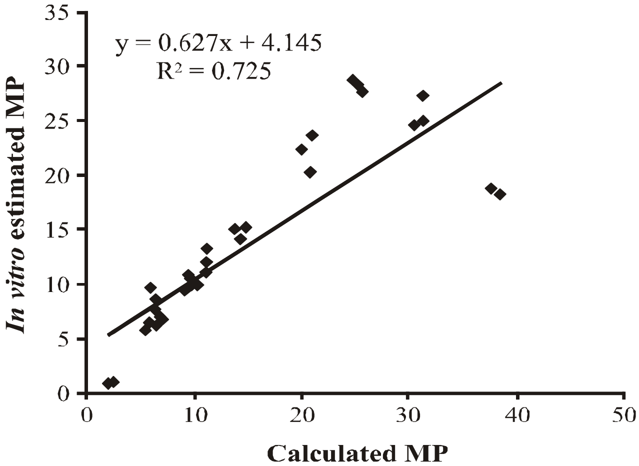

Fig. 1. Regression of in vitro MP on CNCPS protein fraction calculated MP

poor source of lysine. Previous report (Ejeta *et al.,* 1987) of higher lysine% and lower tyrosine% of pearl millet than sorghum support present finding. Lower isoleucine/ leucine ratio in pearl millet than that of sorghum and maize (Ejeta *et al.,* 1987) was in support of present findings. As per McDonald *et al.* (2010) arginine, lysine, methionine, cystine and tryptophan were the main

Table 3. Utilizable amino acid content of the feeds for buffaloes (% CP)

Feed	HIS	ARG	THR	VAL	MET	CYS	ILE	LEU	PHE	LYS	ASP	GLU	SER	GLY	ALA	PRO	TYR
Grains																	
Maize	2.31	4.52	4.43	3.75	2.01	1.78	3.29	10.18	4.82	2.28	3.86	7.13	3.67	1.95	0.44	4.11	2.45
Sorghum red	2.43	4.44	3.41	4.93	1.86	1.34	3.27	9.34	4.02	3.07	6.42	2.49	9.05	4.51	0.86	5.89	6.92
Pearl millet	5.95	4.40	1.98	4.14	1.21	2.33	2.16	3.54	5.15	4.52	4.73	8.69	11.77	12.89	2.50	5.87	3.88
Barley	3.42	4.41	2.60	4.61	1.61	1.11	3.60	7.62	4.14	3.28	6.95	7.62	5.34	4.07	6.01	9.70	6.21
Agro-industrial byproducts																	
Wheat bran	5.99	3.67	1.15	5.67	1.53	2.69	2.70	6.03	8.55	3.40	6.14	8.01	6.48	1.06	2.70	4.73	5.13
Rice bran	5.13	4.41	1.59	2.60	1.74	1.88	1.45	2.24	6.55	5.27	5.37	3.40	3.33	1.81	1.52	12.16	3.47
Gram churi	4.18	2.09	1.23	2.03	1.66	1.18	1.78	2.46	5.60	4.79	4.73	3.32	4.40	1.48	1.29	10.97	2.46
Maize gluten meal	3.83	3.82	1.65	2.60	1.82	0.61	2.70	13.40	7.95	2.63	4.96	4.32	9.22	2.82	4.40	4.51	3.05
Oil cakes																	
DOGNC	5.26	1.80	2.40	1.70	0.74	0.46	1.26	1.74	7.12	3.70	6.50	6.14	5.86	1.44	2.36	7.26	1.76
DOMC	2.20	5.62	4.65	4.69	0.89	2.05	4.00	4.50	6.83	4.59	4.49	10.59	1.47	2.70	1.77	4.09	1.19
CSC	3.30	6.03	2.66	4.19	2.40	1.10	3.73	3.06	6.79	5.19	5.22	9.39	1.90	1.80	0.59	3.40	4.21
SBM	3.60	5.52	1.30	4.99	2.85	1.44	1.32	7.28	4.60	6.42	7.47	9.70	3.09	1.28	1.26	3.27	1.38
Fodder																	
Maize	2.93	4.47	3.55	4.47	1.52	0.37	3.47	6.64	3.31	2.58	9.28	7.00	1.27	4.82	1.28	2.38	1.92
Sorghum	4.51	1.92	0.86	4.30	2.01	1.15	4.15	4.59	3.79	5.59	9.26	7.78	4.73	3.44	1.15	5.94	2.49
Pearl millet	5.46	7.87	1.12	3.46	1.10	1.39	1.86	6.16	7.49	5.20	5.57	6.35	4.45	2.35	2.46	2.21	3.70
Berseem	1.95	5.10	4.98	4.80	1.12	1.20	4.27	6.95	4.45	4.80	8.05	7.32	2.90	7.51	1.07	3.63	1.90
Wheat straw	2.57	1.81	2.32	3.66	1.79	1.72	2.89	4.78	2.70	4.40	9.23	6.48	1.87	2.23	2.22	3.60	2.04

limiting indispensable amino acids in maize and sorghum. As per Merchen and Titgemeyer (1992) maize gluten meal was a poor source of lysine and an excellent source of sulfur AA and leucine which corroborates well with the present findings. Though lower concentration of cysteine was reported in present study, higher methionine content in maize gluten meal resulted in higher concentration of sulphur amino acid in MGM. There are reports indicating high level of valine, isoleucine, leucine, arginine and phenylalanine in groundnut meal-deoiled (Maneemegalai and Prasad, 2011). In the present study, however, content of only phenylalanine was higher in DGNC. Thanaseelaan (2013) observed that rape seed meal (RSM) was a better source of lysine and methionine as compared to GNC (NRC, 1994). In the present study it was observed that DOMC was a better source of lysine than GNC, whereas, similar methionine content was reported in both the cakes.

Much less work has been done regarding the utilizable AA composition of ruminant feedstuffs. Zhao and Lebzien (2002 found a linear relationship between the utilizable crude protein (g/kg) and the uAA (g/kg) estimated from *in vitro* incubations. Similar to present results, intestinal digestible lysine and methionine of 6.7 and 2.7g per kg of SBM were observed in a study by Borucki Castro *et al.* (2007). Sampath *et al.* (2003) reported that UDP fraction of groundnut cake, CSC, maize gluten meal (40%CP), bajra grain and rice polish contained 0.85 and 0.25; 1.65 and 0.34;1.33 and 0.88; 1.48 and 0.11, 0.95 and 0.17% of lysine and methionine, respectively. Less variation was observed in the composition of amino acid in the feed CP and uCP. Findings of Boisen *et al.* (2000) also suggested reduced variation in the concentrations of individual amino acids in the duodenum related to original feed composition. In a study, Merchen and Titgemeyer (1992) found higher proportion of threonine and isoleucine in the duodenal digesta of animals fed on alfalfa fodder. It was suggested that the profile of EAA at duodenal level could be altered by appropriate supply from the supplemental sources. Thus, amino acid composition of protein at duodenum level might depend on variations in the microbial population as well as dietary protein.

CONCLUSIONS

The data generated would be useful in determining the protein quality and requirement of specific amino acid for ruminants. Critical analysis of the variation in amino acid profiles among different classes of feedstuffs *viz.* energy rich (grain), protein rich (oil ckaes), fibrous (fodders), fillers (agro-industrial byproducts, wheat straw) would be useful in precise ration formulation for livestock. The less variation in the amino acid composition of CP and uCP signifies that supplementation of specific amino acid could be modified through diet of animals.

REFERENCES

AFRC, 1992. *Technical Committee on Responses to Nutrients*, Report no. 9. Nutritive requirements of ruminant animals: Protein, Farnham Royal, UK, CABI.

AOAC, 2005. *Official Methods of Analysis*, 18th edn. Association of Official Analytical Chemists, Arlington, Verginia.

Boisen, S., Hvelplund, T. and Weisbjerg, M.R. 2000. Ideal amino acid profiles as a basis for feed protein evaluation. *Livest. Prod. Sci.* 64: 239-251.

Borucki Castro, S.I., Phillip, L.E., Lapierre, H., Jardon, P.W. and Berthiaume, R. 2007. Ruminal degradability and intestinal digestibility of protein and amino acids in treated soybean meal products. *J. Dairy Sci.* 90: 810-822.

Calsamiglia, S. and Stern, M.D. 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. *J. Anim. Sci.* 73: 1459-1465.

Cecava, M.J., Merchen, N.R., Berger, L.L. and Fahey, Jr. G.C. 1988. Effects of dietary energy level and protein source on site of digestion and duodenal nitrogen and amino acid flows in steers. *J. Anim. Sci.* 66: 961-974.

Das, L.K., Kundu, S.S., Kumar, D. and Datt, C. 2014. The evaluation of metabolizable protein content of some indigenous feedstuffs used in ruminant nutrition. *Vet. World.* 7: 257-261.

De Boer, G., Murphy, J.J. and Kennelly, J.J. 1987. Mobile nylon bag for estimating intestinal availability of rumen tutdegradable protein. *J. Dairy Sci.* 70: 977-982.

Ejeta, G., Hassen, M.M. and Mertz, E.T. 1987. *In vitro* digestibility and amino acid composition of pearl millet (*Pennisetum typhoides*) and other cereals. *Proc. Nat. Acad. Sci.* 84: 6016-6019.

Licitra. G., Hernandez, T.M. and Van Soest, P.J. 1996. Standardizations of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.*57: 347-358.

Maneemegalai, S. and Prasad, R. 2011. Evaluation of proximate composition and mineral content of commercially available groundnut and sesame meal. *Agric. Sci. Digest.* 3: 136-139.

McCuistion, K.C., Titgemeyer, E.C., Awawdeh, M.S. and Gnad, D.P. 2004. Histidine utilization by growing steers is not negatively affected by increased supply of either ammonia or amino acids. *J. Anim. Sci.* 82: 759-769.

McDonald, P., Edward R.A., Greenhalgh, J.F.D., Morgan, E.A., Sinclair, L.A. and Wilkinson, R.G. 2010: *Animal Nutrition*, 7th edn. Longman Scientific and Technical UK, pp. 476-530.

Melesse, A., Steingass, H., Boguhn, J. and Rodehutscord, M. 2013. *In vitro* fermentation characteristics and effective utilizable crude protein in leaves and green pods of *Moringa stenopetala* and *Moringa oleifera* cultivated at low and mid-altitudes. *J. Anim. Physiol. Anim. Nutr.* 97: 537-546.

Merchen, N.R. and Titgemeyer, E.C. 1992. Manipulation of amino acid supply to the growing ruminant. *J. Anim. Sci.* 70: 3238-3247.

Moloney, A.P., Woods, V.B. and O'Mara, F.P. 2001. *Determination of the small intestinal digestibility of individual concentrate ingredients using in vitro and in vivo techniques*. In: End of project report: Characterisation of feedstuffs for ruminants: 31-34.

Mondal, G., Walli, T.K. and Patra, A.K. 2008. *In vitro* and *in sacco* ruminal protein degradability of common Indian feed ingredients. *Livest. Res. Rural Dev.* 20 :63.

NRC. 1984. *Nutrient Requirements of Beef Cattle*. 6th edn. National Research Council, National Academy Press, Washington, DC.

NRC. 1994. *Metabolic modifiers: Effects on the nutrient requirements of food-producing animals.* National Research Council, National Academy Press, Washington, DC.

NRC. 2000. *Nutrient Requirements of Beef Cattle.* 7th rev. edn.: Update. National Research Council, National Academy of Sciences. Washington, DC.

NRC. 2001. *Nutrient Requirements of Dairy Cattle:* 7th rev. edn. National Research Council, National Academy of Sciences. Washington, DC.

Promkot, C. and Wanapat, M. 2003. Ruminal degradation and intestinal digestion of crude protein of tropical protein resources using nylon bag technique and three-step *in vitro* procedure in dairy cattle. *Livest. Res. Rural Dev.* 15:2003.

Prusty, S., Kundu, S.S., Kumar, V. and Datt, C. 2013b. Dry matter and neutral detergent fiber degradation kinetics of roughages in relation to carbohydrate and protein fractions. *Indian J. Anim. Nutr.* 30: 374-380.

Prusty, S., Kundu, S.S., Sontakke, U. and Bala, P.A. 2013a. degradation characteristics and energy value of grains, oil seed cakes and agroindustrial by-products. *Indian J. Anim. Nutr.* 30: 381-386.

Sampath, K.T., Chandrasekharaiah, M. and Thulasi, A. 2003. Limiting amino acids in the bypass protein fraction of some commonly used feedstuffs. *Indian J. Anim. Nutr.* 73: 1155-1158.

Sniffen, C., O'Connor, J., van Soest, P. J., Fox, D. and Russell, J. 1992. A Net Carbohydrate and Protein System for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70. 3562-77.

Taghizadeh, A., Safamehr, A., Palangi, V. and Mehmannavaz, Y. 2008. The determination of metabolizable protein of some feedstuffs used in ruminants. *Res. J. Biol. Sci.* 3: 804-806.

Thanaseelaan, V. 2013. Proximate analysis, mineral and amino acid profiles of deoiled rapeseed meal. *Int. J. Food, Agric. Vet. Sci.* 3: 66-69.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Symposium: carbohydrate methodology, metabolism and nutritional implications. In dairy cattle. *J. Dairy Sci*. 74: 3583-3597.

Zhao, G.Y. and Cao, J.E. 2004. Relationship between the *in vitro*-estimated utilizable crude protein and the Cornell Net Carbohydrate and Protein System crude protein fractions in feeds for ruminants. *J. Anim. Physiol. Anim. Nutr.* 88: 301-310.

Zhao, G.Y. and Lebzien, P. 2000. Development of an *in vitro* incubation technique for the estimation of the utilizable crude protein (uCP) in feeds for cattle. *Arch. Anim. Nutr*. 53: 293-302.

Zhao, G.Y. and Lebzien, P. 2002. The estimation of utilizable amino acids of feeds for ruminants using an in vitro incubation technique. *J. Anim. Physiol. Anim. Nutr.* 86: 246-56.

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