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# *In-vitro* methane production potential and *in-sacco* degradability of conventional and non-conventional protein supplements

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ABSTRACT

The study was taken up to assess the *in-vitro* methane and total volatile fatty acids (TVFAs) production; *in sacco* degradability and metabolizable energy (ME) availability from conventional and non conventional protein supplements like mustard cake (MC), deoiled mustard cake (DMC), deoiled groundnut cake (DGNC), soybean meal (SBM), cottonseed cake (CSC), corn gluten meal (CGM), maize oil cake (MOC), tomato pomace (TP) and spent brewers grains (SBG). The CP content varied from 18.2 (TP) to 66% (CGM). TP had the highest EE (11.0%) and lignin (13.5%) content. The net gas production (NGP) varied significantly from 80.7 ml/g DM (CSC) to 236.8 ml/g DM (MOC). The *in-vitro* methane production (expressed as ml/g DM) was the lowest from CSC and CGM, while the highest was observed from SBM and MOC. But when methane was expressed as per cent of NGP or as ml/g digestible OM at $t^{1/2}$, the significantly lowest methane production was observed from TP followed by that from CSC, SBG MC and CGM. The protein supplement with high rumen undegradable protein (RUDP) content produced less methane as compared to the ones that had low UDP content. The TVFA production was the lowest from CSC, which may be due to the lowest degradability of OM and NDF. The digestion kinetic parameters for DM and CP revealed that the rate of degradation was significantly lowest in CGM and highest in DGNC (DM) and TP (CP). The effective degradability (ED) was lowest in CGM and highest was observed in SBM (DM) and DGNC (CP). The rumen UDP as per cent of total protein in CGM was significantly higher than all other protein supplements, it was followed by TP and was lowest in DGNC. It was concluded that the protein supplements with high rumen RUDP value like tomato pomace, cotton seed cake, spent brewer's grains and corn gluten meal had low methane production potential.

**Key words**: Conventional protein supplements, *In-sacco* degradability, *In-vitro* methane production, Non-conventional protein supplements

The enteric methane generated by ruminants is an important greenhouse gas (GHG), which accounts for 89.2% of the total GHG emissions, followed by 9.49% generated from manure (Patra 2012). Methane remains in the atmosphere for only 10 years, but trap 21 times more heat than $CO_2$. Therefore, it is considered as a highly potent GHG and requires immediate interventions to restrict its alarming emissions from livestock. The primary substrates for ruminal methanogenesis are $H_2$ and $CO_2$, produced during fermentation of dietary carbohydrates. Significant quantities of ruminal methane, particularly with high-protein diets, can also arise from microbial fermentation of amino acids (Mills *et al*. 2001). The amount of methane released depends on dietary factors like soluble sugars, dietary lipids, level of feeding, roughage to concentrate ratio, rate of passage of digesta, efficiency of feed conversion, processing and supplementation (Benchaar *et al*. 2001, Bakshi and Wadhwa 2009). Mitigating the enteric methane production in ruminants without altering fibre degradability and animal production is desirable both as a strategy to reduce global greenhouse gases emission and as a means of improving feed conversion efficiency (Dong *et al*. 2010, Martin *et al*. 2010). Both the conventional and non conventional protein supplements used in the formulation of concentrate mixture/complete feed plays key role in the productivity of livestock. Wadhwa *et al*. (2010, 2012) reported that protein supplements like corn gluten meal, highly resistant to microbial fermentation in the rumen can be effectively utilized in the small intestine improving the production performance of the animals (Wadhwa and Bakshi 2009). However, little information is available on the methane production potential of such protein supplements. Therefore, database should be generated regarding rumen undegradable protein (UDP) and methane production potential of protein supplements, so that effective rations could be formulated, which have the profound effect on productivity of ruminants with least

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effect on global warming. The present study was conducted with the objective of evaluating methane production potential of conventional and non-conventional protein supplements.

## MATERIALS AND METHODS

*Procurement of protein supplements*: The samples of conventional oil seed cakes, viz. mustard cake (MC), deoiled mustard cake (DMC), deoiled groundnut cake (DGNC), soybean meal (SBM), cottonseed cake (CSC) and non-conventional protein supplements, viz. corn gluten meal (CGM), maize oil cake (MOC), tomato pomace (TP) and spent brewers grains (SBG) were procured free of cost from local market, starch industry, fruit and vegetable processing industry and brewery.

In-vitro *gas production studies:* The nutritional value of above protein supplements was evaluated by *in vitro* gas production technique (IVGPT; Menke *et al*. 1979, Menke and Steingass 1988). Two sets of samples were incubated, each in triplicate. In the first set about 375 mg of the ground sample (on DM basis) was incubated at 39°C for 24 h in triplicate in 100 ml calibrated glass syringes with buffered rumen fluid for assessing the net gas production, digestibility of nutrients, VFA production and ME availability. Blank and sample of standard hay were run in triplicate with each set. Rumen contents were collected from 3 buffalo calves fitted with permanent rumen fistulae, fed 10 kg green fodder, 4 kg wheat straw and 2 kg concentrate mixture (wheat 25, mustard cake 10, de-oiled mustard cake 20, rice bran 15, de-oiled rice bran 15, wheat bran 12, mineral mixture 2, common salt 1 part each).

In the second set, gas production was recorded at 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72, 84 and 96 h in triplicate. The rate and extent of gas production were calculated by using graph pad prism software to work out $t^{1/2}$. Methane at $t^{1/2}$ was estimated by gas chromatograph equipped with stainless steel column packed with porapak-Q and flame ionization detector. Standard calibration gas consisted of 50% methane and 50% carbon dioxide. The flow rates for nitrogen, hydrogen and zero air were 30, 30, 320 ml/min respectively. From the headspace of each syringe, 100ml gas was collected by puncturing the silicon tube and injected in gas chromatograph for the estimation of methane. Digestible OM was determined at $t^{1/2}$ and accordingly methane production in ml/g digestible OM at $t^{1/2}$ was worked out.

Volatile fatty acids (VFAs) were also estimated by using GLC equipped with a glass column (6 ft length and 1/8 inch diameter) packed with chromosorb 101 (Cottoyn and Boucque 1968). Samples were prepared by adding 0.2 ml of 25% metaphosphoric acid/ml of rumen liquor, allowing it to stand for 2 h followed by centrifugation at 4000 rpm for 7 min. Supernatant was used for estimation of VFA. Standard VFA mixture was prepared by mixing stock solutions (each of 25 mg/ml concentration) of standard acetic acid, propionic acid, butyric acid and distilled water in 1.68, 0.48, 0.24 and 7.24 ml, respectively, to obtain final concentration of 7.0, 1.62, 0.68 mM/100 ml, respectively. The mixture was stored in deep freezer until further use.

In-sacco *degradability:* The *in-sacco* degradability and digestion kinetic parameters were determined (Mehraz and Orskov 1977) by using the above mentioned rumen fistulated buffalo calves. 5g of finely ground (1mm) sample (protein supplement) was placed in the nylon bag (8 cm × 17 cm) stitched with monofilament polyester thread with pore size of 50±10μ. The bags were incubated in the rumen for 2, 4, 6, 8, 10, 12, 24, 36, 48 and 60 h in triplicate. The bags were removed after the stipulated period, washed until rinsing water became colourless; bags were dried in a forced air oven at 60°C for 48 h. The 'zero' h samples were not incubated in the rumen but the bags containing samples as such were washed in the same manner as incubated bags. The disappearance of DM was assessed as the loss in weight of the bag content. The residue was analyzed for DM and CP content. The different physical constants characterizing extent and rate of ruminal degradation, i.e. 'a' rapidly soluble fraction, 'b' insoluble but potentially degradable fraction and 'c' degradation rate constant of 'b' were worked out. The effective degradability (ED) was calculated by using the equation of McDonald (1981). The rumen fill was calculated according to Van Eys (1982).

*Chemical and statistical analysis:* Samples were analysed for proximate (AOAC 2000) and cell wall components (Robertson and Van Soest 1981). Data were analysed by simple ANOVA (Snedecor and Cochran 1994) by using SPSS (2007) Version 16 and the differences in means by using Tukey's b test.

## RESULTS AND DISCUSSION

The chemical composition of protein supplements revealed that organic matter content varied from 86% (spent brewers grains) to 96% (cotton seed cake). The total ash content was the highest in spent brewer grains (SBG) followed by that in maize oil cake and lowest in CSC (Table 1). The CP content varied from 18.2% (TP) to 65.9% (corn gluten meal). The CP content of CGM was in close agreement with the values reported earlier (Wadhwa *et al*. 2010). The TP had the highest amount of ether extract followed by that in CSC and MC. The EE in other protein supplement varied between 1and 4%. The cell wall constitutents' analysis revealed that the NDF and cellulose content of TP and that of SBG was considerably higher than soybean meal and corn gluten meal. The TP had the highest ADF content, while spent brewers grains had the highest hemicellulose content. The lignin content of TP was observed to be the highest amongst the protein supplements evaluated.

The *in vitro* gas production studies revealed that the NGP of various protein supplements varied ($P<0.01$) from 80.7 ml/g DM (CSC) to 236.8 ml/g DM (MOC). The CGM, TP, SBG and DMC were next in a ascending order as compared to CSC as far as fermentation of substrate was concerned (Table 2). The TOMD was highest ($P<0.01$) in CGM and SBM and lowest ($P<0.01$) in CSC and TP. The NDFD

Table 1. Chemical composition of protein supplements, % DM basis

| Parameter | MC | DMC | DGNC | SBM | CSC | CGM | MOC | TP | SBG |
|---|---|---|---|---|---|---|---|---|---|
| Total ash | 6.0 | 5.3 | 8.0 | 7.0 | 4.0 | 5.5 | 9.5 | 4.7 | 14.0 |
| OM | 94.0 | 94.8 | 92.0 | 93.0 | 96.0 | 94.5 | 90.5 | 95.3 | 86.0 |
| CP | 40.4 | 39.2 | 38.3 | 51.0 | 21.5 | 65.9 | 20.0 | 18.2 | 23.56 |
| EE | 7.0 | 1.0 | 1.0 | 4.0 | 9.1 | 2.0 | 4.0 | 11.0 | 3.0 |
| Cellulose | 12.5 | 13.6 | 17.5 | 10.5 | 20.5 | 11.5 | 15.2 | 23.0 | 21.30 |
| NDF | 30.3 | 34.4 | 29.8 | 19.7 | 47.0 | 20.6 | 46.0 | 56.0 | 59.60 |
| ADF | 18.5 | 20.6 | 27.5 | 12.0 | 29.0 | 13.0 | 21.5 | 44.0 | 27.50 |
| HC | 11.8 | 12.9 | 2.3 | 7.7 | 26.4 | 7.6 | 24.5 | 12.0 | 32.10 |
| ADL | 5.0 | 7.0 | 8.5 | 2.0 | 9.4 | 1.5 | 5.5 | 13.5 | 6.2 |

MC, Mustard cake; DMC, deoiled mustard cake; DGNC, deoiled groundnut cake; SBM, soybean meal; CSC, cottonseed cake; CGM, corn gluten meal; MOC, maize oil cake; TP, tomato pomace; SBG, spent brewers grains.

Table 2. *In vitro* digestibility, ME availability and methane production from different protein supplements, %

| Parameter | MC | DMC | DGNC | SBM | CSC | CGM | MOC | TP | SBG | PSE |
|---|---|---|---|---|---|---|---|---|---|---|
| NGP, ml/g DM | $169.58^{d}$ | $158.38^{c}$ | $183.54^{e}$ | $200.32^{f}$ | $80.68^{a}$ | $107.16^{b}$ | $236.76^{g}$ | $153.52^{c}$ | $160.88^{c}$ | 10.63 |
| NDFD,% | $46.87^{b}$ | $51.55^{b}$ | $41.03^{b}$ | $97.64^{c}$ | $21.27^{a}$ | $93.48^{c}$ | $81.71^{c}$ | $35.06^{ab}$ | $56.11^{b}$ | 6.20 |
| TOMD,% | $82.99^{c}$ | $82.64^{c}$ | $81.36^{c}$ | $99.50^{e}$ | $61.76^{a}$ | $98.60^{e}$ | $90.78^{d}$ | $62.40^{a}$ | $70.10^{b}$ | 3.24 |
| $CH_4$, ml/g DM | $30.30^{d}$ | $28.88^{d}$ | $33.30^{e}$ | $44.18^{f}$ | $16.60^{a}$ | $19.52^{b}$ | $43.30^{f}$ | $24.42^{c}$ | $27.90^{d}$ | 2.16 |
| $CH_4$, % of NGP | $17.87^{b}$ | $18.23^{b}$ | $18.24^{b}$ | $22.06^{c}$ | $20.57^{c}$ | $18.22^{b}$ | $18.29^{b}$ | $15.91^{a}$ | $17.34^{ab}$ | 0.42 |
| $t^{1/2}$, hrs | $7.58^{b}$ | $9.12^{d}$ | $7.02^{a}$ | $8.40^{c}$ | $9.23^{d}$ | $23.57^{f}$ | $8.34^{c}$ | $8.92^{d}$ | $11.96^{e}$ | 0.95 |
| $CH_4$, ml/g DOM at $t^{1/2}$ | $8.25^{d}$ | $10.27^{f}$ | $11.69^{g}$ | $10.32^{f}$ | $6.17^{b}$ | $9.70^{e}$ | $12.17^{g}$ | $5.39^{a}$ | $7.20^{c}$ | 0.45 |
| $NH_3$-N, mg/dl | $42.89^{e}$ | $43.94^{e}$ | $52.22^{f}$ | $61.10^{g}$ | $34.32^{d}$ | $30.85^{c}$ | $12.94^{a}$ | $20.46^{b}$ | $20.01^{b}$ | 3.67 |
| PF | $1.80^{a}$ | $1.83^{a}$ | $2.11^{a}$ | $1.35^{a}$ | $2.32^{a}$ | $4.56^{b}$ | $2.04^{a}$ | $2.04^{a}$ | $1.46^{a}$ | 0.22 |
| ME, MJ/kg DM | $10.46^{d}$ | $8.18^{b}$ | $9.32^{c}$ | $11.59^{e}$ | $6.56^{a}$ | $9.24^{c}$ | $10.34^{d}$ | $9.14^{c}$ | $7.89^{b}$ | 0.35 |

NGP, Net gas production; PF, partitioning factor; ME, metabolisable energy; figures with different superscripts in a row differ significantly, $P<0.01$.

digestibility varied significantly ($P<0.01$) from 21.3% (CSC) to 97.6% (SBM). The CGM had highest ($P<0.01$) partitioning factor (PF) compared to all other protein supplements. The PF in all other protein supplement varied from 1.35 (SBM) to 2.32 (CSC) and the differences were statistically nonsignificant ($P>0.05$). The ME availability was highest ($P<0.01$) from SBM, followed by that in MC and MOC, but it was lowest ($P<0.01$) from CSC. The availability of ME from other potential non conventional sources like TP was 39% higher than that available from cotton seed cake. The ammonia nitrogen varied ($P<0.01$) from 12.9 mg/dl (MOC) to 61.1 mg/dl (SBM).

The *in-vitro* methane production (expressed as ml/g DM) was the lowest ($P<0.01$) from CSC and CGM, while the highest ($P<0.01$) was observed from SBM, comparable with that of MOC (Table 2). But when methane was expressed as per cent of NGP or as ml/g digestible OM at $t^{1/2}$, lowest ($P<0.01$) methane production was observed from TP, followed by that from CSC, SBG and MC. The presence of gossypol in CSC might have depressed NGP and methane production as well. The highest ($P<0.01$) methane production was observed from MOC, followed by that from DGNC and SBM. The oiled MC had significantly ($P<0.01$) low methane production from its solvent extracted counterpart i.e. DMC, clearly indicating that the oil in the MC had inhibitory effect on methanogens. The results also clearly indicated that protein supplement with high UDP content produce less methane as compared to the ones had low UDP content. Lee *et al*. (2003) also reported that the methane production was negatively correlated with CP and EE contents of feedstuffs.

The TVFA production was the highest ($P<0.01$) from MOC followed by that from DGNC, with lowest ($P<0.01$) amount of ammonia nitrogen and sufficiently higher fermentation of nutrients (OM and NDF) as indicated by highest NGP (Table 3). On the other hand, the TVFA production was the lowest ($P<0.01$) from CSC, which may be due to the lowest degradability of OM and NDF. The production of acetate from DGNC and that of propionate from MOC was the highest ($P<0.01$) resulting in the lowest ($P<0.01$) A:P ratio in MOC. CSC and SBG followed MOC as far as A:P was concerned. The A:P ratio varied in a very narrow range of 1.97 to 2.07 and the differences were statistically nonsignificant. The relative proportion of VFA revealed that MOC produced the lowest ($P<0.01$) amount of acetate and highest ($P<0.01$) amount of propionate and that of butyrate amongst protein supplements evaluated. All the protein supplements had the capability to produce branched chain VFA's like isovalerate. DMC produced highest ($P<0.01$) amount of valerate, while CGM, MOC and TP did not produce any valerate.

The digestion kinetic parameters of DM revealed that

Table 3. *In vitro* volatile fatty acid production (mM/dl) from protein supplements

| Parameter | MC | DMC | DGNC | SBM | CSC | CGM | MOC | TP | SBG | PSE |
|---|---|---|---|---|---|---|---|---|---|---|
| TVFA | 9.76$^{b}$ | 11.61$^{c}$ | 14.41$^{d}$ | 11.63$^{c}$ | 7.89$^{a}$ | 9.75$^{b}$ | 15.73$^{e}$ | 12.19$^{c}$ | 12.34$^{c}$ | 0.55 |
| Acetate (A) | 5.82$^{b}$ | 6.91$^{c}$ | 8.59$^{e}$ | 7.00$^{c}$ | 4.64$^{a}$ | 5.78$^{b}$ | 8.48$^{e}$ | 7.52$^{d}$ | 7.23$^{cd}$ | 0.30 |
| Propionate (P) | 2.92$^{b}$ | 3.43$^{c}$ | 4.37$^{e}$ | 3.42$^{c}$ | 2.51$^{a}$ | 2.92$^{b}$ | 5.52$^{f}$ | 3.62$^{cd}$ | 3.90$^{d}$ | 0.21 |
| Butyrate | 0.81$^{c}$ | 0.87$^{c}$ | 1.33$^{e}$ | 0.70$^{b}$ | 0.58$^{a}$ | 0.80$^{c}$ | 1.70$^{f}$ | 1.00$^{d}$ | 1.03$^{d}$ | 0.08 |
| A:P ratio | 1.99$^{c}$ | 2.01$^{c}$ | 1.97$^{c}$ | 2.05$^{c}$ | 1.85$^{b}$ | 1.98$^{c}$ | 1.53$^{a}$ | 2.07$^{c}$ | 1.86$^{b}$ | 0.04 |
| *Relative proportion, %* | | | | | | | | | | |
| Acetate | 59.58$^{bc}$ | 59.49$^{bc}$ | 59.63$^{bc}$ | 60.19$^{bc}$ | 58.79$^{b}$ | 59.29$^{bc}$ | 53.86$^{a}$ | 61.65$^{c}$ | 58.57$^{b}$ | 0.50 |
| Propionate | 29.92$^{a}$ | 29.54$^{a}$ | 30.34$^{ab}$ | 29.36$^{a}$ | 31.81$^{c}$ | 29.94$^{a}$ | 35.11$^{d}$ | 29.74$^{a}$ | 31.55$^{bc}$ | 0.42 |
| Butyrate | 8.26$^{cd}$ | 7.47$^{bc}$ | 9.23$^{e}$ | 5.98$^{a}$ | 7.42$^{b}$ | 8.21$^{bcd}$ | 10.77$^{f}$ | 8.20$^{bcd}$ | 8.34$^{d}$ | 0.30 |
| Isovalerate | 0.77$^{c}$ | 1.19$^{d}$ | 1.53$^{e}$ | 2.91$^{f}$ | 1.07$^{d}$ | 1.44$^{e}$ | 0.19$^{a}$ | 0.47$^{b}$ | 0.30$^{ab}$ | 1.10 |
| Valerate | 1.51$^{d}$ | 2.31$^{e}$ | 1.49$^{d}$ | 1.51$^{d}$ | 0.83$^{b}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ | 1.22$^{c}$ | 0.19 |

Figures with different superscripts in a row differ significantly, P<0.01.

Table 4. Digestion kinetic parameters for DM degradability of protein supplements, %

| Parameter | MC | DMC | DGNC | SBM | CSC | CGM | MOC | TP | SBG | PSE |
|---|---|---|---|---|---|---|---|---|---|---|
| 'a' | 45.52$^{f}$ | 29.38$^{bcd}$ | 37.46$^{e}$ | 31.75$^{cde}$ | 34.47$^{de}$ | 26.10$^{bc}$ | 10.36$^{a}$ | 23.97$^{b}$ | 30.50$^{bcd}$ | 1.85 |
| 'b' | 49.95$^{cd}$ | 56.48$^{d}$ | 48.54$^{bc}$ | 67.78$^{e}$ | 34.80$^{a}$ | 51.83$^{cd}$ | 82.77$^{f}$ | 42.17$^{b}$ | 42.37$^{b}$ | 2.74 |
| 'c', /h | 0.046$^{b}$ | 0.088$^{d}$ | 0.142$^{f}$ | 0.057$^{bc}$ | 0.060$^{bc}$ | 0.019$^{a}$ | 0.074$^{cd}$ | 0.116$^{e}$ | 0.065$^{bc}$ | 0.007 |
| UDF | 4.53$^{b}$ | 14.13$^{d}$ | 14.0$^{d}$ | 0.47$^{a}$ | 30.73$^{f}$ | 22.07$^{e}$ | 6.87$^{c}$ | 33.87$^{h}$ | 27.13$^{f}$ | 2.22 |
| ED | 77.76$^{f}$ | 73.33$^{e}$ | 78.71$^{f}$ | 78.84$^{f}$ | 59.02$^{b}$ | 48.31$^{a}$ | 72.18$^{d}$ | 58.67$^{b}$ | 61.60$^{c}$ | 2.04 |
| TD | 64.52$^{b}$ | 77.52$^{e}$ | 84.98$^{f}$ | 69.47$^{c}$ | 70.57$^{cd}$ | 42.85$^{a}$ | 74.68$^{de}$ | 82.27$^{f}$ | 72.20$^{cd}$ | 2.30 |
| PED | 62.93$^{b}$ | 75.61$^{e}$ | 82.88$^{f}$ | 67.75$^{c}$ | 68.83$^{cd}$ | 41.79$^{a}$ | 72.83$^{de}$ | 80.24$^{f}$ | 70.42$^{cd}$ | 2.24 |
| AED | 63.16$^{d}$ | 68.25$^{e}$ | 74.94$^{f}$ | 70.90$^{e}$ | 50.10$^{b}$ | 34.24$^{a}$ | 71.31$^{e}$ | 55.78$^{c}$ | 53.94$^{c}$ | 2.42 |

a, Rapidly soluble fraction; b, potentially degradable fraction; c, degradation rate of b; UDF, undegradable fraction; ED, effective degradability; TD, true degradability; PED, potential extent of digestion; AED, apparent extent of digestion; figures with different superscripts in a row differ significantly; P<0.01.

the rapidly soluble fraction was lowest (P<0.01) in MOC and highest (P<0.01) in mustard cake (Table 4). The insoluble but potentially degradable fraction was highest (P<0.01) in maize oil cake followed by that in soybean meal and lowest in cotton seed cake. The potentially degradable fraction of deoiled mustard cake was degraded at a faster rate (P<0.01) than that of mustard cake. The degradation rate was the lowest (P<0.01) for corn gluten meal (CGM) and highest (P<0.01) for groundnut cake. The spent brewers grains, cotton seed cake, soybean meal and mustard cake had comparative (P>0.05) degradation rate. TP had the highest (P<0.01) undegradable fraction, which may be available for utilization in small intestine. It was followed by CSC and SBG, while SBM had the lowest. The effective degradability which predicts the actual degradation of a fraction based on rate of passage of digesta varied (P<0.01) from 48.3% (corn gluten meal) to 78% (soybean meal). The effective degradability of mustard cake and deoiled groundnut cake was comparable (P>0.05) with that of soybean meal. The tomato pomace, cotton seed cake and spent brewers grain had higher effective degradability (ED) than the corn gluten meal. The true digestibility (TD), and PED and AED of dry matter from corn gluten meal was lowest (P<0.01) and that for deoiled groundnut cake had the highest (P<0.01). The cotton seed cake, tomato pomace and spent brewers grain had higher (P<0.01) apparent extent of digestion as compared to that of corn gluten meal.

The digestion kinetic parameters for CP revealed that MOC had the lowest (P<0.01) and CSC had the highest amount of rapidly soluble fraction (Table 5). The insoluble but potentially degradable fraction was the highest (P<0.01) in MOC and lowest (P<0.01) in CSC. The SBM, TP and SBG had considerably higher (P<0.01) amount of potentially degradable fraction as compared to other protein supplement. CGM had the lowest degradation rate (P<0.01) while highest was observed in TP. The degradation rate of MC, DMC, DGNC and MOC was comparable (P>0.05). The effective degradability was lowest in CGM followed by TP, while mustard cake and deoiled groundnut cake had the highest value. The lower is the ED, higher will be the availability of protein for utilization in small intestine. The rumen UDP was lowest (P<0.01) in CSC and highest (P<0.01) in CGM, which may be available for utilization in small intestine for production purposes. The rumen UDP as per cent of total protein was highest (P<0.01) in CGM followed by that in tomato pomace and it was lowest in DGNC. Solvent extraction improved the level of rumen UDP as deoiled mustard cake had 44% more UDP as per cent of CP in comparison to that of mustard cake. The parameters like TD and PED revealed that CGM no doubt had an edge on other protein supplements. The results pointed towards the potential of protein supplements with

Table 5. Digestion kinetic parameters for CP degradability of protein supplements, %

| Parameter | MC | DMC | DGNC | SBM | CSC | CGM | MOC | TP | SBG | PSE |
|---|---|---|---|---|---|---|---|---|---|---|
| 'a' | $41.08^{f}$ | $33.88^{e}$ | $44.36^{f}$ | $5.03^{b}$ | $60.44^{g}$ | $23.98^{d}$ | $-6.87^{a}$ | $11.07^{c}$ | $19.41^{d}$ | 3.95 |
| 'b' | $57.37^{cd}$ | $58.75^{cd}$ | $53.36^{bc}$ | $83.41^{f}$ | $28.37^{a}$ | $49.46^{b}$ | $99.84^{g}$ | $63.37^{d}$ | $70.19^{e}$ | 1.06 |
| 'c', /h | $0.070^{b}$ | $0.064^{b}$ | $0.069^{b}$ | $0.10^{a}$ | $0.096^{c}$ | $0.010^{c}$ | $0.068^{b}$ | $0.141^{d}$ | $0.099^{c}$ | 0.007 |
| ED | $83.39^{fg}$ | $76.14^{e}$ | $83.88^{g}$ | $71.83^{d}$ | $82.91^{f}$ | $38.21^{a}$ | $66.04^{c}$ | $64.87^{b}$ | $75.50^{e}$ | 2.65 |
| TD | $73.73^{b}$ | $71.90^{b}$ | $73.37^{b}$ | $80.08^{c}$ | $79.21^{c}$ | $28.78^{a}$ | $73.02^{b}$ | $84.87^{d}$ | $79.78^{c}$ | 3.08 |
| PED | $71.91^{b}$ | $70.12^{b}$ | $71.56^{b}$ | $78.10^{c}$ | $77.25^{c}$ | $28.07^{a}$ | $71.21^{b}$ | $82.77^{d}$ | $77.81^{c}$ | 3.00 |
| AED | $74.43^{d}$ | $68.29^{c}$ | $73.89^{d}$ | $72.61^{d}$ | $72.14^{d}$ | $21.67^{a}$ | $69.61^{c}$ | $64.73^{b}$ | $73.38^{d}$ | 3.11 |
| UDP | $6.71^{d}$ | $9.36^{e}$ | $6.17^{c}$ | $14.35^{f}$ | $3.67^{a}$ | $40.71^{g}$ | $6.80^{d}$ | $6.40^{c}$ | $5.77^{b}$ | 2.13 |
| UDP%CP | $16.61^{ab}$ | $23.86^{c}$ | $16.12^{a}$ | $28.17^{d}$ | $17.09^{b}$ | $61.79^{g}$ | $33.96^{e}$ | $35.13^{f}$ | $24.50^{c}$ | 2.65 |

Note: As in Table 4; UDP, rumen undegradable protein; Figures with different superscripts in a row differ significantly, $P<0.01$.

high UDP content like tomato pomace, cotton seed cake, corn gluten meal and spent brewers grains in mitigating enteric methane production without affecting the digestibility parameters.

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