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In vitro Evaluation of Concentrate Mixtures containing Graded Levels of Cottonseed Meal in Buffalo Inoculum

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ABSTRACT

The present study was conducted to assess the chemical composition and *in vitro* nutritional worth of concentrate mixtures containing graded levels of cottonseed meal (CSM) at 0, 3.75, 7.50, 11.25 and 15% replacing SBM @ 0, 25, 50, 75 and 100% on w/w basis replacing soybean meal (SBM) in buffalo inoculum. Crude protein content of concentrate mixtures varied from 20.07% to 20.95%. Ether extract content in concentrate mixtures varied from 5.26% to 5.64%. No significant difference was observed in net gas production, partitioning factor, OM digestibility, NDF digestibility, microbial mass production, efficiency of microbial mass production, short chain fatty acids and metabolizable energy among the concentrate mixtures. The ammoniacal nitrogen (NH_3-N, mg/dl) was higher ($P<0.05$) in concentrate mixture 1 (34.00) and lower ($P<0.05$) in concentrate mixture 5 (27.00) than other concentrates evaluated. The total volatile fatty acid production, hydrogen recovery and hydrogen consumed via CH_4 was similar among the concentrate mixtures evaluated. No significant difference was observed in VFA utilization index among the concentrate mixtures. It was concluded that soybean meal could be replaced by CSM up to 100% in the diet of ruminants without affecting the nutrient digestibility and microbial mass production.

HIGHLIGHTS

- No adverse effect was observed on the nutrient digestibility and microbial mass production in the concentrate mixtures containing graded levels of CSM replacing SBM.
- Ammonia-N showed a declining trend with increasing level of CSM in concentrate mixtures.

Keywords: Cottonseed meal, *in vitro* evaluation, ammoniacal nitrogen, volatile fatty acids

India has the largest livestock population in world. In 20th livestock census, total livestock population in the country is 535.78 million showing an increase of 4.6% over preceding Livestock Census, 2012. Among them are 192.49 million cattle, 109.85 million buffaloes, 74.26 million sheep, 148.88 million goats and 9.06 million pigs. Improving the productivity of farm animals is one of the major challenges as livestock improves food and nutritional security by providing nutrient rich food products, generate income as well as employment and contribute to foreign exchange through exports. To reach the potential productivity, animal nutrition is the main area that needs to be focused on.

India ranks first in the population of buffaloes. According to 20th livestock census (2019), total buffalo population in the country is 109.85 million showing an increase of 1.0% over previous livestock census, 2012. They are also dual-purpose animals, showing good meat characteristics, though their potential for meat still remain unexplored and unexploited. Buffaloes serve as an insurance against the risk of crop failure due to natural calamities so they are considered as a financial asset (Dhanda, 2004).

Cotton is one of the most important fiber and cash crop

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of India and plays a dominant role in the industrial and agricultural economy of the country. In 2018, cottonseed production in India was 9.8 million metric tonnes (MMT) which ranked second after China (10.1 MMT) (FAO, 2018). Cottonseed meal (CSM) is a by-product of the oil industry, i.e., after oil extraction from cotton seeds. CSM is a relatively rich sources of protein (30 to 50%) and amino acids (He *et al*., 2015). Cottonseed meal is a plant protein source, abundant in most parts of the world and is relatively high in protein and generally less expensive per unit of protein than soybean meal. The protein content of cottonseed meal varies from about 22% in meal made from undecorticated cottonseed to 42% in meal made from decorticated seed. The decorticated cottonseed meal contains 41% CP and 78% TDN. Due to increased price of soybean meal, dairy farmers are seeking suitable and viable alternate protein supplement to soybean meal. Therefore, keeping in view the above points, a comprehensive study was taken up to evaluate an alternate feed stuff CSM in the diet of buffaloes by *in vitro* gas production technique.

MATERIALS AND METHODS

Chemical analysis

The concentrate mixtures with graded levels of cottonseed meal (CSM) at 0, 3.75, 7.50, 11.25 and 15% replacing SBM @ 0, 25, 50, 75 and 100% on w/w basis were prepared (Table 1) and analysed for proximate (AOAC, 2005) and cell wall constituents (Van Soest *et al*., 1991).

In vitro evaluation

Male buffaloes fitted with rumen fistulae maintained on 2 kg conventional concentrate mixture (maize-38, mustard cake-15, SBM- 15, deoiled rice bran-12, wheat bran-10, rice polish-7, mineral mixture-2, common salt-1part), 17 kg green fodder, 3 kg wheat straw and *ad lib* urea molasses mineral block were used as a donor for rumen liquor. The rumen contents were collected and then strained through 4 layered muslin cloth. The strained rumen liquor (SRL) was added to the buffer media (containing macro, micro mineral solutions, resazurin and a bicarbonate buffer solution prepared as per (Menke *et al*., 1979; Menke and Steingass, 1988) in 1:2 ratio. The medium was kept at 39ºC in a water bath and flushed with CO_2. Thirty ml of buffered rumen fluid was dispensed into 100 ml calibrated glass syringes containing 375 mg test feed under the anaerobic conditions. Syringes were sealed with rubber tube and plastic clip and placed in a water bath at 39ºC for 24 h. Blank was also run in triplicate with each set which only contained buffered rumen liquor. After 24 h, the volume produced in each syringe was recorded and the contents of syringes were transferred to spoutless beaker, boiled with neutral detergent solution for estimating the OM and NDF digestibility (Van Soest and Robertson, 1988). The amount of gas produced was used to calculate ME. The partitioning factor (PF) was calculated as per the method described by France *et al*. (1993).

Estimation of volatile fatty acids

Volatile fatty acids were estimated using Netchrom 9100 gas chromatograph (Netel, New Delhi, India) equipped with flame ionization detector as per method described by Cottyn and Boucque (1968). The gas column (6 ft length and 1/8 inch diameter) packed with chromosorb 101 was used for the estimation of VFA. The gas flows for nitrogen, hydrogen and zero air were 15, 30, and 300 ml/min, respectively. Temperature of injector oven, column and detector were 250°C, 175°C and 270ºC respectively. Samples were prepared by adding 0.2 ml of 25% metaphosphoric acid per ml of rumen liquor/ contents of *in vitro* syringes, 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 VFAs and distilled water in the proportion of acetic acid 1.68 ml, propionic acid 0.48 ml, isobutyric acid 0.12 ml, butyric acid 0.24 ml, isovelaric acid 0.12 ml, valeric acid 0.12 ml and make the volume to 10 ml to obtain final concentration of acetic acid, 7.0, propionic acid, 1.62; isobutyric acid, 0.34; butyric acid 0.68; isovaleric acid 0.29 and valeric acid 0.29 mM/100 ml. The standard was stored in deep freeze until further use.

Determination of ME availability

The ME value of the substrate was calculated by using the following equation developed by Menke *et al*. (1979).

$$ME\ (kg) = 1.24 + 0.146\ G\ (ml/200\ mg\ DM) + 0.007\ CP + 0.0244\ EE$$

where,

ME = Metabolizable energy, MJ/kg DM

G =Net gas production, ml/200 mg DM

CP = Crude protein, g/ kg

EE = Ether extract, g/kg.

Determination of hydrogen recovery

Hydrogen recovery (%) was estimated as (4M+2P+2B) / (2A+P+4B) × 100, the ratio of hydrogen consumed via CH_4/VFA was estimated as 4M/(2P+2B), where acetate (A), propionate (P), butyrate (B) and methane (M) production was expressed in mmol by Demeyer (1991).

Determination of fermentation efficiency

This was calculated on the basis of the equation worked out by Orskov (1975) and modified by Baran and Zitnan (2002)

$$FE = (0.622a + 1.092p + 1.56b)\ 100/\ (a + p + 2b)$$

Where: *a*, *p*, and *b* express the concentration (µmol) of acetic, propionic and butyric acids respectively in the total concentration of VFA produced. The final results of this equation have been expressed in percentage and show an amount of energy stored in VFAs as a percentage participation of the initial energy.

Determination of VFAs utilization index

This was expressed by non-glucogenic VFAs/glucogenic VFAs ratio (NGGR) according to Orskov (1975).

$$NGGR = (A + 2B + V)\ /\ (P+V)$$

Where A, P, B and V express the concentrations (µmol) of acetic, propionic, butyric, and valeric acids, respectively. Valeric acid is classified as both glucogenic and non-glucogenic VFA because its oxidation creates 1 mole of acetic acid and 1 mole of the propionic acid. Too high NGGR indicates high loss of energy in the form of gases.

Statistical analysis

Data were analysed by simple ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2012) version 21. The differences in means were tested by Tukey's b.

RESULTS AND DISCUSSION

Chemical composition of concentrate mixtures containing graded levels of CSM

The chemical composition of various concentrates with graded level of cotton seed meal is given in Table 2. The OM was 91.66% in concentrate 1, 91.32% in concentrate 2, 91.53% in concentrate 3, 92% in concentrate 4 and 91.98% in concentrate 5. All the concentrates mixtures were iso-nitrogenous as the CP content of concentrate mixtures varied from 20.07% to 20.95% (Table 2). The ether extract content in concentrate mixtures varied from 5.26% to 5.64%. The total ash content was 8.33% in concentrate 1, 8.67% in concentrate 2, 8.46% in concentrate 3, 7.92% in concentrate 4 and 8.06% in concentrate 5.

The NDF content in concentrate mixtures varied from 30.46% to 33.40%, ADF content varied from 13.36% to 15.23% (Table 2). The hemicellulose content varied from 16.24% to 19.04% in the concentrate mixture evaluated. The ADL content varied from 4.36% to 4.80%. The ADICP of the concentrate mixtures ranged from 7.22% to 7.98%. The NDICP in concentrate mixtures varied from 9.12% to 9.88%. The total carbohydrate content in concentrates ranged between 65.09% to 66.24%, indicating that there was almost similar carbohydrate content in the concentrate mixtures.

In vitro evaluation of concentrate mixtures *containing* graded levels of CSM

The net gas production (NGP, ml/g DM/24h) was 196.19, 194.50, 196.94, 195.27 and 192.97 in concentrate mixtures 1, 2, 3, 4 and 5, respectively (Table 3). No significant difference was observed in NGP among the concentrate mixtures. The results are in agreement with those of Abdalla *et al*. (2012) who reported no significant difference in gas production on replacing cottonseed meal with soybean meal. The PF was 3.80, 3.78, 3.75,

Table 1: Ingredient composition of concentrate mixtures (parts/100 parts)

Ingredient	CONC 1 (0% CSM)	CONC 2 (25% CSM)	CONC 3 (50% CSM)	CONC 4 (75% CSM)	CONC 5 (100% CSM)
Maize	34	34	34	34	34
Soybean meal	15	11.25	7.5	3.75	0
Cottonseed meal	0	3.75	7.5	11.25	15
Mustard cake	15	15	15	15	15
Wheat bran	10	15	15	15	15
Deoiled rice bran	17	17	17	17	17
Rice polish	6	6	6	6	6
Mineral mixture	2	2	2	2	2
Salt	1	1	1	1	1

Table 2: Chemical composition of concentrate mixtures containing graded levels of CSM, % DM basis

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5
OM	91.66	91.32	91.53	92.00	91.98
CP	20.07	20.31	20.95	20.52	20.86
EE	5.36	5.26	5.50	5.36	5.64
Total ash	8.33	8.67	8.46	7.92	8.06
NDF	31.40	32.33	33.40	30.46	30.73
ADF	15.16	15.23	14.36	13.93	13.36
Hemicellulose	16.24	17.10	19.04	16.53	17.37
ADL	4.36	4.50	4.73	4.80	4.63
ADICP	7.79	7.22	7.79	7.98	7.41
NDICP	9.31	9.12	9.69	9.70	9.88
TCHO	66.24	65.76	65.09	66.20	65.44

OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, ADL- Acid detergent lignin, TCHO- Total carbohydrates, ADICP- Acid detergent insoluble crude protein, NDICP- Neutral detergent insoluble crude protein.

Table 3: *In vitro* utilization of nutrients of concentrate mixtures containing graded levels of CSM (24 h)

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
NGP, ml/g DM/24h	196.19	194.50	196.94	195.27	192.97	1.07
PF, mg/ml	3.80	3.78	3.75	3.83	3.86	0.04
OMD, %	79.85	80.07	80.68	81.64	81.22	0.33
NDFD, %	41.17	43.69	47.05	44.49	43.80	0.94
MMP, mg	110.60	109.11	109.71	115.98	114.89	1.85
EMMP, %	40.16	39.95	39.51	40.76	41.19	0.56
DMD, %	81.79	82.06	82.32	83.09	82.73	0.28
SCFA, mmole	0.87	0.86	0.87	0.86	0.85	0.00
ME, MJ/kg DM	9.41	9.33	9.53	9.45	9.46	0.04
NH_3-N, mg/dl	34.00[b]	31.00[ab]	30.50[ab]	30.00[ab]	27.00[a]	0.79
Fer CO_2, mmoles	46.86	48.17	48.07	47.70	47.41	0.36
Fer CH_4 mmoles	24.30	25.11	25.27	26.32	25.98	0.36

NGP- Net gas production, PF- partitioning factor, D- digestibility, OM- organic matter, NDF- neutral detergent fibre, MMP- microbial mass production, EMMP- efficiency of microbial mass production, DM- dry matter, SCFA- short chain fatty acids, NH_3-N-ammoniacal nitrogen, Fer CO_2- fermentable carbon dioxide, Fer CH_4- fermentable methane, Means bearing different superscripts in a row differ significantly ($P<0.05$).

3.83, 3.86 mg/ml in concentrate mixtures 1, 2, 3, 4 and 5, respectively and varied non-significantly. The partitioning factor (PF) is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher partitioning factor (PF) means proportionally more of degraded matter is incorporated into microbial mass i.e., the efficiency of microbial protein synthesis is higher. The PF of ruminant diets should be in the range of 2.71-4.4 (Blummel *et al.,* 1997). The PF in the present study was within the suggested range. The OM digestibility (%) in concentrate mixture 1, 2, 3, 4 and 5 was 79.85, 80.07, 80.68, 81.64 and 81.22, respectively. There was no significant difference in OM digestibility among the concentrate mixtures evaluated. Abdalla *et al*. (2012) also reported no significant difference in organic matter true digestibility (OMTD) on replacing cottonseed meal with soybean meal. The NDF digestibility varied non-significantly among the concentrate mixtures. Microbial mass production (MMP) in concentrate mixtures 1, 2, 3, 4 and 5 was 110.60, 109.11, 109.71, 115.98, 114.89 mg and efficiency of microbial mass production (EMMP) was 40.16, 39.95, 39.51, 40.76, 41.19% in concentrate mixtures 1, 2, 3, 4 and 5, respectively (Table 3). The MMP and EMMP results varied non significantly among the concentrate mixtures. The DM digestibility (%) was similar in the concentrate mixtures showing no significant difference. Nasser (2009) also reported to significant difference in true degradability of dry matter (TDMD)

Table 4: *In vitro* volatile fatty acids production (mM/dl) in concentrate mixtures containing graded levels of CSM (24 h)

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
Acetate	2.70	2.64	2.73	2.83	2.81	0.031
Propionate	1.51	1.44	1.47	1.40	1.42	0.022
Isobutyrate	0.06	0.06	0.05	0.06	0.05	0.003
Butyrate	0.27	0.31	0.30	0.28	0.26	0.014
Isovalerate	0.00	0.00	0.00	0.00	0.00	0.000
Valerate	0.00	0.00	0.00	0.00	0.00	0.000
TVFA	4.54	4.45	4.55	4.56	4.55	0.036
A:P	1.79	1.84	1.86	2.02	1.98	0.040
Relative proportion, %						
Acetate	59.38	59.40	60.03	62.00	61.80	0.436
Propionate	33.33	32.24	32.29	30.74	31.27	0.467
Isobutyrate	1.40	1.42	1.03	1.25	1.14	0.069
Butyrate	5.89	6.94	6.65	6.01	5.79	0.284
Isovalerate	0.00	0.00	0.00	0.00	0.00	0.000
Valerate	0.00	0.00	0.00	0.00	0.00	0.000

TVFA- Total volatile fatty acids, A:P- acetate: propionate, Means bearing different superscripts in a row differ significantly (P<0.05).

Table 5: Hydrogen balance of nutrients of concentrate mixtures containing graded levels of CSM (24 h)

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
H- recovery, %	99.05	100.16	99.01	98.88	98.89	0.45
H- consumed via CH_4	3.81	3.83	3.92	3.84	3.82	0.05
FE, %	78.98	78.64	78.56	77.80	78.00	0.20
VFA UI	2.14	2.28	2.27	2.41	2.36	0.05

FE- fermentation efficiency, H- Hydrogen, VFA UI- volatile fatty acids utilization index, Means bearing different superscripts in a row differ significantly (P<0.05).

by which jojoba meal was added to a concentrate diet at 6%, 9% and 18% levels, substituting for cottonseed meal. The short chain fatty acids (SCFA, mmole) production was similar among all the concentrate mixtures and varied from 0.85 to 0.87 (Table 3). The metabolizable energy (ME) was 9.41, 9.33, 9.53, 9.45 and 9.46 MJ/kg DM in concentrate mixtures 1 to 5, respectively. There was no significant difference in ME value among the concentrate mixtures.

The ammonia nitrogen (NH_3-N, mg/dl) was higher ($P<0.05$) in concentrate mixture 1 (34.00) and lower ($P<0.05$) in concentrate mixture 5 (27.00) (Table 3). Ammonia- N showed a declining trend with increasing level of CSM in concentrate mixtures. Concentrate mixtures 2 (31.00 mg/dl), 3 (30.50 mg/dl) and 4 (30.00 mg/dl) had similar NH_3-N concentration. However, Nasser (2009) reported no significant difference in NH_3-N on adding jojoba meal to a concentrate diet at 6%, 9% and 18% levels, substituting for cottonseed meal. The fermentable carbon dioxide (Fer CO_2, mmol) level in the concentrate mixtures containing graded levels was similar to that of control concentrate (conc 1). Concentrate mixture 1, 2, 3, 4 and 5 produced 46.86, 48.17, 48.07, 47.70 and 47.41 mmole fermentable CO_2, respectively. The fermentable methane (Fer CH_4, mmol) in concentrate mixtures varied non significantly and concentrate 1, 2, 3, 4 and 5 produced 24.30, 25.11, 25.27, 26.32 and 25.98 mmol fermentable CH_4, respectively.

The acetic acid content (mM/dl) in concentrate mixture 1 (2.70), concentrate mixture 2 (2.64), concentrate mixture 3 (2.73), concentrate mixture 4 (2.83) and concentrate mixture 5 (2.81) was similar (Table 4). The propionic acid content (mM/dl) in concentrate mixture varied non significantly. There was non-significant difference in isobutyric acid content among the concentrate mixtures. Concentrate mixture 1, 2, 3, 4 and 5 had 0.06, 0.06, 0.05, 0.06, 0.05 mM/dl isobutyric acid, respectively. The butyric acid concentration (mM/dl) in concentrate mixture 1 (0.27), concentrate mixture 2 (0.31), concentrate mixture 3 (0.30), concentrate mixture 4 (0.28) and concentrate mixture 5 (0.26) also varied non significantly.

The total volatile fatty acid production (TVFA, mM/dl) was similar ($P>0.05$) in concentrate mixture 1 (4.54), concentrate mixture 2 (4.45), concentrate mixture 3 (4.55), concentrate mixture 4 (4.56) and concentrate mixture 5 (4.55) and differed non significantly. However, Nasser (2009) reported significant increase ($P<0.05$) in the *in vitro* TVFA concentration when jojoba meal was added at L2 (9%) and L3 (18%) levels substituting cottonseed meal. The acetate: propionate (A: P) was similar among the concentrate mixtures evaluated. Concentrate mixture 1, 2, 3, 4 and 5 had 1.79, 1.84, 1.86, 2.02 and 1.98 mM/dl A:P ratio, respectively. Nasser (2009) also reported similar results where no significant difference was observed in A:P ratio in concentrate mixture having graded levels of jojoba meal replacing cottonseed meal.

The relative proportion (%) of acetic acid in concentrate mixture 1 (59.38), concentrate mixture 2 (59.40), concentrate mixture 3 (60.03), concentrate mixture 4 (62.00) and concentrate mixture 5 (61.80) was similar (Table 4). The relative proportion (%) of propionic acid was 33.33, 32.24, 32.29, 30.74 and 31.27 in concentrate mixture 1, 2, 3, 4 and 5, respectively. The relative proportion (%) of isobutyric acid in concentrate mixture 1, 2, 3, 4 and 5 was 1.40, 1.42, 1.03, 1.25 and 1.14, respectively. The relative proportion of butyrate in concentrate mixture 1 (5.89%), concentrate mixture 2 (6.94%), concentrate mixture 3 (6.65%), concentrate mixture 4 (6.01%) and concentrate mixture 5 (5.79%) was similar among the concentrate mixtures evaluated.

The hydrogen recovery (%) was 99.05, 100.16, 99.01, 98.88, 98.89 in concentrate mixture 1, 2, 3, 4 and 5, respectively (Table 5). The hydrogen consumed via CH_4 was similar among concentrates. Concentrate mixture 1, 2, 3, 4 and 5 had 3.81, 3.83, 3.92, 3.84 and 3.82 hydrogen consumed via CH_4, respectively. The fermentation efficiency (%) in concentrate 1 (78.98), concentrate 2 (78.64), concentrate 3 (78.56), concentrate 4 (77.80) and concentrate 5 (78.00) was similar. The volatile fatty acids utilization index (VFA UI) was 2.14, 2.28, 2.27, 2.41 and 2.36 in concentrate mixtures 1, 2, 3, 4 and 5, respectively. No significant difference was observed in VFA utilization index among the concentrate mixtures. VFA utilization index is non-glucogenic to glucogenic VFA ratio (NGGR).

CONCLUSION

In conclusion, no significant difference was observed in net gas production, partitioning factor, OM digestibility, NDF digestibility, microbial mass production, efficiency of microbial mass production, short chain fatty acids, metabolizable energy, fermentable carbon dioxide, fermentable methane among the concentrate mixtures evaluated. The data conclusively revealed that soybean meal could be replaced by CSM up to 100% on w/w/ basis without affecting the nutrient digestibility and microbial biomass production.

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