DR-1918
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RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume Issue : ()

Chemical Composition, *in vitro* Gas Production and Ruminal Fermentation of Cottonseed Meal and Certain Conventional Protein Sources in Buffalo Inoculum

Siliveru Srinath, Jasmine Kaur, J.S. Hundal **10.18805/ajdfr.DR-1918**

ABSTRACT

Background: The price of conventional protein sources like soybean and peanut has increased markedly in recent years resulting in urgent need for exploring alternate protein sources. Due to increase in availability of cottonseed meal (CSM) in cotton-growing areas of India as compared to other oil seed meals, CSM is becoming one of the major components of concentrate mixture (CM) fed to animals.

Methods: A study was undertaken to assess the chemical composition and *in vitro* nutritional worth of CSM in comparison to conventional oilseed cakes used in livestock feeding.

Result: The CP content of protein sources varied from 47.85% in CSM to 47.76% in soybean meal (SBM). The net gas production (NGP, ml/g DM/24h) in CSM (160.48) was lower ($P<0.05$) than SBM (198.04) and MC (199.55). The metabolizable energy (ME, MJ/kg DM) was higher ($P<0.05$) in MC and lowest ($P<0.05$) in DMC. The partitioning factor (PF, mg/ml), OM digestibility (%), DM digestibility (%), NDF digestibility (%), efficiency of microbial mass production (EMMP, %), fermented CO_2 was reported to be higher ($P<0.05$) in CSM compared to conventional oilseed cakes. The TVFA (mM/dl) production in CSM (6.63) was higher ($P<0.05$) than GNC (6.45) but lower ($P<0.05$) than SBM (6.97), MC (7.11) and DMC (7.25). The fermentation efficiency (FE, %) in CSM (74.70) and GNC (74.97) was higher ($P<0.05$) than conventional oilseed cakes. The VFA utilization index (VFA UI) was highest ($P<0.05$) in SBM (3.81) and lowest ($P<0.05$) in GNC (3.39). The results revealed that CSM has the potential to be used as a protein source for livestock feeding.

Key words: Cottonseed meal, Digestibility, *in vitro* evaluation, Volatile fatty acid.

INTRODUCTION

Livestock industry is an integral part of our country. The buffalo plays a very important role in Indian economy. The population of buffaloes in Punjab is reported to be 4 million (National Livestock Census, 2019). They provide high quality milk and meat and are a source of draught power for smallholders in the country. Productivity of most of animals is less than their genetic potential due to various factors like inadequate feeding and reproductive mismanagement. Another constraint to livestock production is the scarcity of animal feed and fluctuation of the quality and quantity of animal feed supply throughout the year. Quality and quantity of roughages vary seasonally. Plants grow rapidly during the rainy season, their quality in the early season may be good but it declines as they mature rapidly. Ruminants mostly feed on low quality roughages which are poor in energy, protein, vitamins and minerals. Addition of the mill by products can meet the nutritional requirements of animals.

Cottonseed meal (CSM) is an agro industrial byproduct derived from the production of cottonseed oil. The dehulled cottonseed is pressed and solvent extracted to remove oil. Cotton is a rich source of amino acids (He *et al*., 2015). The availability of cottonseed meal in cotton growing areas of the country is increasing, so it can be used widely in animal feeding. Due to increased price of soybean meal, dairy farmers are seeking suitable and viable alternate protein supplement to soybean meal. It is important to examine the use of low-cost feed supplements in which co-products of agro industrial origin are an option for reducing the cost of supplementation. Therefore, keeping in view the above points, a comprehensive study was planned for *in vitro evaluation* of CSM in comparison to conventional protein sources.

Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 001, Punjab, India.

Corresponding Author: Jasmine Kaur, Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 001, Punjab, India.
Email: sachdeva-jasmine@rediffmail.com

How to cite this article: Srinath, S., Kaur, J. and Hundal, J.S. (2022). Chemical Composition, *in vitro* Gas Production and Ruminal Fermentation of Cottonseed Meal and Certain Conventional Protein Sources in Buffalo Inoculum. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DR-1918.

Submitted: 21-03-2022 **Accepted:** 11-07-2022 **Online:** 23-07-2022

MATERIALS AND METHODS

Chemical analysis

An experiment was conducted at Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences

University, Ludhiana. Samples of cottonseed meal and common protein feeds fed to livestock, *viz*., soybean meal, groundnut cake, mustard cake, deoiled mustard cake were collected from local market. The samples were dried in hot air oven (60°C, 24 h) and then grounded to pass through 1.0 mm sieve and stored in plastic containers for chemical estimation. The finely ground feed ingredients (cottonseed meal, soybean meal, groundnut cake, mustard cake, deoiled mustard cake) were analyzed for proximate (AOAC, 2005) and cell wall constituents (Van Soest *et al*., 1991).

In vitro evaluation

Rumen fluid collected from 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 (UMMB) 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 39°C 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 270°C 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 0.12ml, 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,

$$\frac{(4M+2P+2B)}{(2A+P+4B)} \times 100$$

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 are 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 CSM and conventional oil cakes

The chemical composition of ingredients used in the experiment is given in Table 1. The organic matter (OM) content in cottonseed meal (CSM), soybean meal (SBM) and groundnut cake (GNC) was 92.5%, 89.35%, 93.06%, respectively. The OM content in mustard cake (MC) was 93.46% which was higher and OM present in deoiled mustard cake (DMC, 88.08%) was lower than other oil cakes evaluated. Wanapat *et al*. (2013) reported that CSM contained OM of 90.6% which was almost similar to the present study. The CP content of CSM was 47.85% while CP content of SBM was 47.76%. The CP content of GNC, MC and DMC was 43.64, 39.59 and 42.01%, respectively. The CP of CSM in the current study was slightly higher than that reported by Fadel and Ashmawy (2015) (45.05% CP) and Sun *et al*. (2013) (46.2% CP). The ether extract (EE) of CSM and SBM was 1.71% and 1.36%, respectively. The EE content of MC (8.34%) was higher and GNC (0.91%) was lower than other oilseed cakes evaluated. EE content of DMC was 1.25%. The EE content of CSM in the present study was similar to that reported by Silva *et al*. (2009). The total ash content of CSM, SBM, GNC, MC and DMC was 7.50%, 10.65%, 6.94%, 6.53% and 11.91%, respectively. The total ash content in the present study was similar to that reported by Silva *et al*. (2009). The NDF content of CSM (29%) was almost similar to SBM (30.73%) and NDF content of GNC, MC and DMC was 30.06%, 21.4% and 21.8%, respectively. Silva *et al*. (2009) and Wanapath *et al*. (2013) reported 28.21% and 32.3% NDF in CSM which was almost similar to that reported in the present study. The ADF content in CSM, SBM, GNC, MC and DMC was 17.4%, 20.43%, 22.36%, 19.03% and 21.13%, respectively. Wanapath *et al*. (2013) reported 20.7% ADF which was slightly higher than that reported in the present study whereas Silva *et al*. (2009) reported 11.41% ADF which was lower than that in the current study.

The hemicellulose content of CSM, SBM, GNC, MC and DMC was 11.60%, 10.30%, 7.70%, 2.37% and 0.67%, respectively. ADL content in CSM, SBM, GNC, MC and DMC was 6.3%, 4.13%, 7.93%, 6.26% and 7.06%, respectively. The ADICP and NDICP content in CSM was 6.27% and 9.31%, respectively. The ADICP and NDICP in SBM was 7.04% and 16.53%, respectively. The ADICP and NDICP in CSM was lower than SBM. The carbohydrate content was 42.94%, 40.23%, 48.51%, 45.54% and 44.83%, in CSM, SBM, GNC, MC and DMC, respectively.

Table 1: Chemical composition of ingredients used in the experiment, % DM basis.

Parameter	CSM	SBM	GNC	MC	DMC
OM	92.50	89.35	93.06	93.46	88.08
CP	47.85	47.76	43.64	39.59	42.01
EE	1.71	1.36	0.91	8.34	1.25
Total ash	7.50	10.65	6.94	6.53	11.91
NDF	29.00	30.73	30.06	21.40	21.80
ADF	17.40	20.43	22.36	19.03	21.13
Hemicellulose	11.60	10.30	7.70	2.37	0.67
ADL	6.30	4.13	7.93	6.26	7.06
ADICP	6.27	7.04	5.89	6.67	5.13
NDICP	9.31	16.53	12.54	7.79	5.51
TCHO	42.94	40.23	48.51	45.54	44.83

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 2: *In vitro* gas production and digestibility of CSM and conventional oil cakes (24 h).

Parameter	CSM	SBM	GNC	MC	DMC	SEM
NGP, ml/g DM/24h	160.48^{a}	198.04^{b}	173.78^{a}	199.55^{b}	164.95^{a}	4.59
PF, mg/ml	4.91^{c}	3.60^{a}	4.19^{abc}	3.91^{ab}	4.52^{bc}	0.16
OMD, %	85.09^{c}	79.83^{ab}	77.80^{a}	83.88^{c}	81.76^{b}	0.89
NDFD, %	52.44^{c}	41.35^{b}	31.27^{a}	29.59^{a}	26.29^{a}	3.23
MMP, mg	159.44^{c}	98.61^{a}	125.23^{ab}	123.24^{ab}	134.44^{b}	6.76
EMMP, %	53.73^{c}	36.92^{a}	45.81^{bc}	41.91^{ab}	49.56^{bc}	2.03
DMD, %	87.14^{c}	85.31^{b}	81.72^{a}	85.60^{b}	86.72^{c}	0.64
SCFA, mmole	0.71^{a}	0.88^{b}	0.77^{a}	0.88^{b}	0.73^{a}	0.02
ME, MJ/kg DM	9.89^{b}	10.73^{c}	9.82^{b}	11.96^{d}	9.08^{a}	0.27
NH_3-N, mg/dl	70.00^{ab}	64.50^{a}	78.50^{c}	71.00^{b}	72.00^{b}	1.55
Fer CO_2, mmoles	51.11^{b}	49.62^{a}	49.36^{a}	49.77^{a}	49.04^{a}	0.25
Fer CH_4, mmoles	30.64^{b}	31.35^{c}	29.73^{a}	30.55^{b}	31.27^{c}	0.20

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 ($P<0.05$).

In vitro evaluation of CSM and conventional oil cakes

The net gas production (NGP, ml/g DM/24h) in CSM (160.48) was lower ($P<0.05$) than SBM (198.04) and MC (199.55), however, it was similar to that of GNC (173.78) and DMC (164.95) (Table 2). DeVore (2018) also reported lowest ($P<0.05$) *in vitro* gas production in CSM when compared to soyhulls, corn gluten feed (CGF) and wheat middlings. The partitioning factor (PF, mg/ml) was higher ($P<0.05$) in CSM (4.91) than SBM (3.60) and MC (3.91). The PF of various oilseed cakes tested in the present study varied in the following order as CSM>DMC>GNC>MC>SBM. The OM digestibility (%) was higher ($P<0.05$) in CSM (85.09) and MC (83.88) than the other oilseed cakes tested. The OM digestibility (%) of SBM (79.83) was statistically similar to that of GNC (77.80) and DMC (81.76). The OM digestibility of CSM in the present study was higher than that reported by Tripathi *et al*. (2014). The NDF digestibility (%) of CSM (52.44) was higher ($P<0.05$) than SBM (41.35) as well as other oilseed cakes tested. The microbial mass production (MMP, mg) was highest ($P<0.05$) in CSM (159.44) followed by DMC (134.44), GNC (125.23), MC (123.24) and lowest ($P<0.05$) in SBM (98.61). The efficiency of microbial mass production (EMMP, %) was higher ($P<0.05$) in CSM (53.73) and lower ($P<0.05$) in SBM (36.92) than the other oil seed cakes tested. The EMMP was similar ($P<0.05$) in GNC (45.81), DMC (49.56). The DM digestibility (%) was higher ($P<0.05$) in CSM (87.14) and DMC (86.72) than SBM (85.31). The DM digestibility (%) in SBM (85.31) was similar to that of MC (85.60) and was lowest ($P<0.05$) in GNC (81.72). The DM digestibility of CSM (72.64%) in the present study was higher than that reported by Devore (2018).

The short chain fatty acid (SCFA, mmole) production in CSM (0.71) was lower ($P<0.05$) than SBM (0.88), however, it was similar to that of GNC (0.77) and DMC (0.73). The SCFA production in SBM was similar to that of MC. The SCFA production of CSM in the present study was higher than SCFA production in cottonseed cake (0.62 mmole) reported by Raji *et al*. (2017). The metabolizable energy (ME, MJ/kg DM) in CSM (9.89) was higher ($P<0.05$) than DMC (9.08) but it was lower ($P<0.05$) than SBM (10.73) and MC (11.96). However, the ME content of CSM (9.89 MJ/kg DM) was similar to that of GNC (9.82 MJ/kg DM). The results of present study are in accordance with those of Khanum *et al*. (2007) who reported 9.74 MJ/kg DM ME in CSM. The ammonia nitrogen (mg/dl) was lowest ($P<0.05$) in SBM (64.50) and highest ($P<0.05$) in GNC (78.50) among the oilseed cakes tested with intermediate values for CSM (70.0), MC (71.0) and DMC (72.0). The fermentable CO_2 in CSM (51.11) was higher ($P<0.05$) than other oilseed cakes tested *viz*., SBM (49.62), GNC (49.36), MC (49.77) and DMC (49.04). The fermentable CH_4 (mmol) in CSM (30.64) was lower ($P<0.05$) than SBM (31.35) and DMC (31.27), however, it was higher than GNC (29.73). The fermentable CH_4 (mmol) in SBM was similar to that in DMC.

The total volatile fatty acid (TVFA) production of ingredients used in the experiment is presented in Table 3. The acetic acid (mM/dl) production in CSM (4.04) was lower ($P<0.05$) than SBM (4.44), MC (4.37) and DMC (4.62), however, it was similar to that of GNC (3.93). The propionic acid, isobutyric acid and isovaleric acid production varied non-significantly among the protein sources evaluated. The butyric acid (mM/dl) production in CSM (0.69) was higher ($P<0.05$) than SBM (0.60), GNC (0.58) and DMC (0.60), however, it was similar to that in MC (0.67). The results of the present study are in agreement with those of Lamba *et al*. (2014) who reported almost similar values for acetic (4.64mM/dl), propionic (2.51 mM/dl) and butyric acid (0.58mM/dl) in cottonseed cake. The valeric acid (mM/dl) in CSM (0.15) was lower ($P<0.05$) than other protein sources evaluated. The TVFA (mM/dl) production in CSM (6.63) was higher ($P<0.05$) than GNC (6.45) but lower ($P<0.05$) than SBM (6.97), MC (7.11) and DMC (7.25). The

Table 3: *In vitro* VFA production (mM/dl) in CSM and conventional oil cakes (24 h).

Parameter	CSM	SBM	GNC	MC	DMC	SEM
Acetate	4.04^{a}	4.44^{b}	3.93^{a}	4.37^{b}	4.62^{c}	0.09
Propionate	1.33	1.34	1.36	1.39	1.37	0.01
Isobutyrate	0.16	0.12	0.13	0.15	0.14	0.01
Butyrate	0.69^{b}	0.60^{a}	0.58^{a}	0.67^{b}	0.60^{a}	0.01
Isovalerate	0.26	0.28	0.24	0.26	0.27	0.01
Valerate	0.15^{a}	0.19^{b}	0.20^{b}	0.27^{c}	0.24^{c}	0.01
TVFA	6.63^{b}	6.97^{c}	6.45^{a}	7.11^{cd}	7.25^{d}	0.10
A:P	3.03^{ab}	3.30^{c}	2.89^{a}	3.15^{b}	3.36^{c}	0.06
Relative proportion, %						
Acetate	60.90^{a}	63.71^{b}	60.97^{a}	61.41^{a}	63.69^{b}	0.44
Propionate	20.09^{b}	19.29^{ab}	21.13^{c}	19.53^{ab}	18.93^{a}	0.26
Isobutyrate	2.36	1.67	1.97	2.18	1.98	0.12
Butyrate	10.42^{d}	8.63^{ab}	9.06^{bc}	9.46^{c}	8.31^{a}	0.25
Isovalerate	3.98	4.05	3.74	3.67	3.75	0.07
Valerate	2.25^{a}	2.66^{a}	3.13^{b}	3.76^{d}	3.34^{bc}	0.18

TVFA-Total volatile fatty acids, A:P-Acetate: Propionate, means bearing different superscripts in a row differ significantly ($P<0.05$).

Table 4: Hydrogen balance of CSM and conventional oil cakes (24h).

Parameter	CSM	SBM	GNC	MC	DMC	SEM
H- recovery, %	78.16^{c}	75.12^{b}	79.57^{d}	74.73^{b}	73.22^{a}	0.79
H consumed via CH_4	5.03^{b}	4.97^{ab}	4.79^{a}	5.13^{b}	5.04^{b}	0.04
FE, %	74.70^{c}	73.96^{a}	74.97^{c}	74.38^{b}	73.81^{a}	0.15
VFA UI	3.76^{bc}	3.81^{c}	3.39^{a}	3.61^{b}	3.76^{bc}	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).

acetate: propionate ratio in SBM was higher (P<0.05) than CSM, GNC and MC, however, it was similar to DMC. TVFA production in CSM in the present study was slightly lower than that reported by Lamba *et al.* (2014) (CSC: 7.89 mM/dl).

The relative proportion (%) of acetic acid in SBM (63.71) was higher (P<0.05) than CSM (60.90), GNC (60.97) and MC (61.41), however, it was similar to that in DMC (63.69). The relative proportion (%) of propionic acid in CSM (20.09) was similar to SBM (19.29), MC (19.53). The relative proportion of propionic acid was highest (P<0.05) in GNC and lowest (P<0.05) in DMC among the protein sources evaluated. The relative proportion of isobutyric acid and isovaleric acid was similar among the protein sources evaluated. The relative proportion (%) of butyric acid was highest (P<0.05) in CSM (10.42) followed by MC (9.46), GNC (9.06), SBM (8.63) and DMC (8.31). The relative proportion of valeric acid was similar in CSM and SBM and was lower (P<0.05) than other protein sources evaluated.

The H-recovery (%) in CSM (78.16) was higher (P<0.05) than SBM (75.12), MC (74.73) and DMC (73.22), however, it was lower (P<0.05) than GNC (79.57) (Table 4). The hydrogen consumed via methane was lowest (P<0.05) in GNC (4.79). The hydrogen consumed via methane in CSM was similar to SBM, MC and DMC. The fermentation efficiency (%) in CSM (74.70) and GNC (74.97) was higher (P<0.05) than SBM (73.96), MC (74.38) and DMC (73.81). The VFA utilization index (VFA UI) was highest (P<0.05) in SBM (3.81) and lowest (P<0.05) in GNC (3.39). The VFA UI in CSM (3.76) was similar to DMC (3.76).

CONCLUSION

In conclusion, CP and NDF content of CSM was similar to that of SBM. The PF, OMD, DMD, NDFD, EMMP was higher (P<0.05) in CSM than other protein sources evaluated. The results conclusively revealed that cottonseed meal could be used as a potential source of nutrients for livestock feeding.

Conflict of interest: None.

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