In vitro Evaluation of Corn Germ Meal as Ruminant Feed

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ABSTRACT

The present study was taken up to assess the chemical composition and in vitro nutritional worth of corn germ meal (CGM) in comparison to conventional oilseed cakes used in livestock feeding. The CP content of protein sources varied from 18.59% in CGM to 49.41% in soybean meal (SBM). CGM had the highest ether extract (EE) content, neutral detergent fibre (NDF), acid detergent fibre (ADF) and total carbohydrates. However, total ash, acid detergent insoluble crude protein (ADICP) and neutral detergent insoluble crude protein (NDICP) was lowest in CGM. In vitro net gas production in CGM (267.91 ml/g DM/24 h) was higher (P<0.05) than other conventional oil cakes. The digestibility of organic matter varied from 85.12% in DMC (deoiled mustard cake) to 96.19% in SBM. The ME availability was highest (P<0.05) in CGM (9.63 MJ/kg DM). Ammonical nitrogen in CGM was lower (P<0.05) than SBM and GNC (groundnut cake). The total volatile fatty acids (TVFA) production (mM/dl) was highest (P<0.05) in GNC (12.56) and lowest (P<0.05) in CGM (9.31). Methane production was lowest (P<0.05) in CGM than other conventional oil cakes. Hydrogen recovery (%) was higher (P<0.05) in CGM (65.76) and SBM (65.78) than other protein sources tested. Fermentation efficiency (%) was higher (P<0.05) in SBM (77.02) and GNC (76.75) while volatile fatty acids utilization index (VFA UI) was higher (P<0.05) in CGM (2.92) and DMC (2.84) than other protein sources tested. The results revealed that CGM can be used as a potential protein source for ruminants.

Keywords: Corn germ meal, In vitro evaluation, Methane, Volatile fatty acid

Livestock plays an important role in Indian economy. India’s livestock sector is one of the largest in the world with a holding of 11.6% of world population. Contribution of livestock to GDP is 4.1% (Islam et al., 2016). Rearing of livestock depends upon the availability of feedstuffs. Growing population and rapid urbanization has continued to shrink the cultivated land across the India. The increase in build up has reduced the agriculture land by 212.49 square kilometres in India (Kavitha et al., 2015). The replacement of traditional feed sources with the non-conventional feed resources (NCFRs) and crop residues is the good alternative to deal with feed shortage crisis. The NCFRs are credited for being non competitive in terms of human consumption, very cheap to purchase, by-products or waste products from agriculture, farm made feeds and processing industries and are able to serve as a form of waste management in enhancing good sanitation (Areaya, 2018). Recycling, reprocessing and utilization of all or a portion of the wastes, offers the possibility of returning these materials to beneficial use as opposed to the traditional methods of disposal and relocation of the same residues (Amata, 2014).

Corn is the most common and major feed ingredient used for both livestock and poultry. Corn germ meal is the by-product of corn wet milling industry. Corn germ is separated, dried, and processed in a germ plant for extraction of the corn oil. After the oil is extracted, the remaining feed by-product is called corn germ meal (CGM), which can be used as an energy source in animal nutrition (Meyer et al., 2010). Corn germ meal is very palatable and can be used as medium protein and energy ingredient in the diet of ruminants. Being rich in highly digestible amino acids, it offers a great alternative protein
source for swine and poultry (Loy and Wright, 2003). The presence of hemicellulose fiber in corn germ meal at higher levels delivers good hydration and pelleting characteristics to the feed.

Keeping in view the need to explore non-conventional feeds for animals, the present study was contemplated to explore the chemical composition of corn germ meal and its effect on ruminal fermentation in comparison to conventional oil cakes in vitro.

**MATERIALS AND METHODS**

**Chemical analysis**

Samples of common protein feeds fed to livestock, viz. soybean meal, mustard cake, groundnut cake, deoiled mustard cake and unconventional protein supplement, viz. corn germ meal 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 (Corn germ meal, soybean meal, groundnut cake, mustard cake, deoiled mustard cake) were analyzed for proximate (AOAC, 2005) and cell wall constituents (Robertson et al., 1991).

**In vitro evaluation**

Rumen fluid was collected from 3 buffalo calves fitted with permanent rumen fistulae maintained on 2 kg conventional concentrate mixture (maize-20, wheat-15, deoiled mustard cake-10, mustard cake-10, SBM- 15, rice bran-15, deoiled rice bran-12, mineral mixture-2, common salt-1part), 5 kg green and ad lib wheat straw before the morning feeding and immediately strained through muslin cloth to remove large feed particles and transferred to laboratory in pre-warmed thermos. The fluid was diluted (1:2 v/v) with a culture medium containing macro, micro mineral solutions, resazurin and a bicarbonate buffer solution prepared as per (Menke et al., 1979; Menke and Steingass, 1988). The medium was kept at 39°C in a water bath and flushed with CO₂. Thirty ml of buffered rumen fluid was dispensed into 100 ml calibrated glass syringes containing 375mg test feed under the anaerobic conditions. Syringes were sealed with rubber tube and plastic clip and placed in a water bath at 39°C for 24 h. A 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 (VFA) 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 oven 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 hrs 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, butyric acid 0.24 ml to obtain final concentration of acetic acid, 7.0, propionic acid, 1.62; butyric acid 0.68 mM/100 ml. The mixture was stored in deep freeze until further use.

**Estimation of methane**

Two hundred mg of 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 estimation of methane. Methane was estimated in gas liquid chromatography (GLC) (Netchrom 9100) equipped with stainless steel column packed with porapak Q and flame ionization detector. Standard calibration gas (Sigma gases, New Delhi) consisted of 50% methane and 50% carbon dioxide. The gas flow rates for nitrogen, hydrogen and zero air were 30, 30 and 320 ml/min, respectively. From the head space of each syringe, 100µl gas was
collected by puncturing the silicon tube and injected in gas chromatograph for the estimation of methane.

**Determination of hydrogen recovery**

Hydrogen recovery (%) was estimated as \((4M+2P+2B)/(2A+P+4B) \times 100\); 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 (Demeyer, 1991).

**Determination of fermentation efficiency**

Fermentation efficiency (FE) was calculated on the basis of the equation worked out by (Orskov, 1975) and modified by (Baran and Zitnan, 2002)

\[
FE = \frac{0.622a + 1.092p + 1.56b}{a + p + 2b} \times 100
\]

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 VFA utilization index**

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

\[
NGGR = \frac{(A + 2B + V)}{(P + V)}
\]

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 CGM and conventional oil cakes**

The chemical composition of ingredients revealed that CP content of CGM was 18.59% whereas SBM, GNC, MC and DMC had 49.41, 49.16, 41.35 and 42.97% CP, respectively (Table 1). Kumar et al. (2018) reported that maize oil cake contained 18.66% CP, which is similar to that obtained in present study. Anderson et al. (2012) reported that CGM contained 23.64% crude protein, which is higher than that obtained in the present study. The ether extract content of CGM was 13.85% and was higher than other conventional oil cakes. The total ash content of CGM was 1.85% whereas total ash in SBM, GNC, MC and DMC was 9.23, 6.08, 5.90 and 6.30%, respectively. Total ash content of CGM was lower than SBM and MC. The NDF content of CGM was 54.0% whereas total NDF in SBM, GNC, MC and DMC had 18.30, 15.50, 23.20 and 26.70% NDF, respectively. NDF content of corn germ meal was lower than the other ingredients tested. Almeida et al. (2011) and Li et al. (2018) reported that CGM contained 49.29% and 50.30% NDF, respectively, which is almost similar to that reported in the present study. The ADF content of CGM was 12.35% whereas SBM, GNC, MC and DMC had 12.35, 10.55, 18.00 and 18.45% ADF, respectively. CGM contained higher ADF than SBM and MC. Li et al. (2018) reported that CGM contained 14.38% ADF, which is lower than that reported in the present study. The acid detergent insoluble crude protein (ADICP) content of CGM was 3.03% and ADICP of CGM was lower than SBM and MC. The neutral detergent insoluble crude protein (NDICP) content of CGM was 8.56% and NDICP of CGM was lower than SBM but was similar to the NDICP of mustard cake. The total carbohydrate of CGM was 65.71%, which was higher as compared to other oil seed cakes evaluated.

**In vitro evaluation of CGM and conventional oil cakes**

Net gas production (NGP, ml/g DM/24h) was highest (P<0.05) in CGM (267.91) among various protein sources evaluated (Table 2). However, NGP was similar in mustard cake and deoiled mustard cake. Kelzer et al. (2010) also reported that in vitro gas production (IVGP) was higher in CGM (52.1%) as compared to DDGS 1 (dried distillers grains plus solubles that had no heat exposure before
(fermentation) (38.6%), high protein dried distillers grains (HPDDG) (37.5%), wet distillers grains plus soluble (WDGS) (40.7%), wet corn gluten feed (WCGF) (39.0%) and DDGS 2 (dried distillers grains plus solubles that had heat exposure before fermentation) (40.1%). The results of the current study are in accordance with those of Lamba et al. (2014) who reported that NGP (ml/g DM/24h) was highest (P<0.01) in maize oil cake (236.76) than the other protein sources evaluated during the experiment.

The partitioning factor (PF, mg/ml) of CGM (3.32) was similar to that of MC (3.73) and DMC (3.66) but was lower (P<0.05) than SBM (4.09) and GNC (4.44) (Table 2). However, Lamba et al. (2014) reported that maize oil cake had partitioning factor similar to the other protein sources.

The organic matter digestibility (OMD, %) in CGM (87.45) was similar to that of MC (86.98) and DMC (85.12). However, % OMD of SBM (96.19) and GNC (93.91) was higher (P<0.05) than other protein sources evaluated. Similarly, Lamba et al. (2014) also reported that OMD (%) of maize oil cake (90.78) was lower (P<0.01) than SBM (99.50) and was higher (P<0.01) than MC, DMC and deoiled GNC. Kannan et al. (2017) reported that total OMD (%) in SBM (86.30) was higher (P<0.001) than MC (82.17) and GNC (82.99).

The neutral detergent fibre digestibility (NDFD, %) of CGM (77.18) was similar to that of SBM (81.13) and it was higher (P<0.05) than other protein sources (GNC, MC, DMC) evaluated. Lamba et al. (2014) reported that NDFD (%) of maize oil cake (81.71) was similar to SBM (97.64) and was higher (P<0.01) than other protein sources evaluated.

The microbial mass production (MMP, mg) of corn germ meal (108.97) was similar to deoiled mustard cake (119.88). MMP (mg) was highest (P<0.05) in groundnut cake (167.66) followed by soybean meal (151.65) and mustard cake (125.85). The efficiency of microbial mass production (EMMP, %) of CGM (33.64) was similar to that of MC (40.95) and DMC (39.92). However, EMMP (%) of CGM was lower (P<0.05) than that of GNC (50.49) and SBM (46.09).

The dry matter digestibility (DMD, %) of CGM (87.42) was similar to that of MC (86.42) and DMC (84.92). DMD (%) of SBM (95.48) and GNC (93.35) was higher (P<0.05) than other protein sources evaluated. Kannan et al. (2017) reported that total DMD (%) in SBM (84.74) was higher (P<0.001) than MC (80.36), GNC (80.27) and DMC (77.07).

The short chain fatty acids (SCFA, mmole) were higher (P<0.05) in CGM (1.15) than other protein sources evaluated. The ME (MJ/Kg DM) was higher (P<0.05) in corn germ meal (9.63) than the conventional oil cakes evaluated (Table 2). Lamba et al. (2014) reported that ME (MJ/kg DM) of maize oil cake (10.34) was similar

Table 1: Chemical composition of CGM and conventional oil cakes (% DM basis)

| Parameter | CGM | SBM | MC | GNC | DMC |
|-----------|-----|-----|----|-----|-----|
| OM        | 98.15 | 90.77 | 94.10 | 93.92 | 93.70 |
| CP        | 18.59 | 49.41 | 41.35 | 49.16 | 42.97 |
| EE        | 13.85 | 1.13 | 7.06 | 7.37 | 1.88 |
| Total ash | 1.85 | 9.23 | 5.90 | 6.08 | 6.30 |
| NDF       | 54.00 | 18.30 | 23.20 | 15.50 | 26.70 |
| ADF       | 19.20 | 12.35 | 18.00 | 10.55 | 18.45 |
| Hemicellulose | 34.80 | 5.95 | 5.20 | 4.95 | 8.25 |
| ADL       | 1.15 | 0.80 | 7.15 | 2.15 | 7.50 |
| ADICP     | 3.03 | 8.93 | 6.37 | 4.64 | 8.23 |
| NDICP     | 8.56 | 24.50 | 8.73 | 8.41 | 12.03 |
| TCHO      | 65.71 | 40.23 | 45.69 | 37.03 | 48.85 |

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.
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(P<0.01) to MC (10.46) but was lower (P<0.01) than SBM (11.59). The ammonia nitrogen (mg/dl) in CGM (47.50) was lower (P<0.05) than SBM (61.50) and GNC (55.00). Schilling et al. (2017) reported that ammonia concentration was lower in the heifers fed CGM diet as compared to fed DDGS diet (P<0.01). Lamba et al. (2014) reported that in vitro ammonial nitrogen (mg/dl) was lowest (P<0.01) in maize oil cake (12.94) than the other protein (SBM, MC, DMC and deoiled GNC) sources evaluated. Detray (2016) reported that ammonia concentration was lowest in the animals fed CGM diet as compared to animals fed the DDGS diet (P<0.01). However, Kelzer et al. (2009) reported that ammonia nitrogen was similar among the control (0% coproduct), DDGS (15% coproduct) and dehydrated CGM (15% coproduct) treatments in Holstein cows. Treatments were formulated by replacing the portions of forage and concentrate feeds with 15% coproducts.

In vitro volatile fatty acids production in CGM and conventional oil cakes

In vitro acetic acid (mM/dl) production in CGM (5.99) was higher (P<0.05) than SBM (5.81) and MC (5.81) and it was lower (P<0.05) than GNC (7.60) and DMC (6.45) (Table 3). The propionic acid (mM/dl) production in CGM (2.29) was similar to MC (2.24) and DMC (2.32). Lamba et al. (2014) also reported that acetic acid (mM/dl) production in maize oil cake (8.48) was higher (P<0.01) than SBM (7.00) and MC (5.82) and it was similar to deoiled GNC (8.59).

The isobutyric acid (mM/dl) production was lower (P<0.05) in CGM (0.115) than other conventional oil cakes. The butyric acid (mM/dl) production was higher (P<0.05) in GNC (0.515) than CGM (0.489). The butyric acid (mM/dl) production was similar in SBM, MC and DMC. However, Lamba et al. (2014) reported that butyric acid (mM/dl) in maize oil cake (1.70) was higher (P<0.01) than deoiled groundnut cake (1.33). The isovaleric acid (mM/dl) production was lower (P<0.05) in CGM (0.270) than other conventional oil cakes. The valeric acid (mM/dl) production showed similar trend and was lower (P<0.05) in CGM (0.156) than other conventional oil cakes.

The TVFA production (mM/dl) was highest (P<0.05) in GNC (12.56) (Table 3). However, TVFA production (mM/dl) was lowest (P<0.05) in CGM (9.31) among the protein sources evaluated. Detray (2016) also reported that the DDG based diets had more total VFA concentration compared to the CGM diets (P=0.04). Similarly, Schilling et al. (2017) also reported that cattle fed DDGS diets had more total VFA concentration than the cattle fed corn germ meal diets (P=0.04). Contrary to results of the present study, Lamba et al. (2014) reported that TVFA (mM/dl) production in maize oil cake (15.73) was higher (P<0.01) than SBM, MC, DMC and deoiled GNC. A: P ratio was highest (P<0.05) in DMC (2.78). A: P ratio was similar in CGM (2.62) and MC (2.59).

Table 2: In vitro nutrient digestibility of CGM and conventional oil cakes (24h)

| Parameter            | CGM     | SBM     | MC      | GNC     | DMC     | SEM     |
|----------------------|---------|---------|---------|---------|---------|---------|
| NGP, ml/g DM/24h     | 267.91  | 200.93  | 219.71  | 198.52  | 217.79  | 8.36    |
| PF, mg/ml            | 3.32    | 4.09bc  | 3.73ab  | 4.44c   | 3.66ab  | 0.13    |
| OMD, %               | 87.45a  | 96.19b  | 86.98a  | 93.91b  | 85.12a  | 1.46    |
| NDFD, %              | 77.18b  | 81.13b  | 47.19a  | 63.13ab | 47.77a  | 4.94    |
| MMP, mg              | 108.97a | 151.65c | 125.85ab| 167.66c | 119.88a | 7.45    |
| EMMP, %              | 33.64a  | 46.09bc | 40.95ab | 50.49c  | 39.92ab | 1.98    |
| DMD, %               | 87.42a  | 95.48b  | 86.42a  | 93.35b  | 84.99a  | 1.41    |
| SCFA, mmole          | 1.15b   | 0.95a   | 0.97a   | 0.88a   | 0.96e   | 0.03    |
| ME, MJ/kg DM         | 9.63b   | 8.12a   | 8.40a   | 7.81a   | 8.24a   | 0.22    |
| NH3-N, mg/dl         | 47.50a  | 61.50b  | 45.00a  | 55.00b  | 43.00a  | 2.34    |

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, NH3-N-ammoniacal nitrogen, Means bearing different superscripts in a row differ significantly (P<0.05).
The acetic acid (%) was highest (P<0.05) in CGM (64.37) and DMC (63.45) whereas acetic acid (%) was lowest (P<0.05) in SBM (57.71) than other protein sources evaluated (Table 3). In contrast to results in the present study Lamba et al. (2014) reported that acetic acid (%) was lower (P<0.01) in maize oil cake (53.86) among the other protein sources evaluated. The propionic acid (%) was higher (P<0.05) in SBM (26.31) and GNC (26.79) than CGM (24.55). The isobutyric acid (%) was lowest (P<0.05) in CGM (1.242) among the other protein sources evaluated. The butyric acid (%) was highest (P<0.05) in CGM (5.256) than other protein sources. Lamba et al.
(2014) also reported that butyric acid (%) was highest (P<0.01) in maize oil cake (10.77) than other protein sources (SBM, MC, DMC and deoiled GNC) evaluated. The isovaleric acid (%) was highest (P<0.05) in MC (3.547). The isovaleric acid (%) was lower (P<0.05) in GNC (2.786) than CGM (2.910). Lamba et al. (2014) also reported that isovaleric acid (%) was lower (P<0.01) in maize oil cake (0.19) than other protein sources evaluated. The valeric acid (%) was highest (P<0.05) in MC (6.917) and lowest (P<0.05) in CGM (1.678). Detray 2016) also reported that valerate and isovalerate were lower (P<0.01) in CGM diets than DDG diets in Holstein heifers.

**Methane production from fermentation of CGM and conventional oil cakes**

The methane (%) was lower (P<0.05) in CGM (14.52) than other protein sources evaluated (Table 4). Methane production in SBM (23.73) and GNC (22.55) was higher (P<0.05) than MC (20.70) and DMC (19.45). The methane (ml/100mg DM) production was lower (P<0.05) in CGM (3.08) and higher (P<0.05) in SBM than other protein sources evaluated. Lee et al. (2003) also reported that methane (ml/g DM) in CGM (6.07) was lower (P<0.01) than other oilseeds meals (canola meal, soybean meal and coconut meal) evaluated. In contrast to results in the present study, Lamba et al. (2014) reported that methane (ml/g DM) was higher (P<0.01) in maize oil cake (43.30) than MC (30.30) and was similar to SBM (44.18). The results of the present study are in agreement with those of Kannan et al. (2017) who reported that methane production was higher (P<0.001) in SBM than MC, DMC and GNC.

The methane (ml/100mg DMD) was lower (P<0.05) in CGM (3.52) than other protein sources evaluated. Methane (as ml/100mg DMD) in SBM (5.22) and MC (5.09) was higher (P<0.05) than GNC (4.59) and DMC (4.46). The methane (ml/100mg OMD) followed the similar trend and was lower (P<0.05) in corn germ meal than other protein sources evaluated. Methane (as ml/100mg OMD) was higher in SBM (5.71) and MC (5.38) as compared to GNC (4.86) and MC (4.76).

**Hydrogen balance of CGM and conventional oil cakes**

H recovery (%) in CGM (65.76) and SBM (65.78) was higher (P<0.05) than other protein sources evaluated (Table 5). Hydrogen consumed via CH₄ was highest (P<0.05) in GNC (8.73) and lowest (P<0.05) in MC (6.15). H consumed via CH₄ in corn germ meal (6.83) was similar to that of SBM (6.67) and DMC (6.72). Fermentation efficiency (%) of CGM (75.48) was higher (P<0.05) than DMC (74.93) and it was similar to that of MC (75.55). However, SBM (77.02) and GNC (76.75) showed the highest (P<0.05) fermentation efficiency among the protein sources evaluated. The VFA utilization index or non-glucogenic to glucogenic VFA ratio in CGM (2.92) was similar to that of DMC (2.84). The VFA utilization index in CGM was higher (P<0.05) than other protein sources evaluated.

**CONCLUSION**

In conclusion, CGM had higher EE, NDF, ADF and total carbohydrates but lower CP, total ash, NDICP and ADICP than other protein sources evaluated. In vitro net gas production, ME availability and short chain fatty acids were higher (P<0.05) in CGM than other conventional oil cakes tested. TVFA production and methane production was lowest (P<0.05) in CGM. The results conclusively revealed that corn germ meal can be used as a promising source of nutrients for livestock.

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