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Research Article

Effect of Inclusion of Raw or Water Soaked Black Gram Husk based Complete Ration for Sheep in *In-vitro* (RUSITEC)

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ABSTRACT

Incubation trial was carried out with the rumen simulation technique (RUSITEC) to evaluate the inclusion of Black gram husk in the complete ration for sheep. A total of seven experimental complete rations with 11-12% of crude protein were prepared by replacing a test mix with the raw or water soaked husk at 0, 10, 20 or 30% level. The rations were isocaloric and isonitrogenous. The IVDMD, *in vitro* nitrogen degradability, microbial protein synthesis and total short chain fatty acid production were significantly (P<0.01) high at 10% inclusion of raw or 30% inclusion of water soaked black gram husk in the complete rations for sheep. The raw black gram husk could be included up to 10% level in the complete mash ration for sheep. However, through the simple method of overnight water soaking the inclusion level of the black gram husk could be maximized to 30% in complete ration of sheep.

Key words: Black gram husk, Complete ration, RUSITEC, Dry matter, Nitrogen degradability

INTRODUCTION

Acute shortage of grazing and browsing resources in the country can be managed by using complete feed system based on locally available crop residues and agro-industrial byproducts (Yadav, 2001). The concept of complete ration feeding with use of locally available crop residues and agro industry by products seems to be an ideal one.

The availability of agro-industrial by-product in India is varied and abundant but is of limited use because of the lack of information on the nutritive value. During the milling of seeds for preparation of pulses, a significant amount of milling byproduct is available. Such available by product of black gram husk contains broken seed coat, germ and small pieces of broken cotyledons (Jain 1986). Black gram (*Vigna mungo*) husk (BGH) is one such agro-industrial by-products available in substantial quantity as this pulse is grown as cash crop in vast areas of Tamil Nadu. The use of unconventional feed is limited due to the presence of one or more toxic factors including tannins (Kondo *et al*., 2007). Tannin affects digestibility either by binding the digestive enzymes or by binding feed nutrients (Barman and Rai, 2000). Black gram husk is an protein rich (16-18%) feed ingredient and has an ideal calcium phosphorus ratio. The total tannin content of black gram husk ranges from 2.70 to 4.55% with a mean of 3.67±0.31.% When overnight water soaking or calcium hydroxide treatments were carried out the total tannins content in the husk was significantly (P<0.01) reduced to 1.14 (Arulnathan 2003). However, very little information is available in the literature on inclusion of black gram husk in the complete ration for ruminants. Hence a study was carried out focusing on to assess the inclusion level of black gram husk in the complete ration for sheep by using *in vitro* rumen simulation technique (RUSITEC).

MATERIALS AND METHODS

Deactivation of Tannin inBlack gram Husk

Black gram husk was soaked in water 1:5 (W/V) for overnight to reduce the tannin content (Kataria, 1988). After the soaking process the supernatant water was decanted. The soaked samples were again washed twice with plain water followed by rinsing with distilled water. The samples were dried in hot air oven at 60° C for 36 hours and used for further analysis.

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Experimental Complete Rations

The effect of inclusion of black gram husk (raw or water soaked) was studied by replacing the black gram husk at 0, 10, 20 and 30% level of a test mix containing 74% wheat bran and 26% groundnut oil cake in complete rations having the roughage to concentrate ratio of 60:40. The ingredient composition of complete ration is presented in Table 1. The ingredients were ground in hammer mill and seven isocaloric and isonitrogenous complete rations were prepared. The samples were prepared separately by using the sieve and particles less than 850 micron were removed and the complete rations were prepared. The experimental complete rations containing no BGH (CR-1), replacing 10% of the test mix with raw BGH (CR-2), replacing 20% of the test-mix with raw BGH (CR-3), replacing 30% of the test-mix with raw BGH (CR-4), replacing 10% of the test-mix with water soaked BGH (CR-5), replacing 20% of the test-mix with water soaked BGH (CR-6) replacing 30% of the premix with water soaked BGH (CR-7) were prepared. The black gram husk samples and complete rations were analyzed for proximate principles in duplicate as per the methods described in AOAC (1980).

Rumen degradability studies

The complete rations were subjected to *in vitro* dry matter and nitrogen degradability studies in RUSUTEC for 48 hrs. Three measurements were made from two runs for each treatment, thus yielding six observations.

Experimental design

Apparatus: The Semi-continuous culture system developed at the Department of Animal Nutrition Laboratory, Madras Veterinary College was adopted from the "RUSITEC" and run essentially as described by Czerkawski and Breckenridge (1977). It consisted of eight 1 litre capacity reaction vessels immersed in water bath maintained at 39^{o}C.

Preparation of the samples for incubation

The samples of complete ration for studies in RUSITEC were ground through 3 mm sieve. The ground samples were then sieved and particles passing through 2 mm sieve but retained by 0.85 mm sieve were collected (Dong *et al.*, 1997) and stored in air-tight containers for further analysis.

Nylon bag size and pore size

Nylon bags of 12.5x7.5 cm size made up of precious woven monofilament polyester cloth with a specified pore size of 100 µm were used in the present study (Carro *et al.,* 1995).

Sample size

Ten gram of samples on dry matter basis was weighed separately into each nylon bag for incubation in the RUSITEC. The sample size to bag ratio was 15mg/cm^2 (Carro *et al.,* 1995).

Incubation procedure

Rumen digesta was collected from three sheep maintained on grazing. It was thoroughly mixed and transported to the laboratory (within 30 minutes) in a pre-heated vacum flask. The rumen fluid was strained through a double-layered muslin cloth into a CO_2 filled beaker. The solid content in the muslin was squeezed to maximum to get the rumen liquor.

Each reaction vessel was charged with 500 ml of strained rumen liquor and 200 ml of artificial saliva (McDougall, 1948). One nylon bag (pore size 100 µm) containing 80 g of rumen digesta solids (fibrous fraction from the rumen content straining) and another containing 10 g dry matter of feed to be tested were placed into the perforated feed container and the assembly was put into the reaction vessel which was filled up to the brim with distilled water making the total volume of the container to one liter.

Artificial saliva was pumped at a constant ratio of infusion (650 ml/day) into the reaction vessel by a peristaltic pump. The effluent and fermentation gases were collected in effluent collection vessels (containing few drops of saturated $HgCl_2$ solution) and gas collection bags respectively. After 24 hours the solid inoculums were removed and a new bag of feed was placed in the feed container. Thus each reaction vessel at a time contained 2 bags introduced each in 2 consecutive days and removed 48 hours later.

The bag to be removed was allowed to drain, squeezed and washed in artificial saliva in a polyethylene bag. The washings were returned to the respective reaction vessels. The removed bags were further washed and dried at 60^{o}C for 48 hours. Each experiment totally consisted of 7 days adoption period followed by collection period of three consecutive days

Replication

Raw or water soaked black gram husk based complete ration treatments were allotted to each reaction vessel at random. The data were generated from two runs, thus yielding six observationsfor each treatment.

Collection period

The liquid effluent was collected to study the various rumen parameters from the 8th day followed by three consecutive daysThe pH of effluent was measured using digital pH meter. About 4.5 ml of sample from each reaction vessel was taken and 0.5 ml of 50% of Trichloracetic acid was added for ammonia – N (NH_3N) analysis. For measuring the Volatile fatty acids (VFA) concentration 2.5 ml of the effluent was taken and 0.5 ml of 25% of metaphosphoric acid was added and further 10 ml of effluent was stored in the refrigerator for analysis of microbial protein.

***In vitro* dry matter degradability studies**

Loss in weight of nylon bag after every incubation in RUSITEC followed by washing and drying was recorded to calculate dry matter disappearance. The *In vitro* degradability of samples was calculated using the following formula and expressed as%age on dry matter basis.

$$\textit{In vitro}\ \text{degradability} = \frac{\text{(Weight of bag with samples before incubation) – (weight of bag with samples after incubation)}}{\text{Weight of samples}} \times 100$$

The effective degradability of dry matter was calculated at the flow rate of the results of dry matter degraded at various time intervals and were fitted to exponential equation of McDonald, (1981) mentioned as below:

$P = a + b\ (1 - e^{-ct})$

Where,

P = % of degradation at time t,

a = % soluble fraction.

b = Insoluble but potentially degradable as%age

a+b = The value of potential degradability of the material as%age

c = The degradation rate, expressed as%age/hour

(a, b, c are constant in exponential equation).

***In vitro* nitrogen degradability studies**

The nitrogen content of residues obtained was determined in Kjeltec as per AOAC (1980). The residual dry matter in the nylon bag is generally contaminated with significant amount of microbial nitrogen (Nocek *et al.*, 1979). This contaminated nitrogen was estimated by incubation of nitrogen - free cellulosic materials in the nylon bag under similar conditions and appropriate corrections were made prior to calculating the effective degradability (Negi *et al.*, 1988).

Rumen fermentation studies

The pH of effluents collected was determined using digital pH meter. The ammonia nitrogen concentration of the effluents collected was determined colorimetrically as per the method of Weatherburn (1967).The microbial protein content was estimated as per the method of Makker *et al.* (1982).Total and individual VFA concentration of the effluents was measured by gas chromatographic method as per the procedure of Chase (1990). The Netel make Gas Chromatograph Model "Omega QC" was used for this estimation.

Stoichiometry of the rumen fermentation

The following stoichiometric equations were used to predict various patterns (Orskov *et al.*, 1968)

1. $\text{A/P ratio} = \dfrac{\text{Acetate}}{\text{Propionate}}$
2. $\text{Non-Glucogenic ratio (NGR)} = \dfrac{\text{Acetate} + (2 \times \text{Butyrate})}{\text{Propionate}}$

Gas analysis

Gas samples drawn from the total gas produced was fractioned for methane and carbon dioxide (Fievez *et al* 2005).

Statistical analysis

This experiment was adopted a completely randomized design (CRD). The data obtained in different parameters were subjected to statistical analysis as per the procedure of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The proximate composition of black gram husk and experimental complete rations were presented in Table 2. The mean values for crude protein, ether extract, crude fibre, nitrogen-free extract and total ash were 18.21±1.07; 1.36±0.17; 20.29±1.79; 54.60±1.04 and 5.54±0.42% respectively

The proximate composition of black gram husk reported in the present study were in agreement with earlier reports of Jain (1986), Rane *et al.* (1988), Tomar *et al.* (1993), Padmaja (1996) and Sudhakara Reddy *et al.* (2002).

The proximate composition of black gram husk observed in the present study was also comparable to arhar chuni (Paliwal *et al.*, 1981 and Rane *et al.*, 1988) and green gram chuni (Paliwal *et al.*, 1981and Radhakrishna, 2002).

The proximate composition of black gram husk and experimental complete rations were presented in Table 2. The crude protein content of the complete rations ranged between 11.57 ± 1.01 and 12.16 ± 0.89. The range of values for CF, EE, NFE and TA were 21.27 ± 0.58 and 23.31 ± 1.64; 1.79 ± 0.23 and 2.17± 0.21; 48.20 ± 3.96 and 50.51 ± 0.25 and 14.25 ± 1.48 and 15.44 ± 0.64 respectively. The rations were iso-nitrogenous.

***In vitro* dry mater degradability of complete rations**

The IVDMD of the experimental complete rations CR-1 to CR-7 is presented the Table 3. The *in vitro* dry matter degradability at the end of 48 hours of incubation ranged between 50.58 ± 0.67 and 58.14 ± 1.63%. The IVDMD in general showed no variation up to 20% inclusion of the raw husk. The dry matter degradation was the lowest at the highest level of raw black gram husk inclusion (30%) in the complete ration (CR-4). Sudhakara Reddy *et al.* (2002) reported 50% of dry matter disappearance by 12 hours of incubation and further 25% by the end of 48 hours in urad chuni.

Among the complete rations tested the highest level of dry matter degradation, though non-significant was observed at 10% of level inclusion of raw black gram husk. On the other hand water soaking of the black gram husk resulted in the significantly ($P<0.01$) highest level of IVDMD at all levels of inclusion. Thus it was inferred that the inclusion of raw or water soaked black gram huskBGH respectively at 10 or 30% level in the complete ration resulted in better *in vitro* dry matter degradability. The variation in the IVDMD observed among the experimental complete rations could be explained on the basis of *in vitro* nitrogen degradability (Table 3).

***In vitro* nitrogen degradability of complete rations**

The nitrogen degradability of the experimental complete rations CR-1 - CR-7 is reported in Table 3. The nitrogen degradation at the end of 48 hours of incubation ranged between 71.43 ± 1.24 and 81.54 ± 1.08%.

The nitrogen degradability pattern in the experimental complete rations followed the dry matter degradability pattern. The variations observed in the dry matter degradability among the experimental complete rations could be attributed to their nitrogen degradability pattern (Table 4). The higher level of dry matter degradability observed with CR-2 and CR-7 could be attributed (Table 3) to the corresponding significantly higher-level nitrogen degradation.

Table 1: Ingredient composition (%) of complete rations containing raw or water soaked black gram husk replacing the testmix at different levels

Ingredients	CR-1	CR-2*	CR-3*	CR-4*	CR-5**	CR-6**	CR-7**
Black gram husk	0	10	20	30	10	20	30
Test mix***	30	20	10	0	20	10	0
Maize	8	8	8	8	8	8	8
Paddy straw	40	40	40	40	40	40	40
CO-1	20	20	20	20	20	20	20
Mineral mixture	1	1	1	1	1	1	1
Salt	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100

* Raw black gram husk; ** Water soaked Black gram husk; *** Test mix-74 parts Wheat bran and 26 parts Groundnut oil cake

Table 2: Proximate composition of complete rations containing raw or water soaked black gram husk (before incubation) (% Dry matter basis)

Complete ration	Crude protein	Crude fibre	Ether extract	NFE	Total ash
CR-1 (0%)	11.80±0.58	22.03±0.66	1.94±0.07	49.66±2.39	14.57±1.09
CR-2(10%)	11.74±0.32	23.28±1.30	1.93±0.65	48.72±2.03	14.33±1.05
CR-3(20%)	12.16±0.89	23.31±1.64	1.79±0.23	48.24±0.97	14.50±1.50
CR-4(30%)	11.57±1.01	22.88±1.92	2.03±0.21	48.28±2.63	15.24±0.51
CR-5(10%)	11.61±1.13	22.58±2.40	2.17±0.21	48.20±3.96	15.44± 0.64
CR-6(20%)	11.68±0.90	22.92±1.64	1.85±0.06	49.25±1.10	14.30±0.35
CR-7(30%)	11.87±0.82	21.27±0.58	2.10±0.16	50.51±0.25	14.25±1.48

Values in parenthesis indicates the level of inclusion of raw or water soaked black gram husk.Mean value of three observations

Table 3: Dry matter and nitrogen degradability of complete rations containing different inclusion levels of raw or water soaked black gram husk at 48 hours of incubation (% Dry matter basis)

Complete Ration	Dry matter degradability	Nitrogen degradability
CR-1	55.45±1.63^{b}	76.14±0.62^{b}
CR-2	56.11±1.00$^{b\,c}$	78.13±0.95$^{b\,c}$
CR-3	55.61±1.57^{b}	76.52±1.40^{b}
CR-4	50.58±0.67^{a}	71.43±1.24^{a}
CR-5	58.14±1.63^{c}	81.54±1.08^{c}
CR-6	57.85±0.86^{c}	80.83±1.70^{c}
CR-7	57.91±1.13^{c}	81.28±1.26^{c}

^{a,b,c} Mean value with different superscripts in columns differ significantly (P<0.01); n=6

The nitrogen degradability was the lowest at the highest level of raw black gram husk inclusion (30%) and highest at 10% level of inclusion of raw black gram husk. On the other hand water soaking of black gram husk resulted in the significant (P<0.01) and highest level of nitrogen degradability at all levels of inclusion.

The presence of high level of total tannins in the raw husk (Arulnathan 2003) coupled with the high level of lignin in it could be attributed as reasons for the low IVDMD and *in vitro* nitrogen degradability values at high levels of inclusion of the husks. The possible inhibition of the activity of rumen microbes due to high level of tannins (Makkar *et al.,* 1988) and reduced availability of nitrogen and amino acids required for rumen microbial growth (Reed, 1995) might have resulted in the low IVDMD with increased level of inclusion of the raw husk. Reed (1995) reported decline in the IVDMD of tree leaves with increased level of total tannins in them. This argument is re-inforced with the significantly lowest level of ammonia nitrogen, microbial protein synthesis and total short chain fatty acids produced when the CR-4 was incubated (Table 4) in the present study. Results of this study indicated that inclusion of BGH at 10% level in the raw form and 30% level in water soaked form resulted in the highest level of nitrogen degradability.

Rumen Fermentation characteristics of complete rations

The rumen fermentation characteristics of experimental complete rations containing different levels of raw or water soaked black gram husk is presented in Table 4.

pH

The pH of the fermentation medium in the RUSITEC ranged between 6.95±0.03 and 6.84 ± 0.65 with different level of inclusion of raw BGH and 6.98±0.04 and 6.99±0.03 with different levels of inclusion of water soaked husk. The values reported for pH in the present study coincided with the earlier reports of Sudakara Reddy *et al.* (2002) and RadhaKrishna *et al.* (2002) for urad chuni and green gram chuni, respectively. The variation in the pH was however statistically non-significant.

Ammonia nitrogen

The ammonia nitrogen concentrations were significantly the lowest (P<0.01) at the highest level of inclusion of the raw husk (30%) and the highest at 10% inclusion of the raw husk. On the other hand the ammonia nitrogen concentration was significantly high (P<0.01) up to 30% level of inclusion of the water soaked husk. The level of ammonia nitrogen reported in the present study was well within the normal range suggested by Sattar and Roffler (1981). Though the rumen degradation products including ammonia are utilized for microbial protein synthesis in the rumen, the synthetic process is much slower than that of the degradation process (Bhar and Katiyar, 1989). The relatively high ammonia nitrogen level recorded in the present study at 10% inclusion of raw and 30% inclusion of water soaked black gram husk could be explained on the observation of Bhar and Katiyar (1989).

Microbial protein synthesis

The microbial protein synthesis was significantly the lowest (P<0.01) at the highest level of inclusion of the raw husk (30%). It was the highest at 10% inclusion of

Table 4: Rumen fermentation characteristics of complete ration containing different inclusion levels of raw and water soaked black gram husk

Complete diet parameter	CR-1	CR-2	CR-3	CR-4	CR-5	CR-6	CR-7
pH	6.98± 0.04	6.95±0.03	6.95±0.07	6.84±0.65	6.98±0.04	6.96±002	6.99±0.03
Ammonia nitrogen (mg/100ml)	7.34^{b}±0.19	7.71^{bc}±0.06	7.34^{b}±0.14	6.85^{a}± 05	7.81^{c}±0.12	7.94^{c}±0.10	7.89^{c}±0.02
Microbial protein synthesis (mg/100ml)	23.61^{b}±0.32	24.43^{bc}±0.36	23.55^{b}±0.79	20.62^{a}±0.42	25.57^{c}±0.32	25.80^{c}±0.49	26.06^{c}±0.56
Total short chain fatty acids (mmol/day)	43.39^{c}±0.01	44.70^{c}±0.06	41.67^{b}±0.45	39.28^{a}±0.12	44.09^{c}±0.49	44.70^{c}±0.52	44.89^{c} ±0.71
Acetic acid (mmol/day)	25.15^{bc}±0.29	25.78^{c} ±0.16	24.11^{ab}±0.55	23.29^{a}±0.33	25.26^{b}±0.62	25.40^{c}±0.32	25.13^{bc}±0.49
Propionic acid (mmol/day)	12.86^{bc} ±0.145	12.94^{bc}±0.52	12.23^{b}±0.61	11.00^{a}±0.38	12.95^{bcd}±0.09	13.33^{cd}±0.15	13.92^{d}±0.10
Butric acid (mmol/day)	5.38±0.14	5.98±0.30	5.33±0.51	4.99±0.07	5.88±0.04	5.97±0.05	5.84±0.12
A:P ratio	1.95±0.45	1.99±0.09	1.97±0.14	2.12±0.10	1.95±0.06	1.90±0.01	1.80±0.02
Nonglucogenic ratio (NGR)	2.79±0.03	2.925±0.175	2.86±0.27	3.03±0.12	2.86±0.06	2.80±0.00	2.645±0.35
Total gas production (l/day)	1.13^{ab}±0.11	1.29^{cd}±0.01	1.19^{bc}±0.07	1.01^{a}±0.03	1.24^{bcd}±0.02	1.34^{cd}±0.02	1.37^{d}±0.04
CO_2 :CH_4	1.90±0.04	1.90±0.02	1.86±0.08	1.88±0.04	1.85±0.09	1.88±0.04	1.83±0.05

^{a,b,c,d} values with different superscripts in rows differ significantly (P<0.01) n=6

the raw husk. On the other hand the microbial protein synthesis was significantly high (P<0.01) up to 30% level of inclusion of the water soaked husk. The possible availability of more nitrogen and amino acids for the microbes due to low tannins content (Reed, 1995) in the water soaked husk coupled with high level of rumen ammonia concentration might have resulted in the significantly higher microbial protein synthesis when water treated or raw husks were incorporated at 30 or 10% levels respectively in the complete rations.

Volatile fatty acids

The total short chain fatty acids concentration was significantly (P<0.01) the lowest at highest level of inclusion of the raw husk (30%). It was the lowest at 10% inclusion of the raw husk. On the other hand the short chain fatty acids production was significantly (P<0.01) high up to 30% level of inclusion of water soaked husk.

The pattern of TSCFA production observed was similar to the pattern of production of acetic, propionic and butyric acids. The acetate and propionate levels were significantly highest (P<0.01) at 10% inclusion of raw BGH. On the other hand the level of production of both acetate and propionate were high and as well as statically similar up to 30% inclusion of water soaked BGH. The reduced tannin content in the complete rations containing water soaked BGH may be attributed as one of the reasons for the high level of short chain fatty acids production as suggested by Singh (1978). It may also be argued that the relatively more availability NH_3-N and amino acids (Reed, 1995) required for rumen microbial growth, less inhibition on the activity of the rumen microbes due to low tannins (Makker *et al.*, 1988) and higher dry matter and nitrogen degradabilities of the experimental rations (Table 3) might have also contributed to the high level of microbial protein synthesis (Table 4) when water soaked husk was incorporated in the rations. The high level of microbial protein synthesis observed with inclusion and water soaked husks could have resulted in the significantly more production of SCFA also.

The acetate to propionate ratio calculated in the present study ranged between 1.80±0.02 and 2.12±0.10. Czerkawski (1986) reported 2.00 or 1.12 as the ratio between acetate and propionate respectively in high forage or high grain rations. The acetate to propionate ratio was however statistically similar with different levels of inclusion of raw or water soaked husks in the present study.

The trend was also reflected in the NGR of the fatty acids. The NGR ranged between 2.64±0.35 and 3.03±0.12. Orskov (1977) suggested the ideal NGR values between 2.25 and 3.00. In the present study the calculated NGR were within the range suggested by Orskov (1977). Hence it could be expected that feeding of the experimental complete rations could result in the greatest efficiency of growth and fattening in sheep. However, most of the NGR values recorded in the present study were in the range of 2.80 and 2.90 slightly lower than the upper limit prescribed by the Orskov (1977) and hence it could also be expected to produce more methane.

The link between the composition of mixture of SCFA and chemical composition of the rations are not often close because the composition of mixture of fatty acids produced reflects not only the substrate but also the metabolic activity of the rumen microbes (Sutton, 1968). However, taking a holistic view it could be construed that the experimental complete rations CR-2 and CR-7 provided better ideal nutrient make up that resulted in better rumen fermentation characteristics than other rations in the present study.Sutton (1968) observed that the composition and metabolism of the rumen microbial population is dependent on the chemical composition of the diet.

Gas production

The total gas production ranged between 1.13±0.11 and 1.37±0.04 litre per day when the complete rations containing raw or water soaked husks were incubated. The total gas production decreased significantly with increased level of inclusion of the raw husk. On the other hand the gas production increased with increased level of inclusion of the water soaked husk, possibly due to reduced level of total tannins as suggested by McGinty (1969).

Conclusion

From the data collected and analysed in present study it was concluded that the black gram husk was a good protein rich ingredient. When the raw or water soaked black gram husks were incorporated at 0, 10, 20 or 30% level in the complete rations for sheep, it was observed that the in vitro degradabilities of dry matter and nitrogen, microbial protein synthesis and TVFA production were increased at 10% inclusion of raw husk or 30% inclusion of water soaked husk. Hence it was concluded that the raw or water soaked black gram husk could be incorporated respectively at 10 or 30% level in the

complete mash ration for sheep as it replaced the conventional protein source in cost effective manner.

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