INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2000/02–1-2–48–51

Effect of Varying Levels of Formaldehyde and Heat Treatment on *in situ* Ruminal Degradation of Different Vegetable Protein Meals

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ABSTRACT

The study was conducted to determine the effect of different levels of formaldehyde and heat treatment of maize gluten, rapeseed, sunflower and cottonseed meals on rumen undegraded protein (RUP) value in a fistulated male buffalo calf. Protein sources were treated with formaldehyde at 0, 0.5, 1.0 and 1.5%, and also subjected to heat through autoclaving for 0, 30, 45 and 60 minutes. The nylon bags each containing 10 g sample were incubated for 24 h. Maximum RUP value in maize gluten, rapeseed, sunflower and cottonseed meal was observed as 97.09, 89.75, 79.25 and 50.59%, by formaldehyde treatment at 1.0, 1.5, and 1.5, and 1.0%, respectively. While maximum RUP value in maize gluten, rapeseed, sunflower and cottonseed meal was observed as 91.09, 50.79, 15.89 and 64.05%, respectively using heat treatment trough autoclaving for 60 minutes.

Key Words*:* Formaldehyde; Heat treatment; Vegetable protein meals; Rumen degradation

INTRODUCTION

Protein is one of the most expensive constituents of the ruminant's diet. Under many feeding regimens most of the dietary protein fed to ruminants undergoes extensive modification due to microbial activity in the rumen. Consequently, nitrogenous compounds with low biological values are upgraded. Conversely a protein having a high availability and biological value in the non-ruminant may be altered in the rumen to lower availability and biological value, or both. (Peter *et al*., 1971).

Different methods have been used to overcome the degradation of protein in the rumen. Of which the two methods i.e. treatment of protein with "heat" and "aldehydes" are widely used (Orskov, 1982). In the present study *in situ* degradability of different vegetable protein meals was determined using these two techniques.

MATERIALS AND METHODS

Fistulated male buffalo calf used for the study was fed green fodder ad libitum i.e. berseem/oat and a supplement of concentrate mixture for containing maize gluten, rapeseed, sunflower and cottonseed meals. The forage and concentrate was offered twice a day. The following vegetable protein sources were subjected to heat and formaldehyde treatments:

1) Maize gluten meal (60%)
2) Rapeseed meal
3) Sunflower meal
4) Cottonseed meal

The samples of the meals were ground in a laboratory Wiley mill using 2 mm mesh screen.

Formaldehyde treatment. Individual protein sources (100 g) were placed in a dish and treated with formaldehyde at 0.50, 1.00 and 1.50% levels. The formaldehyde solution was sprayed on protein source. After thorough manual mixing the treated material was kept at room temperature for 24 hours. The material was stored in small plastic container for further use.

Autoclaving. The protein sources were oven dried. These were then auto-claved for 0, 30, 45 and 60 minutes at 15 pound steam pressure in a laboratory autoclave.

The nylon bags (pore size 50 μm, bag size 10 cm x 20 cm, bag area 50 mg of DM/cm^2) contained feed samples (10 g each) were incubated for 24 hours in the rumen of male buffalo calf. All the sample bags were soaked in the 39^oC distilled water for 15 minutes before placing into the rumen (Lykos & Varga, 1995). Sample bags were inserted into the rumen in reverse sequence and were removed at the same time to reduce variation associated with washing procedure (Grigsby *et al.,* 1992). The bags were suspended in the rumen of male buffalo calf by nylon strings about 50 cm in length starting at 9.00 a.m. each bag was tied with 50 g stainless steel weight.

All the bags were immediately immersed in ice water upon removal from the rumen to stop microbial activity (Lykos & Varga, 1995). Then, the bags were washed in running tap water till the rinse was clear. The bags were then kept in the oven at 70^oC for 48 hours for drying till the constant weight (Gangadhar *et al.,* 1992). The dry matter and crude protein content of pre and post

incubation feed samples was estimated. Quadruplicate samples were used for each protein source and treatment. Ruminal disappearance of crude protein was computed without correcting for microbial contamination (Griffin *et al.*, 1993). The proximate analysis of protein sources were performed according to AOAC (1984) and presented in Table I.

The data on various parameters were subjected to statistical analysis. The one way analysis of variance (Complete Randomised Design) was used to test the significance of variables. The comparison of means were made by Duncan's multiple range test (Steel & Torrie, 1984).

Table I. Ingredient composition matrix

Composition (%)	Maize gluten meal	Rapeseed Meal	Sunflower meal	Cottonseed meal
Moisture	6.00	8.00	8.00	10.00
Dry matter	94.00	92.00	92.00	90.00
Crude protein	53.58	36.80	32.20	45.00
Crude fibre	1.00	13.50	23.43	15.30
Ether extract	5.34	2.73	3.07	3.12
Crude ash	2.73	10.07	8.93	7.40
NFE	31.35	28.90	24.37	19.18

RESULTS AND DISCUSSION

(a) Formaldehyde Treatment

Maize gluten meal (60%). Maximum RUP value of 97.09% was achieved at 1% formaldehyde treatment as compared with control (66.82%). The RUP values at 0.5 and 1.5% formaldehyde treatment were 93.68 and 89.55, respectively (Table II). Formaldehyde treatments at 0.5 and 1.5% were not different (P>0.5). Whereas, treatments between 1.0 and 1.5% level differed (P<0.05). It was observed that formaldehyde treatment of maize gluten meal at 0.5% level is sufficient and economical for maximum protein protection from rumen degradation. These observations are in accordance with the earlier studies (Titgemeyer *et al*., 1989; Chaturvedi & Walli 1995). These studies also suggest that maize gluten meal, 60% is naturally protected by heat during processing and have good protein bypass characteristics.

Rapeseed meal. The RUP values of rapeseed meal increased (P<0.05) with increase in level of formaldehyde. These observations are in range as reported in earlier studies. Lardy *et al.* (1993) observed 23%, Dakowski *et al.* (1996) estimated the bypass protein value of rapeseed meal to be 27%. Similar trends were observed by Herland (1996). Somewhat higher ruminal escape values (34.8 and 48%) of rapeseed meal were reported as compared with the present study by Subuh *et al*. (1996) and Prabhu *et al*. (1996).

Sunflower meal. The mean values revealed significant effect of different levels of formaldehyde (P<0.01) treatment of sunflower meal. Maximum RUP value of 79.25% was observed at 1.5% formaldehyde treatment. This represented 90.97% unit increase in RUP value as compared with control i.e. 7.15%. The RUP values were 78.67 and 68.90% at 0.5 and 1.0% formaldehyde treatment, respectively. Formaldehyde treatment of sunflower meal at 0.5 and 1.5% was not different (P>0.50), while treatments between 0.5 and 1.0%, 1.0 and 1.5% and all the levels of formaldehyde with control differed (P<0.05). These observations suggest that formaldehyde treatment at 0.5% level is better and economical for achieving highest RUP value of sunflower meal.

Cottonseed meal. Maximum RUP value of cottonseed meal was 50.59% at 1% formaldehyde treatment which is 59.28% unit higher than untreated meal. Prabhu *et al*. (1996) reported this value to be 21%. Narayan and Agrawal (1995) observed the bypass protein value of cottonseed cake of 24.87%. Pena *et al*. (1986) reported this value to be 15.3%. The results revealed that there was no difference (P>0.50) among different treatments, which suggest 0.5% formaldehyde treatment can also be used effectively. Van Soest (1982) observed that formaldehyde treatment decreased the disappearance of protein due to the formation of irreversible cross linkages between amino acids that are resistant to microbial action. These observations suggest that formaldehyde treatment forms acid-reversible cross linkages with amino and amide groups, rendering the protein insoluble at the pH of rumen (5.5-6.5) (Ferguson *et al.,* 1967).

b) Heat Treatment

Maize gluten meal (60%). The values showed significant (P<0.01) effect of different time periods for which maize gluten meal was autoclaved for protein

Table II. Mean ± SE%ages of RUP values of Maize gluten meal (60%), Rape seed meal, Sunflower meal and Cotton seed meal as Influenced by different levels of formaldehyde treatments at 24 hours incubation

Groups	Treatment	Maize Gluten Meal, 60%	Rapeseed Meal	Sunflower Meal	Cottonseed Meal
A, Control	0%	66.82^{c} ±1.61	19.62^{d} ±1.18	7.15^{c} ±0.58	20.6^{b} ±1.39
B	0.5%	93.68^{ab} ±1.06	74.62^{c} ±0.56	78.67^{a} ±3.24	46.29^{a} ±2.33
C	1.0%	97.09^{a} ±3.10	84.75^{b}±0.87	68.9^{b}±3.61	50.59^{a}±1.54
D	1.5%	89.55^{b} ±0.85	89.75^{a}±1.35	79.25^{a}±1.27	49.46^{a}±0.68

Means with different superscripts in the column indicate significant (P<0.05) differences

Table III. Mean ± SE%ages of RUP values of Maize gluten meal (60%), Rape seed meal, Sunflower meal and Cotton seed meal as influenced by different autoclaving time periods at 24 hours incubation.

Groups	Treatment	Maize Gluten Meal	Rapeseed Meal	Sunflower Meal	Cottonseed Meal
A, Control	0 min	66.82^{c} ±1.61	19.62^{d} ±1.18	7.15^{c} ±0.58	20.6^{b} ±1.39
B	30 min	87.27^{ab} ±0.82	47.33^{b} ±1.43	9.61^{b} ±0.51	40.92^{b} ±1.78
C	45 min	84.38^{b} ±1.68	41.53^{c} ±0.60	13.49^{a} ±0.85	43.0^{b} ±3.26
D	60 min	91.09^{a} ±1.31	50.79^{a} ±0.61	15.89^{a} ±1.36	64.05^{a} ±2.82

Means with different superscripts in the column indicate significant (P<0.05) differences.

protection in the rumen (Table III). It was maximum (91.09%) at 60 minutes autoclaving time, which is almost 26.64% unit higher than the untreated value. The RUP values at autoclaving times of 30 minutes and 60 minutes, 30 minutes and 45 minutes did not differ (P>0.50) which suggest that autoclave treatment for 30 minutes may be considered optimum for protein protection of maize gluten meal.

Rapeseed meal. The mean RUP values of heat treated rapeseed meal were higher (P<0.05) than control and maximum (50.79%) was observed at 60 minutes autoclaving. Similar trends was observed by Mckinnon *et al*. (1995), they reported that short duration heating of rapeseed meal to 125°C was an effective method of increasing rumen undegradable protein contents without reducing intestinal digestibility.

Sunflower meal. The maximum RUP value was 15.89% at 60 minutes autoclaving time as compared to untreated value of 7.15%. There was no difference between groups A and B, C and D. The results revealed that autoclaving of sunflower meal for 30 minutes was not effective in the protection of protein degradation whereas, autoclaving for 45 minutes was effective and economical for protein protection.

Cottonseed meal. The RUP values showed significant (P<0.01) effect of different time periods for which cottonseed meal was autoclaved for protein protection. The results revealed that protected protein values increased by increasing the time periods of autoclaving. It reached maximum value of 64.05% at 60 minutes autoclaving time. This is 67.83% units increase than the untreated value. The present findings are in agreement with Tagari *et al.* (1986). They observed that autoclaving of cottonseed for 60 minutes was effective in reducing ammonia N concentration in vitro. Similar observations were recorded by Schroeder *et al*. (1995). They observed that as the temperature and time of processing increased the crude protein disappearance from the rumen decreased in cottonseed cake and the passage of undegraded protein to the small intestine increased. Broderick and Craig (1980) reported that heat treatment of feedstuffs can decrease proteolysis by blocking reactive sites for microbial proteolytic enzymes. The reduction in protein degradation after heating could be due to the denaturation of proteins blocking the lysine and arginine residues being the reaction sites from trypsin-like enzymes of rumen microbes.

CONCLUSION

The findings of the present study suggest that to achieve higher rumen by-pass values maize gluten meal (60%) and sunflower meal should be treated with 5% formaldehyde whereas, for cotton seed meal heat treatment through autoclaving for 60 minutes gave better results. While comparing both treatments formaldehyde treatment is practicable and economical.

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(Received 11 March 2000; Accepted 28 March 2000)