



Optimization of roasting condition for soybean cake evaluated by *in situ* protein degradability and N-fractionation method

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ABSTRACT

The present investigation was carried out to optimize temperature of roasting of soybean over a fixed time period of 30 min to make it a bypass protein. As the temperature of roasting increased, the effective protein degradability of soybean cake decreased and a reasonably good effect was seen when soybean cake was roasted at 160°C for 30 min, as reflected by a marginal increase in UDP content of the cake at 170°C. The ADIN (acid detergent insoluble N) content of the cake also increased with the increase in the roasting temperature and there was hardly any further increase in UDP content after 160°C temperature. Protein fractionation on the basis of solubility in three solvents, namely, phosphate buffer, NDS (neutral detergent solution) and ADS (acid detergent solution) also gave the similar indication as the *in situ* protein degradation. As the temperature of roasting increased, A + B₁ fraction which relates to RDP (rumen degradable protein) fraction decreased and correspondingly, the B₂ fraction, which is the main component of UDP (undegradable dietary protein) increased. On the basis of N degradability in rumen and N solubility results, it could be concluded that the optimum roasting temperature for making soybean cake a good source of bypass protein is 160°C for 30 min.

Key words: ADIN, ADS, Roasting, RDP, Soybean cake, UDP

The whole soybean is a rich source of both energy as well as protein. The protein degradability of soya protein in rumen is moderate and not very high and it can be further reduced if same is subjected to physical (heat) or chemical (formaldehyde) treatment, so that protein proportion of the cake bypass the rumen as undegradable or bypass protein. Protecting highly degradable protein sources like GN cake and mustard cake by formaldehyde treatment have given very good results in terms of increased growth rate and milk production in cattle, buffaloes and goats (Chatterjee and Walli 2003a,b).

Heat treatment of whole soybean also enhances its palatability and the heat also destroys the anti-nutritional factors contained in raw beans. Roasting of soybean is the most common heat treatment given to this proteinous feed and the roasted soybean can be incorporated even in the complete feed blocks, to act as a concentrated source of

energy and protein, for high yielding dairy cows producing 15 to 20 kg of milk per day. Both, the cultivation of soybean, as well as its use as feed for dairy cattle has not been taken up seriously in India, though it a most proteinous high diet for poultry feed.

Formaldehyde treatment of cakes (GN cake, mustard cake and soybean cake) effectively reduces their protein degradability and feeding of such treated cakes have improved the performance of the animals through histopathological experiments in terms of growth and milk production. Thus, the present investigation was under taken to assess the effectiveness of roasting treatment, to optimize roasting condition (roasting temperature for soybean) in terms of bypassability of its protein.

MATERIALS AND METHODS

The roasting of soybean cake was undertaken at a commercial factory situated at Khanna, Punjab. Roasting of the cake was done at different temperatures, viz. 140, 150, 160 and 170°C for 30 min.

Protein quality evaluation: The samples of soybean cake either raw or roasted at different temperatures were evaluated for protein quality, using *in situ* nylon bag technique and *in-vitro* N-fractionation method.

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In sacco *nylon bag technique*: The method of Mehrez and Orskov (1977) and later modified by Orskov and Mc Donald (1979) was used for suspension and removal of nylon bags in the rumen, and for estimating the effective degradability of the raw and heat treated soybean cake.

Pre-weighed nylon bags (9 cm × 15 cm with 40 µ pore size) containing 5 g of milled samples ground to pass through 2.5 mm sieve were tied with nylon threads and fastened tightly to 3 iron chains. The samples in the bags were raw soybean cake and soybean cake roasted at 140, 150, 160 and 170°C for 30 min and the formaldehyde treated cake (1.2 g of HCHO/ 100 g of cake protein). The chains containing the bags were then placed in the rumen of 3 fistulated animals so that the observation for each ingredient could be recorded, in triplicate, in 3 animals. The bags were taken out at regular intervals of time, viz. 8, 12, 24, 36 and 48 h. After removal from the rumen, the bags were washed thoroughly by swirling vigorously in plastic trough filled with a continuous supply of fresh water, till the water in the trough was clear. The bags were drained off to get rid of excess water and then dried in oven at 65 to 70°C for first 24 h. The lumps formed inside the bags were broken manually and the material was thoroughly mixed, followed by drying at 90°C till constant weight. The dried bags after desiccation were weighed again in order to determine the dry matter loss at different hours for each of the cake samples. The residue left in each bag was subjected to nitrogen estimation by micro-Kjeldahl's technique for measuring protein disappearance.

In vitro N-fraction by CNCP system: The method of Chalupa and Sniffen (1996) for partitioning of proteins was adopted by subjecting the feedstuffs to digestion in different solvents / detergents to compare solubility (insolubility) of 5 samples of soybean cake roasted at 140, 150, 160 and 170°C and compared with the raw soybean cake and the HCHO treated cake. All the roasted, HCHO treated and raw cake samples were subjected to analysis of different N fractions, using solubility in phosphate buffer, neutral detergent solvent and acid detergent solvent. From this, the highly degradable fraction, partially degradable fraction and totally undegradable fractions were determined and the values were correlated with sacco nylon bag technique. The phosphate buffer soluble fraction (A + B₁) corresponds to non-protein nitrogen, all globulins and some albumins. The fraction B₂ (PBIN-NDIN) is mostly rest of the albumins and all glutelins. The fraction B₃ (NDIN-ADIN) is mostly prolamins,

extension protein and denatured proteins. Fraction C (ADIN) corresponds to Maillard products and nitrogen bound to lignin, which is totally undigestible in whole of the digestive tract.

RESULTS AND DISCUSSION

Rumen degradability of cake samples

Ruminal DM disappearance: At 8 h incubation, the DM degradability of raw cake as well as cake roasted at different temperatures, viz. 140°C, 150°C, 160°C and 170°C had the lowest value, whereas at 48 h incubation the values were the highest. As the roasting temperature increased, the DM disappearance decreased, and the decrease being higher at higher temperatures, viz. 160°C and 170°C (Table 1).

The raw soybean cake is more degradable in rumen even at 8 h, compared to soybean roasted at different temperatures. Chatterjee and Walli (2003a) found similar trend in DM disappearance between untreated and formaldehyde treated (at different levels) mustard cake and groundnut cake. These results suggest that the roasting of soybean at higher temperature, especially above 150°C, decrease its DM disappearance in rumen.

Ruminal CP disappearance: Crude protein is heterogeneous mixture of non-protein nitrogen (NPN) and true protein. In rumen, non-protein nitrogen and some of the other highly degradable proteins are solubilized quickly and disappear from the nylon bag during incubation. There is a wide variations in the protein degradability of feed proteins, which can be attributed to their molecular structure. Heat treatment (roasting) can cause some changes in the molecular structure of proteins, which can reduce their degradability in rumen.

Raw and roasted soybean cake samples had lower CP degradability value (Table 2) at 8 h incubation and the highest value was at 48 h incubation. CP disappearance also increased as the incubation period increased. However, as the temperatures of roasting increased, CP disappearance decreased. The CP disappearance for raw soybean, was highest for all the time periods in comparison to roasted sample. As temperatures increased, CP disappearance decreased. Similar trend was observed by McNiven *et al.* (1994) on roasting of soybean. The 48 h CP disappearance at 160°C and at 170°C was 59.0±0.84 and 57.0±2.03% respectively.

Table 1. DM disappearance from raw and roasted cake at different temperatures for 30 min

Soybean cake	8 h	12 h	24 h	36 h	48 h
Raw soybean cake	61.11±1.74	72.71±0.41	90.50±0.95	97.01±1.94	98.21±0.11
140°C	58.03±0.86	69.71±0.32	85.0±3.82	93.5±0.64	96.17±0.14
150°C	55.51±0.92	67.98±0.30	87.2±0.65	92.37±0.32	95.13±0.14
160°C	54.42±0.62	66.99±0.20	85.39±0.54	92.0±0.92	95.00±1.45
170°C	52.42±0.32	64.49±0.55	89.50±0.40	92.0±3.06	93.84±0.37

Table 2. CP disappearance from raw and roasted soybean cake at different temperatures for 30 min

Soybean cake	8 h	12 h	24 h	36 h	48 h
Raw soybean cake	25.5±1.83	35.0±0.84	55.0±1.73	70.0±3.61	74.0±1.17
140°C	17.0±0.87	25.0±0.71	52.5±1.39	62.0±0.65	68.0±1.57
150°C	20.0±1.15	35.0±2.38	50.0±6.69	58.0±2.85	64.0±2.58
160°C	22.0±1.99	35.0±2.39	49.0±1.96	57.0±0.92	59.0±0.84
170°C	20.0±1.61	28.0±2.39	42.0±2.37	52.0±2.92	57.0±2.03

Table 3. ECPD, RDP and UDP of raw and roasted soybean cake, roasted at different temperatures for 30 min

Sample	ECPD	CP (%)	RDP (%)	UDP (%)
On DM basis				
Raw	58.50	45.00	26.32	18.67
140°C	50.20	45.00	22.59	22.41
150°C	48.30	45.00	21.73	23.27
160°C	46.00	45.00	20.70	24.30
170°C	45.10	45.00	20.29	24.71

Percent ECPD, RDP and UDP content of raw and roasted Soybean cake at different temperatures: RDP and UDP contents of cakes (% DM basic) roasted at different temperatures are presented in Table 3. Thus, as the roasting temperatures increased, the RDP content decreased and correspondingly the UDP content increased. Chatterjee and Walli (2003a) also reported a similar reverse trend for RDP and UDP values of GNC and mustard cake after subjecting the cake to different levels of formaline treatment, with RDP showing a decreasing trend and UDP an increasing trend.

The results showed that raw soybean is somewhat higher in RDP, whereas after roasting soybean cake, the value decreased uniformly as the roasting temperature increased from 140°C to 170°C. Thus, the UDP content of soybean increased after heat treatment and was highest at 170°C (24.71%), which was only slightly higher than at 160°C (24.30%) of heat treated using the weighted averages of the potential degradability and the computer programme as per Orskov and Mc Donald (1979), the effective protein degradability of the raw and treated soybean were estimated. It can be clearly predict that the ECPD decreased (Table 3) with increasing the temperature (58.50 in raw to 46.00 in roasted at 160°C).

N-fractionation of roasted soyabaen cake samples: The data on different N fractions (% of total N) of cakes is shown in Table 4, A + B₁ fraction corresponds to the phosphate buffer soluble N (PBSN) and the fraction 'C' corresponds to ADIN. B₂ fraction correspond to N fraction which is less degradable in rumen, but mostly degradable in lower tract where as, B₃ is undegradable in rumen and partly digestible in the lower tract. The B₂ fraction (i.e. PBIN-NDIN) in raw soyabeen cake was 63.30%, which subsequently increased with the increase in temperatures, the values being 66.03, 67.90, 70.89 and 71.12%, respectively while, A + B₁ showed a decreasing trend from 35.22% in raw cake to 22.88% in cake roasted at 170°C for 30 min, but there was no definite trend seen in B₃ and C fractions. The A + B₁ fraction appeared to be positively related to ECPD, but B₂ could be negatively related to ECPD. As far B₃ and C fractions, these may be of lesser importance in influencing the ECPD value of feeds, compared to B₂ and A + B₁ fraction. Different N fractions (Table 5) were significant as also reported by Chalupa and Sniffen (1996) from where we can see that A + B₁ fraction is the highly degradable N fraction in rumen, which includes NPN, globulins and some albumins. The B₂ fraction is less degradable N fraction in rumen and partly digestible in the lower tract. It includes mostly albumins and glutelins. The B₃ fraction is least degradable in rumen and includes prolamins, extension proteins and denatured proteins. The C fraction includes Maillard products, N bound to lignin or even condensed tannins, and is almost totally undegradable N fraction not only in rumen, but also in the lower tract. Sharma and Singh (1997) and Mondal and Walli (2003) have given a database on protein fractions for different oil cakes, grains and their by-products. The above authors also indicated that increase in A + B₁ fraction increases the degradability value, whereas the increase in B₂, B₃ and C fraction could

Table 4. Different nitrogen (% of total N) fractions in raw and roasted soybean, roasted at different temperatures for 30 min

Sample (soybean cake)	A + B ₁ (PBSN)	B ₂ (PBIN- NDIN)	B ₃ (NDIN- ADIN)	C (ADIN)	ECPD
Raw (without roasting)	35.22	63.30	0.405	1.08	58.5
140°C	32.28	66.03	0.780	0.81	50.2
150°C	29.67	67.90	2.008	0.482	48.3
160°C	25.88	70.89	2.14	1.09	46.0
170°C/30 min	22.88	71.12	3.36	2.64	45.1

Table 5. Different N fractions and their significance as per Cornell net carbohydrate protein system (CNCPS)

N fractions	Derived as (% of total N)	Significance
A + B ₁	PBSN (phosphate buffer soluble N)	A, NPN (i.e., NH ₃ , NO ₃ , AA peptides etc.); B ₁ , Globulins and some albumins (highly degradable in rumen)
B ₂	PBIN – NDIN (phosphate buffer insoluble N – Neutral detergent insoluble N)	Mostly albumins and glutelins (less degradable in rumen but digestible in lower tract)
B ₃	NDIN – ADIN (NDIN – acid detergent insoluble nitrogen)	Prolamins, extension proteins and denatured proteins (very less degradable in rumen and also less digestible in lower tract)
C	ADIN (acid detergent insoluble nitrogen)	Maillard products, N bound to lignin etc. (Almost totally undegradable in both rumen or lower tract)

Chalupa and Sniffen (1996)

Table 6. Prediction equations to derive effective crude protein degradability (ECPD) of raw, roasted soybean cake from various soluble/insoluble protein fractions

	r	R ²
1. ECPD = 33.63424026 + 0.89824611 (A + B ₁) – 0.174329925 (B ₂) – 0.2541073921 (B ₃) + 1.398296608 (C)	0.73	0.53
2. ECPD = 46.256225949 + 0.8253277191 (A + B ₁) – 0.320238478 (B ₂) – 0.419856296 (C)	0.72	0.52
3. ECPD = 42.73418756 + 0.810303725 (A + B ₁) – 0.251985896 (B ₂).	0.71	0.51
4. ECPD = 22.50895891 + 0.917516113 (A + B ₁)	0.70	0.50

Figures in parenthesis ('R²' values) are multiple regression coefficients and ('r') values are correlation coefficient. Correlation coefficients of A + B₁, B₂, B₃ and C with ECPD were 1.0, –0.52, –0.45, –0.38 and 0.70, respectively.

decrease the ECPD value of feed stuffs. Chatterjee and Walli (2003a) evaluated formaline treated MC and GNC for different N fractions by CNCPS system as well as effective protein degradabilities of the same cakes by *in sacco* method.

Prasad and Mudgal (1979) also reported that nitrogen solubility of the roasted soybeans was lower than that of the raw soybeans. Faldet *et al.* (1992) reported that heat treatment of soybeans had little effect on ADIN content, except for soybeans heated at 160°C for 90 and 120 min. Thus, the effect of heat treatment is a function of both temperature and time of heat exposure. At higher temperature like 160°C for 30 min there is an increase in the ADIN content of the cake, as seen in the present study (Table 4).

Prediction equation for ECPD: Prediction equations were drawn on the basis of correlations at different N fractions with the ECPD, to derive effective crude protein degradability (ECPD) of raw and roasted soybean (Table 6). The correlation co-efficient 'r' value shows that the prediction of effective protein degradability based on N fractions, using different combinations, in multiple regression equation were high, it varied from 0.73, when all the four N fractions, viz. A + B₁, B₂, B₃ and C, to 0.71, when only A + B₁ fraction was considered.

Sharma and Singh (1997), Chatterjee and Walli (2003b) and Mondal and Walli (2003) also found very high correlation coefficients to predict the effective protein degradability from different N fractions obtained by Lab method.

On the basis of the protein degradability and N-fractionation of soybean cake, it was concluded that roasting at 160°C for 30 minutes was the optimum time-temperature combination for soybean to make it a good bypass protein.

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REFERENCES

- Chalupa W and Sniffen C J. 1996. Protein and amino acid nutrition in lactating dairy cattle—today and tomorrow. *Animal Feed Science and Technology* **58**: 65–75.
- Chatterjee A and Walli T K. 2003a. Economics of feeding formaldehyde treated mustard cake as bypass protein to growing buffalo calves. *Indian Journal of Dairy Science* **56** (4): 241–44.
- Chatterjee A and Walli T K. 2003b. Effect of formaldehyde treatment on effective protein degradability and *in vitro* post-ruminal digestibility of mustard cake. *Indian Journal of Animal Nutrition* **20** (2): 143–48.
- Faldet A, Martin O, Larry D, Satter and Broderick A G. 1992. Determining optimal heat treatment of soybeans by measuring available lysine chemically and biologically with rats to maximize protein utilization by ruminants. *Journal of Nutrition* **122**: 151–60.
- McNiven M A, Hamilton R M G, Robison P H and DeLeeuw J W. 1994. Effect of flame roasting on the nutritional quality of common cereal grains for non-ruminants and ruminants. *Animal Feed Science and Technology* **47** (1–2): 31–40.

- Mehrez A Z and Orskov E R. 1977. A study on the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *Journal of Agricultural Science* (Cambridge) **88**: 645.
- Mondal G and Walli T K. 2003. *In vitro* post-ruminal protein digestibility of chemicals used in feed ingredients and prediction for CNCV based protein fractions. *Indian Journal of Animal Nutrition* **20** (3): 339–44.
- Orskov E R. and McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *Journal of Agricultural Science* (Cambridge.) **92**: 499.
- Prasad C S. and Mudgal V D. 1979. Effect of feeding protected proteins on the growth rate, feed utilization and body composition of goats. *Indian Journal of Dairy Science* **32** (4): 104.
- Sharma R and Singh B. 1997. N solubility and dietary protein fractions of various feedstuffs. *Indian Journal of Animal Nutrition* **14**: 139–42.

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