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Effect of feeding protected protein on productive performance in dairy cows

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ABSTRACT

An experiment was conducted on lactating crossbred cows for assessing feeding effect of heat treated (HTSBM) and formaldehyde treated (FTSBM) soybean meal on productive performance of crossbred cows. Untreated soyabean meal (UTSBM) served as control group and HTSBM and FTSBM as treatment groups. Feeding of roughage and concentrate mixture having soybean meal (untreated) was offered in UTSBM group; while concentrate mixture with heat treated and formaldehyde treated soybean meal was offered to HTSBM and FTSBM group, respectively (n=8). Results showed that feeding HTSBM and FTSBM did not have significant effect on average dry matter (DM) intake (kg/Day) of cows. The cows fed HTSBM and FTSBM diets had a higher dry matter ($P<0.05$) and crude protein ($P<0.01$) digestibility than cows fed UTSBM diet. However digestibility of all other nutrients did not differ significantly among groups. Heat and formaldehyde treatments reduced ($P<0.01$) rumen degradable protein and increased ($P<0.01$) undegradable dietary protein in soybean meal. The average daily milk yield and fat corrected milk yield (kg/Day) was significantly higher ($P<0.01$) in HTSBM (9.01 ± 0.14 and 9.42 ± 0.17) and FTSBM (8.93± 0.14 and 9.30 ± 0.18) groups compared to UTSBM (7.70 ± 0.98 and 8.12 ± 0.16) group. The milk parameters like fat, protein, lactose, total solid and solid not fat did not differ significantly among groups. The feed cost per kg of milk was lower in HTSBM and FTSBM groups compared to UTSBM group.

Keywords: Crossbred cows, Milk yield, Protected proteins, Rumen degradable protein, Undegradable dietary protein

Scientific and balanced feeding of dairy cattle is very important for successful dairy farming. A well balanced ration must fulfill the nutritional need of energy, protein, vitamins and minerals in animals for optimal health and production. Out of all the nutrients, protein has a vital role in growth, production and repair of body tissue. Thus, it is essential that efficient measures are taken to ensure their effective utilization by the animals. Microbial protein has a significant contribution in supplying amino acid to the host animal but it may not be sufficient to meet the requirement of high producing dairy animals. To meet the dietary requirement, ruminants depends largely on the proportion of ruminally undegraded protein (RUP) having high total digestibility. In such situation, by pass proteins or protected protein can act as an important source of amino acids to meet the increased demand of early lactating or fast growing animals.

The proteineous oil cakes having higher rumen degradability produce higher amount of ammonia, which gets excreted as urea through urine. Moreover, for conversion of ammonia to urea in liver, animal has to spent energy. Therefore, to increase the energy efficiency of protein utilisation of these feeds, the protein needs to protected from ruminal degradation so that it can be absorbed from small intestine of animal for synthesis of tissue protein as well as gluconeogenesis in liver (Walli, 2005). Therefore, these oil cakes need to be treated to protect its protein from excessive microbial degradation so that the animal gets the benefit of its high quality protein and amino acid profile. Heat treatment and formaldehyde treatments of oil cakes are efficient methods to protect their protein supplements. Feeding bypass protein in ruminants cause significant reduction in wasteful ammonia production in the rumen from dietary proteins and an increase in proportionate production and in the supply

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of amino acids to the host ruminants for production and reproduction purposes (Walli, 2008). Various researchers (Dosky *et al.* 2012; Tiwari *et al.* 2018) observed improved milk production and milk composition on feeding bypass protein in dairy animals.

To further explore these concepts, an experiment was conducted to evaluate the effects of feeding untreated, heat treated or formaldehyde treated soybean meal (as a form of protected protein) on the productive performance of crossbred cows. The findings from this study aim to contribute to the understanding of how different protein treatments affect dairy cow performance, with the potential to enhance dairy production systems overall.

MATERIALS AND METHODS

Ethical statement: The study was conducted in accordance with the ethical standards and guidelines of the Institutional Animal Ethics Committee (IAEC) of ICAR-Research Complex for Eastern Region, Patna, India.

Experimental procedure: A feeding trial was conducted in the Instructional Livestock Cattle Farm, ILF(C), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam for 120 days. Twenty four crossbred cows (Holstein Friesian × Jersey) based on milk yield and parity was selected for the experiment with eight animals in each group using Randomized block design (RBD) technique and they were allotted to three dietary treatments i.e. UTSBM, HTSBM and FTSBM. The experimental animal diets were formulated based on the guidelines provided by ICAR (2013) using Para, Napier, Paddy straw and concentrate mixture. The concentrate ration was prepared by using conventional feed ingredients consisted of maize 47 parts, wheat bran 20 parts, rice polish 15 parts, soybean meal 15 parts, mineral mixture 2.5 parts and common salt 0.5 parts. Feeding of roughage and concentrate mixture having untreated soybean meal was offered in UTSBM group; while concentrate mixture with heat treated soybean meal and formaldehyde treated soybean meal was offered to HTSBM and FTSBM group, respectively. The chemical composition of the rations used for different experimental groups has been given in table 1. The roughage to concentrate ratio of 60:40 was maintained for all the diets.

For heat treatment, the soybean meal was first spread in aluminium tray. Then the trays were placed in hot air oven and the soybean meal was heated for two hours at temperature of 140°C (Faldet *et al.* 1992). For formaldehyde treatment of soybean meal, formaldehyde was used at the rate of 1.2 g formaldehyde per 100g crude protein (Sirohi *et al.* 2013). For practical purpose 13.5 ml of (37%) formalin was poured used per kg of soybean meal, mixed thoroughly with formalin and then packed in a sealed plastic bag for 7 days. Afterwards, it was aerated before being fed to cows

Data collection: During the experimental period, all animals were given free access to clean drinking water and weighed quantity of feeds was offered twice a day at morning and evening throughout the feeding trial. A seven day digestibility trial was conducted at the end of the feeding experiment. Daily feed intake was measured by weighing the feed offered and the refusals within a 24-hour period throughout the experimental phase. Feed samples and residuals from each cow were collected and quantified daily. Similarly, the total faeces excreted by each cow were collected and weighed every day. All daily samples of feed offered, feed residues, and faeces corresponding to each treatment were pooled and thoroughly mixed before being subsampled for further analysis. The proximate analysis of the feeds, fodders and faeces were carried out as per AOAC (2007). Fibre fraction was fractioned according to the method of Van Soest *et al*. (1991). In vitro crude protein (CP) degradability, undegradable dietary protein (UDP) and rumen degradable protein (RDP) of soybean meal were estimated as per Licitra *et al.* (1998).

Daily milk yield of individual cow was measured using weighing balance. Milk composition of each animal was determined at interval of 15 days using ultrasonic milk analyzer. Fat corrected milk (FCM) was calculated according to Overman and Gaines (1933).

Statistical analysis: The experimental data were presented using basic statistics viz., mean and standard

Table 1. Chemical composition (% DM) of concentrate mixtures (UTSBM, HTSBM and FTSBM), paddy straw, green fodder used during feeding trial (on percent DM basis)

Particulars	Concentrate mixtures			Para	Napier	Paddy Straw
	UTSBM	HTSBM	FTSBM			
Dry matter	88.87	89.15	88.96	18.53	22.38	90.11
Organic matter	90.10	90.55	90.48	88.21	87.39	80.70
Crude protein	16.10	16.00	15.98	9.92	9.40	2.89
Ether extract	2.47	2.61	2.55	1.70	2.69	0.91
Crude fibre	10.62	10.54	10.49	26.83	23.55	32.31
NFE	60.91	61.40	61.46	49.76	51.75	44.59
NDF	21.92	22.24	21.85	56.12	71.20	69.92
ADF	14.51	14.60	14.47	34.63	43.10	49.62
Total ash	9.90	9.45	9.52	11.79	12.61	19.30

NFE-Nitrogen-free extract; NDF-Neutral detergent fibre; ADF-Acid detergent fibre

error. For analysis of data, one way ANOVA techniques were used and data were analyzed using SPSS software version 20.

RESULTS AND DISCUSSION

This research assessed the effect of heat treated and formaldehyde treated soybean meal in the feed of crossbred lactating cows on feed intake, nutrient digestibility, rumen degradability of protein, milk yield and its composition and feed cost.

The inclusion of heat treated and formaldehyde treated soybean meal to the diet had no significant impact on dry matter intake (DMI) per day, dry matter intake per 100 kg body weight and dry matter intake per kg of metabolic body size (Table 2). Heat treated soybean meal has been shown to increase the availability of RUP, which in turn enhanced nutrient intake and milk yield. However, previous studies found that while heat treatment can improve nutrient intake, it does not necessarily affect DMI significantly (Chesini *et al.* 2022; Pereira *et al.* 2021). This aligns with the findings of the current experiment where no significant difference was observed in DMI between control and heat treated group.

In support of the present findings, replacing dried distillers grains with solubles with heat treated soybean meal in forage-based growing cattle diets found no significant differences in average daily gain, dry matter intake, or gain:feed ratio among the treatments (Gullickson *et al.* 2023). Savari *et al*. (2024) also observed that the inclusion of heat treated soybean meal as a rumen undegradable protein source in diet of lactating Holstein cows did not affect the dry matter intake when compared with fish meal and meat meal, under iso-nitrogenous dietary conditions. This implies that heat treated soybean meal do not have adverse effect on dry matter intake, which is consistent with the current data showing no significant differences in dry matter intake. Likewise, although higher formaldehyde treatment levels soybean meal on sheep performance improved dry matter consumption, the differences were not statistically significant when compared to soybean meal which was not treated (Arifuddin *et al.* 2022). This corroborates current research showing that formaldehyde treatment has no significant effect on dry matter intake of cows.

The apparent digestibility of dry matter (DM) and crude protein (CP) increased significantly in both HTSBM and FTSBM groups as compared to UTSBM group (Table 2). This suggests that both treatments are equally effective in enhancing nutrient digestibility. The higher digestibility of DM in HTSBM and FTSBM groups could be a result of the removal of anti-nutritional factors and the improvement of protein availability with these treatments. Similarly, heat treatment of soybean meal has been reported to enhance the intake of NDF and CP, which are important for enhanced nutrient utilization and absorption (Chesini *et al.* 2022). Likewise, Patel *et al*. (2012) also observed higher DM and CP digestibility on feeding by pass protein in buffalo heifer. Heat treatment and formaldehyde treatment both enhance the rumen undegradable protein (RUP) content, which is less prone to degradation in the rumen and more available for absorption in the intestines (Chesini *et al.* 2022; Fessenden *et al.* 2020). This results in higher CP digestibility, as observed in the current study. In addition, efficiency of microbial protein synthesis in the rumen has been reported to be increased by soybean meal treated with enzymes, which also raises CP and amino acid absorption in small intestine (Ansia *et al.* 2021).

Inclusion of HTSBM and FTSBM in the diet did not affect the digestibility of ether extract (EE), crude fiber (CF), NFE, organic matter (OM), NDF and ADF. Lack of significant differences among groups in terms of OM, CF, EE, NFE, NDF and ADF digestibility (Table 2) indicated that treatments improved protein digestibility without negatively effecting the digestibility of other nutrients. This is in agreement with studies of Chesini *et al.* (2022); Fessenden *et al.* (2020) Cueva *et al.* (2023) and Marques

Table 2. Average dry mater intake and apparent nutrient digestibility of organic nutrients in different experimental groups

Parameters	Experimental groups			P value
	UTSBM	HTSBM	FTSBM	
Dry matter intake (kg/Day)	10.90 ± 0.04	11.10 ± 0.07	10.97 ± 0.07	0.12
Dry matter intake per 100kg body weight (kg)	3.34 ± 0.07	3.40 ± 0.07	3.35 ± 0.05	0.78
Dry matter intake /W $^{0.75}$ kg (g)	141.92 ± 2.21	144.44 ± 2.22	142.32 ± 1.31	0.63
Apparent nutrient digestibility (%)				
Dry matter	60.05^{a} ± 0.82	63.30^{b} ± 0.31	62.73^{b} ± 0.31	0.04*
Organic matter	60.01 ± 0.81	62.07 ± 0.34	61.59 ± 0.30	0.06
Crude protein	63.43^{a}± 0.81	63.43^{b}± 0.81	66.89^{b}± 0.48	0.01**
Ether extract	71.89 ± 2.27	72.92 ± 0.75	73.12 ± 1.01	0.827
NFE	57.43 ± 0.83	58.91 ± 0.61	58.48 ± 0.39	0.288
NDF	57.74 ± 1.84	59.16 ± 2.44	58.64 ± 1.27	0.870
ADF	58.47 ± 0.10	60.24 ± 0.60	59.75 ± 0.92	0.362

Means in a row with different superscripts (a, b, c) differ significantly at 5% probability level

Table 3. Crude protein, crude protein degradability, RDP and UDP percentages of untreated, heat treated and formaldehyde treated soybean meal

Particulars	Untreated soybean meal	Heat treated soybean meal	Formaldehyde treated soybean meal	P value
Crude Protein (%)	45.02 ± 0.01	45.03 ± 0.01	45.05 ± 0.03	0.64
Crude protein degradability (%)	58.80^{b} ± 0.03	50.01^{a} ± 0.02	50.01^{a} ± 0.03	<0.001**
Rumen degradable protein (%)	26.47^{b} ± 0.02	22.52^{a} ± 0.01	22.53^{a} ± 0.02	<0.001**
Undegradable dietary protein (%)	18.55^{a} ± 0.01	22.51^{b} ± 0.01	22.52^{b} ±0.01	<0.001**

Means in a row with different superscripts (a, b, c) differ significantly at 5% probability level

et al. (2023) where treated soybean meal enhanced protein digestibility but did not have significant impact on digestibility of other nutrient

The *in vitro* protein degradability of feed, particularly soybean meal, can be significantly influenced by various treatments such as heat and formaldehyde. In the present investigation, it was observed that the RDP fraction was significantly higher (P<0.01) in untreated soybean meal compared to HTSBM and FTSBM (Table 3). The proportion of undegradable dietary protein (UDP) was noticeably increased in soybean meal subjected to heat and formaldehyde treatments (Table 3). These findings suggest that both heat and formaldehyde treatments can effectively reduce RDP while enhancing UDP levels in soybean meal.

The reason behind the reduction in RDP and increase in UDP may be denaturation and cross-linking of proteins which reduce their accessibility to proteolytic enzymes (Okon *et al.* 2023). This aligns with various studies investigating effect of different treatment on protein degradability. For instance, protease from *Streptomyces griseus* has been found to effectively degrade protein in untreated feed but its efficacy diminishes in treated feeds due to structural alteration in proteins (Okon *et al.* 2023; Roe *et al.* 1991). Due to these treatments, protein undergo structural changes which modify protein matrix and impact solubility and accessibility. As a result, enzyme can not penetrate and hydrolyze the modified protein structure which reduce the rate of degradation (Okon *et al.* 2023; Assoumani *et al.* 1992). It has been observed that highly soluble proteins are generally more prone to enzymatic degradation, while those containing cross-linking disulfide bonds or other structural alterations are more resistant (Tomankova *et al.* 1995). This is consistent with the higher UDP observed in soybean meal treated with heat and formaldehyde in current investigation, as these treatments probably cause structural alterations. The increase in UDP percentages with corresponding reduction in RDP percentages in HTSBM and FTSBM indicated that these treatments were effective in increasing the undegradable protein content. This could results in improved protein utilization and overall performance in dairy cows, as affirmed by the findings from related studies (Paula *et al.* 2017; Kaufman *et al.* 2018; Chesini *et al.* 2022).

Milk and milk composition: The inclusion of treated soybean meal in the diets of dairy cows has been the focus of extensive research due to its ability to enhance milk production and composition. Particularly, researches on FTSBM and HTSBM has been performed to know their effects on milk and fat corrected milk (FCM) yield.

In the present study, the average daily milk and FCM yield of cows of HTSBM and FTSBM groups increased significantly (P<0.01) compared to those in UTSBM, while there is no significant difference in milk fat, protein, lactose, total solids, and solids not fat percentages among the groups (Table 4). This finding aligns with several studies that have assessed the impact of treated soybean meals on milk production and composition. A study on Karadi ewes revealed that feeding formaldehyde treated diets significantly increased daily milk yield and improved the yield of milk fat, protein and lactose compared to untreated basal diets (Mustafa, 2021). The improvement is explained by the protection of dietary proteins from

Table 4. Average milk yield, FCM yield, milk compositions and feed cost in different experimental groups

Parameters	UTSBM	HTSBM	FTSBM	P value
Milk yield (kg/Day)	7.70^{a} ± 0.98	9.01^{b} ± 0.14	8.93^{b} ± 0.14	<0.01**
FCM yield (kg/Day)	8.12^{a}± 0.16	9.42^{b} ± 0.17	9.30^{b} ± 0.18	<0.01**
Milk fat (%)	4.34 ± 0.20	4.30 ± 0.19	4.27 ± 0.20	0.74
Milk protein (%)	3.39 ± 0.05	3.42 ± 0.04	3.44 ± 0.06	0.18
Lactose (%)	4.51 ± 0.02	4.52 ± 0.04	4.54 ± 0.02	0.36
Total solid (%)	13.72 ± 0.40	13.76 ± 0.22	13.77 ± 0.21	0.94
Solid not fat (%)	9.29 ± 0.14	9.27 ± 0.13	9.33 ± 0.12	0.62
Total feed cost per day per animal (Rs.)	252.77	250.38	264.37	-
Feed cost per kg of milk (Rs.)	32.83	27.79	29.60	-

FCM, fat corrected milk

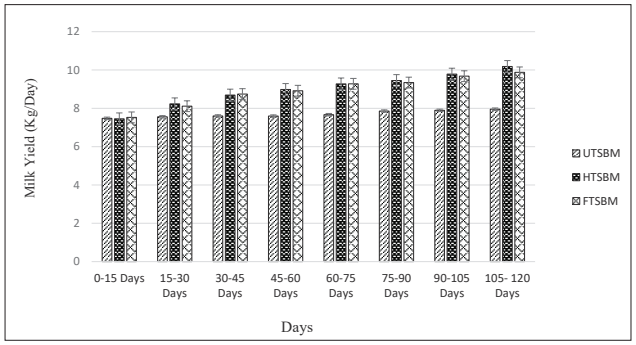

Fig. 1. Graphical representation of average milk yield by different experimental groups

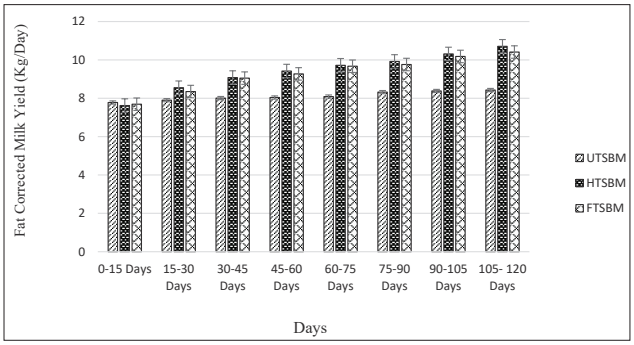

Fig. 2. Graphical representation of average fat corrected milk yield by different experimental groups

ruminal degradation, thus increasing the availability of amino acids for absorption in small intestine, which leads to higher milk production. Similarly, in dairy goats, the replacement of soybean meal with formaldehyde treated sesame meal resulted in the highest milk yield and energy corrected milk yield among all the dietary groups (Firozi *et al.* 2023). These results indicated that formaldehyde treatment effectively enhances the nutritional profile of the feed which improves lactational performance.

Heat treatment of soybean meal also benefited milk production. HTSBM could also improve milk production in mid lactation cows. The heat treated group also showed increase in energy corrected milk and protein yield (Chesini *et al.* 2022). The heat treatment may enhance the RUP concentration, which yielded a more consistent supply of amino acids necessary for milk production. Similarly, in another study, inclusion of heat treated soybeans in the diet increased the production of milk. This reflects improved nitrogen utilization and increased milk production efficiency (Pereira *et al.* 2021).

While heat treatment and formaldehyde treatment of soybean meal have been shown to improve milk yield, studies suggested that such treatments do not necessarily modify the compositional attributes of milk. In consistent with this, a study conducted on lactating cows investigating the effect of feeding formaldehyde treated soybean meal observed improved milk yield while the constituents of milk such as percentages of fats and proteins were comparable among different treatment groups. (Chore *et al.* 2017). Similar findings were reported by Petzel *et al*. (2024), who observed that feeding roasted soybeans to lactating cows enhanced energy corrected milk yield relative to unroasted soybeans, without affecting the proportions of milk fat, protein, lactose or solids-not-fat. This implies treated soybean meals improve milk yields but not the basic milk composition. Moreover, an experiment on mid-lactation cows supplemented with heat treated soybean meal or high-protein corn distillers grains did not show significant effect in the milk fat yield and concentration among the treatment groups (Chesini *et al.* 2022). This favors the idea that the main benefit of feeding treated soybean meals is its potential to enhance milk yield but not changing the milk composition. In contrast, a study by Firozi *et al.* (2023) on dairy goats substituting soybean meal with formaldehyde treated sesame meal reported significant increase in milk yield, as well as higher percentage of milk fat, protein and lactose. This variation might be due to differences in the metabolic responses of goats compared to cows, as well as the specific type of formaldehyde treated meal used.

Feed cost: The feed cost per kilogram of milk was found to be less in HTSBM and FTSBM group than in UTSBM group (Table 4). This is consistent with earlier studies that have investigated the effects of various treatments of soybean meal on performance and feeding cost of dairy cows. Gidlund *et al.* (2015) compared the impact of feeding soybean meal and heat-moisture treated canola meal (TCM) on milk yield and methane production of dairy cows. The findings reported that cows receiving TCM rations, which can be viewed as similar to formaldehyde treated soybean meal in terms of treatment aim, had more milk and milk protein output than those receiving SBM rations. The enhanced efficiency of milk production in cows fed on treated meal implies a better use of the dietary protein and this can be translated into lower feed costs per kilogram of milk. Likewise, another research observed that adding heat treated soybean meal to the diet of mid lactation dairy cows improved nutrient intake, milk production and reduced feed cost (Chesini *et al.* 2022). The trial proved that the cows fed with HTSBM consumed more NDF, CP and EE than the cows fed with untreated soybean meal (CON). This higher nutrient consumption would have helped to increase the milk yield seen in the cows fed heat treated soybean meal. The study also discovered that heat treated soybean meal fed cows had a reduced sorting index for feed particles, which would mean improved feed utilization and possibly reduced wastage of feed, which could help to lower feed costs per kilogram of milk produced.

The results of this study reveal that feeding of heat treated and formaldehyde treated soybean meal gave pronounced advantages over untreated soybean meal in lactating crossbred cows. The benefits of both treatments were comparable against ruminal alterations to soybean protein, as reflected by comparable nutrient utilization and milk yield. But a comparison between the two treatments showed that the cost of milk production was lower in the heat treated group compared to the formaldehyde treated

group. Thus, heat treatment of soybean meal is suggested as a more economical approach for increasing milk production efficiency in lactating animals.

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