RESEARCH ARTICLE

Effect of Feeding Formaldehyde Treated Rapeseed Meal on Metabolic Profile of Surti Buffalo Heifers

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Abstract

Present experiment was conducted on Surti buffalo heifers to compare the graded level (0%, 0.25%, 0.50%, and 1% CP) of formaldehyde (HCHO)-treated rapeseed meal (RSM) in compounded concentrate mixture (CCM) on various metabolic indices. A total of twenty Surti buffalo heifers (16-18 months old; 164.68±5.43 kg) were divided into 4 homogeneous groups of 5 individuals each. The CCM was formulated using formaldehyde-treated rapeseed meal (@ 0%, 0.25%, 0.50%, or 1% HCHO-treated RSM CP), damaged wheat, corn grit, cottonseed extract, rice polish (fine), deoiled rice bran, rice flake bran, molasses, urea, calcite powder and common salt. FT-0 group was fed with CCM having untreated RSM, while FT-25, FT-50 and FT-100 groups were fed CCM having 0.25 %, 0.5 % and 1% CP from HCHO treated rapeseed meal. Results revealed non-significant differences in the serum total protein, albumin, globulin, cholesterol, albumin: globulin ratio and aspartate transaminase and alanine transaminase activities among different groups of heifers. However, serum urea concentration decreased ($p<0.01$) due to increased formaldehyde treated RSM (0, 0.25, 0.5 and 1%) in the CCM. Serum T_3 level was found elevated ($p<0.05$) in the FT-50 and FT-100 groups, which revealed that formaldehyde treatment (@ 0.5 % and 1.0 %) had effectively destroyed the glucosinolate in rapeseed meal. Decreased serum urea and increased T_3 levels of FT-50 and FT-100 groups indicated the effectiveness of formaldehyde treatment to protect the rapeseed meal without altering other blood metabolites.

Key words: Formaldehyde (HCHO), Metabolic profile, Rapeseed meal, Surti buffalo heifers.

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Introduction

Protein is an important component of ruminant diet due to its significant contribution in tissue synthesis leading to growth. Rapeseed meal (RSM) has a high concentration of crude protein (averaging 36.3% of DM) and metabolizable energy (averaging 130 MJ/kg DM) (Kaldmae *et al*., 2010) as well as a well-balanced amino acid profile (Xiong *et al.,* 2012). Therefore, RSM is an excellent source of protein for animal feed. It has the greatest amount of rumen degradable total amino acids (Wang *et al.,* 2016), converted to ammonia in rumen which is used by rumen microbes for their own protein synthesis and rest is lost to circulation and converted to urea in liver and excreted via urine (McDonald *et al*., 2010). Moreover, RSM contains high concentrations of glucosinolate (GLS), known for a long to reduce the uptake of iodine, producing iodine efficiency (Burel *et al.,* 2000), hypertrophy of liver, kidney and thyroid (Tripathi *et al*., 2001), which could lead to lower feed conversion efficiency, impairment of growth, fertility, and reproduction in females (Burel *et al.,* 2001; Conaway *et al.,* 2002) and reduced testosterone levels and impaired semen quality in males (Pattanaik *et al.,* 2004)

HCHO treatment of dietary protein is one of the most common and exploited technique to protect it from ruminal degradation (Kanjanapruthipong and Buatong, 2002) and proven as novel method to limit these glucosinolates (Das and Singhal, 2005) through binding with it (Sahoo, 2002). It could be helpful in improving the bioavailability of amino acids along with prevention of ammonia-N loss of dietary protein (Kamalak *et al.,* 2005; Walli, 2005). Being a chemical compound, one might suppose that HCHO may affect the metabolism of nutrients and thus may exhibit

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pathological conditions in animals. In view of above concept, a feeding trial was conducted to assess the effect of feeding formaldehyde treated rapeseed meal on metabolic profile of Surti buffalo heifers.

Materials And Methods

Following approval of IAEC, 20 Surti buffalo heifers of 16-18 months of age with a mean body weight of 165±5.43 kg were selected from the elite herd of the Livestock Research Station, Navsari Agricultural University, Navsari (India). Experimental heifers were divided into four homogenous groups (five animals per group) based on their body weight. Heifers were housed in a confined, ventilated shed with a concrete floor and the facility of individual feeding and watering. The animals were let loose to exercise for 2 h in the morning and 1 h in the afternoon in an open padlock, during which they had free access to fresh, clean, wholesome drinking water. All the animals were vaccinated against common contagious diseases and dewormed with broad-spectrum anthelmintics before the onset of the experiment. Proper ethical care and management procedures were followed during the entire period of the experiment.

All the animals were fed individually to meet their protein and energy requirement for growth as per ICAR (1998). The ingredients of CCM included damaged wheat (4.0%), corn grit (4.0%), cottonseed extract (3.5%), rice polish (fine, 9.9%), deoiled rice bran (47.1%), rice flake bran (5.0%), molasses (11.0%), urea (1.0%), Calcite powder (2.0%) and common salt (2.0%) with rapeseed meal (10.0%) as protein source with formaldehyde treated RSM @ 0, 2.5, 5 and 10 % in FT-0, FT-25, FT-50 and FT-100 CCM, respectively. Animals were fed with basal diet consisting 5 kg green roughage and *ad libitum* dry roughage, along with required quantity of respective above CCM.

About 5 mL of blood sample was collected in sterilized vaccutainers from each animal in the morning (before watering and feeding) on 150th day (5 month) of experiment. Blood samples were centrifuged at 650 x g for 15 min and the serum collected was stored at -40^{o}C till further analysis. The serum concentrations of glucose, total protein, albumin, globulin, A:G ratio, triglycerides, total cholesterol, urea nitrogen (SUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by colorimetric method using spectrophotometer (Model No. 4001/1 Gensys 20, Thermo Fisher Scientific, USA) at appropriate wavelength using respective analytical kits from Randox Laboratories Pvt. Ltd., Mumbai. Thyroid hormones, *viz.*, triiodothyronine (T_3) and thyroxine (T_4) were assessed-by using the analytical kits of Omega Diagnostic Ltd., Mumbai.

The data on different blood biochemical parameters was analyzed by using one way analysis of variance for treatment effects (Snedecor and Cochran, 1994). Pair-wise mean differences between groups were compared by Tukey's post-hoc test for significance at *p<0.05, **p<0.01.

Results and Discussion

The result revealed non-significant effect of feeding different levels of formaldehyde treated rapeseed meal on blood indices like serum glucose, total protein, albumin, globulin, A/G ratio, cholesterol, ALT and AST (Table 1). These findings indicated that the different dietary treatments used had no influence on these parameters. Many researchers have also recorded similar results for above blood indices

Table 1: Effect of formaldehyde treated protein on metabolic profile of Surti buffalo heifers

Parameter studied	Dietary treatments				P-Value
	Control	FT-25	FT-50	FT-100	
Glucose (mg/dL)	54.42±3.45	56.28±1.90	54.32±1.51	57.90±1.87	0.659
Urea (mg/dL)	19.31^{a}±0.59	18.90^{a}±0.55	17.73^{ab}±0.27	16.53^{b}±0.37	0.030*
Cholesterol (mg/dL)	76.34±2.21	78.26±1.30	77.94±1.47	77.28±1.13	0.836
Protein fraction (mg/dL)					
T. protein (mg/dL)	6.54±0.19	6.57±0.18	6.74±0.18	6.81±0.18	0.693
Albumin (mg/dL)	3.06±0.12	3.10±0.13	3.16±0.10	3.20±0.10	0.841
Globulin (mg/dL)	3.47±0.22	3.46±0.24	3.58±0.22	3.60±0.16	0.952
A/G	0.90±0.09	0.92±0.09	0.90±0.08	0.89±0.05	0.997
Hepatic enzyme activity (U/L)					
ALT (U/L)	54.86±1.85	53.51±1.50	52.29±2.28	52.83±1.85	0.794
AST (U/L)	97.12±1.83	96.47±2.13	93.63±2.45	94.34±2.05	0.617
Thyroid hormone (nmol/L)					
T_3 (nmol/L)	1.85^{a}±0.02	1.87^{ab}±0.02	1.99^{bc}±0.04	2.01^{c} ±0.03	0.01**
T_4 (nmol/L)	38.08±1.03	38.29±1.08	40.37±0.96	40.75±1.23	0.223

Means bearing different superscript (a,b,c) in a row differ significantly at *p<0.05 or **p<0.01.

following feeding of HCHO treated dietary protein in the ration of dairy animals (Akbar *et al.,* 1999; Jadhav *et al.,* 2018). The experimental animals were reared under uniform managemental conditions and fed on isocaloric and isonitrogenous diet. Above all, there exists a strong homeostasis mechanism in mammals, which tries to prevent any deviation in internal environment from the normality and tries to maintain a stable and relatively constant situation.

In the present study, feeding formaldehyde treated rapeseed meal had no significant effect on blood glucose, which concurred with earlier reports on cattle (Tyagi *et al.,* 2009; Jadhav *et al.* 2018) and lactating Murrah buffalo (Akbar *et al.,* 1999). Similar were the observations of Shamoon *et al.* (2009) on feeding formaldehyde-treated soyabean meal to Awassi lambs. The reason may be a high metabolic rate of utilization of glucose and homeostatic mechanism of animal body that does not allow appreciable change in glucose level. However, contrary to these, Puri *et al.* (2004) reported increased blood glucose level on feeding of formaldehyde-treated mustard cake in buffalo calves. Non-significant differences in serum cholesterol ($p>0.05$) observed among control and treatment groups in present study (Table 1) also corroborated well with the reports of Shelke *et al.* (2011), Puri *et al.* (2004) and Jadhav *et al.* (2018) in dairy animals.

The non-significant differences observed in total serum protein concentrations between groups fed HCHO treated RSM compared well with feeding of HCHO treated mustard cake in buffalo calves (Puri *et al.* 2004), in buffalo heifers (Jadhav *et al.,* 2018), in lactating Murrah buffaloes (Akbar *et al.,* 1999) and mustard cake & ground nut cake in pregnant Murrah buffalo (Shelke *et al.,* 2011). Albumin, globulin concentrations and A/G ratio in the present study were within the normal range and did not differ significantly due to treatments. Many earlier researchers (Puri *et al.*, 2004; Shelke *et al.,* 2011; Jadhav *et al.,* 2018) have also reported similar non-significant effect of formaldehyde treated protein on albumin, globulin content or albumin: globulin ratio.

Significantly lower ($p<0.05$) serum urea nitrogen (SUN) in FT-50 and FT-100 (17.7±0.27 and 16.53±0.37) groups in comparison to FT-0 (control) and FT-25 (19.31±0.59; 18.90±0.55) groups found in the present study (Table 1) indicated that the HCHO treatment of dietary protein was effective in protecting the ruminal degradation of protein. These results concurred well with the reports of Sahoo and Walli (2005) and Jadhav *et al.* (2018). Several other researchers also recorded a decline ($p<0.05$) in SUN level of ruminants fed with different percentage of undegradable protein (UDP) or bypass protein (Akbar *et al.,* 1999; Shelke *et al.,* 2011). On the contrary, Puri *et al.* (2004) reported increase in BUN on feeding of HCHO treated mustard cake in buffalo calves. These discrepancies might be attributed to different ingredients used as sources of bypass protein which might have affected the degradation kinetics in the rumen and subsequent utilization in the lower gut after bypassing the rumen and absorption into the system as amino acids (Kumar *et al.,* 2006). The results of present experiment indicate that there was gradual decrease in SUN level in HCHO treated groups (FT-50 and FT-100), reflecting the efficiency of HCHO treatment to protect the protein from rumen microbial degradation. Higher SUN in control and FT-25 groups, indicated less efficient utilization of dietary nitrogen for microbial protein synthesis due to higher ammonia level. Feeding rumen protected protein not only results in more supply of amino acid, but also saves energy loss in urea synthesis (Shelke *et al.,* 2011)

Serum ALT, AST concentrations were numerically lower ($p>0.05$) in HCHO treated groups (FT-50 and FT-100, Table 1). Like our findings no-significant difference in ALT and AST on feeding HCHO treated protein in buffalo heifers (Jadhav *et al.,* 2018) and in buffalo calves (Puri *et al.,* 2004) have been documented. Tiwari and Kumar (2011) observed non-significant effect on ALT but with significant reduction of AST in crossbred heifers fed with undegradable protein (UDP), High variability in AST values and a smaller number of animals per treatment might have not allowed the data to attain the significance point in our study. These can also be correlated with low SUN in HCHO treated groups, where liver may be supposed to have lesser stress for formation and elimination of urea. This indicates the lower stress (health benefit) on liver for conversion of ammonia to urea in animals fed with protected (bypass) protein. The variable effects on hepatic enzymes recorded by different workers could be attributed to different model of animals used, their age and sex, physiological condition, feeding regimen, protein supplements and method of protein protection

Interestingly, the serum levels of thyroid hormones (T_3 and T_4) were significantly higher ($p<0.05$) in FT-50 and FT-100 groups compared to control and FT-25 groups (Table 1), indicating formaldehyde treatment had effectively controlled the glucosinolate. These findings match with Jadhav *et al.* (2018). Earlier researcher (Sahoo, 2002; Das and Singhal, 2005) also have stated that formaldehyde treatment has emerged as a novel method of limiting the glucosinolate by binding to it. The lowest levels of serum thyroid hormones (T_3 and T_4) in control group were attributed to glucosinolate present in rapeseed meal, which is metabolised to isothiocyanates, thiocyanates, oxazolidinethiones and nitriles by myrosinase in rumen and compete with iodine (Burel *et al.,* 2001). The reduced absorption of iodine leads to iodine deficiency (Burel *et al.*, 2000), hypertrophy of the liver, kidney and thyroid (Tripathi *et al.,* 2001). Several researchers have suggested that the presence of rapeseed meal in the diet of ruminants impaired thyroid function caused by the myrosinase activity in rapeseed meal (Tripathi *et al.,* 2001). High glucosinolates in diet resulted into growth retardation, reduced feed conversion, and impairment of growth, fertility and reproduction (Burel *et al.,* 2001; Conaway *et al.,* 2002). However, Lardy and Kerley (1994) reported that use

of rapeseed meal didn't affects triiodothyronine hormone. Present findings suggested that formaldehyde treatment efficiently limit the glucosinolate in rapeseed meal and is better for overall health and performance of buffalo heifers.

Conclusion

From the overall evaluation of the results obtained in the present study, it could be observed that serum T_3 level was increased ($p<0.01$) and blood urea level was decreased ($p<0.05$) in FT-50 and FT-100 groups (0.5% and 1% CP of formaldehyde treated rapeseed meal) without altering other metabolic indices and hepatic enzymes. These results suggest that formaldehyde treatment of rapeseed meal effectively limits the glucosinolate and protects protein from rumen degradation. Hence, it could be recommended that use of formaldehyde for protection of dietary protein from rumen degradation is safe without any adverse effect.

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