Indian J. Anim. Nutr. 2022. 39 (3): 306-313
doi: 10.5958/2231-6744.2022.00037.8

Indian Journal of Animal Nutrition

Replacement of Green Fodder with Corn Silage: Effects on Nutrient Utilization, Antioxidant Status and Immune Response in Hariana Heifers

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ABSTRACT

The systemic concentrations of key blood metabolites associated with feed intake, growth, nutrient partitioning, and utilization are potential physiological markers of the dietary variation. The aim of this study was to determine the effect of green fodder replacement with corn silage on biomarkers of nutrient metabolism, antioxidant potential, and immune response in growing Hariana cattle. A total of 18 growing Hariana calves were randomly assigned into three groups (having 6 calves in each group) on body weight (130±3.0 kg) and age basis (14±1.5 months). Experimental heifers either received a basal diet devoid of corn silage (control) or were fed on TMR of which 50% ($Silage_{50\%}$) and 100% ($Silage_{100\%}$) green fodder (DM basis) were replaced with corn silage. In the present study, plasma total protein and albumin concentration showed higher ($P<0.05$) levels in animals receiving a diet containing 0% silage than $Silage_{50\%}$, and $Silage_{100\%}$ groups. However, green fodder replacement with corn silage did not exert any effect on biomarkers of energy and lipid metabolism, antioxidant, and immune response and plasma mineral levels showed the non-significant ($P>0.05$) effect of green fodder replacement with corn silage. The results of this study indicated that the replacement of green fodder with corn silage did not exert any adverse effect on the blood biomarkers of nutrient metabolism, antioxidant potential, and immune response therefore can be used as a substitute of green fodder in growing Hariana cattle.

KEYWORDS: Antioxidant, Biomarker, Cattle, Corn Silage, Green Fodder, Immunity

Article received: 19 June 2022; Article accepted: 24 September 2022

INTRODUCTION

The critical issue currently faced by the Indian dairy sector is a huge shortage quality fodder that has left livestock vulnerable. In future, a persistent crisis of green fodder can be the major factor that limits the productive potential of dairy animals. Various approaches have been tried to maintain the round-the-year fodder availability. Among these, silage is one of them. Silage is the green succulent roughage preserved under controlled anaerobic fermentation by compacting green cereal crops in air and watertight silo. Well-Fermented silage is readily consumed by animals and may improve their health and production characteristics (Varadyova et al., 2010). Recent studies on silage production indicate that it could replace conventional fodder without any ill effect on the performance of dairy animals (Chaudhary et al., 2014). In a well-managed system, where losses are low, the silage dry matter content, digestibility and ME content will be similar or slightly lower and crude protein content might be similar to parent fodder (Kaiser and Piltz, 2004). Preference for cereal green fodder including maize, sorghum, pearl millet, etc. is due to it has more sugar content than protein, as sugar is utilized in the fermentation process to make lactic acid by microorganisms (Nazli et al., 2019).

Blood metabolites have been used to monitor and evaluate the health and nutritional status of animals (Cherdthong et al., 2014) and may explain, in part, the biological variation in efficiency. A major factor that affects the blood biomarkers of animals is dietary composition. Therefore, a corollary to this concept exists in the utilization of diets, suggesting that when different diets are fed to cattle, the analyses of blood biomarkers should be able to differentiate

between the efficiencies of the utilization of diet. Considering the potential of silage and limitation of green fodder availability, the present study was designed to investigate the effect of replacement of green fodder with corn silage on biomarkers of nutrient metabolism, antioxidant potential, and immune response in growing Hariana cattle.

MATERIALS AND METHODS

Animal care procedures were approved (approval number, 121/IAEC/18) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India. A total of 18 growing Hariana heifers were selected from the cattle herd maintained at LFC, DUVASU, Mathura. All heifers were housed in a well-ventilated shed having the proper arrangement for individual feeding and watering without having access to the other animal's diet. Deworming of all the experimental animals was done before the start of the experiment by oral administration of Fentas bolus (Intas Pharmaceuticals Pvt. Ltd., India) at the dose level of 10 mg/kg body weight. Animals were let loose every fortnightly for exercise. Selected heifers were randomly assigned into three groups ($n=6$) on body weight (130±3.0 kg) and age basis (14±1.5 months). Heifers either fed on basal TMR consisted of compounded concentrate: green fodder: wheat straw in a proportion of 40: 40: 20 (control) or TMR of which 50% green fodder (DM basis) was replaced with corn silage ($Silage_{50\%}$) and TMR of which 100% green fodder (DM basis) was replaced with corn silage ($Silage_{100\%}$). Ingredients and nutrients composition of TMR fed in different groups are presented in Table 1. TMR was prepared daily by hand mixing and offered at 0900 h in all tests. The calves were fed the TMR in such an amount that at least 5% refusals were left daily per animal. Fresh drinking water was offered *ad libitum* twice daily at 0800 h and 1700 h.

Table 1. Composition of TMR fed during experimental period (% DM basis or as mentioned)

Particular	Treatment[α]		
	Control	$Silage_{50\%}$	$Silage_{100\%}$
Ingredient composition, g/kg DM			
Berseem fodder	40	20	0
Corn silage	0	20	40
Wheat straw	20	20	20
Mustard oil cake, solvent extract	12.8	12.8	12.8
Ground barley grain	10.4	10.4	10.4
Gram chuni	8	8	8
Wheat bran	8	8	8
Micronutrient mixture[β]	0.8	0.8	0.8
Nutrient composition			
DM	59.89	62.27	64.65
CP	16.74	14.70	12.66
EE	3.17	3.04	2.92
Total ash	8.87	7.88	6.89
NDF	46.76	47.59	48.42
ADF	25.26	25.94	26.62
ADL	4.42	4.40	4.37
Ca	1.11	1.13	1.15
P	0.44	0.74	1.03

[á]Control, TMR without corn silage; $Silage_{50\%}$, TMR of which 50% green fodder was replaced with corn silage and $Silage_{100\%}$, TMR of which 100% green fodder was replaced with corn silage.

[â]Micronutrient mixture consisted (kg^{-1}) of 700,000 IU of vitamin A, 70,000 IU of vitamin D3, 250 mg of vitamin E, 190 g of Ca, 90 g of P, 50 g of Na, 19 g of Mg, 1.2 g of Cu, 9.6 g of Zn, 1.5 g of Fe, 6.0 g of Mn, 325 mg of I, 150 mg of Co, 10 mg of Se.

The representative samples of TMR offered and residue left was dried in a hot air oven at 60°C till a constant weight was attained and ground in a Wiley mill to pass a 1-mm sieve. Processed samples were pooled animal-wise and stored at the dry place for chemical analysis. The representative samples of TMR offered and residue left and feces were analyzed for nutrients composition (AOAC, 2005) and fibre fraction (Van Soest et al., 1991).

Peripheral blood samples were collected in heparinized vacutainer tubes (BD Franklin, USA) by venipuncture of anterior vena cava at 0, 30, 60, and 90 days post-treatment. Collected blood samples were analyzed for biomarkers of nutrient metabolism, antioxidant potential and immune response blood biochemical attributes. A fraction of whole blood samples were used for the analysis of superoxide dismutase (SOD; Madesh and Balasubramanian, 1998) and catalase (CAT; Aebi, 1984) activity. Remaining amount of blood samples was centrifuged at 3000 rpm for 30 min to separate the plasma from packed erythrocytes. Plasma samples were stored at -20°C until further analysis of total protein, albumin, PUN, creatinine, glucose, triglycerides, cholesterol, total antioxidant status (TAS), total immunoglobulin (Total Ig), Ca and P were determined colorimetrically by the automated biochemical analyzer (BS-120 Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co. Ltd.). NEFA and BHBA concentrations were determined to assess the energy status of the animals at periodic intervals. Estimation of NEFA and BHBA were done by using a "Bovine specific ELISA kit" (Cayman's Chemical Company, Ann Arbour, Michigan, USA). TAS was measured as ferric reducing antioxidant power (FRAP) assay procedure described by Benzie and Strain (1999). The total Ig in the plasma samples of calves was estimated by the zinc turbidity method (McEwan et. al., 1970).

Data of the study were subjected to analysis of variance using the General Linear Model (GLM) procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA). The effect of green fodder replacement with corn silage on blood biomarker was tested using the following model:

$$Yijk = \mu + Ti + Dj + (T \times D)ij + eijk$$

Where; *Yijk* is the dependent variable, *ì* is the overall mean of the population, *Ti* is the mean effect of the treatment, *Dj* is the mean effect of day of sampling (j=0, 30, 60 and 90 days of dietary treatment), *(T×D)ij* is the effect of the interaction between treatment and period and *eijk* is the unexplained residual element assumed to be independent and normally distributed. Individual animals were used as the experimental unit for all data. The pair-wise comparison of means was carried out using "Tukey's honest significant difference (HSD) test". Significance was determined at $P<0.05$ and the values are presented in the tables. Error bars in figures depict standard error. The data were also analyzed for correlation coefficient (r) and coefficient of variation (CV).

RESULTS AND DISCUSSION

In the present study, plasma concentrations of total protein, albumin, globulin, PUN, and creatinine were used as protein metabolism biomarkers (Table 2). Mean plasma total protein and albumin values showed a significant effect ($P<0.05$) between groups and reported highest in the control group. The periodic changes in plasma globulin, PUN, and creatinine concentration during 90 days study period showed a non-significant effect of treatment. Effect of green fodder replacement with corn silage showed a negative correlation with plasma total protein, albumin, and creatinine level while plasma globulin and PUN concentration showed a weak positive correlation with treatment.

Table 2. Effect of green fodder replacement with corn silage on biomarkers of nutrients metabolism

Parameter	Treatment			SEM	P value	r¥	CVβ
	Control	Silage$_{50\%}$	Silage$_{100\%}$				
Biomarkers of protein metabolism							
Total protein concentration, g/l	7.21[b]	6.87[ab]	6.74[a]	0.16	0.042	-0.009	0.079
Albumin level, g/l	4.26[b]	4.06[ab]	3.78[a]	0.098	<0.001	-0.037	0.104
Globulin level, g/l	2.95	2.81	2.96	0.038	1.000	0.015	0.029
PUN level, mg/100 ml	32.0	29.7	31.3	1.53	0.141	-0.064	0.139
Creatinine, mg/100 ml	1.23	1.15	0.86	0.25	0.168	-0.098	0.181
Biomarkers of energy and lipid metabolism							
Glucose level, mg/100 ml	48.7	49.7	51.8	1.05	0.079	0.410	0.031
Cholesterol level, mg/100 ml	93.6	93.9	91.8	0.24	0.809	-0.191	0.028
Triglyceride level, mg/100 ml	30.3	32.2	31.2	1.09	0.787	0.111	0.030
NEFA concentration, nmol/l	0.108	0.107	0.105	0.006	0.552	-0.056	0.124
BHBA concentration, mmol/l	0.25	0.26	0.25	0.009	0.969	0.082	0.076

SEM, standard error of mean, Mean with different superscript in a row differs significantly (P<0.05), ¥Greater (r > 0) or lower (r < 0) than zero value of Pearson square correlation coefficient shows positive or negative correlation between RFI and attributes whereas, zero (r = 0) value of Pearson square correlation coefficient shows no correlation among RFI and attributes, ßCoefficient of variation.

The systemic concentrations of key blood metabolites associated with feed intake, growth, nutrient partitioning, and utilization are potential physiological markers of feed efficiency (Nkrumah et al., 2007). The wide variation is noticed among reviewed values, which may be due to the plane of nutrition, kinds of feedstuffs used in feeding, age, and status of animals. No information is available regarding the effect of replacement of fodder with silage on plasma metabolites. Plasma total protein and albumin concentration showed higher values in heifers receiving a diet containing no silage than Silage$_{50\%}$ and Silage$_{100\%}$ groups. Higher plasma total protein and albumin levels in the CS$_{0\%}$ group might be due to the higher protein content and metabolizability compared to CS$_{50\%}$ and CS$_{100\%}$ groups. Regarding the effect of alteration in the composition of diet on protein metabolism, Tufan et al. (2016) fed as ad libitum, one of the following roughage sources; a) hay, b) grass silage, c) corn silage or d) 50% grass silage + 50% corn silage mixes on a DM basis and found no effect on plasma total protein, albumin, and glucose in beef cattle. Belyea et al. (1974) conducted a study to find the effects of silage diets on health, reproduction and blood metabolites of dairy cattle. They fed corn silage, corn

silage plus hay-crop silage and corn silage plus hay and noted that globulin content of corn silage plus hay-crop silage and corn silage plus hay group was higher than corn silage group. However, plasma total protein content was higher in the corn silage plus hay group. Mahmoud and Ebeid (2014) found that partial or complete substitution of complete berseem with corn silage did not affect plasma globulin values and values ranged from 4.00 to 4.61 g/100 ml. Serum urea concentration is a marker of protein intake and digestion, degradation of protein sources, and energy availability in the rumen (Roseler et al., 1993). In the present study, similar PUN concentrations in low and high efficiency calves and showed no effect of replacement of green fodder with corn silage. Higher PUN in silage fed groups was reported by Belyea et al. (1974) and (Prewitt et al., 1971) in dairy cows. In accordance with the findings of the present study, Mahmoud and Ebeid (2014) found that partial or complete substitution of complete berseem with corn silage did not affect plasma creatinine values and values ranged from 1 to 2 mg/100 ml.

In the present study, the plasma concentration of glucose, cholesterol, triglycerides, NEFA and BHBA were used as biomarkers of energy and lipid metabolism (Table 2). Statistical analysis revealed a non-significant ($P<0.05$) effect of replacement of green fodder with corn silage showed a non-significant effect of treatment on energy and lipid metabolism. Mean plasma glucose, cholesterol and NEFA concentration showed weak negative while mean plasma triglycerides and BHBA concentration showed a weak positive correlation with treatment.

Green fodder replacement with corn silage did not exert any effect on studied biomarkers of energy and lipid metabolism. Tufan et al. (2016) fed as a diet containing different levels of silage and found no effect on plasma glucose levels in beef cattle. No difference in plasma glucose concentration was also observed by Persichetti et al. (2014) in Holstein cows, with the inclusion of the different levels of high moisture corn silage in the diet. In accordance with the findings of the present study, Mahmoud and Ebeid (2014) found that partial or complete substitution of complete berseem with corn silage did not affect plasma total lipids and values ranged from 378.80 to 622.10 mg/100 ml. Chen et al. (2015) also observed no difference in serum concentrations of cholesterol and triglycerides among treatments having a different proportion of forage. However, Lazarrov and Vilianova (1972) observed that corn silage diets have been associated with lower total blood lipids and total cholesterol. BHBA and NEFA is the product of tissue fatty acid catabolism, and systemic concentrations increase in proportion to the degree of fat mobilization. These two biomarkers are used to access the energy balance of animals. Belyea et al. (1974) found that plasma cholesterol showed large increases for all three groups reflecting increased grain intake while the decline in plasma NEFA in the corn silage and corn silage plus hay-crop silage groups apparently reflected increasing energy intakes as DMI gradually approached energy requirements.

In the present study, SOD, CAT, and FRAP (TAS) were used as biomarkers of antioxidant status whereas; plasma total Ig level were used as a biomarker of an immune response (Table 3). No significant differences in the blood SOD, CAT activity, TAS and plasma total Ig was observed between $CS_{0\%}$, $CS_{50\%}$, and $CS_{100\%}$ groups. SOD activity, TAS, and Plasma total Ig showed negative whereas; CAT activity showed a positive correlation with treatment. No significant difference in the mean plasma Ca and P concentrations were observed between control, $Silage_{50\%}$, and $Silage_{100\%}$ groups (Table 3). Plasma Ca levels showed a weak negative while plasma P showed a weak positive correlation with treatment. No information is available regarding the effect of replacement of green fodder with corn silage on antioxidant status and immune response. Si et al. (2018) found that supplementation of different levels of *Broussonetia papyrifera* silage did not affect the activity of antioxidant status and immune response.

Table 3. Effect of green fodder replacement with corn silage on biomarkers of antioxidant and immunity

Parameter	Treatment			SEM	P value	r[¥]	CV[β]
	Control	$Silage_{50\%}$	$Silage_{100\%}$				
SOD activity, µmol MTT formazan/mg Hb	0.46	0.42	0.42	0.054	0.093	-0.084	0.054
CAT activity, nmol/ml	163.8	172.7	168.4	23.15	0.093	0.162	0.118
TAS activity, mmol/l	1.14	1.15	1.13	0.04	0.729	-0.151	0.052
Total Ig level, mg/ml	22.0	20.9	20.6	0.90	0.904	-0.372	0.057
Ca level, mg/100 ml	10.1	9.38	10.09	0.35	0.672	-0.031	0.044
P level, mg/100 ml	5.53	5.40	5.80	0.23	0.762	0.113	0.036

No significant difference in the mean plasma Ca and P concentrations was observed between control, $Silage_{50\%}$, and $Silage_{100\%}$ groups. Little and Manston (1972) compared corn silage and alfalfa hay as forages for dairy cattle and found slightly higher P and slightly lower Ca in the blood of the corn silage cows but found within physiological range as reported by Singh et al. (2016) in growing Hariana cattle.

CONCLUSIONS

The findings of this study revealed that the replacement of green fodder with corn silage did not exert any effect on biomarkers of energy and lipid metabolism, antioxidant and immune response, and plasma mineral levels. However, plasma concentrations of total protein and albumin showed higher in the control group compared to $Silage_{50\%}$ and $Silage_{100\%}$ groups could be due to higher protein content in berseem fodder. In conclusion, the replacement of green fodder with corn silage did not exert any adverse effect on the blood biomarkers, therefore, can be used as a substitute of green fodder.

ACKNOWLEDGMENTS

The authors would like to thanks the staff of the Department of Animal Nutrition and Livestock Farm Complex, DUVASU, Mathura, India. The authors also gratefully acknowledge Dr. R.K. Yadav for assistance during the analysis of biochemical attributes. The fund for this study was provided by the University under the Indian Council of Agricultural Research Grant, New Delhi, India.

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