Effects of Soybean and Rice Bran Oil Supplementation on Lactation Performance and Milk Fatty Acid Profile in Surti Goats

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ABSTRACT

An experiment was conducted to study the effect of soybean and rice bran oil supplementation on lactation performance and milk fatty acid profile in Surti goats. Twenty-four multi-parous Surti lactating goats were distributed into four groups on the basis of live body weight, parity and previous lactation yield for 150 days. Treatment groups were classified as basal diet supplemented without any oil (CON), or supplemented at 3% of dry matter intake (DMI) through soybean oil (SBO), rice bran oil (RBO) or a blend of rice bran and soybean oil at 50:50 (SRBO). Basal diet consisted of compound concentrate mixture, green jowar and pigeon pea straws. Milk yield (kg/d), fat concentration (%) and yield (g/d), fat corrected milk (FCM) and energy corrected milk (ECM) yield (kg/d) were significantly (P<0.05) increased in oils supplemented groups as compared to control. Concentration of milk solid not fat (SNF), protein and lactose remained similar (P>0.05) amongst treatments but their yield (g/d) was significantly (P<0.01) improved in oils supplemented groups as compared to control. Feed efficiency in terms of milk yield/DMI and FCM/DMI significantly (P<0.05) improved in SBO, RBO and SRBO as compared to CON. Supplementation of both soybean oil and rice bran oil either alone or in combination significantly reduced short chain fatty acid (SCFA), medium chained fatty acid (MCFA), saturated fatty acid (SFA) with increased long chain fatty acids (LCFA) and poly unsaturated fatty acids (PUFA) in milk. Oil supplementation increased (P<0.01) milk content of oleic acid (C18:1 ω-9) and linoleic acid (C18:2 ω-6) in SBO, RBO and SRBO as compared to CON. However, linolenic acid (C18:3 ω-9) content remained non significant amongst treatments. Various lipid quality indices like atherogenicity index and thrombogenicity index were significantly (P<0.01) improved in all the oil supplemented groups as compared to CON indicating improved nutritional quality of milk.

Key words: Milk fatty acid, Rice bran oil, Soybean oil, Surti goat

INTRODUCTION

Dairy goat farming is gaining importance in Indian sub- continent as it provides food and nutritional security to the millions of small and marginal farmers (Haenlein, 2001). Goat milk and milk products play important role in human nutrition due to its nutritional advantage with regards to smaller size of fat globules, lower amount of casein and higher mineral content over cow milk (Attaie and Richter, 2000; Gomes *et al.,* 2013). Amongst goat milk composition, milk fat is much is an important component due to its physical, nutritional and organoleptic properties. A characteristic goatish (musky) flavour of goat milk and milk products is attributed to its fatty acids (C4:0 - C10:0), which is about twice the amount of compared to cow milk fat (Ibrahim and Soryal, 2014). Goat milk production and composition, especially (FAs profile and flavour) is a genetic trait, but it can be effectively altered by the dietary supplementation with lipids (Nudda *et al.,* 2006). During last decade, there is an increased interest among esearchers to reduce saturated fatty acids and enrich with beneficial polyunsaturated fatty acids (PUFA) or conjugated linoleic acid (CLA), which could offer potential health benefits to consumers (Bouattour *et al.,* 2008). Fatty acid content in goat milk is affected by the fat content, type of predominant fat and physical form of the dietary fat supplement. Vegetable fat supplements are usually more effective in the form of free oil than in the form of seeds at increasing the milk FA content (Nudda *et al.,* 2014). Plant oils from different oilseed sources have different FA compositions and accordingly exert different effects on milk FA profile (Gómez-Cortés *et al.,* 2008). In goats, supplementing PUFA rich vegetable oils like soybean oil (Almieda *et al.,* 2019; Li *et al.,*

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2009) and rice bran oil (Maia *et al.,* 2006) has been shown to affect milk yield and milk FA composition in terms of ω -3, ω -6 and CLA. However, to the best of our knowledge, there is meager information about response of soybean oil and rice bran oil supplementation in local Indian goat breeds. Considering this, an experiment was planned to study the effect of soybean oil and rice bran oil supplementation on production performance and nutritional quality of milk in Surti goats.

MATERIALS AND METHODS

The present study was carried out at the Department of Animal Nutrition, Navsari, India and the experimental animals were kept at Livestock Research Station (LRS), Navsari Agricultural University, Navsari, Gujarat, India. Twenty-four (n=24) lactating Surti goats were randomly distributed into four groups on the basis of their live body weight (28.10±1.04 kg), parity (4.47±0.47) and previous lactation yield (133.39±5.00 kg) during complete lactation of 150 days. Treatment groups were classified as basal diet supplemented without any oil (CON), or supplemented at 3% of DMI either through soybean oil (SBO), rice bran oil (RBO) or a combination (50:50) of both (SRBO). Basal diet consisted of compound concentrate mixture, green jowar and pigeon pea straws. Compounded concentrate mixture mixed with additive was offered at 08.30 h, green sorghum at 10.00 h and 16.00 h while pigeon pea straws were fed during night hours. Experimental animals were individually fed during entire experiment of 150 days according to ICAR (2013). Drinking water was available at all the time during experiment Dry matter intake was recorded fortnightly throughout the experimental period. Samples of feed ingredient were analysed for proximate composition according to the methods of AOAC (2005) and fiber fractions were estimated following methods of Van soest *et al.* (1991). Samples of feed and oils were analysed for fatty acid composition according to methods of O'Fallon *et al.* (2007).

Goats were hand milked twice a day at morning (08.30 h) and evening (16.00 h) and daily milk yield was recorded. Morning and evening milk samples were

pooled and used for chemical composition at each fortnight. Milk samples were analysed for fat, solid not fat (SNF), protein and lactose using infrared spectrophotometry (MilkoScan™ FT1 120). Samples for milk fatty acid were collected from each animal were stored at -20°C in the airtight plastic bottle container until laboratory analysis.

Fatty acid methyl esters (FAME) was prepared by base-catalysed methanolysis of glycerides (KOH in methanol) according to international standards (ISO-IDF, 2002) and stored in vial at -20°C for further analysis. FAME was analysed on gas chromatography mass spectrometer (GCMS-QP 2010 Plus) equipped with an auto sampler injector. The FAME was separated in 60 m capillary column (60 m x 0.25 mm x 20 um) film thickness and helium was used as carries gas at a flow rate of 1 mL/min. The injector and detector temperatures were 250ºC and 240ºC, respectively. The temperature programme was followed as: The initial temperature held at 100ºC for 5 min after sample injection, then programmed to increase at 2°C per min to 240°C and held there for 5 min. Sample (1uL) were injected by split injection (split ratio 50:1). The mass spectrometer was operated at source temperature of 230ºC with scan range of 50-1000 m/z. Identification of FAME was performed from the retention time by using standards of 37 individual FAME (Supelco, Bellefonte, PA).The peak areas in the chromatograph were calculated and normalized using response factors.

Statistical Analysis

Data for milk yield and composition were analysed using the PROC MIXED procedure of SAS with repeated measures (version 9.3; SAS Institute Inc., Cary, NC) using Tukey's HSD (honestly significant difference) multiple comparison test and following statistical model was used.

$$Y_{ijk} = ì + A_k + D_i + T_j + (D_i \times T_j) + e_{ijk}$$

Y_{ijk} = dependent variable; ì = overall mean; A_k = random effect of animals; D_i = fixed effect of diet, T_j = fixed effect of time; e_{ijk} = residual error

The statistical model contained fixed effects of treatment, period and their interactions, random effects of the animal and residual error. Parameters related to

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fatty acid analysis were analysed by one-way ANOVA using tukey's HSD. Differences were declared significant at P<0.05, with values of P<0.10 being interpreted as a trend towards significance.

RESULTS AND DISCUSSION

Data pertaining to proximate and fatty acid composition of experimental feed is presented in Table 1. The concentrate: roughage ratio was tried to maintain at 30:70 throughout experimental period. The diets were iso-nitorgenous but due to addition of oil it was not isocaloric (Adeyemi *et al.,* 2015; Bouattor *et al.,* 2008).

Milk yield (kg/d) was significantly (P<0.05) higher in SBO (+25.97%), RBO (+32.46%) and SRBO (+10.38%) as compared to CON. However, amongst the oil supplemented groups, RBO showed highest increase in milk production followed by SBO and SRBO. Increased available metabolisable energy might be the main reason behind the increased milk yield with oil supplementation. Gómes-Cortés *et al.* (2008) concluded that increased milk yield in goat was due to greater energy content of the oil supplemented diets and not by a greater DMI. This would be confirmed by the strong positive relationship between energy intake and milk yield (Titi and Rahman, 2013). Various scientists found increased milk yield with vegetable oils supplementation in goat (Bernard *et al.,* 2009; Hassan *et al.,* 2020; Ibrahim *et al.,* 2020; Mele *et al.,* 2008) and sheep (Ferreira *et al.,* 2018; Nudda *et al.,* 2015). In contrast to present result, many other research workers (Bouattour *et al.,* 2008; Lunsin *et al.,* 2012; Bernard *et al.,* 2015; Almieda *et al.,* 2019) found no effect of PUFA rich oil supplementation in dairy animals. Differing responses of milk yield to dietary fat might relate to the genetic potential of breed, supply of energy from the basal diet and effect on dry matter

Table 1. Proximate and fatty acid composition of experimental diet

Attributes	CON	SBO	RBO	SRBO
Dry matter	67.37	64.69	64.69	64.69
on % dry matter basis				
Organic matter	85.10	82.20	82.20	82.20
Crude Protein	12.09	11.95	11.95	11.95
Ether Extract	2.55	5.44	5.44	5.44
Neutral detergent fiber	50.35	49.82	49.82	49.82
Acid Detergent fiber	36.98	36.63	36.63	36.63
ME (MJ/kg DM)	10.36	11.08	11.07	11.07
FA composition (g/100 g FA)				
C10:0	0.65	0.49	0.49	0.49
C12:0	0.79	0.60	0.60	0.60
C14:0	1.27	0.98	1.05	1.01
C16:0	21.24	18.55	20.03	18.83
16:1	0.15	0.13	0.16	0.14
C18:0	11.00	9.53	8.86	9.38
C18:1	14.77	16.63	20.02	18.69
C18:2	25.26	30.96	26.72	29.17
C18:3	9.27	8.61	8.33	8.32
C20:0	0.79	0.69	0.83	0.60
C22:0	2.24	1.79	1.77	1.79
C24:0	1.42	1.10	1.19	1.14

intake (DMI) and nutrient partitioning (Manso *et al.,* 2011).

Milk fat concentration and yield increased (P<0.01) in SBO, RBO and SRBO as compared to CON, but values remained unaffected with time. Many researchers had found combined effect of increased milk fat concentration along with milk production (Bernard *et al.,* 2009; Bernard *et al.,* 2015; Morsy *et al.,* 2015, Li *et al.,* 2016), while some reported only increased milk fat content (Bouattour *et al.,* 2008, Li *et al.,* 2009) with PUFA rich oils supplementation in lactating goats. In contrast, Almieda *et al.* (2019) and Reyes *et al.* (2018) found similar milk fat and milk production in goats. The response of milk fat secretion is usually higher during early lactation because *de novo* lipogenesis is usually more active after the lactation peak than before it. After the lactation peak, dietary FA would probably be partitioned more to adipose tissues (Chilliard *et al.,* 2003). Neither diet nor sampling time had significant (P>0.05) effect on milk SNF, protein and lactose concentration. However, SNF, protein and lactose yields were highest in RBO followed

Table 2. Production performance of Surti does supplemented with vegetable oil

Parameters	CON	SBO	RBO	SRBO	SEM	P value		
						Diet	**Time**	**DxT**
DMI (g/d)	975.31	1016.27	1008.91	994.54	28.42	0.751	<0.001	0.998
Yield								
Milk (kg/d)	0.77[b]	0.95[ab]	1.02[a]	0.85[ab]	0.06	<0.005	<0.001	0.987
Fat (g/d)	28.16[c]	47.19[ab]	52.11[a]	39.83[b]	2.88	<0.001	0.322	0.959
SNF (g/d)	62.18[b]	78.11[ab]	80.47[a]	66.46[ab]	4.58	<0.012	0.110	0.987
Protein (g/d)	23.54[b]	29.80[ab]	33.63[a]	25.88[ab]	1.96	<0.002	0.638	0.947
Lactose (g/d)	31.69[b]	40.09[ab]	41.79[a]	34.65[ab]	2.54	<0.019	0.265	0.945
Total solid (g/d)	92.20[b]	125.33[a]	128.81[a]	94.00[b]	8.82	<0.002	0.171	0.974
FCM (kg/d)[1]	0.73[c]	1.10[ab]	1.19[a]	0.94[bc]	0.08	<0.001	0.291	0.978
SCM (kg/d)[2]	0.70[c]	1.02[ab]	1.09[a]	0.86[bc]	0.06	<0.001	0.204	0.972
ECM (kg/d)[3]	0.64[c]	1.19[ab]	1.42[a]	0.79[bc]	0.12	<0.001	0.265	0.885
Concentration (%)								
Fat	3.70[b]	4.97[a]	5.02[a]	4.69[a]	4.60	<0.001	0.239	0.652
SNF	8.10	8.08	7.84	7.85	7.95	0.062	0.265	0.836
Protein	3.11	3.02	3.19	3.03	3.09	0.053	0.084	0.984
Lactose	4.09	4.38	3.98	4.03	4.12	0.507	0.797	0.748
Milk energy output (MJ/d)[4]	2.15[c]	3.17[ab]	3.47[a]	2.70[bc]	0.19	<0.001	0.401	.957
Milk energy content (MJ/kg)[5]	2.82[c]	3.30[ab]	3.37[a]	3.17[b]	0.05	<0.001	0.379	0.577
Milk efficiency								
MY/DMI	0.81[b]	1.08[a]	1.09[a]	0.87[ab]	0.07	<0.014	<0.001	0.996
FCM /DMI	0.77[b]	1.20[a]	1.27[a]	0.96[ab]	0.08	<0.001	<0.003	0.994

D, Diet; T, Time; DxT, Diet and time interaction; SEM, Standard error of mean; [abc]-Means with different superscript in a row differ significantly; [1]Fat corrected milk (kg/d) = 0.4*milk (kg) + 15*fat (kg) (Tyrell and Reid, 1965); [2]Solid corrected milk (kg/d) = 12.3*fat (kg) + 6.56*SNF (kg) – 0.0752*milk yield (kg) (Tyrell and Reid, 1965). [3]Energy corrected milk (kg/d) = Milk (kg/d)*[38·3*fat (g/kg) + 24·2*protein (g/kg) + 16·54*lactose (g/kg) + 20·7]/3140 (Sjaunja *et al.,* 1991); [4]Milk energy content (MJ/kg) = 4.184*2·204*[(41·63*fat (g/100g) + 24·13*protein (g/100g) + 21·60*lactose (g/100g)–11·72)/1000] (Tyrell and Reid, 1965); [5]Milk energy output (MJ/d) = milk energy (MJ/kg)*milk yield (kg/d) (Morsy *et al.,* 2018)

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Table 3. Milk fatty acid composition (% FAME) of experimental animals supplemented vegetable oil

FA	CON	SBO	RBO	SRBO	SEM	P VALUE
C4:0 butyric	0.06[c]	0.05[c]	0.16[b]	0.26[a]	0.02	0.001
C6:0 caproic	0.58	0.47	0.60	0.71	0.03	0.093
C8:0 caprylic	1.52	1.03	0.93	0.95	0.09	0.071
C10:0 capric	5.00[a]	3.34[ab]	2.60[b]	2.47[b]	0.32	0.009
C12:0 lauric	3.03[a]	1.71[b]	1.39[b]	1.35[b]	0.20	0.002
C14:0 myristic	7.65[a]	4.95[b]	4.26[b]	4.10[b]	0.42	0.003
C15:0 pentadecanoic	1.32[a]	0.83[b]	0.77[b]	0.91[b]	0.05	0.001
C16:0 palmitic	22.99[a]	18.48[b]	20.20[b]	18.74[b]	0.47	0.001
C16:1 palmitoleic	0.71	0.70	0.76	0.76	0.10	0.996
C17:0 heptadecanoic	1.34[a]	0.80[b]	0.70[b]	0.77[b]	0.06	0.001
C18:0 stearic	12.86[b]	17.46[a]	18.27[a]	18.00[a]	0.69	0.007
C18:1 ω-9 oleic	35.09[b]	38.91[ab]	40.76[a]	40.49[ab]	0.81	0.036
C18:2 ω-6 linoleic	1.44[b]	3.02[a]	2.83[a]	2.86[a]	0.16	0.001
C18:3 ω-3 linolenic	0.24	0.30	0.28	0.26	0.01	0.139
C20:0 arachidic	0.58	0.54	0.67	0.69	0.02	0.058
C20:4 ω-6 arachidonic	0.13	0.17	0.13	0.16	0.01	0.307
C20:5 ω-3 eicosenoic	0.02	0.02	0.01	0.01	0.01	0.064
C22:0 behenic	0.23	0.20	0.19	0.23	0.01	0.539
C22:6 ω-3 docosahexaenoic	0.06	0.05	0.05	0.05	0.01	0.844
C24:0 lignoceric	0.08	0.06	0.08	0.08	0.01	0.145
Others	4.52	6.07	3.54	5.45	0.47	0.252
SFA	57.24[a]	49.90[b]	50.83[b]	49.25[b]	0.99	0.007
UFA	37.69[b]	43.17[ab]	44.82[a]	44.58[a]	0.93	0.012
MUFA	35.80[b]	39.61[ab]	41.52[a]	41.25[a]	0.86	0.050
PUFA	1.89[b]	3.56[a]	3.31[a]	3.34[a]	0.16	0.001
ω-6	1.57[b]	3.19[a]	2.96[a]	3.01[a]	0.13	0.001
ω-3	0.32	0.37	0.34	0.32	0.01	0.427
ω-6/ ω-3	5.06[b]	8.78[a]	8.87[a]	9.78[a]	0.58	0.009
SCFA (4-10)	7.15[a]	4.88[b]	4.29[b]	4.38[b]	0.42	0.038
MCFA (12-16)	35.71[a]	26.67[b]	27.38[b]	25.86[b]	1.04	0.001
LCFA (>16)	52.62[b]	62.39[a]	64.79[a]	64.31[a]	1.35	0.001
C16:1 desaturase index[1]	0.04[a]	0.03[b]	0.03[b]	0.03[b]	0.01	0.049
C18:1 desaturase index[2]	0.73[a]	0.69[b]	0.69[b]	0.69[b]	0.01	0.029
D5 desaturase index[3]	0.86[b]	0.90[a]	0.92[a]	0.92[a]	0.01	0.010
D6 desaturase index[4]	0.09[a]	0.05[b]	0.04[b]	0.05[b]	0.01	0.001
Atherogenicity index[5]	1.56[a]	0.94[b]	0.87[b]	0.82[b]	0.09	0.001
Thrombogenicity index[6]	2.04[a]	1.70[b]	1.70[b]	1.64[b]	0.06	0.050
h/H index[7]	1.23[b]	1.85[a]	1.82[a]	1.92[a]	0.08	0.001
Elongase[8]	0.65[b]	0.75[a]	0.74[a]	0.75[a]	0.01	0.001
Peroxidisability index[9]	4.49[b]	6.66[a]	6.24[a]	5.90[a]	0.21	0.001

[a,b,c] -Means with different superscript in a row differ significantly (P<0.05); [1]16:1 desaturase index=C16:1/(C16:1+C16:0) (Kelsey *et al.,* 2003); [2]18:1 desaturase index=C18:1/(C18:1+C18:0) (Kelsey *et al.,* 2003); [3]Delta 5 desaturase index =C20:4 n-6/C20:3 n-6+ C20:4 n-6 (Nudda *et al.,* 2008); [4]Delta 6 desaturase index=C20:3 n-6/C18:2 n-6+ C20:3 n-6 (Nudda *et al.,* 2008); [5]Atherogenicity index=(12:0+4*14:0+16:0)/(MUFA+PUFA) (Ulbricht and Southgate, 1991); [6]Thrombogenicity index (TI)=[(C14:0+C16:0+C18:0)/ (0.5*MUFA+0.5*PUFAn6 + *PUFAn3+ (PUFAn3/PUFAn6))] (Ulbricht and Southgate, 1991); [7]h/H(hypocholesterolaemic/ hypercholesterolaemic)index=(C18:1n9+C18:2n6+C20:4n6+C18:3n3+C20:5n3+C22:6n3)/(C14:0+C16:0) (Santos-Silva *et al.,* 2002); [8]Elongase= C18:0+C18:1 c9/ (C16:0 +C16:1+ (C18:0+C18:1c9))(Oliviera *et al.,* 2014); [9]Peroxidisability index (PI)=(0.025*monoenes) + (1*dienes) + (2*trienes) + (4*tetraenes) + (6*pentaenes) + (8*hexaenes) (Erickson *et al.,* 1992)

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by SBO, SRBO and CON. Total solid content was increased in SBO and RBO as a consequence of increased fat, protein and lactose content. Many earlier reports (Manso *et al.,* 2011; Lunsin *et al.* 2012; Ferriera *et al.,* 2018; Almieda *et al.* 2019) indicated statistically similar milk composition parameters like protein and lactose. In contrast, Nudda *et al.* (2015) reported that supplementation of linseed oil in ewes improved milk protein and lactose contents. FCM yield (kg/d) was significantly (P<0.01) improved with diet in RBO (1.19), SBO (1.10), and SRBO (0.94) as compared to CON (0.73) but time effect remained non- significant (Table 2). SCM and ECM yields were significantly (P<0.01) higher in oil supplemented groups but remained unaffected (P>0.05) by time. Titi and Rahman (2013) supplemented sunflower oil and soybean oil and observed increased ECM yield for ewes. However, Bouattour *et al.* (2008) found non-significant difference in ECM yield in soybean oil supplemented group as compared to control in goat. Milk energy output (MJ/d) and milk energy content (MJ/kg) significantly (P<0.01) increased due to oil supplementation. Feed efficiency in relation to milk yield and FCM yield was significantly (P<0.01) improved in

SBO, RBO, and SRBO diet as compared to CON. This is due to increased milk production without any change DM intake which is indicative of improved feed efficient utilization. Morsy *et al.* (2015) also found increased feed efficiency as a consequence of sunflower and soybean oils supplementation in goats.

Data on effect of inclusion of soybean oil and rice bran oil on milk fatty acid profile is presented in table 3. Both the oils either alone or in combination significantly decreased (P<0.001) short and medium chain FA and increased (P<0.001) long chain FA in the milk. Decreased short and medium chain fatty acids concentrations in oil supplemented groups were consistent with previous studies in lactating goats (Bernard *et al.,* 2015; Almieda *et al.,* 2019; Pascual *et al.,* 2019), sheep (Nudda *et al.,* 2015; Ferreira *et al.,* 2018) and cows (Lunsin *et al.,* 2012). Supplementation of PUFA rich oils increases the concentration of CLA isomer (*trans*-10, *cis*-12), which is a powerful inhibitor of the *de novo* fatty acids synthesis in mammary gland as it causes reduction of lipogenic enzymes (acetyl-CoA carboxylase and fatty acid synthetase) (Palmquist *et al.,* 2005). In addition, capric, caproic and caprylic fatty acids are responsible for the characteristic odor of goat

Table 4. Pearson correlation between milk fat concentration and milk fatty acid composition

	OA	LA	LnA	SFA	MUFA	PUFA	n6:n3	SCFA	MCFA	LCFA	AI	TI
Fat	-0.04	0.41*	0.29	-0.11	-0.07	0.42*	0.27	-0.01	-0.27	0.44*	-0.13	-0.01
OA		0.42*	0.20	-0.84**	0.99**	0.41*	0.31	-0.80**	-0.66**	0.83**	-0.86**	-0.93**
LA			0.22	-0.61**	0.37	0.99**	0.84**	-0.52**	-0.80**	0.70**	-0.65**	-0.42*
LnA				-0.24	0.19	0.28	-0.28	-0.24	-0.31	0.30	-0.30	-0.23
SFA					-0.84**	-0.61**	-0.48*	0.84**	0.84**	-0.80**	0.94**	0.92**
MUFA						0.37	0.27	-0.78**	-0.62**	0.78**	-0.85**	-0.94**
PUFA							0.82**	-0.52**	-.081**	0.70**	-0.66**	-0.42*
n6:n3								-0.42*	-0.64**	0.55**	-0.51*	-0.29
SCFA									0.84**	-0.90**	0.93**	0.74**
MCFA										-0.91**	0.93**	0.67**
LCFA											-0.94**	-0.73**
AI												0.87**
TI												

OA, oleic acid; LA, linoleic acid; LnA, lLinolenic acid; SFA, saturated fatty acid; MUFA, mono unsaturated fatty acid; PUFA, polyunsaturated fatty acid; SCFA, short chain fatty acid; MCFA, medium chain fatty acid; LCFA, long chain fatty acid; AI, atherogenicity index, TI, thrombogenicity index, *P<0.05; **P<0.01; ***P<0.001

milk and meat products, which may create consumers resistance for milk and their products (Chilliard *et al.,* 2003). Therefore, reduction of SCFA and MCFA may be an important aspect to increase acceptability of these products by consumers. Furthermore, MCFA reduction is desirable because the lauric, myristic and palmitic acids are associated with cardiovascular diseases (Ferreira *et al.,* 2018). Addition of both PUFA rich vegetable oils either alone or in combination decreased (P<0.05) SFA content and increased (P<0.01) UFA content in the milk. Stearic (C18:0), oleic (C18:1 ω-9) and linoleic acids (C18:2 ω-6) were increased in all three oils supplemented groups as compared to control, whereas content of linolenic acid (C18:3 ω-3) remained similar among groups. Increased LCFA (>18 C) in milk might due to higher dietary intake of C18:2 from soybean oil and rice bran oil in treatment groups. Grummer (1991) indicated that it is easier to increase the stearic acid content of milk fat with MUFA and PUFA rich supplements than to provide stearic acid (C18:0) *per se*. Increased C18:0 and C18:1 fatty acid in oil supplemented group may be a consequence of biohydrogenation of unsaturated C18 FA in the rumen from diet and action of desaturase in mammary gland. C18:0 is a major regulating factor of mammary lipid secretion and is positively correlated with milk fat content in goats (Chilliard *et al.,* 2006), which was reflected in higher milk fat percentage in both oil supplementation as compared to control in the present study.

The activity of Δ^9 desaturase is essential in the process of UFA synthesis which catalyses the introduction of a cis-double bond between carbons 9 and 10 of SFAs with a chain length of 10 to 18 carbons. The product: precursor ratio has been used as a possible indicator of Δ^9 desaturase activity (Lock and Gransworthy, 2002). The desaturase indices for C16:1, C18:1 and Δ^6 desaturase were decreased (P<0.05) for SBO, RBO and SRBO as compared to CON. This might be attributed to increased C18:0 concentrations in the milk, probably arising from ruminal biohydrogenation of linoleic acid (Ferreira *et al.,* 2018). Observed ratio of ω-6:ω-3, which indicates balance between essential fatty

acids, was significantly higher in all the oil supplemented groups as compared to control though it was within normal limit (Bouattour *et al.,* 2008; Manso *et al.,* 2011). According to Simpuolus *et al.* (1999), recommended range of the ω-6: ω -3 for human health is between 5:1 and 10:1.

Atherogenicity index was reduced (P<0.01) in SBO, RBO and SRBO as compared to CON. Reduction in short and medium chain fatty acids might be responsible for the decreased atherogenicity index in the milk (Ibrahim *et al.,* 2020, Bouattour *et al.,* 2008). This index involving the supposed unhealthy saturated FA is considered to be an indicator of risk for coronary heart disease (Ulbricht and Southgate, 1991). Thrombogenicity index (TI), which indicates a tendency for clots to form in the blood vessels, was significantly lower in oil supplemented groups as compared to control. Relatively high hypocholesterolaemic/ hypercholesterolaemic (h/H) ratio is desirable, because higher the h/H ratio, lower the proportion of hypercholesterolaemic fatty acids like C14:0 and C16:0 and their effects on low density lipoprotein (LDL) increases (Osmari *et al.,* 2011). In present findings, h/H ratio was significantly (P<0.001) increased in SBO, RBO and SRBO as compared to CON.

Elongase, responsible for elongation and extension of long chain fatty acid, was significantly (P<0.01) increased in all three oil supplemented groups as compared to control. Peroxidizability index is used to access the stability of PUFA included in food products and higher the PI value, greater the protective potential for coronary artery disease (Janina *et al.,* 2020). In present findings, PI was significantly higher in oil supplemented groups as compared to control, which was highest in SBO followed by RBO, SRBO and CON.

Pearson correlation between milk fat concentration and milk fatty acid composition is presented in table 4. Significant positive correlation was found between milk fat and PUFA (r=0.42) especially with linoleic acid (r=0.41). This might be due to both the oils are ω -6 PUFA rich which was reflected in milk fatty acid profile. Manso *et al.* (2011) found positive correlation (r=0.67) between linoleic acid and PUFA

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content of milk. Further, atherogenicity index (r=-0.86) and thrombogenicity index (r=-0.93) were also negatively correlated with linoleic acid. ω-6: ω-3 ratio was also negatively correlated with atherogenicity index (r=-0.51).

CONCLUSIONS

Soybean oil and rice bran oil supplementation either alone or in combination increased milk yield and its fat content and thus improved the lactation performance of Surti goats. Further, addition of soybean oil and/or rice bran oil showed significant improvement in nutritional quality of milk with respect to FA composition of milk and various lipid quality indices. However, results were more promising in individual oil supplemented groups as compared to mixture of oil.

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Fodder Quality and Yields of Mung Bean as Influenced by Different Weed Management Practices

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ABSTRACT

A field experiment was laid out in Randomized Block Design consisted of eight treatments with three replication to find out suitable weed management in summer mung bean (*Vigna radiata*) under zero tillage condition during summer season of 2019-20 under irrigated condition at ICAR-NDRI, Karnal, Haryana. To evaluate the quality of fodder in terms of, organic matter, total ash, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, acid detergent lignin. The result showed that weed free (T_2) plots recorded higher (P<0.05) green and dry fodder yield, followed by Shaked (Propaquizafop + Imezathyper) (T_6) and Pendimethalin fb one HW at 20 days after showing (T_4), T_2, T_6 and T_4 recorded higher green fodder yield by 59.32, 40.12 and 39.86% over weedy check (T_1). Among weed control treatments, T_4 recorded significantly higher crude protein, ether extract and dry matter content was at par on T_6 and significantly (P<0.05) superior over T_1. Same trends were also observed in CP, EE and dry matter yield (q/ha). The treatments T_4 and T_6 also enhanced fodder quality by reducing neutral detergent fiber, acid detergent fiber and acid detergent lignin content over weedy check. Thus, application of Shaked (Propaquizafop + Imezathyper) at 2 l/ha at 20 days post-sowing would increase yield and quality of summer mung bean fodder under zero tillage condition.

Key words: Crude protein, Fodder yield, Fodder quality, Summer mung bean, Weed management

INTRODUCTION

Agriculture plays major role in Indian economy and it contributes about 17.0% of GDP and about 70% of the population is dependent on agriculture and allied activities for their livelihood (Anonymous, 2019). According to 20th livestock census; India has a livestock population of 535.78 millions showing an increase of 4.6% over 19th livestock census. According to it, total number of milch animal (cow and buffalo) is 125.34 million, which is the largest in the world (Tamta *et al.,* 2019; Anonymous, 2020). India has the highest livestock population and ranks number one in milk production (176.35 Million in 2018-19) (Ali, 2007 and Patel *et al.,* 2016). However, the productivity of Indian cattle is lower than global average (Mallikarjun *et al.,* 2019). One of the major reasons behind lower productivity is scarcity of quality green fodder. The problem may be resolved by mixing of legume fodder with cereal fodder crop (Patil *et al.,* 2009 ; Hindoriya *et al.,* 2019).

Mung bean (*Vigna radiata*) is an important short duration legume crop in India. It is quite versatile crop that can be grown for seeds, green manure, and forage crop. Cultivation of mung bean also enhanced physical, biological and chemical properties of soil. Further, soil fertility could be improved through biological nitrogen fixation with symbiotic association with rhizobium from the atmosphere (Peoples *et al.,* 1995). Mung bean forage could supply adequate amount of nutrients to support adult maintenance in sheep (Garg *et al.,* 2004). Mung bean straw can be mixed with rice and wheat straw to make a bulky component in sheep and goat diets. In a comparison of sheep and goat feeding, mung bean straw was found to be palatable to both species with no deleterious effects on animal health (Khatik *et al.,* 2007). DM digestibility of mung bean straw (64%) fed to ewes *ad libitum* was similar to that of the straws of alfalfa, groundnut and cowpea, and was higher as compared to that of pigeon pea straw (54%). Feeding ewes with mung bean straw increased overall DM

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intake from 12.6 to 18.9 g/kg body weight/day (McMeniman *et al.,* 1988).

In the Northern India, productivity and forage quality of forage decline during mid- to late summer. Solutions to seasonal forage shortages during the summer have traditionally included the use of annual warm-season species for pastures, hay, or silage. Non-traditional crops that could be used as fodder during this period include summer-annual legumes such as mung bean. Forage yields of the crop in the northern western region of India have ranged from 2 to 4 t/ha (Ligon, 1945). A potential advantage of mung bean is that it can be sown as late as May or June, and hence may produce a satisfactory forage yield during the time when forage is in short supply in the region. No issue with palatability or anti-nutritional factors have been reported in mung bean.

Weeds are one of the most important factors to reduce yield and quality of fodder mung bean during summer and rainy season. Being a small growing-period crop, it faces serious weed competition right from the early growth stages (Pandey *et al.,* 1999). The critical period of weed competition in mung bean crop was reported during the 25-30 days (Raghvani *et al.,* 1985; Singh *et al.,* 2021). Mung bean yield may be reduced by up to 50-80 % due to weeds depending upon cultivars, soil moisture level, and other environmental conditions (Kumar *et al.,* 2006; Ali *et al.,* 2011). Therefore, there is a need to find out efficient weed management strategy to appreciate higher growth and yield. The progressive transformation of agriculture concerning intensive use of herbicides is gaining momentum in recent years due higher efficacy and lower cost of such herbicides (Butter *et al.,* 2008). Therefore, chemical weeding under such situation turns out to be indispensible and can be the good alternative to hand weeding (HW). Chemical weed confer an excellent alternative to manual as well as mechanical weeding and supply weed-free environment during early growing stage up to 30-35 days (Dungarwal *et al.,* 2003, Das and Yaduraju, 2011, 2012). Keeping the above information in view, the present study was undertaken to assess the effect of different weed management practices fodder yield and quality of summer mung bean cultivated under zero tillage condition.

MATERIALS AND METHODS

The experiment was conducted at Agronomy Research Farm, ICAR-National Dairy Research Institute, Karnal, and Haryana, located at an altitude of 245 m above mean sea level in Trans Indo-Gangetic Plain of India during summer season of 2019-20. The experiment was conducted following randomized block design (RBD) consisting of eight treatments and three replication *viz.*, T_1-Weedy check, T_2 –Weed free, T_3 – Pendimethalin (PE) at 0.75 kg/ha, T_4-Pendimethalin fb HW at 20 DAS, T_5-Imezathyper (POE) at 75 g/ha at 20 DAS, T_6-Shaked (Propaquizafop+ Imezathyper) (2 l/ha) as POE, T_7 -Pendimethalin at 0.75 kg/ha fb Imezathyper at 75 g/ha at 20 DAS and T8-Pendimethalin at 0.75 kg/ha fb Quizolofop ethyl at 50 g/ha at 20 DAS. The soil of experiment field was clay loam in texture having neutral pH (7.32), medium in Walkley-Black organic carbon (0.53%) and low in $KMnO_4$ oxidizable nitrogen (164 kg/ha), medium in 0.5 M $NaHCO_3$-extractable phosphorus (19.5 kg/ha) and 1N NH_4OAC extractable potassium (227.7 kg/ha) content.

Data on yield was recorded at harvest. Five plants for each treatment were taken for recording the various data. To estimate the quality of fodder crop, representative samples were oven-dried at 60°C to constant weight to determine dry matter (DM) content, was ground using a Wiley mill to pass through a 1 mm sieve and were stored into an airtight polythene bags till further analysis. Proximate principles namely crude protein (CP), crude fiber (CF), ether extracts (EE) and ash was determined according to AOAC (1990). Neutral detergent fibre, acid detergent fiber and acid detergent lignin were measured following methods of Van Soest *et al.* (1991). Non- fiber carbohydrate (NFC) was calculated by following formula:

$$NFC = 100 - (crude\ protein\% - NDF\% - ash\% - crude\ fat\ \%)$$

Data on growth yield and quality parameters were subjected to analysis of variance given by Gomez and Gomez (1984).

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RESULTS AND DISCUSSION

The data presented in Fig. 1 showed that the use of weed management significantly (P<0.05) influenced the green and dry fodder yield. Weed free plots recorded the highest green and dry fodder yield (63.30 and 18.25 q/ha) followed by Shaked (Propaquizafop + Imezathyper) at 20 DAS (55.67 and 14.73 q/ha) treated plots. Weed free treatment and Shaked as POE at 20 DAS recorded (59.32 and 40.12%, respectively) higher green fodder over weedy check. Thus, efficient weed control by herbicides combined with HW influenced yield. Raman and Krishnamoorthy (1999) found the similar results. Variation in yield components of mung bean could be due to difference in growth parameters such as dry matter production. The DM production was outcome of growth parameters like plant height, number. of branches/ plant, number of leaves and LAI. Higher growth attributes lead to higher DM production leading to higher yield.

To provide reasonable diet to the animal, protein is very significant constituents of animal feed because of it play prime role to enhance productivity of animals as protein is used for build- up of new tissue as well as repairing injured tissue. The data on dry matter and crude protein content of mung bean fodder are mentioned in Table 1. The results revealed that weed free plots maximum (P<0.05) dry matter and crude protein (28.83 and 15.16%) followed by Pendimethalin as PE fb one
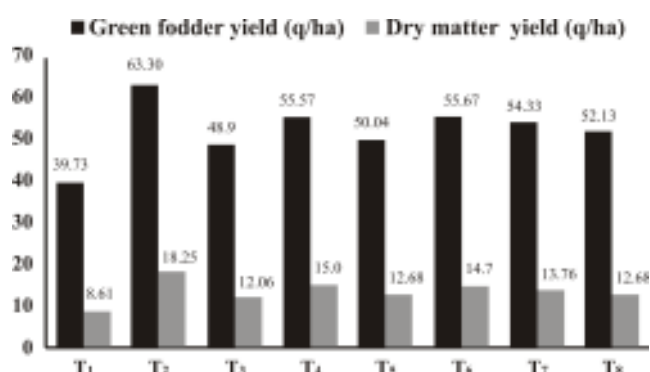

Fig. 1. Effects of different weed management practices on green and dry fodder yield

HW at 20 DAS (27 and 14.19%). Other treatment plots also observed significantly higher (P<0.05) dry matter and crude protein content over weedy check. Weedy check recorded significantly lowest dry matter (21.67 and 12.20%). Similar results were also observed by Peer *et al.* (2013). Weed free, Pendimethalin as PE fb one HW at 20 DAS and shaked managed plots recorded lowest weed growth and crop weed competition for air (CO_2), leading to increased photosynthesis activities of plants, which helped generate greater dry matter accumulation (Srinivashu *et al.,* 2004; Ginwal *et al.,* 2019)). Additionally, less crop weed competition for nutrients might have aided higher absorption of nutrient by crop that reflected in higher amino acid and crude protein content.

Data pertaining to organic matter and total ash

Table 1. Effects of different weed management practices on dry matter and crude protein content of fodder mung bean

Treatments	Dry matter (%)	Crude protein (%)
Weed check	21.67	12.20
Weed free	28.83	15.16
Pendimethalin (PE)	24.67	13.23
Pendimethalin (PE) fb one hand weeding	27.00	14.19
Imezathyper (POE) at 20 days after sowing	25.33	13.37
(Propaquizafop + Imezathyper) as POE	26.4	13.95
Pendimethalin (PE) fb Imezathyper (POE)	25.33	13.67
Pendimethalin (PE) fb Quizolofop ethyl	24.33	13.47
SEm (±)	0.55	0.29
CD (P=0.05)	1.66	0.89

SEm, standard error of mean; CD, critical difference

Table 2. Effects of different weed management practices on total ash, organic matter (OM), and ether extract (EE) content of fodder mung bean

	Ash(%)	OM(%)	EE(%)
Weed check	11.27	88.73	2.41
Weed free	12.17	87.83	2.65
Pendimethalin (PE)	11.34	88.66	2.48
Pendimethalin (PE) fb one hand weeding 20 DAS	12.16	87.84	2.62
Imezathyper (POE) at 20 DAS	11.65	88.35	2.53
(Propaquizafop + Imezathyper) as POE at 20 DAS	11.61	88.39	2.61
Pendimethalin (PE) fb Imezathyper (POE) at 20 DAS	12.02	87.33	2.42
Pendimethalin (PE) fb Quizolofop ethyl at 20 DAS	11.89	88.11	2.54
SEm (±)	0.26	0.26	0.06
CD (P=0.05)	NS	NS	0.08

DAS, days after sowing; SEm, standard error of mean; CD, critical difference

content of mung bean fodder are presented in Table 2 which showed non-significant effect of weed management treatments, however, weed free plots recorded significantly (P<0.05) higher ether extract (2.65%) content which was at par on Pendimethalin as PE fb one HW at 20 DAS (2.62%) and Shaked at 20 DAS (2.61%). Increasing EE content boost availability of fat soluble vitamins *i.e*. A, D, E and K. Ether extract recorded higher under those treatments, which have low crop-weed competition due to efficient management of weed during critical competition period. Data presented on table 3 show that CP and EE yield was significantly affected due to different weed management practices. Weed free plots recorded significantly (P<0.05) higher CP and EE yield (2.77 and 0.48 q/ha) which was at par on Pendimethalin as PE fb one HW at 20 DAS and Shaked at 20 DAS. Total ash yield did not vary significantly due to different weed management treatments. Higher total ash yield was recorded in weed free treatment followed by Pendimethalin as PE fb one HW at 20 DAS and Shaked at 20 DAS.

The data pertaining to NDF, ADF and ADL (Table 4) revealed that weedy check plots recorded significantly (P<0.05) higher NDF, ADF and ADL

Table 3. Effects of different weed management practices on crude protein (CP), ether extract (EE) and total ash yield

Treatments	CP yield (q/ha)	EE yield (q/ha)	Total ash yield (q/ha)
Weed check	1.05	0.21	2.44
Weed free	2.77	0.48	3.51
Pendimethalin (PE)	1.60	0.30	2.79
Pendimethalin (PE) fb one hand weeding 20 DAS	2.13	0.39	3.28
Imezathyper (POE) at 20 DAS	1.70	0.32	2.95
(Propaquizafop + Imezathyper) as POE at 20 DAS	2.05	0.37	3.07
Pendimethalin (PE) fb Imezathyper (POE) at 20 DAS	1.88	0.33	3.04
Pendimethalin (PE) fb Quizolofop ethyl at 20 DAS	1.71	0.32	2.85
SEm (±)	0.04	0.01	0.09
CD (P=0.05)	0.78	0.11	NS

DAS, days after sowing; SEm, standard error of mean; CD, critical difference

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Table 4. Effects of different weed management practices on neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicelluloses and cellulose content of fodder mung bean

Treatments	NDF (%)	ADF (%)	ADL (%)	Hemi cellulose (%)	Cellulose (%)
Weed check	41.97	31.06	5.67	10.91	22.43
Weed free	37.00	28.07	4.72	8.93	23.35
Pendimethalin (PE)	40.15	28.53	5.17	11.62	23.37
Pendimethalin (PE) fb one HW 20 DAS	37.16	27.57	4.97	9.59	22.60
Imezathyper (POE) at 20 DAS	41.41	30.21	5.30	11.20	25.64
(Propaquizafop + Imezathyper) as POE at 20 DAS	38.00	28.87	4.93	9.13	26.16
Pendimethalin (PE) fb Imezathyper (POE) at 20 DAS	37.33	29.60	5.18	7.73	24.26
Pendimethalin (PE) fb Quizolofop ethyl at 20 DAS	37.50	27.00	4.83	10.50	22.17
SEm (±)	1.95	1.10	0.19	0.65	0.97
CD (P=0.05)	NS	NS	NS	NS	NS

DAS, days after sowing; SEm, standard error of mean; CD, critical difference

(41.97, 31.09 and 5.67%, respectively) followed by Imezathyper as POE (41.41, 30.21 and 5.30%, respectively). Weed free plots recorded lowest (P<0.05) NDF, ADF and ADL value (37, 28.07 and 4.72%, respectively). Herbicides application did not affect NDF and ADF content in pasture (Cinar *et al.,* 2013). The total NDF (%) content of crop were decided by crop morphology, genetic constituents and agronomic managements. Low NDF, NDF and ADL content indicates good quality fodder. Inverse relationship is there between lignin content and digestibility. Propaquizafop + Imezathyper as POE at 20 DAS recorded higher cellulose content, whereas application of Pendimethalin (PE) recorded higher hemicellulose content as compared to other treatments (Table 4).

NFC is made-up of starch, soluble fiber and simple sugars. Data pertaining to NFC and TDN are presented on Table 5. TDN yield was significantly (P<0.05) affected by treatments and higher value was recorded in weed free (39.20 q/ha) plots followed by plots treated with Shaked as POE at 20 DAS (34.28 q/ha) and Pendimethalin (PE) fb one HW 20 DAS (34.20). Higher NFC and TDN value indicate higher fodder quality. Weed free condition improve plant growth and health, this ultimately leads to higher fodder quality (Yadav *et al.,* 2016).

Table 5. Effects of different weed management practices on non- fiber carbohydrate (NFC), total digestible nutrient (TDN) and total digestible nutrient yield of mung bean

Treatments	NFC (%)	TDN (%)	TDN yield (q/ha)
Weed check	33.71	59.52	23.65
Weed free	34.71	61.93	39.20
Pendimethalin (PE)	34.37	60.82	29.74
Pendimethalin (PE) fb one HW 20 DAS	35.35	61.54	34.20
Imezathyper (POE) at 20 DAS	32.51	60.02	30.03
(Propaquizafop + Imezathyper) as POE at 20 DAS	35.52	61.57	34.28
Pendimethalin (PE) fb Imezathyper (POE) at 20 DAS	36.14	60.66	32.96
Pendimethalin (PE) fb Quizolofop ethyl at 20 DAS	36.13	61.83	32.23
SEm (±)	0.26	1.66	0.26
CD (P=0.05)	NS	NS	4.56

DAS, days after sowing; SEm, standard error of mean; CD, critical difference

Table 6. Correlation matrix of green fodder yield *vs.* fodder quality

Pearson	Correlations										
	GFY	**CP**	**EE**	**TA**	**NDF**	**ADF**	**ADL**	**HC**	**Cellulose**	**OM**	**DM**
GFY	1										
CP	.986**	1									
EE	.772*	.822*	1								
TA	.819*	.809*	0.562	1							
NDF	-.837**	-.786*	-0.56	-.84**	1						
ADF	-0.627	-0.611	-0.641	-0.59	.765*	1					
ADL	-.894**	-.866**	-.801*	-.710*	.856**	.875**	1				
HC	-0.64	-0.578	-0.2	-0.69	.749*	0.15	0.414	1			
Cellulose	0.211	0.151	0.155	-0.13	0.104	0.35	-0.01	-0.2	1		
OM	-0.662	-0.604	-0.174	-.88**	.770*	0.33	0.472	.841**	0.026	1	
DM	.961**	.982**	.834*	.751*	-0.7	-0.54	-.80*	-0.52	0.271	-0.55	1

**.Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed); GFY, green fodder yield; CP, crude protein; EE, ether extract; TA, total ash; NDF, neutral detergent fiber; ADF, acid detergent fiber, ADL, acid detergent lignin; HC, hemi cellulose, OM, organic matter, DM, dry matter

Correlation studies (Table 6) indicates that the fodder quality of mung bean i.e., CP (r=0.986), EE (r=0.772), TA (r=0.819) and DM (r=0.961) content were positively correlated with green fodder yield. Cellulose content was also positively correlated with fodder yield, but the association was moderate (r=0.211). Green fodder yield was negatively correlated with the contents of NDF (r= -0.837), ADF (r= -0.627), ADL (r= -0.894) and HC (r= -0.64). Quality parameter like CP, EE, DM and TA increase with increase in green fodder yield, however, fiber fractions like NDF, ADF and ADL are inversely related with green fodder yield. Correlation matrix also indicates that CP and other quality parameters were inversely related with fiber fractions.

CONCLUSION

Based on the present finding it may be concluded that application of Shaked (Propaquizafop + Imezathyper) at 2 l/ha at 20 DAS may be recommended to obtain higher yield and fodder quality of summer mung bean under zero tillage condition.

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Combined Effect of Bel and Curry Leaves Supplementation on the Performance of Anestrous Cattle

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ABSTRACT

To assess the effect of bel and curry leaves supplementation on the performances of anestrous cattle, 40 cross bred cattle (24 heifers and 16 cows) were divided into four groups of 10 animals each (containing 06 heifers and 04 cows) following completely randomised design. Dietary treatments were as follows: Group T_1: control (concentrate mixture and paddy straw), group T_2: group control diet + dried bel leaves at 50 g/animal/day, group T_3: control diet + dried curry leaves at 50 g/animal/day, and group T_4: control diet + dried bel leaves at 50 g/animal/day and dried curry leaves at 50 g/animal/day. The experiment was continued for 60 days. The growth performances were measured in terms of body weight and average daily gain (ADG). Blood collection was done at the end of the experiment for assessment of blood biochemical and antioxidant status of the animals. Results revealed significant (P<0.05) improvement in antioxidant status and reproductive performances and decreased (P<0.01) total cholesterol and triglycerides concentration in treatment group as compared to control group. Higher percentage of conception was achieved in group T_4 (50%) followed by group T_3 (30%) and group T_2 (20%), the least percentage was in group I (0%). Therefore, it was concluded that supplementation of bel and curry leaves prevent stress and improved the reproductive status of anestrous cattle.

Key words: Anestrous, Bel, Curry, Stress, Reproduction

INTRODUCTION

Reproductive disorders are the major bottleneck in exploiting the fullest production potential of livestock. Anestrous is one of the most common functional reproductive disorders occurring almost in all species of livestock that adversely affect the economics of production. It lowers the reproductive rate of heifers by delaying the entry into the breeding herds, decreases the number of total calves and milk yield per cow resulting in significant economic loss to the small and marginal farmers as well as dairy industry as a whole (Chaudhari *et al.,* 2012). Now-a-days, various medicinal plants have brought future promise to treat reproductive disorders in livestock as being cheaper, efficacious and safer alternatives to costlier hormones. In early human civilizations, the problem of infertility was treated using herbs and other traditional ways. *Murraya koenigii* (Curry leaf) and *Aegle marmelos* (Bel) leaves have been documented in traditional Indian practice to promote fertility in animals. Leaves of these

plants promoted hypocholesterolemic effects and helpful against oxidative stress and lipid peroxidation action (Mitra *et al.,* 2012). Bel and curry leaves have been demonstrated individually to augment the reproductive function in laboratory rats, anestrous goats and buffaloes (Kumar, 2008; Jondhale *et al.,* 2009). Thus, it was hypothesized that a combination of these two leaves would further improve the reproductive performance. Keeping in view the magnitude and complexity of the problem, the present experiment was designed to asses the combined effect of bel and curry leaves supplementation on the growth, blood metabolites, oxidant/antioxidant status and reproductive performance in cross bred cattle.

MATERIALS AND METHODS

Forty anestrous cattle (24 heifers above 3 years of age and 16 post-partum anestrous cows from 2nd to 3rd parity) with no physiological and anatomical abnormality were selected from Kakatpur block in Puri district of Odisha. These animals were maintained on

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diet comprising of concentrate mixture and paddy straw. Animals were dewormed with broad spectrum anthelmintic (Fenbendazole at 10 mg/kg body weight). All the animals were kept in a well-ventilated sheds with individual feeding and watering arrangements. The concentrate mixture consisted of 25% crushed maize, 30% soya bean meal, 42% wheat bran, 2% mineral mixture and 1% common salt. Animals were divided into four groups of 10 animals each (containing 06 heifers and 04 cows) following completely randomised design. Dietary treatments were Group T_1: control (concentrate mixture and paddy straw), group T_2: control diet supplemented with dried bel leaves at50 g/animal/day, group T_3: control diet supplemented with dried curry leaves at 50 g/animal/day, and group T_4: control diet supplemented with dried bel leaves at 50 g/animal/day and dried curry leaves at 50 g/animal/day. The experiment was continued for 60 days. All the animals were maintained as per the standard management practices. The green bel and curry leaves were collected and washed thoroughly and sun dried for 48 h. The dried leaves were grinded into powder with the help of vertical grinder and stored in 50 g plastic bags at room temperature. The grinded leaves were fed to the animals by mixing with jaggery. The chemical composition of concentrate mixture, paddy straw, dried bel and curry leaves were estimated as per AOAC(1995).The body weight of the animals was recorded in monthly interval during the experimental period using Johnson's formula (1940).

After 60 days of the experimental feeding, blood samples (10 ml) were collected aseptically from each animal by jugular vein puncture in the morning (before watering and feeding), into clean and dry test tubes and kept in slanting position for 45 minutes at room temperature. The blood samples were then centrifuged at 3000 rpm for 15 minutes to harvest serum and stored at -20°C for further analysis. The serum biochemical parameters like glucose, total protein, urea, triglycerides and total cholesterol were estimated in automated biochemistry analyser (Turbo Chem 100, CPC Diagnostic Pvt. Ltd., Chennai, India) by following

standard procedure. Likewise, whole blood (2 ml) was collected from each animal into sterilized micro-centrifuge tube containing 0.30 ml of acid citrate dextrose (ACD, citric acid 8.0 g: sodium citrate 22.0 g and dextrose 25.0 g and volume made to 1 litre in distilled water) as anticoagulant. The blood samples were centrifuged at 3000 rpm for 10 min at 4°C and plasma and buffy coat were separated. The resulting erythrocyte pellet was washed thrice with phosphate buffer saline (PBS; disodium hydrogen phosphate 13.65 g, sodium dihydrogen phosphate 2.43 g and sodium chloride 10 g dissolved in 800 ml distilled water, pH adjusted to 7.4 and volume made to 1litre) (Yagi *et al.,* 1989). RBC diluted to 1:1 in PBS was used for the estimation of haemoglobin. For the estimation of catalase, super oxide dismutase (SOD) and lipid peroxidation (LPO), 1 ml of the 1: 1 diluted RBCs in PBS were mixed with 9 ml distilled water to prepare RBC haemolyzate of 1:20 dilution. Lipid peroxidation in RBC hemolyzate was determined following method of Placer *et al.* (1966); wherein, the concentration of malonaldialdehyde (MDA) in nmol of MDA/mg haemoglobin was calculated using the extinction coefficient of 1.56×10^8 (Utley *et al.,* 1967). Catalase (CAT) was assayed in erythrocytes by the method of Bergmeyer (1983). Super oxide dismutase (SOD) activity of RBC haemolyzate samples was measured using nitro blue tetrazolium as a substrate after suitable dilution according to Marklund and Marklund (1974) with certain modifications as suggested by Minami and Yoshikawa (1979).

All animals were regularly monitored for the onset of heat. The heat was detected by behavioural symptoms (Layek *et al.,* 2011). Animals exhibiting the sign of heat were per-rectally inseminated artificially by the local veterinary assistant surgeon. Pregnancy diagnosis was conducted routinely per rectum at 45 days post-insemination. Data obtained were subjected to one-way analysis of variance using Software Package for Social Sciences (SPSS) version 17.0 (2008) and comparison among treatment means was made by Duncan's multiple range test (Duncan, 1955) with

Table 1. Chemical composition of feeds offered to anestrous cattle (%DM basis)

Attributes	Concentrate mixture	Paddy straw	Bel leaves	Curry leaves
Organic matter	90.10	89.40	90.86	86.43
Crude Protein	19.40	2.01	5.82	7.35
Crude fibre	12.77	38.90	15.56	12.80
Nitrogen Free extract	55.13	46.39	67.58	61.67
Ether extract	2.80	2.10	1.90	4.61
Total Ash	9.90	10.60	9.14	13.57

significance level of P<0.05.

RESULTS AND DISCUSSION

Data on the chemical composition of concentrate mixture, paddy straw, bel and curry leaves are presented in Table 1. The crude protein (CP) content of the concentrate mixture and paddy straw was 19.40 and 2.01 per cent respectively (Table 1). The bel leaves contained 5.82% CP; 15.56% crude fiber (CF) and

similar (P>0.05) in all the treatment groups (Table 2) indicating that supplementation of bel and curry leaves have no effect on body weight gain of anestrous cattle. This finding was in agreement with the observations of significant decrease in body weight of rats fed bel leaves powder (Asghar *et al.*, 2018). Sahoo *et al.* (2001) reported that feeding of polyherbal preparation containing *Withania somnifera, Sphaeranthus indicus,*

Table 2. Body weight changes in experimental animals

Days	Treatments				P value
	T_1	T_2	T_3	T_4	
Initial (0 day), kg	321.7±17.35	324.8±17.56	325. 7±19.73	327.0±19.76	0.998
Final (60 days), kg	332.8±17.75	336.3±17.55	336.5±20.28	338.7±19.9	0.901
Gain (kg)	11.16±1.54	11.50±1.23	10.83±0.76	11.67±1.09	0.762
ADG (g)	186.0±24.31	191.7±19.65	180.5±12.47	194.5±17.56	0.743

9.14% total ash. Curry leaves contain 7.35% CP, 12.80% CF and 13.57% total ash respectively (Table 1). Jain *et al.* (2017) also observed that *Murraya koenigi* contain 2.1-12.5% protein, 9.70-13.06% total ash and 1.35-1.82% of acid insoluble ash. Average daily gain was

Loranthus falcata, Scrophularia lielzzi, Panax ginseng, Nyctanthes arbor-tristis, Phyllanthus emblica, Mimosa tenuiflora, Ocimum tenuiflorum and *Tinospora cordifolia* to Black Bengal goat in the last month of the pregnancy resulted in significantly higher

Table 3. Serum biochemical profile of cross bred animals

Parameter	Treatments				P value
	T_1	T_2	T_3	T_4	
Glucose(mg/dl)	54.14±4.09	53.61±3.17	55.01±3.53	52.06±3.48	0.937
Total Protein (g/dl)	6.81±0.03	7.05±0.22	6.80±0.02	6.79±0.03	0.311
Urea (mg/dl)	34.40±7.12	28.11±3.12	33.20±5.62	38.90±3.08	0.441
Triglyceride (mg/dl)	81.42[a]±1.81	68.60[b]±3.53	76.06[b]±1.74	70.72[b]±0.85	0.002
Total cholesterol (mg/dl)	154.34[a]±10.28	131.82[b]±5.22	114.75[b]±8.10	125.01[b]±5.61	0.009

[a,b]Means bearing different superscripts in a row differ significantly (P<0.05)

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Table 4. Antioxidant enzyme status of anestous cattle supplemented with bel and curry leaves

Parameter	Treatments				P value
	T_1	T_2	T_3	T_4	
Catalase (U/mg Hb)	$1.57^a \pm 0.08$	$1.76^b \pm 0.12$	$1.68^b \pm 0.11$	$1.92^c \pm 0.20$	0.035
SOD (U/mg Hb)	$19.16^a \pm 2.12$	$22.11^b \pm 4.05$	$23.20^b \pm 3.77$	$23.90^b \pm 1.68$	0.013
LPO (μmol MDA formed / mg Hb)	$3.41^c \pm 0.11$	3.11 ± 0.24^b	$2.96^a \pm 0.12$	$3.04^a \pm 0.19$	0.030

[a,b]Means bearing different superscripts in a row differ significantly (P<0.05)

birth weight and absence of kid mortality.

The data regarding blood glucose, total protein, urea, triglycerides and total cholesterol at 60 days of experiment are presented in Table 3. Among the different serum biochemical parameters, the triglycerides and total cholesterol were significantly (P<0.01) lower in treatment groups than control. Higher concentration of genistein, diphenolic phytoestrogens present in *Murraya koenigii* might be responsible for this effect (Ramadan *et al.*, 2015). Thomas *et al.* (2017) suggested that bio active compound present in the plant was responsible for depleting total cholesterol levels by reducing fat absorption in the digestive system and increasing fat excretion into faeces. Krupanidhi *et al.* (2016) observed that plant secondary metabolites like flavonoids present in A. *marmelos and M. koenigii* are responsible to maintain the normal lipid profile in rats through high lecithin acetyl transferase activity, which regulates blood lipids concentration.

Supplementation of bel and curry leaves significantly (P<0.05) increased erythrocytic catalase, SOD levels and decreased (P<0.05) lipid peroxidation as indicated by fall in MDA levels in all the treatments than control animals (Table 4). Bel and curry leaves have potential to protect against oxidative stress.

These plants has been chemically characterised to be rich in alkaloids, polyphenols, flavonoids and chlorophyll. The antioxidant properties of alkaloids, poly phenols, flavonoids and phytols (breakdown of chlorophyll) from different herbal sources are used as nutritional supplements against oxidative stress. (Tachibana *et al.,* 2001; Ningappa *et al.,* 2008).

The overall conception rates in different treated groups were found to be 20, 30 and 50% respectively (Table 5). Supplementation of either bel or curry leaves improved the conception rate that corroborated well with the finding of Kumar *et al.* (2009) in anoestrus buffaloes and Das *et al.* (2016) in delayed pubertal heifers. The maximum conception rate was found in combined bel and curry treated group than other treatments. Pharmacological active compounds like koenimbine, murrayacine, girinimbine, koenigine, koenine, mahanimbine, murrayanol and mahanine present in these leaves have higher antimicrobial, anti-inflammatory, and antioxidant properties (Handral *et al.,* 2012), which may be responsible for increased conception in treated animals. Presence of genistein in the plant may be another contributing factor which bind with estrogen receptor and thus stimulating development and maintenance of reproductive organs with estrous

Table 5. Distribution of conceived animals in different treatment at the end of experiment

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Total number of animal	10	10	10	10
Number of animal comes to estrus	1	4	4	6
Number of animal conceive	0	2	3	5
Conception rate (%)	0	20.00	30.00	50.00

induction (Kumi-Diaka *et al.,* 1998).

CONCLUSION

Supplementation of dried bel (50 g) and curry leaves (50 g) per animal/per day improved the antioxidant status and reproduction rate and reduced the blood cholesterol and triglycerides concentration of anoestrous cattle.

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Effect of Soybean Straw and Seaweed (*Sargassum johnstonii*) Based Total Mixed Ration on Feed Intake, Digestibility and Rumen Fermentation in Crossbred Cows

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ABSTRACT

An experiment was conducted to evaluate the effect of soybean straw and seaweed supplementation on feed intake, nutrient digestibility and rumen fermentation in lactating crossbred cows. Six Holstein Friesian × Kankrej (50:50) crossbred cows were used in an experiment based on switch over design for 135 days with three periods each of 45 days duration. The animals received three treatments as total mixed ration *viz.* T_1: TMR with compound concentrate mixture (60%) and wheat straw (40%); T_2: TMR with compound concentrate mixture (60%), wheat straw (20%) and soybean straw (20%); T_3: TMR with 8% seaweed (*Sargassum johnstonii*). Dry matter (DM) and digestible crude protein intake were at par in T_1 and T_2, but significantly (P<0.05) lower in T_3 while total digestible nutrients intake differed significantly (P<0.05) among all groups. There was no significant difference in nutrient digestibility except lower (P<0.05) DM digestibility in group T_3. Rumen fermentation parameters were also similar among the treatment groups except that total volatile fatty acids (TVFA) concentration was significantly lower (P<0.05) in group T_3. Microbial protein synthesis was also similar in all the groups. The study revealed low feed intake, TVFA and dry matter digestibility in seaweed supplemented group.

Key words: Digestibility, Rumen fermentation, Seaweed, Soybean straw, TMR

INTRODUCTION

Seaweeds are macroalgae having different shapes, sizes, colour and composition, and are inhabitant of littoral zone of aquatic ecosystem (Bast, 2014). Seaweeds are rich sources of essential amino acids, vitamins like A, B_1, B_2, B_3, B_{12}, C, D, E, ω-3 fatty acids and various biologically active compounds essential for life and maintaining good health (Yende *et al.,* 2014). In India, seaweeds are less utilized as traditional livestock feed but nowadays research is aimed to use seaweed resources as feed additive and supplement (Singh *et al.,* 2015; Munde *et al.,* 2019; Sharma and Datt, 2020; Maheswari *et al.,* 2021). However, response of seaweed supplementation depends on many factors like species and physiological satatus of the animal, species and doses of the seaweed, and processing methods employed during the preparation of seaweed meal (Makkar *et al.,* 2016). Fibrous crop residues are valuable feed resources, but poor nutritive value and digestibility restrict their utilization. Legume straw are better than cereal straws due to more protein and energy content as well as more digestibility and less methane production (Beauchemin *et al.,* 2008, Prajapati, 2016; Khare *et al.,* 2018; Jasvantgiri, 2019). Considering the benefits of feeding of legume straw and seaweeds on animal performance it would be pertinent to evaluate both these feed ingredients as feed supplement for ruminants. Hence, the present study was undertaken to study the effect of seaweed and soybean straw based TMR on dry matter and nutrient intake, nutrient digestibility and rumen fermentation in crossbred dairy cows.

MATERIALS AND METHODS

The experiment was conducted on six Holstein Friesian X Kankrej (50:50) crossbred cows using switch over design with three periods of 45 days each. After 45 days, the animals were switched over to another treatment. Thus, each treatment had 6 observations. However, one animal was removed from the experiment due to sickness hence data were compiled for 5 animals in each treatment. Permission was granted by Institutional Animal Ethics Committee (IAEC/310/

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Table 1. Ingredient composition (%) of total mixed rations

Ingredients	T_1(Control)	T_2(Soybean Straw)	T_3(Seaweed)
Wheat straw	40.0	20.0	40.0
Soybean straw	0.0	20.0	0.0
Compound Concentrate Mixture	46.0	40.0	46.0
De oiled Rice Bran	8.0	10.0	0.0
Seaweed (*S. johnstonii*)	0.0	0.0	8.0
Molasses	5.0	9.0	5.0
Mineral mixture	1.0	1.0	1.0

ANRS/2019) to conduct the experiment. The dried biomass of seaweed was procured from local vender in Veraval, Gujarat. The treatments were T_1: TMR with concentrate mixture and wheat straw (60:40); T_2: TMR with 20 % each of wheat straw and soyabean straw and 60% concentrate mixture and T_3: TMR (60:40) with seaweed (*Sargassum johnstonii*) at 8%. The ingredients composition is presnted in Table 1. Quantity of TMR offered was to meet nutrient requirement as per ICAR (2013) feeding standards. The quantity of TMR required each day was offered in two instalments *i.e.* half in morning and remainder in the evening.

Daily feed intake and leftover were recorded for each animal. The DM and nutrient intake were calculated. Body weight (BW) was measured fortnightly in morning before feeding and watering using electronic weighing machine. In the last week of each period, a digestion trail was conducted on all the animals to determine digestibility of nutrients. Representative samples of feed and faeces were collected and analysed for proximate principles as per AOAC methodology (AOAC, 2016) and fibre fraction by Van Soest method (Van Soest *et al.,* 1991). Rumen liquor was collected at 0, 3 and 6 hrs post feeding through stomach tube against negative pressure created by suction pump and strained through four-layer muslin cloth for chemical analysis. Rumen pH was estimated immediately after collection by portable digital pH meter. The total and soluble nitrogen (N) was estimated by Kjeldahl's method, non-protein nitrogen by trichloroacetic acid precipitation, ammonia N by Pearson and Smith (1943) and total volatile fatty acid (TVFA) by steam distillation method using Markham micro distillation apparatus. Urine samples (100 ml) from individual animal was collected in each period and analysed for allantoin, uric acid and creatinine using spectrophotometer (Young and Conway, 1942). Purine derivatives (PD) were measured by spot sample test based on the fact that excretion of creatinine is constant throughout a day, therefore, creatinine was used as an internal marker for estimation of PD (Chen *et al.,* 1992). Daily excretion of creatinine was considered as 0.98 mmol/kg $W^{0.75}$ and microbial N supply was calculated from the daily urinary PD excreted (IAEA, 1997).

The experimental data were analysed as per methods of Snedecor and Cochran (1994) by analysis of variance (ANOVA) using General Linear Model (GLM) procedure with the help of SAS software programme.

RESULTS AND DISCUSSION

The intake of dry matter (DM), crude protein (CP), digestible crude protein (DCP) and total digestible nutrients (TDN) are presented in Table 2. Average daily DM intake (DMI) was significantly (P<0.01) lower by 10.35% in T_3 than T_1. Similar trend was also observed for DMI (% of BW) which decreased by 8.75 % in cows fed seaweed compared to control group. The DMI was less in T_3 which may be due less palatability of seaweed. This was evident from the observations that in each period, irrespective of animals, the DMI decreased in T_3 group in last two weeks of period. This indicated less acceptance of seaweed based TMR by animals for longer period. Due to less DMI, the nutrient intake also decreased in T_3. The intake of CP, DCP and TDN were lower by 13.5, 11.1 and 11.2 % respectively

Table 2. Nutrient intake and body weight changes in crossbred cows

Parameter	T₁	T₂	T₃	SEM	P -value
DMI (kg/d)	12.56[A]	12.25[A]	11.26[B]	0.109	0.0004
DMI (kg/100 kg BW)	3.20[A]	3.08[B]	2.92[C]	0.021	0.0003
CPI (kg/d)	1.48[A]	1.38[A]	1.28[B]	0.032	0.0007
DCPI (kg/d)	0.90[A]	0.88[A]	0.80[B]	0.009	0.0006
TDNI (kg/d)	6.44[A]	6.15[B]	5.72[C]	0.049	0.0002
Body weight (kg)	398.90	399.72	401.37	0.109	0.6747
Body weight gain (kg/d)	0.53[A]	0.57[A]	0.24[B]	1.905	0.0499

[A,B,C] Mean having different superscript in a row differ significantly; DMI, dry matter intake; CPI, crude protein intake; DCPI, digestible crude protein intake; TDNI, total digestible nutrient intake

in T_3 than T_2. The literature regarding feeding seaweed to cows has shown variable responses. Roque *et al.* (2019) observed significant (P<0.001) decrease in dry matter intake of Holstein cows by 10.8 and 38.0 % at low (0.5%) and high (1%) level of *Asparagopsis armata* inclusion, respectively compared to control group. Kinley *et al.,* (2020) also reported 10.8 % reduction in DM intake in steers fed ration with 0.05% *Asparagopsis taxiformis*. However, other reports did not find any significant difference in DMI of cows (Baek *et al.,* 2015; Hong *et al.,* 2015; Sharma and Datt, 2020) and buffaloes (Maheswari *et al.,* 2021) when fed with seaweed products. Similarly, Singh *et al.* (2015) observed that supplementation of brown seaweed (*Sargassum wightii*) in the diet of lactating Sahiwal cows to the extent of 20 % in concentrate mixture did not cause any significant difference in DM, DCP, and TDN intake and body weight changes among treatment groups. No adverse effect of soyabean straw on DM and nutrient intake was observed in present study.

Mudgal *et al.* (2010) also reported non-significant difference in DM intake in crossbred cows when soybean straw replaced wheat straw up to 50 and 75% level. Similarly, feeding of Lucerne straw (Lunagariya *et al.,* 2016; Jasvantgiri, 2019) and groundnut straw (Prajapati, 2016; Sherasia *et al.,* 2018) based TMR did not result in any significant change in DM, DCP and TDN intake in large ruminants compared to only wheat straw based TMR. Khare *et al.* (2018) reported significantly higher intake of DM, DCP and TDN in cows with 20% inclusion level of soyabean straw.

Data pertaining to the apparent digestibility of nutrients is presented in Table 3. There was no significant difference in digestibility of organic matter (OM), CP, ether extract (EE), crude fibre (CF), and nitrogen-free extract (NFE) among treatments. However, DM digestibility was significantly (P<0.05) lower in T_3 by 21.54 % than T_1. Optimum DM and nutrient intake are necessary for better rumen fermentation and substrate degradation. In our study,

Table 3. Digestibility coefficient of nutrients

Parameter	T₁	T₂	T₃	SEM	P value
DM	56.80[A]	49.65[B]	46.73[B]	1.07	0.02
OM	55.99	52.62	56.28	1.27	0.14
CP	62.81	57.51	62.31	2.10	0.208
EE	70.53	68.37	68.11	2.09	0.68
CF	42.72	38.94	46.09	2.18	0.13
NFE	52.53	48.56	50.57	1.40	0.19

[A,B,] Mean having different superscript in a row differ significantly; DM, dry matter, OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; NFE, nitrogen-free extract

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Table 4. Rumen fermentation parameters in crossbred cows

Particular	T_1	T_2	T_3	SEM	P value
pH	6.87	7.04	7.00	0.06	0.180
Total N	91.93	100.49	91.00	4.85	0.360
NH$_3$N	18.36	19.06	18.43	1.05	0.878
Soluble N	13.30	13.46	13.22	0.31	0.870
NPN	29.09	28.31	28.16	0.64	0.570
TVFA	13.93[A]	14.62[A]	12.53[B]	0.36	0.010

[A,B] Mean having different superscript in a row differ significantly; NPN, non-protein nitrogen; TVFA, total volatile fatty acids

the DM and nutrient intake were significantly lower in seaweed (T_3) fed group which may have affected rumen fermentation and DM digestibility. This is further confirmed from lower TVFA production in T_3. Replacement of 20 % of mineral mixture in concentrate with *Sargassum wightii* (Singh *et al.,* 2015) and *Kappaphycus alvarezii* based seaweed product (Sharma and Datt, 2020) in dairy cows and supplementation of tropical seaweed based formulation in lactating buffaloes (Maheswari *et al.,* 2021) did not cause any adverse effect on digestibility of nutrients. However, Bendary *et al.* (2013) reported better digestibility of nutrients with supplementation of seaweeds at 50 g/day in Friesian cows. Supplementation of soybean straw (T_2) in TMR replacing wheat straw had no adverse effect on digestibility of nutrients. Khare *et al.* (2018) also reported no significant effect of supplementing soyabean straw up to 20% in the ration of crossbred cows on digestibility of nutrients. Sherasia *et al.* (2018) in cattle and Chaudhari (2019) in crossbred calves also reported

no adverse effect of replacing wheat straw by groundnut straw, respectively on digestibility of nutrients which corroborates with the present findings.

The average values of rumen pH, nitrogen fractions and total volatile fatty acids (TVFA) are depicted in Table 4. All the parameters, except TVFA, were at par among the groups and within the normal range. Volatile fatty acids in rumen are produced during anaerobic fermentation of feeds and fodder. Total volatile fatty acid production was significantly (P<0.01) reduced in T_3 group which may be due to less DM and nutrient intake and digestibility of DM. Hong *et al.* (2015) also reported decrease in VFA production at 24 h of incubation in Holstein cows at 4% level of brown seaweed. However, Bendary *et al.* (2013) observed higher TVFA and low NH$_3$-N in Friesian cows supplemented sea weeds at 50 g seaweed/day. Kinley *et al.* (2020) in beef steers and Li *et al.* (2018) in sheep observed no adverse effect of supplementation of seaweed (*Asparogopsis spp.*) on rumen fermentation. Legume straws are likely to improve rumen

Table 5. Microbial protein synthesis in crossbred cows

Parameter	T_1	T_2	T_3	SEM	P value
Allantoin (mmol/l)	4.17[A]	3.39[B]	3.87[B]	0.13	0.020
Uric acid (mmol/l)	0.34	0.41	0.37	0.02	0.220
Creatinine (mmol/l)	1.89[A]	1.74[A]	2.47[B]	0.09	0.040
Total PD excreted (mmol/l)	186.57	206.58	178.53	14.8	0.556
PDC index	190.36	210.80	190.38	15.1	0.556
Absorbed purine (mmol/l)	205.47	226.53	194.85	17.3	0.581
Microbial protein supply (g/d)	149.38	164.69	141.66	12.5	0.580

PD, purine derivatives

fermentation due to their better nutritive value and digestibility. However, in present study, replacement of wheat straw by soyabean straw did not reveal any effect on rumen fermentation. Similar to our findings, Sherasia *et al.* (2018) in crossbred cattle and Prajapati (2016) in Surti buffalo observed no significant difference in rumen fermentation parameters due to feeding of ground nut straw based straw TMR as compared to wheat straw based TMR.

Urinary PD measurement is an indirect method for estimating rumen microbial N supply to small intestine (Chen *et al.,* 1992). The microbial N was more or less similar in all the groups (Table 5). Allantoin concentration was 23.0 % and 7.75% higher (P<0.05) in T_1 due to higher DM intake and digestibility. Creatinine excretion was more in seaweed group than others which contradicted with other studies as linear relationship had been observed in DMI with creatinine excretion (Chizzotti *et al.,* 2008; Whittet *et al.,* 2019). The total PD excretion was apparently higher (>10%) in T_2 group as compared to T_1 and T_3 group. Sherasia *et al.* (2018) reported increase in microbial protein (P<0.01) synthesis in crossbred cattle when 50% of wheat straw in TMR was replaced by groundnut straw.

CONCLUSIONS

It may be concluded that supplementation of soyabean straw in TMR replacing 50 % wheat straw has no adverse effect on nutrient intake, digestibility, rumen fermentation and microbial protein synthesis. However, incorporation of brown seaweed (*S. johnstonii*) at 8% in TMR has adverse effect on palatability due to which DM intake, digestibility and VFA production was adversely affected. Hence, further research is needed to increase palatability of seaweed and to determine optimum level of incorporation so that it can be effectively utilized in livestock ration.

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Effect of High, Medium and Low Grain Proportions on Buffalo Rumen Ecosystem

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ABSTRACT

The aim of this study was to detect the major shifts in fermentation, enzymes activities and microbial communities by feeding diet with high, medium and low grain. Buffaloes were adapted to 3x3 switch over design at 64%, 44% and 23% maize grain in diet fed at 90% of dry matter (DM) intake respectively for 21 days. At the end of each treatment, rumen fluid was collected at 0 h and 4 h after feeding. We performed qPCR based absolute quantification of selected rumen microbes in rumen fluid of buffalo. No significant difference was observed in rumen microbial community and enzymatic activity, however, gas produced (ml/ g DM) and *in vitro* digestibility (IVTD %) were significantly lower (P<0.05) in high grain group in comparison with low grain group. Ammonia nitrogen and lactic acid concentrations were significantly (P<0.01) higher in high grain than low grain diet. From above experiment we conclude that there are changes in rumen fermentation due to change in grain proportions, which should be closely monitored during the dietary shifts in ruminants.

Key words: Diet, Enzyme, Real time PCR, Rumen, VFA

INTRODUCTION

The complex symbiotic microbiota of the rumen is responsible for breakdown of plant fibre for maintenance and production of ruminants. This microbiota is highly responsive to changes in diet, age, antibiotic use, and the health of the host animal. Further, it varies according to geographical location, season, and feeding. The incorporation of concentrates in ruminant diets is intended to increase the energy, protein, minerals, and vitamins intake of the animal. The feed utilization, productive efficiency, and the fibre digestion may depend on the nature of the concentrate (Morand-Fehr and Sauvant, 1987). However, long-term feeding of a high-concentrate diet causes a decreased ruminal pH value due to the accumulation of volatile fatty acids (VFA) and lactic acid, and, a chronic digestive disorder known as sub-acute ruminal acidosis may occur (Chen *et al.,* 2012). Thus, an insight into the underlying mechanism that regulate rumen microbial community and fermentation would be desirable. Hence, the present study was conducted to evaluate three different levels of grain in concentrate for its effect on rumen fermentation, microbial profile and *in vitro* digestibility.

MATERIALS AND METHODS

The buffaloes used in this experiment were housed at Animal Nutrition Shed, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India. The shed was well ventilated with separate provision for feeding and watering. All procedures regarding animal handling and treatments within this study were approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA, Ministry of Environment and Forest, GOI).

The experiment was carried out on three fistulated adult male Murrah (average body weight 415±10.2 kg) buffaloes in an experiment based on 3×3 switch over design. The animals were fed concentrate (Table1) to meet their requirements (ICAR, 2013) and were divided in three groups, high, moderate and low grain. The proportion of maize grain in ration of high, medium and low grain groups was at 64%, 44% and 23% of DM intake respectively. After feeding for 21 days, the rumen liquor and content (for enzyme) of buffaloes were collected at 0 and 4 h of post-feeding on two consecutive days. The samples were transported to the laboratory in an ice bucket and immediately processed for further sample preparation.

An *in vitro* feed fermentation study was carried

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Table1. Ingredient composition of concentrate fed to buffaloes

Ingredient	High Grain	Medium Grain	Low Grain
	Ingredient composition (g/kg fed basis)		
Wheat bran	-	9	-
Maize	88	75	67
Deoiled soyabean meal	8	11	26
Mineral mixture	2.5	3	4.5
Salt	1.25	1.5	2.5

out utilizing variety of substrates, and the rumen liquor of experimental buffaloes was used as inoculum. The substrate tested were: hays (maize, oat and berseem), roughages (wheat straw, paddy straw and sugarcane bagasse) and mixed diets comprising of different ratios of concentrate mixture and wheat straw (75:25, 50:50 and 25:75). The substrates were dried and ground to pass 1 mm sieve. Exactly weighed substrate (200 mg±10 mg) was transferred in already calibrated syringes of 100 ml capacity in which 30 ml of medium including 10 ml rumen liquor was dispensed anaerobically following standard protocol (Menke and Steingass, 1988). The syringes were incubated for 24 h at 39°C. Each treatment was repeated in triplicate for two consecutive days. After 24 h of incubation, total gas was measured by recording the displacement of piston in the syringe and subtracted from the gas produced in blank syringes to get net gas production. Methane was estimated using gas chromatograph (Nucon-5765) fitted with flame ionization detector (Agarwal *et al.,* 2008). The incubated medium was used for volatile fatty acid (VFA) estimation (Cottyn and Boucque, 1968). Lactic acid was estimated using procedure of Baker and Summerson (1941). After 24 h incubation, the syringe contents were transferred quantitatively by repeated washings with neutral detergent solution in

Table 2. Primers for real time PCR for absolute quantification of rumen microbes

Primer Name	Primer Sequence	Amplicon Size	Annealing Temp	Reference Reference
Bacteria	F-5'CGGCAACGAGCGCAACCC-3' F-5'CCATTGTAGCACGTGTGTAGCC-3'	130	60	Denman, and McSweeney,
Fungi	GAGGAAGTAAAAGTCGTAACAAGGTTTC CAAATTCACAAAGGGTAGGATGATT	110	60	2005
Protozoa	316f, 52 -GCTTTCGWTGGTAGTGTATT-32 ; 539r, 52 -CTTGCCCTCYAATCGTWCT-32	223	55	Sylvester *et al.,* 2004
Methanogen	F 5'-TTC GGT GGA TCD CAR AGR GC-3'R 5'-GBA RGT CGW AWC CGT AGA ATC C-3	140	60	Denman, and McSweeney, 2005
Ruminococcus albus	F-5'CCCTAAACAGTCTTAGTTCG-3'R-5' CCTCCTTGCGGTTAGAACA-3'	175	60	Kobayashi and Koike, 2001
Ruminococcus flavefaciens	F5'CGAACGGAGATAATTTGAGTTTACTTAGG3' R-5'CGGTCTCTGTATGTTATGAGGTATTA-3'	132	60	Tajima *et al.,* 2001
Fibrobacter succinogenes	F-5'GTTCGGAATTACTGGGCGTAAA-3' R-5'CGCCTGCCCCTGAACTATC-3'	121	60	
Butyrivibrio fibrisolvens	F-5'TCTGGAAACGGATGGTA-3' R-5'CCTTTAAGACAGGAGTTTACAA-3'	284	52	

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spout less beaker and *in vitro* true digestibility (IVTD) was estimated as per the procedure of Van Soest *et al.* (1991) and expressed as g/kg.

5 g of whole rumen content collected from 3 buffaloes was transferred to 100 ml beakers and mixed with PBS (pH 6.8) at 5 ml/g, carbon tetrachloride (1 ml/g) and 0.4% of lysozyme solution (1 ml/g) as described by Agarwal *et al.* (2000). The protein content of the enzyme samples was estimated following the method of Lowry *et al.* (1951). Enzyme activities were expressed as µmoles of reducing sugars per minute per ml of enzyme samples under assay conditions. Specific activity of enzymes was expressed as IU per mg protein.

Total genomic DNA from the rumen liquor was extracted following the procedure of (Yu and Morrison (2004) by using Qiagen stool kit. For preparation of standard curve, the purified PCR product was cloned using specific primer and the plasmid was serially diluted to make a standard curve after calculating copy number (Ritalahti *et al.,* 2006). The amplification reactions were performed in a total volume of 20 µl, containing 2 ng of template DNA, 10 µl of 2X Kappa SYBR master mix, 0.6 µl of each primer (10 µM) and nuclease free water (to make up 20 µl of volume). The

primer sequences and conditions were as described in table 2. The relative quantification of different microbial groups (total bacteria, total fungi, total protozoa, methanogen, Ruminococcus albus, R. flavefaciens, Fibrobacter succinogenes and Butyrivibrio fibrololvens) was done with real-time PCR (CX1000 Touch, BIORAD). The qPCR was standardized for absolute quantification of bacterial cell in per gram of rumen content. For preparation of standard curve, the purified PCR product using specific primer was cloned in pGEMT easy vector (Promega) and transformed in Escherichia coli. The plasmid with insert was extracted and copy number was calculated. The plasmid was serially diluted to make standard curve and the copy number was calculated against respective primers given is Table 2.

Statistical analysis

All data were analyzed as using general linear model of SPSS (SPSS, 2016), with contrast analysis for *in vitro* experiments, to analyze the effect of substrate, inoculum and their interaction. The means were compared using Duncan multiple comparison test. Variability in the data is expressed as the standard error means (SEM) and a probability level of P<0.05 was considered to be statistically significant and the P

Table 3. *In vitro* **fermentation pattern with different ratio of concentrate and roughage**

Substrate(S)		Diet (Grain)			SEM	P Value		
		High	**Medium**	**Low**		**S**	**Diet**	**S*Diet**
Gas produced (ml)/g DM								
Hay	Mean	93.38[b]	106.54[ab]	116.46[a]	2.84	0.001	0.007	0.983
Roughage	Mean	75.55[a]	52.37[b]	66.43[ab]	3.34	0.001	0.024	0.875
Mixed diet	Mean	107.11[c]	128.52[b]	164.04[a]	3.06	0.001	0.001	0.944
***in vitro* true digestibility (IVTD)**								
Hay	Mean	52.82[b]	60.64[a]	63.12[a]	0.68	0.011	0.000	0.059
Roughage	Mean	38.66[b]	45.56[a]	39.40[b]	0.99	0.014	0.012	0.269
Mixed diet	Mean	51.97[b]	58.56[b]	71.16[a]	1.23	0.022	0.000	0.591
Methane (ml)/g Digested DM								
Hay	Mean	32.69	31.98	33.97	0.90	0.000	0.661	0.963
Roughage	Mean	38.34[a]	24.58[b]	32.39[ab]	1.46	0.000	0.002	0.918
Mixed diet	Mean	37.41	40.13	41.28	1.21	0.000	0.351	0.574

[a-b]Mean values within a row with unlike superscript letters were significantly different for each dietary treatment (P<0.05)

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values between 0.05 and 0.10 were considered as a trend. For simplicity of data presentation, mean value for substrates (hay, roughage and mixed diet are presented.

RESULTS AND DISCUSSION

There are currently a number of methane mitigation strategies being investigated to reduce emissions from ruminants, including improved genetics and overall health of animals (potentially leading to reduced herd sizes while maintaining milk yields), use of supplements that reduce methane emissions and immunization against methanogens. However, the most potential strategy is better feed and feeding systems *i.e.* dietary manipulation of the rumen microbiome. In our study, we observed a decrease in methane production in medium grain group (roughage as substrate), which indicates that dietary composition plays major role in deciding methane production. With the information that rumen microbes are very resilient to changes in rumen, manipulation of diet may be used as a successful strategy to decrease methane production in rumen.

In this study, total gas production (ml/g DM) in 24 h increased significantly in low grain diet by 23.7% when compared with high grain treatment whereas medium grain diet is comparable with both diets, (Table 3). Within the three hays, maize showed higher gas production (P<0.01) when compared with other two hays (oat and

Table 4. Microbial profile with different ratio of concentrate and roughage

	Time (h)	Diet (Grain)			Mean	SEM	P value		
		High	Medium	Low			Diet (D)	Time (T)	D*T
Total Bacteria	0	10.09	10.12	10.14	10.12	0.049	0.964	0.665	0.957
	4	10.15	10.18	10.15	10.16				
	Mean	10.12	10.15	10.14					
RA	0	5.70	5.77	5.66	5.71	0.094	0.962	0.476	0.946
	4	5.62	5.55	5.55	5.58				
	Mean	5.66	5.66	5.61					
FS	0	8.68	8.92	9.03	8.87	0.079	0.893	0.803	0.402
	4	8.94	8.79	8.77	8.83				
	Mean	8.81	8.85	8.90					
Methanogen	0	8.08	8.29	7.75	8.04	0.067	0.061	0.710	0.394
	4	8.28	8.08	7.92	8.09				
	Mean	8.18	8.19	7.83					
RF	0	6.52	6.40	6.51	6.48	0.094	0.837	0.661	1.00
	4	6.60	6.48	6.60	6.56				
	Mean	6.56	6.44	6.56					
Total Fungus	0	7.11	7.10	7.18	7.13[a]	0.064	0.827	0.021	0.863
	4	7.52	7.36	7.45	7.44[b]				
	Mean	7.32	7.23	7.31					
Protozoa	0	8.31	8.18	7.70	8.06	0.183	0.504	0.637	0.964
	4	8.41	8.29	8.01	8.24				
	Mean	8.36	8.24	7.85					
BF	0	7.11	7.08	7.08	7.09	0.071	0.822	0.286	0.889
	4	7.33	7.14	7.27	7.24				
	Mean	7.22	7.11	7.17					

[a-b]Mean values within a column with unlike superscript letters were significantly different for each dietary treatment (P<0.05); RA, *Ruminococcus albus*; RF, *R. flavefaciens*, FS, *Fibrobacter succinogenes*; BF, *Butyrivibrio fibrisolvens*

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Table 5. *In vitro* metabolites with different ratio of concentrate and roughage

Substrate(S)		Diet (Grain)			SEM	P Value		
		High	**Medium**	**Low**		**S**	**Diet**	**S*Diet**
NH₃N (mg/dl)								
Hay	Mean	10.89[a]	6.69[b]	5.72[b]	0.69	0.656	0.009	0.996
Roughage	Mean	12.71[a]	6.11[b]	5.61[b]	0.70	0.999	0.000	0.990
Mixed diet	Mean	12.05[a]	7.25[b]	6.92[b]	0.70	0.833	0.007	1.000
Total VFA (mM/dl)								
Hay	Mean	5.41	5.05	6.10	0.17	0.24	0.53	0.46
Roughage	Mean	5.46	5.00	5.31	0.26	0.49	0.08	0.07
Mixed diet	Mean	5.88	5.71	6.05	0.24	0.23	0.07	0.12

[a-b]Mean values within a row with unlike superscript letters were significantly different for each dietary treatment (P<0.05)

berseem). In roughage substrates, gas production increased (P<0.01) by 30.68% in high grain diet in comparison with medium grain diet and wheat straw shows high gas production (P<0.01) with respect to paddy straw and sugarcane bagasse (Table 3). The IVTD was increased (P<0.01) by 8.27% for berseem hay in comparison with maize and oats hay (Table 3). IVTD of sugarcane begasse was increased (P<0.01) by 15.27% as compared to wheat straw and paddy straw, medium grain diet showed higher IVTD (P<0.01) in comparison to other two diets (20:80 and 80:20 concentrate: roughage). Methane production (ml/g digested DM) was lower by 35.89% in medium grain diet (P<0.01) roughages as compared to high grain diet (Table 3).

Feeding of concentrate ensures optimum maintenance and production in ruminants. However, how the level of concentrate especially grain, affects the rumen fermentation, enzymes and microbes is key factor for optimum production and maintenance of animals. In this study, the decrease in feed degradability observed in our study is in agreement with reports from other studies (Steen *et al.,* 2002; Huuskonen *et al.,* 2007; Keady *et al.,* 2007), which indicate adverse effect of higher level of grain on rumen microbes.

The population density of total bacteria, *F. succinogenes, R. flavefaciens* and *R. albus, methanogens* and fungi was similar (P>0.05) in all the three diets. There was an effect of post-feeding time on some microbes as the population of total fungi was

higher (P<0.02) at 4 h post feeding as compared to 0 h feeding (Table 4). The depression in fibre digestibility in the rumen and in the total digestive tract from inclusion of rapidly fermentable carbohydrate, such as concentrate had been reported before (Fahmy *et al.,* 1984; Huhtanen and Jaakkola, 1993) in grass silage based diet, and the depression rate increased sharply after 60% level of concentrate. In contrast to our findings, feeding of different concentrate roughage ratios was found to have no drastic effect on rumen environment (Wanapat and Pimpa, 1999).

The activities of various rumen enzymes like carboxymethylcellulase, xylanase, α-glucosidase, β-glucosidase, amylase and acetyl esterase were affected neither by the diet nor by the post feeding period (Table 6). Ammonia nitrogen production was higher (P<0.05) in high grain fed buffaloes as compared to low grain fed buffaloes (Table 6). Similar observations were made with *in vitro* ammonia nitrogen with all three substrate classes (Table 5). Production of total volatile fatty acids (VFAs) was similar (P>0.05) among treatments in both *in vivo* and *in vitro* experiment.

Higher Ammonia nitrogen and lactic acid concentration observed in the rumen liquor of high grain fed buffaloes in the present study is in agreement with Ogata *et al.*(2019) who reported high lactic acid concentration in cattle fed with high-concentrate diet. In our study the ruminal lactic acid concentrations were higher during high grain diet under both *in vivo* and *in*

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Table 6. Specific enzyme activity and certain metabolites (*in vivo*) with different ratio of concentrate and roughage

	Time (H)	Diet(Grain)			Mean	SEM	P value		
		High	Medium	Low			Diet (D)	Time (T)	D*T
CMCase	0	2.13	2.57	1.97	2.22	2.26	0.91	0.87	0.81
	4	2.44	2.19	2.27	2.30				
Mean	2.28	2.38	2.12	2.26					
MCCase	0	1.10	1.13	1.16	1.13	1.02	0.83	0.51	0.89
	4	0.88	0.71	1.15	0.91				
	Mean	0.99	0.92	1.15	1.02				
Amylase	0	3.18	4.98	3.62	3.92	3.79	0.95	0.88	0.85
	4	3.61	3.22	4.12	3.65				
	Mean	3.39	4.10	3.87	3.79				
α-Glucosidase	0	7.48	5.44	5.12	6.01	5.43	0.70	0.67	0.93
	4	6.13	5.63	2.80	4.85				
	Mean	6.80	5.54	3.96	5.43				
β-Glucosidase	0	0.48	0.38	0.28	0.38	0.40	0.44	0.61	0.82
	4	0.50	0.38	0.41	0.43				
	Mean	0.49	0.38	0.34	0.40				
Xylanase	0	6.80	7.18	6.14	6.70	6.59	0.98	0.87	0.83
	4	6.77	5.87	6.80	6.48				
	Mean	6.78	6.52	6.47	6.59				
Protease	0	60.22	66.61	65.92	64.25	69.06	0.85	0.57	0.97
	4	64.61	77.37	79.65	73.88				
	Mean	62.42	71.99	72.78	69.06				
Acetyl esterase	0	5.73	5.36	5.32	5.47	5.12	0.85	0.55	0.77
	4	4.21	5.78	4.32	4.77				
	Mean	4.97	5.57	4.82	5.12				
Urease	0	0.90	1.48	0.81	1.06	1.03	0.66	0.86	0.73
	4	0.77	1.06	1.15	0.99				
	Mean	0.83	1.27	0.98	1.03				
NH$_3$N(mg/dl)	0	9.72	5.39	4.64	6.58	0.56	0.005	0.758	0.975
	4	9.73	5.83	5.24	6.93				
	Mean	9.72[a]	5.61[b]	4.94[b]					
Lactic Acid	0	1.26	1.28	1.15	1.23	0.03	0.044	0.932	0.333
(mg/dl)	4	1.40	1.21	1.06	1.23				
	Mean	1.33[a]	1.25[ab]	1.11[b]					
TVFA(mM/dl)	0	9.19	7.72	10.64	9.18	0.63	0.184	0.252	0.065
	4	11.15	10.14	11.55	10.94				
	Mean	10.17	8.93	11.10					
A/P	0	3.66	3.75	3.80	3.73	0.12	0.057	0.232	0.059
	4	3.39	3.50	3.34	3.41				
	Mean	3.52	3.63	3.57					

[a-b]Mean values within a row with unlike superscript letters were significantly different for each dietary treatment (P<0.05)

vitro conditions. High ammonia production in rumen with high grain may be due to proliferation of high ammonia producing microbes (HAP), which rapidly ferment concentrate to produce ammonia in rumen. In the present study, the volatile fatty acid profile did not change during *in vivo* and *in vitro* study. Similar to our findings, Hoover *et al.* (2006) reported that the proportion of VFAs (acetate, propionate, and butyrate) was not affected by energy sources. However, Wanapat and Pimpa, (1999) and Oagata *et al.* (2019) reported that VFA in the rumen liquor increased with the increase in the proportion of concentrate mixture in the diet of animals. Animals on the 'hay only' diet showed a more efficient redirection of H_2 into other microbial products in comparison to hay: concentrate diet. A metabolomic study showed that there was an increase in the levels of amino acids, organic and nucleic acids in the liquid phase of the rumen contents in diets, along with decreased methanogenesis, which suggests that there may be enhanced microbial protein synthesis with an alteration in the rumen microbiota, with an increase in the ratio of Bacteroidetes and Firmicutes and a decrease in Archaea and Synergistetes (Martinez-Fernandez *et al.,* 2016). Mathews *et al.* (2019) conducted a study with animals fed either a hay: roughage diet or a hay: concentrate diet in the ratio of 60:40. Additionally an anti-methanogenic compound (chloroform) was also included in the diet. Results showed that with increasing levels of chloroform, there was an increase in H_2 expelled and CH_4 production decreased.

In our study, rumen microbial profile remained unaltered with the three levels of grain, with equally similar observation in rumen enzymes pattern. This may be due to the resilient nature of rumen microbes (and enzymes produced by them) which adapts very rapidly to changing interventions.

CONCLUSIONS

From the present study, it may be concluded that rumen fermentation and its functions are strongly driven by dietary interventions, and thus affects optimum production in animals. Further, a higher grain level is not advised in ruminant animals as it affects the rumen fermentation in unfavorable manner.

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Effect of Plane of Nutrition on Nutrient Utilization in Pre-partum Murrah Buffaloes and Birth Weight of Their Calves

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ABSTRACT

The present study was conducted to ascertain the effect of feeding a higher level of metabolizable energy and metabolizable protein on feed intake, nutrient utilization, and optimizing their calves' birth weight. Eighteen pregnant Murrah buffaloes in the last trimester of pregnancy were distributed into three groups (n=6) based on average body weight (629.69±15.05 kg) and lactation number (3.27±0.05). The feeding trial was conducted 90 days before prepartum. Group T_1 (Control) was fed as per ICAR (2013) recommendation, whereas animals in group T_2 (HME) were offered 30% higher energy and group T_3 (HMP) were offered 40 % higher protein than ICAR (2013) recommendation. Concentrate mixture and green fodder were offered to an individual animal as per experimental protocol. Dry matter intake (DMI) was similar among the groups. Average daily body weight gain was higher (P<0.05) in HMP, followed by HME and control group. It was observed that apparent digestibility coefficients (%) of dry matter, organic matter, ether extract, neutral detergent fibre and acid detergent fibre were higher (P<0.05) in groups HMP and HME as compared to control, the differences between HMP and HME were non-significant. Birth weight of calves was higher (P<0.01) in HMP than HME groups as compared to control, however, the best response was observed in group HMP. It was concluded that the higher levels of protein in the diet of pregnant Murrah buffaloes during last trimester can produce increased calf birth weight compared to high levels of energy.

Key Words: High energy, High protein, Murrah buffalo, Digestibility, Birth weight of calf

INTRODUCTION

The buffaloes is considered as the main dairy animals in India, contributing 51% to the country's total milk production (BAHFS, 2015). In the world, India is the leader in buffalo population with 108.7 million buffalo heads, which is 56.75 per cent of the world's buffalo population (20th livestock census, 2019). Diversity of the buffaloes in India is represented by 19 registered and well-defined breeds along with non-descript populations. Murrah is the prominent buffalo breed in the country, followed by Surti, Mehesana and Jafarabadi. (Vohra *et. al.,* 2016).

Maternal nutritional status is one of the extrinsic factors programming nutrient partitioning and ultimately growth, development, and function of the major fetal organ systems (Wallace *et al.,* 1999; Godfrey and Barker, 2000). Generally, puberty in Murrah buffalo heifers is attained at the age of 34 months. This delay in puberty is due to mainly two reasons; first reason underfeeding during the growing period with a lower average daily gain. Secondly, it is also due to lower birth weight. The

growth of calves depends on birth weight. Therefore, birth weight is a major factor in reducing time taken to attain puberty and the age of first calving. If the mother is severely underfed during the last three months of pregnancy, it might affect the young, causing death *in utero* or reducing viability at birth (Mc Donald, 2002). Therefore, proper nutrition is required during this phase. It was hypothesized that feeding of higher energy and protein than that recommended by ICAR (2013) during advanced pregnancy might be beneficial. The specific objective of the study was to monitor the effects of feeding 40% higher levels of dietary protein and 30% higher levels of dietary energy than the recommendation of ICAR (2013) during the last trimester of pregnancy on nutrient utilization in dame and the birth weight of the calves.

MATERIALS AND METHODS

Eighteen Murrah buffaloes in last trimester of pregnancy were distributed into three groups (n=6) based on average body weight (629.69±15.05 kg) and

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lactation number (3.27±0.05). These animals were divided into three groups (six animals in each group) based on the body weight and lactation number as T_1 (Control), T_2 (HME) and T_3 (HMP). Physical compositions of different concentrate mixtures are presented in Table 1. The animals in control group were fed as per ICAR (2013) feeding standards. Buffaloes in HME group were fed 30% more energy while protein and other nutrients were fed as per ICAR (2013) feeding standards. Animals in HMP group were fed 40% more protein while energy and other nutrients were fed as per ICAR (2013) feeding standards. All three groups were fed mineral mixture and common salt at 60 g/day and 30g/day, respectively. All animals were fed their respective concentrate mixture, green fodder and wheat straw in the form of total mixed ration (TMR) Before

Table 1. Chemical composition of fodder and feed ingredients

Nutrients	Maize grain	Soybean meal	Maize fodder	Sorghum fodder	Berseem fodder	Wheat straw	Control	HME	HMP
Dry matter	90.12± 0.31	90.32± 0.62	21.50± 0.43	22.45± 0.36	17.45± 0.49	90.89± 0.87	89.37± 0.24	90.22± 0.32	90.79± 0.48
				on % DM basis					
Organic matter	94.80± 0.47	94.60± 0.65	90.53± 0.52	90.29± 0.45	90.53± 0.13	89.97± 0.62	92.30± 0.45	92.84± 0.48	92.96± 0.32
Crude protein	9.80± 0.31	45.50± 0.23	10.13± 0.34	9.55± 0.25	18.50± 0.25	4.15± 0.26	19.50± 0.36	20.50± 0.36	27.30± 0.41
Ether extract	4.11± 0.13	1.70± 0.15	2.16± 0.16	2.04± 0.95	2.10± 0.17	1.05± 0.27	3.20± 0.18	3.40± 0.29	3.30± 0.26
NDF	22.05± 0.45	18.01± 0.36	62.70± 0.39	63.10± 0.56	48.10± 0.44	77.70± 0.38	21.20± 0.93	20.80± 0.54	20.70± 0.24
Ash	5.20± 0.32	5.40± 0.17	9.47± 0.48	9.71± 0.35	9.47± 0.39	10.03± 0.37	-	-	-
ME (MJ/kg)	-	-	8.24± 0.77	-	-	6.90± 0.17	6.30± 0.25	8.25± 0.26	5.90± 0.48
MP (g/kg)	-	-	6.45± 0.27	-	-	0	16.5± 0.74	17.5± 0.78	23.80± 0.79
Cell wall constituent analyses of fodder and feed ingredients (% DM basis)									
NDICP	1.97± 0.56	9.2± 0.42	3.02± 0.24	3.39± 0.16	6.39± 0.45	2.08± 0.77			
ADICP	0.5± 0.39	0.9± 0.24	1.78± 0.39	2.19± 0.67	3.29± 0.28	1.03± 0.37			
ME(MJ/kg)	12.61± 0.37	14.72± 0.28	8.24± 0.77	7.87± 0.56	8.83± 0.23	6.90± 0.17			
MP(g/kg)	6.24± 0.77	27.75± 0.47	6.45± 0.27	5.50± 0.37	11.02± 0.29	0 -			
TDN (%)	78.45± 0.36	79.29± 0.25	59.95± 0.46	52.42± 0.67	55.54± 0.58	41.53± 0.33			

NDF, neutral detergent fibre; ADF, acid detergent fibre; ME, metabolizable energy; MP, metabolizable protein; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; HME, 30% higher ME than ICAR (2013); HMP, 40% higher protein than ICAR (2013)

the start of the experimental feeding, animals were dewormed for control of both internal and external parasites. Fortnightly body weight was taken during experimental duration of 90 days.

A metabolic trial was conducted around 30 days prior to parturition to determine the nutrient digestibility. Representative samples of feed offered, faeces voided, and urine excreted were collected and brought to the laboratory for suitable aliquoting (Schneider and Flatt, 1975) of biological samples. The feed, left over residues, and faecal samples were analyzed for their proximate composition (AOAC, 2005) and cell wall constituents (Van Soest *et al.,* 1991). Neutral detergent insoluble CP (NDICP), acid detergent insoluble CP (ADICP), total digestible nutrients (TDN), metabolizable energy (ME) and metabolizable protein (MP) were calculated according to NRC (2001).

Blood samples were collected from the jugular vein using a heparinized vacutainer in the morning before offering food. Plasma was obtained by centrifuging at 3000×g for 10 min and frozen at -20ºC for later analysis of blood parameters. IgG was quantified by coomercial kit (Bioassay Laboratory technique ELISA kit) following manufacturer's instruction.

Data on various parameters (body weight, DMI) were analyzed by one way ANOVA method of Snedecor and Cochran (2004) using the Statistical Analysis System (2000). Data are presented as mean ± SE. Differences with probabilities (P<0.05; P<0.01) were considered significant.

RESULTS AND DISCUSSION

The detailed chemical composition of feed ingredients is presented in Table 1. Dry matter Intake (DMI) and digestibility coefficients of dry matter (DM), organic matter (OM0, crude protein (CP), ether extract (EE), neutral detergent fibre (NDF) and acid detergent fibre (ADF) are presented in table 2. The DMI in HME and HMP was higher (P<0.05) than in the control. Since the gestation period advances, dry matter requirement also increases. From the third fortnight onwards, the dry matter intake increased in all three groups, and it
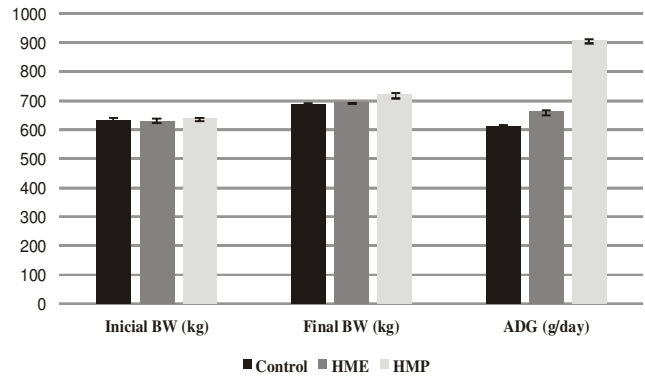

Fig. 1. Bodyweight and body weight gain in various groups

gradually decreased before 10-15 days of parturition. Jabbar, (2004) found that DMI increased on both higher energy and higher protein levels of the feed than the control group in lactating Nili-Ravi buffaloes. The increasing dietary energy density improved DM intake (Vazquez-Anon *et al.,* 1997). Based on the above reviews, it was inferred that the higher energy and higher protein levels in the feed increases the DMI. In contrast, another report indicated that there was no effect of varying energy and protein levels on DM intake (Broderick, 2003).

Apparent digestibility of CP, NDF and ADF were higher (P<0.05) in HMP followed by HME as compared to control. Similarly, El-Ashry *et al.* (2003) reported that the buffaloes fed with the diet containing higher energy level recorded highest digestibility of DM, CP, CF and EE. Similarly, in the present study, the digestibility of nutrients in the HME group was found to be significantly higher than control. It is known that apparent digestibility of CP increased with increased dietary level of protein (Das and Singh, 1999; Lohakare *et al.* 2006; Singh *et al.,* 2009). Nevertheless, Malik *et al.* (1998) reported that the digestibility of CP was not affected by the levels of feed intake. Data pertaining to nitrogen balance are presented in table 2. N excretion in faeces and N excretion in urine was affected by different levels of ME and MP in the diet. The nitrogen retention was found higher (P<0.05) in group HMP followed by HME and control. Increased nitrogen intake at constant dietary energy level did not increase nitrogen digestibility but increased nitrogen retention

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Table 2. Intake and digestibility of nutrient, and nitrogen balance

Particulars	Control	HME	HMP
Body weight (kg)	653.50±9.10	648.34±3.60	657.29±8.30
DMI (kg/d)	17.39[a]±0.70	17.54[ab]±0.90	17.74[b]±0.40
DMI (% BW)	2.68±0.36	2.72±0.25	2.69±0.92
CPI (g/d)	1766.12±38.15	1727.29±36.89	2480.13±31.23
CPI (g/100kg BW)	2.70±0.40	2.67±0.60	3.78±0.70
TDN intake (kg/d)	8.05±1.34	10.98±0.24	7.96±1.75
MPI(g/100kg BW)	143.23±4.80	144.22±4.73	197.19±9.38
MEI(MJ/100kg BW)	18.44±0.12	25.54±0.44	18.68±0.35
Digestibility (%) of nutrients			
Dry matter	62.36[a]±0.6	65.25[b]±0.7	65.73[b]±0.8
Organic matter	67.59[a]±0.2	71.22[b]±0.4	72.33[b]±0.8
Ether extract	67.28[a]±0.7	72.67[b]±0.4	71.05[b]±0.5
Crude protein	61.27[a]±0.8	63.17[b]±0.5	69.84[c]±0.2
Neutral detergent fibre	52.58[a]±1.1	50.67[ab]±0.1	55.81[b]±0.4
Acid detergent fibre	31.71[a]±0.8	32.61[ab]±0.4	35.19[b]±0.3
Nitrogen balance			
Total N intake (g/d)	292.9[a]±5.90	297.4[a]±6.70	314.5[b]±7.70
N excreted in faeces (g/d)	129.17±11.95	133.89±9.45	141.18±8.75
N excreted in urine (g/d)	139.02±6.74	132.99±4.57	134.26±15.89
Total N excreted (g /d)	268.19±7.73	266.88±5.07	275.44±11.39
Total N retention (g/d)	24.71[a]±2.39	30.52[b]±2.18	39.06[c]±3.85

DMI, dry matter intake; CPI, crude protein intake; MPI, metabolizable protein intake; MEI, metabolizable energy intake; [a,b,c] Means bearing different superscripts in a row differ significantly (P<0.05); HME, 30% higher ME than ICAR (2013); HMP, 40% higher protein than ICAR (2013)

(Diaz *et al.,* 2001). Singh *et al.* (2009) reported that increased nitrogen retention with 20% higher protein in Bhadawari buffalo calves. Lohakare *et al.* (2006) reported decrease nitrogen retention in crossbred calves fed with a low protein diet. N balance was significantly (P<0.05) higher in HME group as compared to the control, which indicates the high energy increases the utilization of nutrients. Chaudhari *et al.* (2020) reported

similar findings in lactating Murrah buffaloes.

The average body weight gain differed significantly (P<0.05) among the groups (Figure-1). Bodyweight increased significantly (P<0.05) in HMP group as compared to HME group and control. Lohakare *et al.* (2006) reported higher average daily gain in crossbred calves fed diet containing high metabolizable protein. The present study also coincides with the

Table 3. Average birth weight of calves and total immunoglobulin (mg/dl) level of calves and Murrah buffaloes

Particulars		Control	HME	HMP
Birth weight (kg)		31.26[a]±0.30	33.18[b]±0.37	36.28[c]±0.40
Immunoglobulin (mg/dl)	Buffaloes	32.68[a]±0.57	32.81[a]±0.49	34.20[b]±0.35
	Calves	31.70[a]±0.31	32.56[a]±0.42	35.76[b]±0.51

[(a,b,c)] Means bearing different superscripts in a row differ significantly (P<0.01); HME, 30% higher ME than ICAR (2013); HMP, 40% higher protein than ICAR (2013)

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review that the increasing dietary energy density improved weight gain in lactating Holstein cows (Broderick, 2003). Malik *et al.* (1998) found a higher average daily gain (g/d) in crossbred heifers fed 20% and 40% higher CP. Other report also indicates that higher protein had a significant influence on body weight gain (g/d) than high energy since the protein is essential for muscle repair and growth (Sletmoen-Olsen *et al.,* 2000).

Data on the average birth weight of Murrah buffalo calves are presented in Table 3. The calf birth weights in HMP group was higher (P<0.01) than HME group and control. The calf birth weight in HMP group was about 14% higher than the control and 8.9% higher than that observed in the HME group.Improved birth weight was reported by Gunn *et al.* (2013) and Bolze *et al.* (1985) due to feeding of a high protein diet, and by Schoonmaker *et al.* (2003) and Radunz *et al.* (2010) due to feeding of high energy diet.

Total immunoglobulin level (Table 3) in buffaloes was significantly higher (P<0.01) in group HMP than HME and Control. In contrast of the present study, a negative correlation was found between serum IgG of pre-partum cows and colostral IgG by Toghyani *et al.* (2015). Present findings corroborated with the report that the maternal colostrum had a significant effect on the total immunoglobulin status of the calves because of the increased immunoglobulin concentration in the colostrum of cows fed with high protein diet (Francisco *et al.,* 1993; Toghyani *et al.,* 2015).

CONCLUSION

From the present study, it is evident that feeding of diets containing higher protein and energy during the last trimester of pregnancy resulted in higher birth weight of calves of Murrah buffaloes. But the protein seemed to be more important than energy during this period. Hence it can be concluded that the higher levels of protein in the diet can produce increased calf birth weight compared to high levels of energy.

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Effect of Betaine Supplementation on Growth Performance of Growing Murrah Buffalo Calves

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ABSTRACT

This experiment was conducted to study the effect of betaine (BET) supplementation on growth performance of Murrah buffalo calves. Twenty-one Murrah buffalo calves with body weight (BW) of 98.70±1.31 kg and age of 8.12±0.55 months were selected and randomly distributed into three groups, 7 calves in each group. The diet of all three groups was the same except that the diet of respective group additionally supplemented with 0, 7, and 14 g/d BET during 90 d of the experiment period. The BW and average daily dry matter intake (DMI) were recorded at fortnightly intervals. Blood samples were taken on fortnightly intervals for the estimation of liver enzymes. There was an improvement ($P<0.05$) in the BW and average daily gain (ADG) in calves receiving 7 or 14 g/d BET. However, DMI was unaffected with the supplementation BET. Feed conversion ratio (FCR) was significantly ($P<0.05$) lower in group supplemented with betaine either at 7 or 14 g/d than in the control group. No effect of dietary BET addition in the diets of calves was observed on the alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The results of our study indicate that supplemental BET may play a positive role in regulating the growth of growing Murrah buffalo calves.

Key words: Betaine, Buffalo calves, Growth, Liver enzymes

INTRODUCTION

In order to accelerate growth, improve immunity and liver function of the buffalo calves, various feed additives are used in diets of calves.- Betaine is a trimethyl derivative of the amino acid glycine and widely found in a variety of plants in nature. There is increasing evidence that it is a highly valuable feed additive and may produce positive effects on animal performance (DiGiacomo *et al.,* 2016; Dunshea *et al.,* 2019). Betaine acts as a methyl group donor, and an organic osmolyte, has the ability to improve growth performance in animals. Betaine protects cells from osmotic stressors and allows them to continue normal metabolic activities. Betaine plays an important function in the amino acid, lipid metabolism and antibody production in animals (Zulkifli *et al.,* 2004). Methyl groups are very important in the metabolism of all animals and these methyl groups cannot be synthesized in the body, and must be supplied through diets. Betaine acts as a methyl donor and thus may play an important role in protein synthesis (Sun *et al.,* 2008). Chand *et al.* (2017) found that dietary supplementation of 1.5% and 2% betaine increased the feed intake and body weight gain (BWG) and reduced the FCR of fast-growing broilers. Singh *et al.* (2015) observed a significant increase in feed intake and BWG of broilers fed with diet contained betaine. Mishra *et al.* (2019) reporterd that supplementation of betained reduced serum concentrations of cholesterol and triglycerides in pigs. However, response of supplementary betaine depends on various factors like species, life stage, basal diet and doses of betaine (Mishra *et al.,* 2020) and any blanket recommendation may be misleading. Hence, this experiment was conducted to evaluate the of effect of betaine supplementation on growth performance of buffalo calves.

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MATERIALS AND METHODS

The experimental procedures and protocols were approved by the University Animal Ethics Committee (Reg.No.2058/GO/Re/SL/19/CPCSEA) and CPCSEA, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

The experiment was conducted on Livestock Research Center-1, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. Twenty one Murrah buffalo calves of similar BW (98.70±1.31 kg) and age (8.12±0.55 months) were utilized in this experiment and randomly distributed into three groups of 7 each. The feeding schedule was same for all groups except that diets are supplemented with betaine at 0.0 (B0), 7 (B7), and 14 g/d/calf in respective groups for 90 d of the study period. The BET was supplemented in form of BET hydrochloride (purity 98%, Chemkart, Mumbai, India). The calves were offered diet in the form of total mixed ration (TMR) containing concentrate, fodder (berseem and oat), and straw (wheat and paddy) in a ratio of 45:35:20, respectively to meet their nutrient requirement (Table 1) as per the recommendation of NRC (2001). The concentrate mixture contained 53% yellow maize, 10% wheat bran, 16% mustard cake, 16% groundnut cake, and 3% vitamins and minerals premixes. TMR was prepared daily and offered two times a day at 08:00 h, and 18:00 h. Clean and fresh tap water was offered *ad libitum* to the animals. Experimental calves were kept in tie barn conditions with an arrangement of individual feeding and watering.

Bodyweight was recorded using digital electronic balance before the start of the experiment, and thereafter at fortnightly intervals at 7.00 AM before feeding and watering. Bodyweight gain (BWG) was calculated by subtracting the BW of the previous fortnight from current fortnights. DMI was calculated at fortnightly intervals by subtracting refused feed from offered feed. The FCR was calculated by dividing feed intake with BWG. The samples of TMR were collected fortnightly, composited at the end of the experiment, dried

(65 °C for 48 h), and stored for further analysis. The dried samples of TMR were analyzed for DM, total ash, crude fiber, and crude protein using AOAC (1995) procedures. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated by the method of Van Soest *et al.* (1991).

Blood samples from jugular vein were collected on 0, 15, 30, 45, 60, 75, and 90 d of the experiment period in EDTA containing vials before feeding and watering at 7.30 AM. Thereafter, blood samples were centrifuged at 3000 rpm for 30 min. Plasma was collected and stored at -20ºC in Eppendorf tubes till further analysis. Alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline

Table1. Ingredient and chemical composition of TMR fed during the study period

Attributes	Content (mg/kg)
Ingredient composition of TMR on DM basis	
Wheat straw	100
Paddy straw	90
Berseem fodder	140
Oat fodder	70
Yellow maize (ground)	320
Wheat bran	67
Mustard cake	100
Groundnut cake	100
Mineral and vitamin premix[a]	13
Betaine hydrochloride[b]	Vaiable[c]
Chemical composition of TMR (g/kg)	
Dry matter	784
On dry matter basis	
Organic matter	901
Ash	99
Crude protein	160
Crude fiber	30.0
Neutral detergent fiber	460
Acid detergent fiber	256

[a]Premix composition per kg: vitamin A 32 lac IU, vitamin D_3 6 lac IU, vitamin E 300 IU, vitamin K 400 mg, vitamin B_2 2 g, vitamin B_{12} 6 mg, niacinamide 4 g, calcium pantothenate 1 g, choline chloride 120 g, Ca 300 g, P 36 g, Na 20 g, Cu 800 mg, I 800 mg, Fe 8 g, Mn 22 g, Zn 20 g, Co 40 mg; [b]Betaine hydrochloride (purity 98%, Chemkart, Mumbai, India); [c]upplemental betaine in diet 7 g and 14 g/day/calf

phosphatase (ALP) in the plasma samples were measured by commercial kits (ERBA diagnostics Mannheim Germany).

The data of different variables were analysed by MIXED Model of SPSS software (SPSS for Windows, V 19.0; SPSS, Inc., Chicago, IL, USA)The means were compared by applying 'Duncan's Multiple Range Test'.

RESULTS AND DISCUSSION

Bodyweight on 3, 4, 5, and 6th fortnights of the study period was higher (P<0.05) in B14 than B7 and control groups. ADG was significantly (P<0.05) higher in B14 than both the groups on all fortnights of the study period. There was no significant effect of supplemental BET on mean DMI. The mean FCR was lower (P<0.05)

Table 2. Effect of betaine supplementation on growth performance of Murrah buffalo calves

Variables	Fortnight	Treatment			SEM	P value		
		B0	B7	B14		T	F	T × F
BW, kg	0	95.76	98.60	101.75	1.31	0.001	0.001	0.075
	1	100.38[a]	104.84[ab]	110.17[b]	1.32			
	2	104.42[a]	111.72[ab]	118.37[b]	1.34			
	3	110.34[a]	118.46[b]	126.85[c]	1.31			
	4	113.60[a]	124.68[b]	135.00[c]	1.32			
	5	119.69[a]	130.49[b]	143.20[c]	1.26			
	6	123.81[a]	137.00[b]	151.50[c]	1.28			
	Mean	109.71[a]	117.97[b]	126.69[c]	1.31			
DMI, kg/day	1	2.68	2.62	2.68	0.04	0.411	0.075	0.895
	2	2.81	2.76	2.84	0.04			
	3	2.95	2.93	2.99	0.04			
	4	3.09	3.09	3.17	0.04			
	5	3.23	3.23	3.33	0.04			
	6	3.37	3.39	3.50	0.04			
	Mean	3.02	3.00	3.08	0.04			
ADG, kg/day	1	0.31[a]	0.38[b]	0.43[c]	0.03	0.000	0.048	0.327
	2	0.33[a]	0.42[b]	0.42[b]	0.03			
	3	0.34[a]	0.40[b]	0.47[c]	0.03			
	4	0.33[a]	0.38[b]	0.43[c]	0.03			
	5	0.33[a]	0.39[b]	0.45[c]	0.03			
	6	0.34[a]	0.40[b]	0.44[c]	0.03			
	Mean	0.33[a]	0.40[b]	0.44[c]	0.03			
FCR	1	8.65[b]	6.89[a]	6.23[a]	0.62	0.000	0.072	0.478
	2	8.52[b]	6.57[a]	6.76[a]	0.68			
	3	8.68[c]	7.33[b]	6.36[a]	0.80			
	4	9.36[c]	8.13[b]	7.37[a]	0.72			
	5	9.79[c]	8.28[b]	7.40[a]	0.63			
	6	9.91[c]	8.48[b]	7.95[a]	0.75			
	Mean	9.15[b]	7.61[a]	7.01[a]	0.70			

B0, control group; B7, betaine supplemented group (7 g/d/calf); B14, betaine supplemented group (14 g/d/calf); BW, body weight; DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio; SEM, standard error mean; Means bearing different superscript (a, b, and c) in a row differ significantly at P<0.05

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in both B7 and B14 groups than the control Table 2).

BET supplementation with dose 7 g/d or 14 g/d in growing calves improved the BW, ADG, and feed efficiency. In accordance with the present study, Lakhani *et al.* (2020) reported the positive effect of BET supplementation on BW, ADG, and feed efficiency of crossbred heifers. The findings of the current study are concurring with the results of Nofal *et al.* (2015), they revealed that BW and WG increased in growing chicks that received supplemental BET. BET supplementation improved growth due to its osmo-protective properties that increase nutrient digestibility, supporting intestinal cells and the growth of intestinal microbes (Eklund *et al.*, 2005). Block *et al.* (2004) reported higher ADG, as well as subcutaneous fat thickness in steers fed on diets, supplemented with BET and it might be due to the lipotropic nature of BET. BET plays a potential role in the absorption of digested nutrients by protecting intestinal cells and intestinal microbes from osmotic variation (Ratriyanto *et al.*, 2009).

However, other studies (Wang *et al.*, 2010; Peterson *et al.*, 2012) reported no effect of BET supplementation on BW, ADG, and feed: gain ratio. The contrary effect of the dietary additive of BET on BW, ADG, and feed:

Table 3. Effect of betaine supplementation on energy metabolites and liver function of Murrah buffalo calves

Variables	Fortnights	Treatment			SEM	P value		
		B0	**B7**	**B14**		**T**	**F**	**T × F**
ALT, IU/L	0	17.41	17.02	17.12	0.38	0.123	0.090	0.179
	1	17.31	17.79	16.57	0.34			
	2	17.03	15.93	15.83	0.37			
	3	17.04	17.30	16.50	0.35			
	4	17.94	16.46	16.41	0.37			
	5	17.92	17.89	15.80	0.34			
	6	17.90	16.98	15.35	0.38			
	Mean	17.51	17.05	16.23	0.36			
AST, IU/L	0	127.31	127.21	127.11	1.70	0.868	0.306	0.696
	1	126.98	126.95	126.94	1.82			
	2	127.60	125.16	125.13	1.52			
	3	127.28	127.46	126.38	1.61			
	4	128.05	127.82	126.75	1.75			
	5	127.95	127.94	126.95	1.44			
	6	128.51	127.43	126.79	1.63			
	Mean	127.67	127.14	126.58	1.64			
ALP, IU/L	0	8.49	8.14	8.16	0.28	0.074	0.085	0.713
	1	8.70	8.93	8.55	0.26			
	2	8.78	8.89	8.96	0.28			
	3	9.35	9.30	8.74	0.17			
	4	9.83	8.63	8.77	0.21			
	5	9.48	8.74	8.67	0.24			
	6	9.18	9.12	8.82	0.27			
	Mean	9.12	8.82	8.67	0.25			

B0, control group; B7, betaine supplemented group (7 g/d/calf); B14, betaine supplemented group (14 g/d/calf); ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; SEM, standard error mean

gain ratio may be due to differences in form, ruminal availability, and crude protein content in basal diets (Fernandez-Figares *et al.,* 2002). The inclusion of BET at either 7 g/d or 14 g/d in the diets of growing calves did not affect DMI, which corroborates with the findings of Peterson *et al.* (2012) in lactating Holstein dairy cows. Similar to the present findings, Wang *et al.* (2019) reported that DMI was unaffected with BET supplementation in the diets of post-partum dairy cows. Plasma activity of ALP, ALT, and AST were not influence by supplementation of betaine (Table 3). In agreement with the present findings, Yusuf *et al.* (2018) reported no changes in the concentration of ALT and ALP in finishing male broilers fed diets supplemented with BET at 1.5 and 3.0 g/kg diet. Zhang *et al.* (2014) reported that ALT was unaffected in dairy cows fed on diets supplemented with BET at 10, 15, and 15 g/d. Consistent with the present findings, Mishra *et al.* (2019) reported no effect of BET on liver enzymes (ALT, AST, and ALP) of crossbred (*Landrace × Desi*) sows.

CONCLUSION

This study suggests that dietary supplementation of betaine at 7 and 14 g/d enhanced BW, ADG, and gain: feed ratio but had no significant effect on DMI, ALT, AST, and ALP, which indicate that the growth performance of buffalo calves supplemented with BET might be boosted up.

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Effect of Long time Feeding of Cotton Seed Cake (*Gossypium sp.*) on Blood Profile, Testicular Biometry and Semen Attributes in Growing Barbari Goats

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ABSTRACT

A long term (319 days) feeding trial was conducted on eighteen male growing Barbari goats (age approx. 5 m and mean BW 10.37±0.19 kg). Animals were distributed into three groups (Gr I, Gr II and Gr III) of six each as per completely randomized design and were fed with concentrate pellet and Bengal gram (*Cicer arietinum*) straw to meet their nutrient requirement. Gr I was fed with concentrate pellet (having linseed (*Linum usitatissimum)* cake as protein source), while animals in Gr II and Gr III were fed with T_1 (having equal proportion of linseed and cotton seed (*Gossypum* sp.) and T_2 (having cotton seed (*Gossypum* sp.) cake as protein source, respectively. Testicular biometry was measured at 9 and 12 months of age. Blood was collected at the end of experimental feeding and semen was collected at around 13 months of age. No statistically (P>0.05) significant difference was reported in testicular breadth and circumference between different groups of goats. Hematology and different blood metabolites were similar in all three groups of goats. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and super oxide dismutase (SOD) activity was also statistically similar (P>0.05) in all three groups of goats. Testosterone concentration (ng/ml) was 11.97 for Gr I, 12.17 for Gr II and 11.96 for Gr III. No statistically significant (P<0.05) difference was reported in the serum testosterone level in different group of goats. The colour of semen from Gr I was creamy yellowish while in other groups (Gr II and Gr III) it was creamy. Gossypol feeding had no detrimental effect on consistency, mass motility, progressive motility and viability of sperm in Gr II and Gr III. Malondialdehyde (MDA) in seminal plasma was significantly (P<0.05) increased by feeding of cotton seed cake. The study inferred that long time feeding of cottonseed cake (*Gossypium* sp.) had no deleterious effect on blood and semen attributes in goats.

Key words: Cotton seed cake, Goats, Gossypol, Semen, Testosterone

INTRODUCTION

Protein supplements, those conventionally used in goat feeding includes oil meals of ground nut, soybean meal, linseed and til *etc.*, which are costly and they are mostly utilized in the pig and poultry ration. Cottonseed cake (CSC) is consumed as an alternative ingredient to soy bean meal because of its low cost and accessibility in areas, where it is grown. India is one of the largest producers of cotton seed cake in the world with annual production of around 75 to 90 Lakh MT. All the cotton seed cake produced in the country is consumed domestically. Market value of this commodity is approximately 73 billion dollars. However, cotton seed contains a potential toxic compound, gossypol. This gossypol is a phenolic compound produced by pigment glands found in cotton roots, branches, leaves, and seeds (Cheeke, 1998). A number

of toxic effects of gossypol limit the use of cottonseed meal as a feed ingredient. However, in farm animals, acute toxicity of gossypol is little; there are substantial chronic effects, especially in monogastric animals like pig and poultry. Ruminants can endure higher levels of gossypol as free gossypol has been reported to bind to proteins in the rumen (Randel *et al.,* 1992; Schneider *et al.,* 2002).This complexed gossypol is non-toxic in nature. The effects of gossypol are accumulative and may appear suddenly after a variable period of ingestion in non-ruminants (Patton *et al.,* 1985) as well as ruminants (Cheeke, 1998; Kerr, 1989). Keeping these facts in background, this study was conducted to observe the effect of long duration feeding of different level of cotton seed cake (*Gossypol*) on testicular biometry, blood and semen attributes in male Barbari goats.

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MATERIALS AND METHODS

Feeding trial was conducted at animal experimental unit of Animal Nutrition Management and Product Technology Division of ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura, India. Geographically, the institute is located at 27° N latitude, and 78° N E longitude on 176 m above the sea level. Eighteen male Barbari goats (age approx. 5 months and average body weight 10.37 kg ± 0.19) were distributed into three groups (Gr I, Gr II and Gr III) of six each as per completely randomized design. Three types of concentrate pellet was formulated and prepared using different oil cakes using feed pelleting machine. Type I pellet contained 55% crushed barley (*Hordeum vulgare)*, 25% linseed (*Linum usitatissimum)* cake, 17% wheat (*Triticum aestivum*) bran, 2% mineral mixture and 1 % salt. In type II and type III pellets, 50 and 100% of linseed cake was replaced with cotton seed (*Gossypum* sp.) cake. All these pellets were made iso-nitrogenous. Animals of Gr I was fed with type I concentrate pellet while Gr II and Gr III was fed with type II and type III pellet, respectively. Each animal was treated for external and internal parasite before start of the experimental feeding and was regularly dewormed during the experimentation. Water was provided *ad lib* during experimental feeding.

The duration of the experimental feeding was 319 days (approx. 10.5 months) from 5 months to 15.5 months age. Animals were fed with Bengal gram (*Cicer arietinum)* straw and concentrate pellet at 60:40 ratios to fulfill their nutrient requirements. Animals of Gr I was fed with type I concentrate pellet while those of Gr II and Gr III was fed with type II and type III pellet, respectively. The animals were fed with concentrate pellet at 8 AM and after consumption of pellet they were fed gram straw *ad libitum*. Green fodder was provided two days in a week.

At the end of experimental feeding (at 319 days of experimental feeding) about 10 ml blood was collected from each goat. The site of collection was jugular vein and time was 0 h post feeding. Out of 10 ml, 8 ml blood was used for serum separation by taking blood into a clean and dry glass test tube and keeping it

in slanting position for 45 minutes to separate serum. Fresh blood (2 ml) was kept in Eppendorf tube containing anticoagulant (EDTA) for hematological analysis. For serum separation, blood samples in test tube were brought to the laboratory and centrifuged at 3000 rpm for 15 min to separate serum. Serum obtained were stored in small plastic Eppendorf tubes (2 ml) at -20^0 C till further metabolites analysis. The whole blood was analyzed for hematological parameters using hematology analyzer (Melet Schloesing Laboratories, France) standardized for goats as per manufacturer protocol.

Serum was tested for different metabolites using commercial diagnostic kits (Autospan, Span diagnostic LTD.). Concentration of glucose, total protein, total albumin, triglycerides, urea, HDL cholesterol were quantified using end-point assay using double beam spectrophotometer (UV-Vis spectrophotometer, Optizen, 3220UV, Mecasys.co. Ltd, Korea). The company written procedure on kits leaflet was strictly followed for analysis.

Superoxide dismutase (SOD) activity in serum samples was estimated by using SOD determination kit by SIGMA-ALDRICH Co. LLC. Free radical scavenging activity was measured using 2, 2-diphenyl2-picryl-hydrazyl (DPPH) (Blois,1958). All the tests were performed in triplicates and the results were averaged. Testosterone was estimated in the serum using BG TESTO ELISA kit using competitive enzyme immuno-assay technique utilizing goat specific anti-testo antibody and TESTO-HRP conjugate. Testicular biometry was measured at 9 and 12 months of age of goats. Length and circumference was recorded using measuring tape while breadth was measured using vernier caliper. All the units are in centimeter.

After attainment of 13 months of age, animals were trained for mounting and after 4 weeks of training each animal was subjected to semen ejaculation. Semen ejaculates from each buck were collected twice at weekly intervals with the help of artificial vagina during morning time. A dummy non-oestrous doe was utilized for buck mounting, and semen was collected into the graduated cups. Semen was tested immediately

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after collection for colour, consistency, mass motility and concentration. Just after collection, semen was maintained in hot water bath at 37°C and subjected to evaluation. Each ejaculated volume was measured using graduated collection cup. Mass motility was measured at low power magnification (10×) using a compound microscope with neat semen on thermo stage maintained at 37°C. Semen samples with mass motility greater than 3 were diluted and after dilution evaluated for progressive motility, live/dead count and abnormalities. Diluted semen (10 µl) was placed on a grease free clean pre-warmed slide (37°C) with cover slip and observed under 40×magnification of phase contrast microscope for evaluating the progressive motility. The average values of two experts were recorded for determining the progressive motility. Live and dead sperm count was measured as per standard staining procedure (Hancock, 1951). A drop of diluted semen mixed with 2-3 drops of stain (Eosin (0.67 g/100 ml) and Nigrosin (5 g/100 ml)) was incubated at 30°C for 1 min. Then smears made on pre-warmed slides were allowed to dry at 30°C. Then smear was examined under high power lens of the phase contrast microscope. Same staining technique was used for measuring the sperm abnormalities and about 200 sperm were counted to calculate the per cent abnormal sperm in the semen. Malondialdehyde concentration, as an index for lipid peroxidation (LPO) in the semen samples, was estimated using the thiobarbituric-acid reaction (Esterbauer and Cheeseman, 1990).

Statistical analysis

The data collected in the study were analyzed by ANOVA as per Snedecor and Cochran (1989) according to a completely randomized design using statistical software package (SPSS). Individual animals were considered as experimental units. The difference between means was considered significant at 95% significance level (P<0.05).

RESULTS AND DISCUSSION

The aim of present study was to evaluate the effect of long duration feeding of CSC having gossypol as a toxic constituent on hematology, overall health and semen attributes in male goats. Acute toxicity of gossypol is less, while chronic effects are reported especially in mono gastric animals like pig and poultry. Ruminants in which rumen is not fully functional are also susceptible to gossypol toxicity (Randel *et al.,* 1992). They can tolerate higher levels of gossypol as free gossypol because free gossypol bound to proteins to form complex in the rumen (Coppock *et al.,* 1987, Randel *et al.,* 1992, Schneider *et al.,* 2002), thereby lowering the toxicity of gossypol. There may also be other incompletely defined mechanisms for rumen detoxification of gossypol involved. Free gossypol content in CSC (*Gossypum sp.*) used as ingredient in concentrate pellet preparation was estimated and found to have 0.125 mg/g. The average dose of consumed free gossypol in Gr I was nil, in Gr II 0.25 mg/kg BW/day and in Gr III 0.50 mg/kg BW/day. None of the goats used in experimentation exhibited any sign of toxicity during the period of study. Animal growth rate in different group of goats were also studied (data not shown), but no significant difference in the body weight gain was found in control and treatment group of goats. Body weights and fortnightly body weight changes were not significantly (P>0.05) different among groups.

There was no significant (P>0.05) difference in hematological parameters like Blood cell counts, hemoglobin, PCV, HCT and differential WBC counts between different groups of goats. Different leukocytes were in the normal range. Blood hemoglobin (g/dl) was 7.64 for Gr I, 7.51 for Gr II and 7.84 for Gr III. No adverse effect on total RBC, WBC, thrombocytes count and differential WBC count was reported. (Table1).

Free gossypol has been reported to bind dietary Fe in the small intestine and thus reduce its absorption and retention (Lindsey *et al.,* 1980).In our study all the hematological variables were within the normal reference range for goats. Hematocrit, packed cell volume and hemoglobin, indicators of dietary Fe adequacy, were in normal range. Even though the experimental rations used in this study contained a certain amount of free gossypol, there was no adverse effect on hematological parameters by feeding of gossypol in growing goats. However, increased

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erythrocyte fragility was observed in beef heifers fed a diet with cottonseed meal (Gray *et al.,* 1993, Velasquez-Pereira *et al.,* 1998) and in goat (Luginbuhl *et al.,* 2000). However, the doses were quite higher in earlier cases. No significant difference was reported in serum glucose, total protein, serum urea, cholesterol, triglycerides and HDL in different groups of goats (Table 1). Serum calcium (mg /dl) was 8.54 for Gr I, 8.80 for Gr II and 9.09 for Gr III. Serum antioxidant activity (DPPH) and antioxidant enzyme SOD was also measured. There was no significant difference between different groups of goat. DPPH (% inhibition) was 67.34 for Gr I, 63.11 for Gr II and 62.57 for Gr III while SOD (% inhibition) was 93.03 for Gr I, 93.33 for Gr II and 94.31 for Gr III. There was no significant (P>0.05)

difference in serum biochemical parameters *viz.*, glucose, total protein, urea, triglycerides, cholesterol, HDL and calcium among different groups suggesting that the cotton seed cake containing gossypol had no adverse effect on physiological functions of body. However, serum albumin was significantly higher (P<0.05) in groups of goats fed with cotton seed cake. This might be due to more bypass protein fraction in cotton seed cake, making more protein available at intestinal level.

There was no significant difference in DPPH (% inhibition) and SOD activity among the different groups suggesting no adverse effect of gossypol feeding on antioxidant status of the animals. Testosterone concentration (ng/ml) was 11.97 for Gr I, 12.17 for Gr

Table 1. Effect of gossypol feeding on hematology, serum metabolites, antioxidant activity and testosterone of Barbari goats

Parameters	Gr I	Gr II	Gr III	Mean	Sig
WBC (m/mm^3)	8.52±0.92	6.98±0.90	10.35±1.08	8.61±0.61	0.076
RBC (M/mm^3)	12.36±0.53	12.26±0.31	12.35±0.39	12.32±0.23	0.951
HCT (%)	18.41±0.76	19.08±0.68	18.28±1.26	18.53±0.51	0.813
MCV (fl)	14.97±0.35	15.61±0.42	15.34±0.7	15.30±0.30	0.709
Hb (g/dl)	7.64±0.25	7.51±0.18	7.84±0.17	7.66±0.12	0.576
THR(m/mm^3)	325.85±22.77	418.28±49.52	361.57±61.00	368.57±27.23	0.397
MCHC (g/dl)	30.57±0.41	28.88±0.41	30.44±0.45	29.96±0.29	0.21
Lymphocytes (%)	64.77±2.69	64.01±2.17	58.60±2.05	62.46±1.41	0.153
Monocytes (%)	10.07±1.09	9.87±1.14	11.04±1.19	10.32±0.63	0.746
Granulocytes (%)	25.20±1.90	26.11±1.38	30.35±1.43	27.22±1.00	0.075
Serum metabolites					
Glucose (mg/dl)	69.67±3.63	74.01±3.39	72.47±3.19	72.05±1.91	0.666
Total protein (g/dl)	6.37±0.23	6.34±0.38	6.81±0.06	6.51±10.10	0.105
Albumin (g/dl)[*]	3.58±0.09[b]	4.37±0.24[a]	4.78[a]±0.31	4.24±0.17	0.007
Serum urea (mg/dl)	27.17±2.33	28.80±2.70	28.10±1.72	28.02±1.26	0.882
Cholesterol (mg/dl)	79.46±1.31	75.70±1.67	78.06±2.72	77.74±1.14	0.422
Triglycerides (mg/dl)	92.28±2.12	106.28±7.00	124.00±6.23	107.52±4.20	0.003
HDL (mg/dl)	55.52±3.31	56.55±2.20	55.96±3.03	56.01±1.58	0.969
Calcium (mg/dl)	8.54±0.05	8.80±0.38	9.09±0.59	8.74±0.27	0.34
Antioxidant activity					
DPPH (% inhibition)	67.34±2.01	63.11±1.62	62.57±2.64	64.34±1.26	0.251
SOD (% inhibition)	93.03±2.93	93.33±2.78	94.31±3.15	93.52±1.62	0.953
Testosterone (ng/ml)	11.97±1.44	12.17±4.40	11.96±2.84	12.37±2.03	0.51

[*]Mean with different superscript in a row differ significantly (P<0.05)

Table 2. Effect of gossypol feeding on testicular biometry and fresh semen quality of Barbari goats

Parameters	Gr I	Gr II	Gr III	Mean	Sig
Body weight (kg)	28.0±0.64	27.18±0.96	26.54±1.51	27.24±0.62	0.254
Testis length (cm)	11.64[a]±0.32	11.50[a]±0.36	12.71[b]±0.28	11.95±0.21	0.032
Testis breadth(cm)	7.49±0.14	7.43±0.13	7.87±0.20	7.60±0.10	0.170
Testis Circumference(cm)	21.47±0.40	20.98±0.42	22.20±0.52	21.55±0.27	0.192
Semen Colour	Creamy Yellowish	Creamy	Creamy		
Mass motility	3.30±0.13	3.69±0.08	3.60±0.15	3.51±0.07	0.056
Consistency	1.66±0.08	1.78±0.08	1.75±0.09	1.73±0.05	0.579
Progressive motility (%)	73.12[b]±3.03	79.64[a]±1.69	73.50[ab]±3.0	75.38±1.56	0.018
Viability (%)	79.69±2.18	85.90±2.79	84.20±2.12	83.06±1.05	0.24
Abnormality (%)	15.62±0.85	14.14±0.74	15.18±1.06	14.96±0.78	0.35
Seminal plasma MDA (nmol/ml)	9.83[b]±0.83	12.07[ab]±1.67	15.03[a]±1.98	11.97±0.92	0.034

[*]Mean with different superscript in a row differ significantly (P<0.05)

II and 11.96. Male sex hormone testosterone concentration was also similar among all the groups showing no detrimental effect of gossypol on testo sterone production. Though effects of gossypol are accumulative and may appear suddenly after a variable period of ingestion affecting male reproductive parameters in non-ruminants (Randel *et al.,* 1992) in our study no effect on male sex hormone was reported as reported by Qian and Wang (1984) in cross bred bull. This may be due to binding of free gossypol in the rumen of ruminants.

Morphometric analysis on the testes of any species or breed is helpful in anticipation of sperm production as well as storage potentials and fertilizing ability of the breeder male. Testicular biometry was studied at 9 and 12th months of age to assess the effect of gossypol feeding. No adverse effect on testicular biometry was reported in goats. At 12 months of age, testicular length was significantly higher (P<0.05) in Gr III (12.71 cm) as compared to Gr I (11.64 cm) and Gr II (11.50 cm). No statistically significant difference was reported in testicular breadth and circumference between different groups of goats (Table 2). Qian and Wang (1984) also reported that testicular morphology remained unaltered in cross bred bull, however, Nassir *et al.* (2014) reported that higher levels (30%) of cottonseed cake-based diets showed some negative effect on testicular morphometry in red Sokoto bucks.

But in the present study the level of cotton seed cake was much lower (5 and 10% of diet).

The colour of semen from Gr I was creamy yellowish while in the other groups (Gr II and Gr III) it was creamy. The mass motility was 3.30 for Gr I, 3.69 for Gr II and 3.60 for Gr III. The consistency was also similar in all three groups of Barbari goats being 1.66 for Gr I, 1.78 for Gr II and 1.75 for Gr III. No detrimental effect on sperm progressive motility and viability was reported after incorporation of CSC (*Gossypium sp* in the ration of growing goats after long time feeding. Malondialdehyde concentrations (nmol/ml) was significantly increased (P<0.05) in seminal plasma by feeding of cotton seed cake in goats. This showed that the lipid peroxidation was increased due to gossypol ingestion however no adverse effect on serum antioxidant enzyme SOD was reported (Table 1). Gossypol has proven deleterious action on spermatic mobility (Chongthammakun *et al.,* 1986; Hong *et al.,* 1989), blocking the production, release and use of ATP in these cells (Ueno *et al.,* 1988). Besides, abnormal spermatozoids are formed in animals exposed to gossypol for the reason that ultra-structural abnormalities, mainly in the mitochondrial membranes (Haffer, 1983). Gossypol seems to stop spermatogonial cells entry into meiotic prophase. In this study, no morphological or mobility abnormalities was found in goats fed with cottonseed cake. The possible

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explanation for the absence of deleterious effects in goats, due to low intake of gossypol from cotton seed cake and non-absorbance of protein bound gossypol by the gastrointestinal tract of the ruminants. Ruminal micro biota of adult goats is capable to detoxify the gossypol by binding it to proteins (Calhoun *et al.,* 1995).

CONCLUSION

Present study concluded that long time feeding of low level of cotton seed cake in goats had no adverse effect on hematology, serum antioxidant status and metabolites. There was also no negative effect on semen attributes and testosterone hormone.

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Effects of Inorganic and Nano Copper Supplementation on Growth Performance, Nutrient Utilization and Mineral Availability in Growing Sahiwal Heifers

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ABSTRACT

This study was conducted to evaluate the effect of inorganic and nano copper supplementation on feed intake, nutrient utilization, growth performance and mineral availability in growing Sahiwal heifers. Twenty four growing Sahiwal heifers were randomly allocated into four groups having six heifers in each groups and fed for 120 days. Feeding regimen was similar in all the groups except that treatment groups were supplemented with 0.0 mg copper, 10.0 mg inorganic copper, 5.0 and 10.0 mg of nano copper per kg dry matter (DM; ppm) in four respective groups. Nutrients requirement of experimental heifers were met by feeding concentrate mixture, berseem and wheat straw as basal diet. Group fed on basal diet supplemented with 0.0 mg copper/kg DM served as a control. Experimental heifers were monitored daily for DM intake (DMI) and fortnightly body weight change. At the end of the study, a digestion trial of 7 days was conducted to study the effect of inorganic and nano copper supplementation on nutrients utilization. Feed intake, nutrient digestibility and growth performance were similar in all experimental groups fed on basal diet with or without supplemental Cu. Dietary supplementation of inorganic Cu and nano Cu did not exert any effect on absorption of Ca, P, Zn and Fe in different groups. However, absorption of Cu in nano Cu supplemented groups were higher (P<0.05) than other groups. It was concluded nano Cu can be used in the diet at 5 ppm for feeding in growing cattle as it exerts similar effects as showed by 10 ppm of inorganic Cu.

Key words: Growth Performance, Nutrient Utilization, Mineral Availability, Nano copper

INTRODUCTION

Copper is an essential trace element of animals. It can effectively maintain the stability of the internal environment, and is closely related with haematopoiesis, metabolism, growth, reproduction, and other important life activities (Ognik *et al.,* 2016). Copper entered to the body in the form of sulphate is dissociated in the digestive tract to ion form, which is then absorbed in the small intestine (Nose *et al.,* 2006). Feed ingredients are commonly deficient in Cu; hence, the commercial diet should provide the essential amount of Cu in a biologically dynamic form, which depends on the physical and chemical properties of the form of the supplement in which the Cu is given in the diet. It can ensure the growth and decline in the homeostasis and peroxides damage to the body (Song *et al.,* 2014). Cu deficiency has been linked to a variety of clinical signs, including pale coat, poor fleece, anemia, spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelinization of the spinal cord, impaired reproductive performance, decreased resistance to infectious disease, diarrhoea and generalized ill-health (Tessman *et al.,* 2001). Copper sulphate is the main Cu source in the diet of livestock; which shows poor bioavailability caused by the presence of ingredients that can inhibit absorption. Thus, inorganic mineral administration in animal feed poses a risk to the environment, as excretion of high mineral levels contaminates soil and water. Traditionally, inorganic copper salts have been used in feed formulation (Aksu *et al.,* 2010), causing significant pollution to the soil and water (Jackson *et al.,* 2003). The bioavailability of Cu salts is very low, and approximately 80% of Cu is excreted in the faeces (McDowell, 1992) and consequently, its effect on soil microorganisms, plants and aquatic species is currently

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one of the crucial environmental concerns causing the environmental pollution (Leeson, 2009).

Copper nano particle (Cu-NP) is one of the nano metal which is prepared for application in various fields, so if the copper absorption is enhanced, the copper supplementation and excretion amount may be reduced. It has been documented that Cu-NP has beneficial effects on the animal performance and could be used to replace copper sulphate (Miroshnikov *et al.,* 2015). The unique bioactivity of Nano Cu is mainly due to the particle size and the large surface area. The size is important for penetration through for which a pore structure of a cellular membrane is required, thus affecting the number of proliferating cells (Salata, 2004). Nano particles can bypass conventional physiological ways of nutrient distribution and transport across tis-sue and cell membranes, as well as protect compounds against destruction before reaching their targets. Nano copper has several advantages over copper sulphate, including improved efficacy, lower dosage with improved results, no interference with other ingredients, and last but not least it reduces environmental excretion (Ognik *et al.,* 2016). This study was aims to investigate the effect of dietary nano-copper supplementation on growth performance, nutrient utilization and mineral availability of indigenous heifers.

MATERIALS AND METHODS

Animal care procedures were approved (approval number, IAEC/18/14) 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 24 growing Sahiwal heifers were selected from the cattle herd maintained at Livestock Farm Complex (LFC), DUVASU, Mathura. Experimental heifers were randomly assigned into four groups (N=6) on body weight (100.20±4.84) and age (12-15 months) basis, and the duration of the experiment lasted 120 days. Heifers either received a basal diet devoid of supplemental Cu (control) or were supplemented with 10 ppm of inorganic Cu as copper

sulphate pentahydrate ($CuSO_4.5H_2O$, molecular weight 249.68, minimum assay purity 99%, Central Drug House (CDH) Pvt. Ltd. New Delhi), 5 ppm and 10 ppm of nano Cu as cupric Oxide Nano-powder (CuO, molecular weight 79.54, APS: 40 nm, SSA: 80 m^2/g, minimum assay purity 99%, Sisco Research Laboratories (SRL) Pvt. Ltd. Taloja, Maharashtra). The nutrient requirements of experimental heifers were met by feeding total mixed ration (TMR) consisted of concentrate: green berseem fodder: wheat straw in the proportion of 50:35:15 following NRC (2001) guidelines. Concentrate mixture used for feeding of experimental heifers consisted of 26 parts barley grains, 20 parts wheat bran, 20 parts gram chuni, 32 parts mustard oil cake and 2 parts mineral mixture.

To ensure that each animal consumed the requisite amount of Cu, the calculated amount of $CuSO_4.5H_2O$ and CuO nano powder was mixed with barley flour and prepared the premix (at 2ppm Cu/g of barley flour) and offered prior to providing the ration. TMR was prepared daily by hand mixing and was offered at 09:00 h and 18:00 h. The animals were provided with fresh and clean drinking water free of choice twice daily at 08:00 h and 17:00 h. Experimental 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.

Feeds and fodders consumption was recorded daily by weighing TMR offered and orts left, and dry matter intake (DMI) was calculated and recorded daily according to the DM content of the diet. Body weight of the experimental animals was recorded at the onset of experiment, and subsequently at 15, 30, 45, 60, 75, 90, 105 and 120 days post-Cu supplementation by using computerized weight management system (Leotronic Scales Pvt. Ltd., India). Heifers were weighed for 2 consecutive days in the morning at 06:00 h before offering feeds, fodders, and water. The average of two days was considered as body weight for that fortnight and considered for average daily gain (ADG), feed-to-gain ratio (FCR), and feed conversion efficiency (FCE). The representative samples of feeds and fodders offered and orts left were dried in a hot air oven at 60°C

till a constant weight was attained and then ground in a Wiley mill to pass a 1-mm sieve. The samples were analyzed for DM (method 973.18c), CP (method 4.2.08), ether extract (EE; method 920.85), and total ash (TA; method 923.03) following standard procedures (AOAC, 2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the procedures described by Van Soest *et al.* 1991. Ca and P in feeds, fodders, orts left, and fecal samples were analyzed by Talapatra *et al.* 1940 and volumetric method, respectively. Cu, Zn and Fe content in samples of feeds and fodders, and orts left were analyzed by Atomic Absorption Spectrophotometer (AAS; Perkins, USA). Ingredients and chemical composition of the

Table 1. Ingredients and chemical composition of total mixed ration

Ingredients composition	Content (g/kg DM or as depicted)
Berseem fodder	350
Wheat straw	150
Barley grains	130
Wheat bran	100
Gram chunni	100
Mustard oil cake	160
Mineral mixture and vitamin premix[α]	10
Copper supplement[β]	Variable
Chemical composition	
Dry matter, g/kg of diet	620.9
on dry matter basis	
Crude protein	161.6
Ether extract	33
Neutral detergent fiber	534.5
Acid detergent fiber	274.3
Calcium	11.6
Phosphorus	6.4
Copper, mg/kg DM	8.75
Zinc, mg/kg DM	39.40
Iron, mg/kg DM	296.49

[α]Mineral and vitamin premix contained (kg⁻¹): vitamin A, 700000 IU; vitamin D_3, 70,000 IU; vitamin E, 250 mg; nicotinamide, 3.0 g; Ca, 190 g; P, 90 g; Na, 50 g; Zn, 9.6 g; Fe, 1.5 g; Mn, 6.0 g; I, 325 mg; Co, 150 mg; Se, 10 mg; Mg, 19.0 g; [ã]In inCu₁₀ group- 10.0 mg/kg Cu as $CuSO_4.5H_2O$, nanoCu₅ and nanoCu₁₀ group 5 and 10.0 mg/kg Cu as nano copper oxide, respectively

basal diet fed during the experimental period is depicted in Table 1. The Cu content of basal diet without supplemental Cu was estimated at 8.75 mg Cu/kg DM.

The data for measured variables were subjected to analysis of variance (ANOVA) using the mixed model repeated measure procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA). The effect of treatments, days in trial and their interaction on performance variable like weight gain was analyzed by using following model:

$$Yijk = μ + Ti + Dj + (T × D)ij + eijk$$

where Yijk is the dependent variable, μ is the overall mean of the population, Ti is the mean effect of the Cu, Dj is the mean effect of days of sampling (j = 0, 30, 60, 90 and 120 days of dietary treatment), (T × D)ij is the effect of the interaction between treatment and days of trial and eijk is unexplained residual element assumed to be independent and normally distributed. The effect of treatments on nutrients digestibility and mineral metabolism was analyzed by using one-way ANOVA with significance defined at P<0.05. Individual animal was used as the experimental unit for all data. The statistical difference between the means was determined by using ''Tukey's honest significant difference (HSD) test.''

RESULTS AND DISCUSSION

Effect of inorganic and nano Cu supplementation on dry matter intake and growth performance are depicted in Table 2. Dietary supplementation of inCu and nanoCu in heifers had no significant effect on DMI. DMI increased with the increase in the body weight of the experimental heifers. Percent DMI (kg/100 kg BW) in different groups showed similar effect of inorganic and nano Cu supplementation. The variation between the groups for mean ADG (g) were not significantly different (P>0.05). FCR and FCE were used as feed efficiency measures which were not significantly different (P>0.05). Similar to present findings Cu supplementation had no effects on average daily gain and daily feed intake in lambs (Cheng *et al.,* 2008; Dezfoulian *et al.,* 2012)), heifers (Mullis *et al.,* 2003; Vaswani *et al.,* 2018), Simmental steers (Engle and Spears, 2001) and goat kids (Waghmare *et al.,* 2014).

Table 2. Effect of inorganic and nano copper supplementation on dry matter intake and growth performance

Parameters	Supplemental Cu (mg/kg DM)				SEM	P value		
	Cu_0	in Cu_{10}	nano Cu_5	nano Cu_{10}		Treatment (T)	Period (P)	T×P
DMI (kg/day)	4.08	4.31	4.29	4.30	0.20	0.303	0.198	0.998
DMI (% BW)	3.17	3.23	3.18	3.24	0.14	0.383	0.574	0.998
ADG (g/day)	517.86	528.27	535.71	546.13	40.53	0.487	0.698	0.887
FCR	9.12	9.01	8.77	8.72	0.72	0.559	0.147	0.928
FCE	0.124	0.127	0.131	0.132	0.009	0.337	0.451	0.922

DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio; FCE, feed conversion efficiency

In contrary to the present findings, a growth improvement response has been reported in castrated *Black Bengal* kids (Mondal and Biswas, 2007) and Cashmere goats (Zhang *et al.,* 2008). Some studies compared the inorganic forms of Cu with Cu-NP and the latter showed an improvement in the growth performance of piglets (Gonzales-Eguia *et al.,* 2009; Chang *et al.* (2018). Results obtained in this study also demonstrate that intake of DM, CP, DCP, TDN and ME in control, inCu$_{10}$, nanoCu$_5$ and nanoCu$_{10}$ groups of

Table 3. Effect of inorganic and nano Cu supplementation on nutrient intake during digestion trial

Nutrient	Supplemental Cu (mg/kg DM)				SEM	P value
	Cu_0	$inCu_{10}$	$nanoCu_5$	$nanoCu_{10}$		
Dry matter intake						
kg/day	4.93	5.01	5.06	5.09	0.18	0.585
kg/100 kg BW	3.28	3.31	3.26	3.27	0.04	0.364
g/kg BW	32.56	33.11	32.59	32.65	0.37	0.362
g/kg $W^{0.75}$	114.08	115.68	114.87	114.79	1.21	0.816
Crude protein intake						
g/day	769.41	767.09	775.75	801.24	31.38	0.598
g/100 kg BW	506.01	506.14	497.69	511.62	3.04	0.300
g/kg BW	5.06	5.06	4.98	5.12	0.03	0.297
g/kg $W^{0.75}$	17.67	17.69	17.56	18.01	0.19	0.943
Digestible crude protein intake						
g/day	567.54	562.21	563.44	594.89	27.53	0.643
g/100 kg BW	369.15	367.87	360.49	375.88	3.96	0.954
g/kg BW	3.69	3.68	3.61	3.76	0.04	0.954
g/kg $W^{0.75}$	12.93	12.89	12.73	13.27	0.25	0.729
Total digestible nutrient intake						
kg/day	3.24	3.32	3.31	3.47	0.15	0.746
kg/100 kg BW	2.14	2.18	2.11	2.20	0.03	0.591
g/kg BW	21.37	21.80	21.14	21.10	0.30	0.592
g/kg $W^{0.75}$	74.59	76.32	74.64	77.58	1.15	0.799
Metabolizable energy intake						
Mcal/day	11.72	12.02	11.95	12.55	0.54	0.746
Mcal/100 kg BW	7.73	7.88	7.64	7.95	0.11	0.593
kcal/kg BW	77.28	78.82	76.44	79.53	1.09	0.592
kcal/kg $W^{0.75}$	269.71	275.95	269.88	280.52	5.23	0.799

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heifers were statistically non significant (P>0.05) which is depicted in Table 3. Mudgal *et al.* (2007) did not observe any effect on CP and DCP intakes in the buffalo calves supplemented with 10 ppm of Cu and 0.3 ppm of Se.

Data on the apparent nutrient digestibility across 7 days digestion trial is shown in Table 4. Use of nanoparticles, including Cu nanoparticles, as feed additives can improve the digestion and absorption of nutrients in livestock (Hill and Li., 2017). The present study showed that, digestibility of DM, OM, EE, CF, NFE and fibre fractions (NDF and ADF) was similar and there was no effect on digestibility of nutrients (P>0.05) during digestion trial. In agreement with present findings, earlier finding reported no effect on digestibility of nutrients in kids (Waghmare *et al.,* 2014), Chokla rams (Shinde *et al.,* 2013), Cashmere goats (Zhang *et al.,* 2008) and in heifers (Vaswani *et al.,* 2018) by dietary supplementation of Cu.

Mondal and Biswas (2007) reported that supplemental copper sources had no effect on DM, OM, NFE digestibility whereas, supplementation of Cu-proteinate significantly improved digestibility of EE and CF as compared to CuSO$_4$.Similarly, an increasing trend in digestibility of DM and OM in Cu-proteinate supplemented kids (Datta *et al.,* 2007) and digestibility of crude fat and energy in pigs (Zhang *et al.,* 2008;

Gonzales-Eguia *et al.,* 2009) was reported. Dezfoulian *et al.* (2012) found that copper source had a significant effect on digestibility of nutrients except EE, as proteinate source resulted in higher digestibility of DM, CP, NDF, ADF, NFC and OM in lambs.

The data regarding Ca, P, Cu, Zn and Fe absorption observed during digestion trial has been presented in Table 5. Mean values for Cu absorption in inorganic Cu supplemented group was significantly higher (P<0.05) than control group and lower than nano Cu supplemented groups whereas; mean values for Cu absorption in nano Cu supplemented group was significantly higher (P<0.05) than both control and inorganic Cu supplemented group. Cu has been reported to interact or influence the metabolism of a number of other elements. In present study, Cu absorption showed positive correlation with supplemented inorganic and nano Cu levels and was found highest in 10 ppm nano Cu supplemented group whereas, no effect of inorganic and nano Cu supplementation was observed on absorption of Ca, P, Zn and Fe. It has been found that changes in essential trace mineral digestibility in the gastrointestinal tract are primary mechanisms for maintaining trace mineral homeostasis (King *et al.,* 2000). Copper absorbed by animals is mainly stored in liver (Zatulovskaia *et al.,* 2015). Different forms of Cu differ in their solubility, availability and effects on

Table 4. Effect of inorganic and nano Cu supplementation on nutrient digestibility (% or as mentioned)

Nutrient	Supplemental Cu (mg/kg DM)				SEM	P value
	Cu$_0$	inCu$_{10}$	nanoCu$_5$	nanoCu$_{10}$		
Initial body weight (kg)	151.33	149.83	153.83	155.00	6.46	0.603
Final body weight (kg)	154.83	153.50	157.67	158.67	6.49	0.618
Body weight gain (kg)	3.50	3.67	3.83	3.67	0.29	0.470
Average daily gain (g)	500.00	523.81	547.62	523.81	41.83	0.470
Dry matter	59.77	59.61	60.03	59.70	0.85	0.495
Organic matter	65.97	65.80	66.06	65.81	0.74	0.544
Crude protein	73.04	72.72	72.41	73.48	0.74	0.483
Ether extract	82.10	83.29	81.54	84.07	0.53	0.836
Crude fibre	44.70	45.73	46.37	44.79	1.16	0.940
Nitrogen free extract	72.04	70.55	71.41	72.48	1.43	0.447
Neutral detergent fibre	51.78	51.07	52.44	51.09	1.13	0.174
Acid detergent fibre	37.78	39.41	39.49	39.12	1.26	0.417

animal per-formance. The increased Cu bioavailability likely results in an increase in the activities of enzymes involved in nutrient metabolism, resulting in better energy digestibility (Scott *et al.,* 2018b). The results of the present study are similar to the observations of Shen *et al.* (2020) who reported that nano-Cu supplementation for up to 10 days, the Cu content in blood significantly increased in goats and the concentration of Fe significantly decreased (P<0.01) and the effect of $CuSO_4$ group was significantly lower than that in nano-Cu group (P<0.01). Similar pattern of Cu retention observed in organic Cu supplemented animals (Shinde *et al.,* 2013). In agreement to present study, Gonzales-Eguia *et al.* (2009) reported that Cu availability was significantly improved and the faecal copper level was

reduced in nano Cu fed group as compared to $CuSO_4$ fed group in piglets. Copper supplementation at 30 mg/kg significantly increased liver Cu concentration in heifers compared with Cu supplementation at 15 mg/kg (Yost *et al.,* 2002). The lowest amount of additional Cu in the form of nanoparticles increased the apparent digestibility of Cu and Zn in turkeys. Therefore, the environmental burden of excreted Cu was substantially reduced along with decreasing dietary Cu levels but to a lesser extent when copper sulfate was replaced with Cu nanoparticles (Jankowski *et al.,* 2020).

In contrary to the present study, Dezfoulian *et al.* (2012) reported that Cu supplemented groups had a lower percentage of Cu absorption compared to the control group. Moreover, liver and plasma Cu

Table 5. Effect of inorganic and nano Cu supplementation on mineral absorption

Mineral	Supplemental Cu (mg/kg DM)				SEM	P value
	Cu_0	$inCu_{10}$	$nanoCu_5$	$nanoCu_{10}$		
Calcium						
Intake (g/day)	54.10	55.33	54.57	57.20	2.17	0.766
Voided in faeces (g/day)	37.63	37.53	36.44	38.82	1.95	0.577
Absorption (g/day)	16.47	17.80	18.13	18.39	1.17	0.093
Absorption (%)	31.47	32.14	31.29	32.04	2.43	0.219
Phosphorus						
Intake (g/day)	29.80	30.48	30.06	31.51	1.19	0.766
Voided in faeces (g/day)	12.73	12.56	12.61	13.22	0.53	0.520
Absorption (g/day)	17.07	17.92	17.45	18.29	1.07	0.954
Absorption (%)	56.89	58.07	56.63	57.50	1.70	0.253
Copper						
Intake (mg/day)	40.83	98.82	70.48	102.20	6.11	0.109
Voided in faeces (mg/day)	36.49	86.89	59.16	85.09	5.19	0.150
Absorption (mg/day)	4.34[a]	11.93[b]	11.32[b]	17.11[c]	1.04	0.008
Absorption (%)	10.68[a]	12.32[b]	16.11[c]	16.81[c]	0.57	0.014
Zinc						
Intake (mg/day)	524.07	535.97	528.57	554.10	20.98	0.766
Voided in faeces (mg/day)	411.64	413.68	415.68	422.65	15.36	0.690
Absorption (mg/day)	112.43	122.29	112.89	131.45	12.94	0.571
Absorption (%)	20.94	21.73	20.94	22.04	2.20	0.778
Iron						
Intake (mg/day)	1542.34	1577.36	1555.60	1561.26	60.46	0.823
Voided in faeces (mg/day)	1109.99	1086.18	1078.82	1054.65	38.28	0.138
Absorption (mg/day)	432.35	491.19	476.78	506.62	36.62	0.220
Absorption (%)	28.11	30.60	29.79	31.13	1.53	0.906

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concentrations in heifers were not greatly influenced by different supplemental Cu sources (Rabiansky *et al.,* 1999). The *in vitro* study suggests that copper accumulated in the intestines reduces absorption of calcium and zinc, but does not affect iron absorption (Ognik *et al.,* 2016). Cu excretion was lower in chickens treated with Cu-NP as compared to the $CuSO_4$ treated groups (Scott *et al.,* 2018). Our results are suggesting that the nano form of Cu (CuO) was more efficient than inorganic Cu (Cu sulphate) in influencing the Cu metabolism in Sahiwal heifers. Study contributes to further understanding the application of nano Cu and inorganic Cu mineral diets as a nutritional strategy for heifers to reduce dietary faecal excretion and thus Cu pollution in soil and water.

CONCLUSION

It was concluded that supplementation of Cu either from inorganic or by nano source did not exert any adverse effect on growth performance, nutrient utilization and absorption of most of the minerals except Cu. Cu absorption was better in nano Cu supplemented animals than non supplemented or inorganic Cu supplemented group. Findings of the present study revealed that level of 5 ppm nano Cu can be used for feeding in growing cattle as it exerts similar effects as showed by 10 ppm of inorganic Cu.

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Effect of Rice Distiller Dried Grain Solubles (RDDGS) With or Without Enzyme Supplementation on Performance and Biochemical Parameters on Commercial Layer Chicken

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ABSTRACT

The objective of this study was to evaluate the effect of rice distiller dried grain solubles (RDDGS) with or without enzyme supplementation on performance of commercial layer chicken. Two hundred forty commercial layer chickens at 26 weeks age were randomly allocated to 10 treatments, each treatment containing 6 replicates and 4 layers in each replicate. A standard layer ration (crude protein 18%, metabolizable energy 2600 kcal/kg diet) was offered to all birds. Cocktail enzyme was supplemented at 250 g/ton of feed. The feed and water were provided *ad lib* during the entire experimental period. The birds were raised in cages under uniform management and fed the respective diets from 27th to 42nd weeks of age. The study revealed that 15%RDDGS with enzyme supplementation was more effective among all the dietary treatments in terms of performance and serum biochemical variables in White Leghorn (WL) layer during the entire experimental period. It was concluded that with enzyme supplementation RDDGS can be included up to 15% in layer diets without affecting the performance and serum biochemical parameters

Key words: Body weight, Feed intake, Feed conversion ratio, White Leghorn layer

INTRODUCTION

Rice distiller dried grain with solubles (RDDGS) is the byproduct of the processing of rice alcohol industry which is produced from the distillation of fermented rice. During processing, rice is cooked at 131°C and 2.6 kg/m² pressure and yeast is added to the cooked rice for fermentation (Huang *et al.,* 1999). Then the alcohol is distilled from fermentation liquor, the leftover is known as rice distillers dried grains with solubles (RDDGS). Corn, wheat, barley and sorghum are the commonly used cereals used for bioethanol production. In the present context, rice as substrate for bioethanol production is increasing due to its relative lower price, higher production and easy availability, there by leading to increased availability of RDDGS. It comprises of 65% of distiller's grain and 35% of solublse (Babcock *et al.,* 2008). Most of the research work on distillers grain (DDGS) with soluble are on corn, wheat, barley and sorghum DDGS. Very scanty information is available in literature regarding the feeding value of RDDGS in poultry. So, there is need to explore its nutritive value,

RDDGS contains 47% CP and 3500 kcal/ kg of ME (National Research Council, 1989; Chiou *et al.,* 1996). It is also more nutritious than the cereal grains from which it is made up of, as it contains other nutrients recovered from the fermented grains. It is also high in protein compared to rice, and more in energy and less in fiber than its other byproduct like rice bran or rice polishing. The final product also contains yeast residue. It does not contain any antinutritional factor, as might be the case with trypsin inhibitors in soybean, commonly used protein source in poultry ration. The cost of RDDGS is always less than that of the soy bean meal. Further, supplementation of exogenous enzymes may improve nutrient utilization (Deniz *et al.,* 2013). Considering these points, this experiment was designed to evaluate the effect of RDDGS with or without cocktail enzymes on performance of layers.

MATERIALS AND METHODS

Two hundred forty commercial layer chickens at 26 weeks age were randomly allocated to 10 treatments, each treatment having 6 replicates and 4 layers in each

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replicate. A standard layer ration (CP 18%, ME 2600 kcal/kg diet) was offered to birds in T_1 (without enzyme) and T_2 (with enzyme). Experimental diets from T_3 to T_6 were formulated with four levels of RDDGS without enzyme (5%, 10%, 15% and 20%). Experimental diets from T_7 to T_{10} were formulated with four levels of RDDGS with enzyme (5%, 10%, 15% and 20%). Cocktail enzyme was supplemented at 250 g/ton of feed. The feed and water were provided *ad lib* during the entire experimental period (27 to 42 weeks). Parameters such as daily hen day egg production, egg weight, weekly feed intake and feed conversion ratio were measured.

Eggs were collected two times a day. The sum of the two collections along with the number of birds alive on each day was recorded and summarized at the end of the each period. Eggs were collected at the end of each period for three consecutive days and the average egg weights were recorded. Hen day egg production in per cent was calculated by dividing the total number of eggs laid every day by number of hens survived during each day. Feed intake/ period (g) were calculated by subtracting the residual feed/ period (g) from the total feed (g) offered during the period. Feed intake /hen/day (g) was calculated accordingly. On cumulative basis of feed intake, feed intake/hen/d was arrived at the end (feed intake/hen days) by considering the mortality in that period. The feed conversion ratio (FCR) was calculated (feed intake /egg mass). The egg weight (g) was recorded to the nearest 0.1 g accuracy in each of the replicate. The weight of all the eggs produced during last three consecutive days of each laying period was recorded to calculate the mean egg weight of each treatment group. Six eggs were collected from each treatment to determine the average egg weight. The egg mass (g) per day was calculated by multiplying the average egg weight by the total no of eggs produced in each replicate and was expressed as g/hen.

Blood samples were collected at the end of the experiment from the wing vein, serum was collected, and stored frozen till analysis. Cholesterol in serum was measured using a commercial kit following manufacturer's instruction. The data were analysed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 15th version and comparison of means was done using Duncan's multiple range test (Duncan, 1955) and significance was considered at P<0.05.

RESULTS AND DISCUSSION

The per cent hen day egg production (Table 1) in White Leghorn layers was higher (P<0.05) in the birds fed diets containing 15% RDDGS with enzyme as compared to the RDDGS without enzyme (5, 10 and 15%) and control group during first and second periods. During the periods P3 (35-38 weeks) and P4 (39-42 weeks) egg production was higher (P<0.05) in 15% RDDGS with enzyme group as compared to groups fed 5 and 10% RDDGS with or without enzyme. Lower egg production was noticed in the control and 20% RDDGS fed group irrespective of enzyme supplementation during all periods. During the overall period (27-42 wks), egg production was significantly higher (P<0.05) in groups fed 15% RDDGS supplemented with enzyme as compared to control group, and lower egg production was observed in groups fed 20% RDDGS irrespective of enzymes supplementation. Data indicate that RDDGS can not be included in layer ration at more than 15% level of inclusion. These findings are similar to many previous studies (Roberson *et al.,* 2005; Lumpkins *et al.,* 2005; Pineda *et al.,*2008; Swiatkiwicz and Koreleski, 2006; Deniz *et al.,* 2013; Jiang *et al.,* 2013; Abd El-Hack *et al.,* 2015) which recommended a usage rate of 15% DDGS in laying hen diets to maintain egg production. The differences in daily egg production were observed among the dietary treatments even at greater inclusion levels of RDDGS. The findings were similar to Lumpkins *et al.* (2005) and Roberson *et al.* (2005). Swiatkiewicz and Koreleski (2006) reported a reduction in egg production in hens fed 20% DDGS in phase II of egg production, but not during phase I. Egg production

results indicate no negative effects of DDGS on hen performance at 15% level of inclusion.

Significant (P<0.05) difference was observed in feed intake (Table 2) during first (27-30wks), second (31-34wks), third (35-38wks), fourth (39-42wks) and over all period (27-42weeks). Feed intake was significantly (P<0.05) significantly lower in control as compared to other groups indicating no adverse impact of RDDGS on feed intake. Similarly, other research workers (Roberson *et al.,* 2005; Roberts *et al.,* 2007a,

Table 1. Ingredient and nutrient (%) composition of commercial layer diets (27-42 weeks)

	Control	Control +E	5% RDDGS +NE	10% RDDGS +NE	15% RDDGS +NE	20% RDDGS +NE	5% RDDGS +E	10% RDDGS +E	15% RDDGS +E	20% RDDGS +E
Maize	57.7	57.7	54.8	51	47.7	44.35	54.8	51	47.7	44.35
SBM	27	27	21.9	17	11.6	6.6	21.9	17	11.6	6.6
RDDGS	0	0	5	10	15	20	5	10	15	20
DORB	0.5	0.5	3.5	7	10.9	14.1	3.5	7	10.9	14.1
Enzyme (E)	0	0.025	0	0	0	0	0.025	0.025	0.025	0.025
Stone grit	12.03	12.03	12.06	12.1	12.15	12.16	12.06	12.1	12.15	12.16
DCP	2.01	2.01	2.01	2	2.01	2.01	2.01	2	2.01	2.01
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Soda.bicarb	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
DL-Methionine	0.13	0.13	0.1	0.06	0.02	0.01	0.1	0.06	0.02	0.01
Lysine HCL	0.01	0.01	0.1	0.19	0.29	0.38	0.1	0.19	0.29	0.38
TMM	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
AB2D3K	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
B Complex	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Coccistat	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antibiotic	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Toxin Binder	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tylosine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
TOTAL	100.3	100.3	100.4	100.3	100.6	100.6	100.4	100.3	100.6	100.6
Estimated nutrient content on dry matter basis										
ME (kcal/ kg)	2601	2601	2608	2599	2602	2601	2608	2599	2602	2601
CP (%)	18.06	18.06	18.03	18.09	18.00	18.00	18.03	18.09	18.00	18.00
Ca (%)	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80
Av.P. (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lysine(%)	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Methionine(%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
NaCl (%)	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36

E, enzyme; NE no enzyme; SBM, soybean meal; RDDGS, rice distillers dried grains with soluble; soda bicarb, sodium bicarbonate, TMM, trace mineral mixture; ME, metabolizable energy; CP, crude protein; av P, available phosphorus; Vitamin premix provided per kg diet: vitamin A, 200000 IU; vitamin D_3, 3000 IU; vitamin E, 10 mg; vitamin K, 2 mg; riboflavin, 25 mg, vitamin B_1 1 mg, vitamin B_6, 2 mg, vitamin B_{12}, 40 mg, and niacin 15 mg; *TMM provided per kg diet: manganese, 120 mg, zinc 80 mg, iron 25 mg, copper 10 mg, iodine 1mg and selenium 0.1mg.

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Table 2. Effect of dietary inclusion of rice distiller dried grain with solubles (RDDGS) with enzyme on percent hen day egg production (%) in White Leghorn layers

Treatment	RDDGS% in diet	Enzyme at 250 g/ton of feed	Age in weeks				Mean
			P1 (27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	90.03	85.42	89.73[a]	91.97[ab]	89.29[ab]
T_2	0	+E	91.22	89.58	90.77[a]	92.86[a]	91.11[a]
T_3	5	-E	91.82	93.60	89.44[a]	90.92[ab]	91.44[a]
T_4	10	-E	93.90	90.92	88.69[a]	90.03[ab]	90.89[a]
T_5	15	-E	91.22	87.50	91.37[a]	91.67[ab]	90.44[a]
T_6	20	-E	88.10	88.69	81.25[b]	82.00[c]	85.01[b]
T_7	5	+E	94.20	88.39	91.52[a]	92.56[ab]	91.67[a]
T_8	10	+E	93.15	92.86	90.77[a]	90.18[ab]	91.74[a]
T_9	15	+E	96.28	96.28	92.02[a]	92.02[ab]	94.15[a]
T_{10}	20	+E	91.81	90.77	87.50[a]	87.65[b]	89.44[ab]
N			6	6	6	6	6
P Value			0.265	0.302	0.001	0.001	0.017
SEM			0.658	0.930	0.609	0.597	0.520

[a-c]Mean bearing at least one common superscript in a column differ significantly (P<0.05); [P1-P4] P1, period 1; P2, period 2; P3, period 3 and P4, period 4; RDDGS, rice distillers dried grain with solubles +E: with enzyme, - E: without enzyme

b; Masa'deh 2011; Deniz *et al.,* 2013) had reported that inclusion of corn distilleries dried grain soluble up to 15% of the diet did not affect feed intake. Abd El-Hack *et al.* (2015), observed that increasing DDGS level up to 16.5% was associated with an increase in feed intake.

The results revealed that, there was no significant (P>0.05) difference in feed conversion ratio (feed intake/egg mass) (Table 3) during period-P2

Table 3. Effect of dietary inclusion of rice distiller dried grain with solubles (RDDGS) with enzyme on feed intake in White Leghorn layers

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1 (27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	97.65[b]	98.15[c]	101.1[c]	100.3[d]	99.3[d]
T_2	0	+E	97.97[b]	99.73[bc]	103.1[bc]	103.9[bcd]	101.2[cd]
T_3	5	-E	103.9[ab]	107.4[a]	110.1[a]	111.0[a]	108.1[a]
T_4	10	-E	106.3[a]	107.0[a]	107.0[ab]	108.0[ab]	107.1[ab]
T_5	15	-E	103.5[ab]	106.5[a]	106.5[ab]	106.3[bc]	105.7[abc]
T_6	20	-E	107.2[a]	108.8[a]	98.50[c]	95.98[d]	102.6[bcd]
T_7	5	+E	104.1[ab]	105.0[ab]	109.9[a]	107.5[ab]	106.6[ab]
T_8	10	+E	105.6[a]	107.4[a]	107.9[ab]	106.5[abc]	106.8[ab]
T_9	15	+E	106.6[a]	109.1[a]	100.7[c]	102.1[cd]	104.6[abc]
T_{10}	20	+E	104.7[a]	105.7[ab]	107.5[ab]	107.2[ab]	106.3[ab]
N			6	6	6	6	6
P Value			0.014	0.003	0.001	0.001	0.001
SEM			0.731	0.743	0.709	0.686	0.559

[a-c] Mean bearing at least one common superscript in a column differ significantly (P<0.05); P1, period 1; P2, period 2; P3, period 3 and P4, period 4; RDDGS, rice distillers dried grain with solubles +E: with enzyme, - E: without enzyme

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(31-34 weeks), whereas, significant (P<0.05) difference was observed in period-P1 (27-30 weeks), period-P3 (35-38 weeks), period-P4 (38-42 weeks) and over all period (27-42 weeks) of age. The overall feed conversion ratio (feed intake/ egg mass) was significantly (P<0.05) improved in group fed 15% RDDGS without enzyme. The results are in agreement with the findings of earlier research workers (Roberson *et al.,* 2005; Ghazalah *et al.,* 2011; Romero *et al.,* 2012; Swiatkiewicz *et al.,* 2013) which indicated that inclusion of DDGS at the level of 200 g/kg with enzyme (xylanase and phytase) in the diet had no effect on feed intake and feed conversion ratio, and DDGS may be incorporated in the diet of laying hens without any negative effects. Gupta *et al.* (2020) observed significant (P<0.01) improvement in FCR with enzyme supplementation. It was reported that RDDGS can be used up to 100 g/kg diet of laying hens along with enzyme supplementation for better productivity of layer hens. Higher methionine content in DDGS diet may be

associated with higher egg mass and better FCR per kilogram egg production. DDGS is also rich source of water-soluble vitamins, microelements, and other biologically active substances like nucleotides, mannanoligosacharids, β-1, 3/1,6-glucan, inositol, glutamine, and nucleic acids, which may be associated with higher egg production performance due to beneficial effect on the health of animals (Gupta *et al.* 2020).

In periods 1 and 2, there was no significant difference in the egg weight in different dietary treatments. However, during period 3, 4 and overall periods the egg weight was significantly (P<0.05) higher in groups fed diets containing 15% RDDGS supplemented with enzyme. The results are similar to that of other reports (Masa'deh, 2011; Romero *et al.,* 2012; Gupta *et al.,* 2020). There was a significant (P<0.05) decrease in cholesterol (Table 4) levels among different dietary treatments. A lower serum cholesterol level was noticed in the group fed 15% RDDGS

Table 4. Effect of dietary inclusion of rice distiller dried grain with solubles (RDDGS) with enzyme on feed conversion ratio in White Leghorn layers

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1 (27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	1.980[b]	1.896	2.029[bc]	1.959[d]	1.966[cd]
T_2	0	+E	1.956[b]	1.801	2.044[bc]	2.014[cd]	1.954[d]
T_3	5	-E	2.018[b]	1.939	2.131[ab]	2.061[bcd]	2.037[bcd]
T_4	10	-E	2.063[b]	1.893	2.181[a]	2.145[ab]	2.070[abc]
T_5	15	-E	2.009[b]	1.836	1.946[c]	1.979[d]	1.942[d]
T_6	20	-E	2.096[b]	1.894	2.182[a]	2.154[ab]	2.082[ab]
T_7	5	+E	2.069[b]	1.866	2.205[a]	2.186[ab]	2.081[ab]
T_8	10	+E	2.030[b]	1.902	2.242[a]	2.219[a]	2.098[ab]
T_9	15	+E	2.079[b]	1.991	2.111[ab]	2.104[abc]	2.071[abc]
T_{10}	20	+E	2.267[a]	2.084	2.215[a]	2.124[abc]	2.172[a]
N			6	6	6	6	6
P Value			0.014	0.236	0.001	0.001	0.001
SEM			0.018	0.022	0.016	0.016	0.013

[a-c] Mean bearing at least one common superscript in a column differ significantly (P<0.05); P1, period 1; P2, period 2; P3, period 3 and P4, period 4; RDDGS, rice distillers dried grain with solubles +E: with enzyme, - E: without enzyme

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Table 5. Effect of dietary inclusion of rice distiller dried grain with solubles (RDDGS) with enzyme on serum cholesterol level in White Leghorn layers

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Cholesterol (mg/dl) 42 weekAge
T_1	0	-E	179.00[ab]
T_2	0	+E	185.53[a]
T_3	5	-E	174.47[abc]
T_4	10	-E	178.10[ab]
T_5	15	-E	144.62[de]
T_6	20	-E	153.37[cde]
T_7	5	+E	173.80[abc]
T_8	10	+E	174.38[abc]
T_9	15	+E	140.53[e]
T_{10}	20	+E	143.40[de]
N			6
P Value			0.001
SEM			2.777

[a-c] Mean bearing at least one common superscript in a column differ significantly (P<0.05); P1, period 1; P2, period 2; P3, period 3 and P4, period 4; RDDGS, rice distillers dried grain with solubles +E: with enzyme, - E: without enzyme

supplemented with enzyme, whereas higher (P<0.05) cholesterol level was observed in control. The results are in agreement with the findings of Gupta *et al.* (2017) which reported that feeding of DDGS manifested lowering effect on serum lipid profile.

CONCLUSIONS

Feeding of RDDGS at 15% level of incorporation along with enzyme supplementation was more effective among all the dietary treatments in terms of improved hen day production and feed conversion ratio with cholesterol lowering effect. It was concluded that incorporation of RDDGS at 15% in the layer ration with enzyme supplementation improved performance.

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Effect of Edible oil on Lipid metabolism and Expressions of Scavenger Receptor Class-B Type 1 in Rabbit Liver

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ABSTRACT

This experiment was conducted to investigate the effect of edible oil on HDL and scavenger receptor class-B type 1 in rabbit liver. SR-B1 is extremely expressed in various tissues, including the liver, where its pronouncement is modulated by different mechanisms, which includes the transcriptional activity of nuclear receptors. Eighteen albino rabbits were fed with rabbit food pallets for 21 days, of which 06 were fed normal diet (Group-I), and the other 06 were fed with edible oil at 2 ml/kg + normal feed (Group-II), third group of 06 rabbits were fed edible oil at 5 ml/kg + normal feed (Group-III). Estimation of HDL levels in serum and the expression levels of scavenger receptor class-B type 1 genes in rabbit liver were determined by using real-time fluorescent quantitative PCR, ELISA and Western blot. The expressions of SR-B1 mRNAs, protein levels and SR-B1 activity in Group-II and group - III were significantly over expressed as compared with those in Group-I. Low HDL levels were observed in serum of Group -III compared with Group-II and Group-I. It was concluded that edible oil showed regulative effect on liver by a potential mechanism of overexpression SR-B1 genes, protein abundance, activity of SR-B1 and low concentrations of high density lipoprotein (HDL) in serum, accordingly holding down the cholesterol synthesis in rabbit liver.

Key words: Cholesterol, Edible oil, High density lipoprotein, Scavenger receptor class-B type 1

INTRODUCTION

Lipids are vital component and major sources of energy second to carbohydrates (Acton *et al.,* 1996). They play specific roles in membrane signaling events. Some of the lipids are indicators and also take vital role in cell development. Concentration of lipids could represent physiological conditions of cells (Rigotti *et al.,* 1997). Cholesterol takes an essential role for the cellular functions, in addition to maintenance of plasma membrane fluidity and integrity. It is acquired from plasma lipoproteins by selective cholesterol uptake pathway (Glass *et al.,* 1983). The High-density lipoprotein (HDL) is very important cholesterol carrying lipoprotein. It has two essential roles; first it improves reverse cholesterol transport (RCT) and it regulates inflammation. The scavenger receptor, class B type I (SRB1) is a crucial cholesterol receptor for HDL that could mediate particular HDL lipid uptake into cells. It was first characterized by Acton *et al.* (1996). SRB1 was recognized as the first HDL receptor and it was focused to perform in the selective

transport and adjustment of cholesterol and lipids (Acton *et al.,* 1996, Lee *et al.,* 2017). This is significant, as HDL cholesterol levels are shown to be inversely related with a risk of stroke (Glass *et al.* 1985). SRB1 plays major role in production of CE for steroid hormone synthesis, as well as regulating plasma metabolism of cholesterol. SRB1 can bind HDL through interconnection with its apolipoproteins (Rigotti *et al.,* 1997). Animals that do not express SRB1 have enhanced levels of plasma concentrations of cholesterol associated with enlarged HDL elements with increased levels of unesterified cholesterol content and rehabilitated apolipoprotein composition (Rigotti *et al.,* 1997; Miettinen *et al.,* 2001; Mardones *et al.,* 2001; Braun *et al.,* 2003; Van *et al.,* 2004). Unesterified and esterified cholesterols are unchanged in hepatocyte but reduced biliary cholesterol level (Varban *et al.,* 1998; Miettinen *et al.,* 2001). Hepatic clearance of HDL cholesterol from blood is controlled by hepatic SR-BI expression in mice (Out *et al.,* 2004; Brundert *et al.,* 2005; Kozarsky *et al.,* 1997). In contrast, up-regulation

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of SR-B1 expression in liver of mice results in decreased levels of HDL cholesterol from blood and enhanced biliary secretion compared to down regulation levels of SRB1 (Wang *et al.,* 1998; Ueda *et al.,* 1999; Zhang *et al.,* 2005). Modern studies modeling RCT from macrophages have established that hepatic SRB1 plays major role in driving this process (Temel *et al.,* 2002). Down regulation of SRB1 gene expression results in reduced cholesterol storage in steroidogenic cells and ovaries (Rigotti *et al.,* 1997; Miettinen *et al.,* 2001), due to the main function of SRB1 in HDL cholesterol consumption by steroidogenic cells (Mardones *et al.,* 2020). Numerous studies indicated the major physiological role played by SRB1 in cellular consumption of HDL cholesterol including cholesterol ester, free cholesterol and bioactive lipids, such as α-tocopherol (vitamin E) (Luo *et al.,* 2010). In this study, we have investigated the effect of palm oil on rabbit liver and mRNA expression in up- regulation of SRB1 confirmed by quantitative RT-PCR analysis, Western blot and ELISA as well as measured HDL levels in blood of experimental rabbits.

MATERIALS AND METHODS

Eighteen Male albino rabbits weighing ~1750 g aged 3-4 months, obtained from a breeding farm of Bangalore were individually housed in 80 cm × 60 cm × 60 cm rabbit cages under standardized temperature between (15-21°C), humidity (45-60%) and light (12 h light-dark) and dark period for 21 days with a mixed diet of pellets, green leafy vegetables, and water *ad libitum.* Animals were divided into 3 groups with 6 rabbits in each group. The group- I was kept as control, with a normal diet and water supplied regularly. Group- II was supplemented with 2 ml/kg of body weight dose of palm oil and group- III animals were given 5 ml/kg BW of palm oil for 21 days. After experimental period, they were sacrificed and serum used for HDL assessment, extracted liver tissues used for ELISA, Western blot and qRT-PCR.

After 21 days experimental period incisions were made into their thoracic cavities. Blood withdrawal was performed in the morning at around 8:00–9:00 am,

following a 12-h overnight fast. The blood was collected by heart puncture and allowed to clot in sample vials. Blood was centrifuged (10 min, 1500×g, 4 °C), the supernatant was harvested by simple aspiration subsequently used until analysis of high density lipoprotein. The automated analyzer Hitachi – 7170 was used and measured concentration of HDL in serum sample.

SRB1 activity of liver tissue was measured with the ELISA kit (R&D Systems, Minneapolis) according to the manufacturer's instructions. Briefly, samples and a diluted series of standards were incubated with a mouse monoclonal antibody to SR-B1 and SR-B1 conjugate in a 96-well plate at room temperature for 3 h. After washing systematically, substrate solution and stop solution were added, and the optical density of each well at 450 nm was was determined. For expression levels of SRB1 in rabbit, proteins were collected from liver of each experimental group. For quantitative analysis, the same amount of protein (30 μg) was loaded for each sample. Proteins were separated on 4–15% SDS-PAGE and relocated to a nitrocellulose membrane. Membranes were incubated overnight with 5% milk TBS-Tween solution at 4°C, washed and incubated for 2 h with rabbit primary polyclonal antibody SRB1. After second incubation with a peroxidase conjugated IgG secondary antibody was accomplished. The bound antibody bands were detected using Uptight US HRB Blot chemiluminescence as well as β-actin was used as control. Densitometries analyses of protein bands in the Western blots were done using G: Box Imager software. Relative band intensities were derived by using the software to calculate integrated optical density for each band, and then each band was normalized with the integrated optical density of the corresponding β -actin band. The bands were read in the air dried membrane.

RNA extraction and cDNA synthesis: (Trizol method): Liver tissue samples of all three groups were frozen in liquid nitrogen, followed by RNA isolation using Trizol Reagent (Thermo fisher Scientifics) under aseptic conditions. Concentrations of RNA were quantified by the optical density at 260 nm wavelengths

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Table 1. Sense and antisense primers of SRB1 & β-actin.

Gene	Primers	Temp
SR-B1	Sense: 5'-AGGGTGTTCGAAGGCATCC-3'	58 °C
(128-bp product)	Antisense: 5'-GACCCGTTGGCAAACAAAGT-3'	
β-actin	Sense: 5'-CATGCCATCCTGCGTCTGGACC-3'	62 °C
(169-bp product)	Antisense: 5'-TACTCCTGCTTGCTGATCCACATCTGC-3'	

using the Nanodrop ND-1000. Purity was checked for RNA quantity in sample and cDNA was synthesized using applied biosystem kit following manufacturer's instructions.

Real-Time qRT-PCR: Each sample was evaluated in triplicate alongside with negative control. qRT-PCR was used to amplify the target genes using the cDNA according to the manufacturer's instructions. Briefly, the RT Mix was prepared as per the manual by preparing 10 μl of cDNA sample, a final PCR volume of 20 μl containing 10 μl of ready master mix having, 10X RT random primers, 25X dNTP Mix, 10X RT buffer, MultiScribe™ Reverse Transcriptase, RNAse inhibitor, and nuclease-free water. On a Real-Time PCR System 7500 (Applied Biosystems), the reaction was performed. The sense and anti-sense primers used are tabulated (Table 1). The PCR Setup included 5.0 μl of template, 1.0 μl of forwarding Primer, 1.0 μL of Reverse Primer, 12.5 μl of master mix (PowrerUp SYBR Green), and 5.5 μL of PCR water making up to a total volume of 25 μl.

All the experiments were performed on three different occasions and data are presented as mean ± SD. The data was analyzed by one-way ANOVA. Data were statistically evaluated with SPSS/10 software. A value of $^*P<0.05$ was considered to indicate a significant variation among groups.

RESULTS AND DISCUSSION

The findings show that SR-B1 protein abundance in Group-III and group-II animals are significantly ($P<0.05$) higher than that found in group-I (Fig. 1). β - Actin was used as positive control by using the technique Western blot in the Rabbit liver with definite antibodies. In Lane III and Lane II no significant changes of Beta Actin have seen in comparison to Lane I

SR-BI (60kDa)   Catalog No. AB137829

Fig. 1. Expression of SR-B1 in Lane I SR-B1 (catalog No. AB137829) examined as untreated (control) group; Lane II SR-B1 with 2 ml/kg dose; Lane III of 5 ml/kg dose

(Fig. 2). Outcomes of the present work showed the relationship between palm oil supplementation with hepatic over-expression of SRB1 in As compared to control, Group-III manifested reduced plasma levels of HDL. The effect of edible oil on HDL values is presented in Fig 6. HDL levels were significantly lower

Beta Actin (42kDa)   Catalog No. AB8227

Fig. 2. Expression of Beta Actin (catalog no AB8227) in Lane I in untreated (control) Group; Lane II (2 ml/kg) and Lane III (5ml/kg) treated group

in Group-III rabbits as compared to control animals. In Group-II rabbits, HDL level decreased when compared to control group, but compared to Group-III the HDL levels increased which is due to moderate effect of edible oil at 2 ml/kg. HDL mediated cholesterol
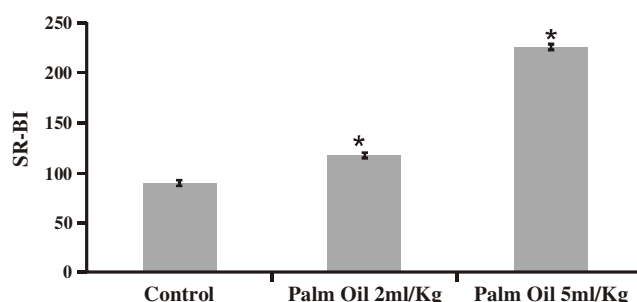

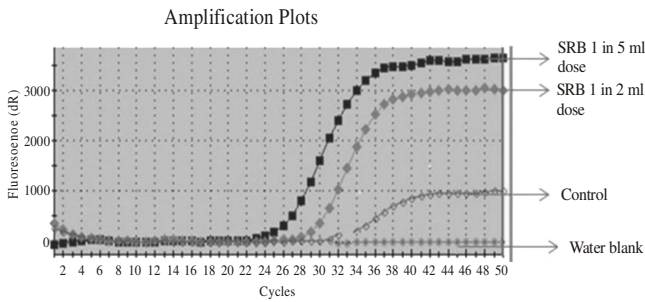**Fig. 3: Effect of palm oil on rabbit liver SRB1 level was quantified using ELISA.**

Fig. 4. Quantification of SRB1 found in rabbit liver using the RT-PCR method shows an increase in level of SRB1 in liver tissue of group-III

elimination is the standard restrictive step of RCT. In experimental animals, supplemented with high fat and high cholesterol diets overcome SR-B1 expression and enhanced the levels of HDL (Ji *et al.,* 1997). The effect of oil supplementation on serum SRB1 levels was assessed by ELISA. Enhancement in liver SRB1 levels was observed when oil was supplemented for the long term. Precisely, medium and high-dose of oil produced 1.8, 2.7 fold (P<0.05) augmentation in serum SRB1 levels, respectively, when compared with control (Fig 3). The gene expression of SRB1 in liver was analyzed by qRT-PCR. Palm oil supplementation may increase the gene expression of the SRB1 in Group-II and Group-III compared to group-I. The results of this study are similar to our previous study in which we observed an increase in the expression of PPARγ and PPAR α after 21 days of experimental feeding of polyphenol rich palm oil (Ahamed *et al.,* 2021). PPAR genes also
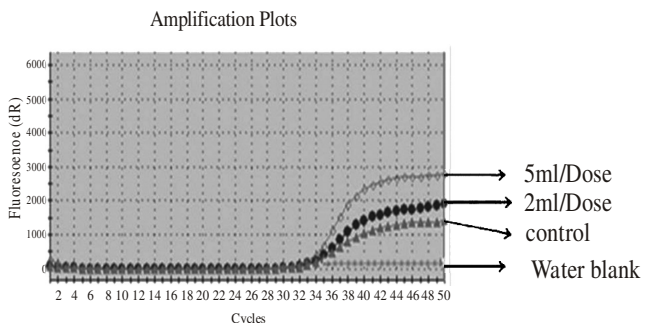
Fig. 5. Quantification of β-Actin found in rabbit liver using the RT-PCR method. Level of β-Actin was found more in 5 ml/kg oil supplemented rabbits (Group-III)

was reported to enhance the expression of SR-B1 (Babiker *et al.,* 1997). Expressions of mRNA for SR-B1 and β-actin are presented in Fig 5. Concentrations of RT-PCR products for SR-B1 were significantly increased in the tissues of oil supplemented group (Fig 4). The SRB1 plays key role in the metabolism of HDL cholesterol and CE in the liver. This receptor also aids in enabling the preliminary step of HDL arbitrate cholesterol efflux (Zhang *et al.,* 2005). In our study, overexpression of SR-B1 in liver might have enhanced the macrophage (Arai *et al.,* 1999). We report here that SR-B1 mRNA expression was increased in Group-III (5 ml/kg palm oil treated animals) and SRB1 protein abundance compared to remaining two groups. Slight increased amount of SRB1 protein was observed in group-II compared to control groups, but compared to group-III lesser protein level was observed. In agreement with our study, high expressions of SRB1 in the liver, while decreasing levels of HDL in plasma was reported earlier (Wang *et al.,* 1998; Kozarsky *et al.,* 2000). Whereas dietary edible oil in hypercholesterolemic
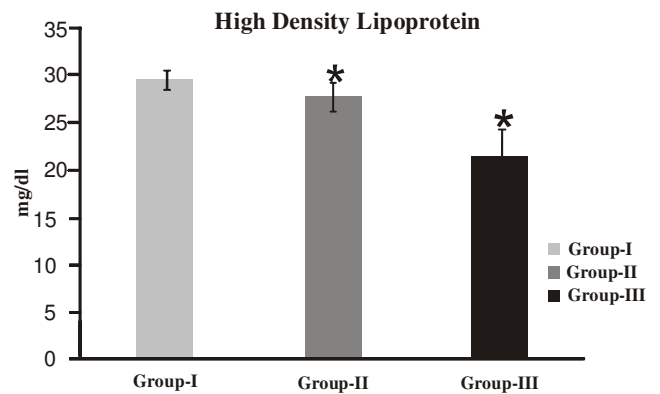

Fig. 6. All values are expressed in Ct Mean ± SD (n=6), the values were analyzed with ANOVA *= P<0.05; Group-I Control animals, Group-II 2 ml/kg palm oil + feed, Group-III 5ml/kg palm oil + feed

rabbits (Group-II andGroup-III) increases SRB1 protein levels, previous studies demonstrated that it correlates with reduced plasma HDL compared to control group rabbits. In a comparable study reduced HDL levels in plasma and over expression of SRB1 was found in transgenic mice controlled by the apolipo

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protein A-I promoter (Rigotti *et al.,* 2003). This demonstrated that over expression of liver SRB1 may promote RCT. It is well identified that overexpression of SRB1 enhance the rate of consumption of HDL by the liver (Ueda *et al.,* 1999; Ji *et al.,* 1999; Alam *et al.,* 2001). These studies described SRB1 may directly arbitrate essential role in liver HDL metabolism, in resolving HDL concentrations and in regulating cholesterol concentrations in plasma and thus may influence the development of atherosclerosis and gallstone disease.

CONCLUSION

In conclusion, our study showed that supplementation of high concentration of edible oil in feed leads to imbalance of cholesterol levels in experimental rabbits and also resulted in over-expression of SRB1 gene in liver of experimental rabbits.

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Nutritional Evaluation of Potato Meal on Growth Performance, Nutrient Utilisation, Blood Parameters and Economics in Growing Pigs

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ABSTRACT

The experiment was conducted for nutritional evaluation of potato meal as an energy source in growing pigs. For the study, 12 pigs were distributed into 3 treatment groups for 60 days, each treatment having 4 pigs. Three treatments comprised of T_1 - control diet, T_2 -potato meal replacing 25% of crude protein contributed by maize and T_3 - potato meal replacing 50% of crude protein contributed by maize. The pigs of all experimental groups were weighed individually at 15 days intervals. Digestion trial and blood collection was done at the end of the experiment. Control diets fed pigs had better (P<0.05) body weight gain, final body weight, average daily gain and FCR as compared those of groups T_2 and T_3. Blood parameter showed no significant difference among the groups, whereas blood parameters remained within the permissible limit. It was concluded that as the amount of potato meal increased, the gain in body weight and the digestibility of nutrients decreased.

Key words: Digestibility, Maize, Pigs, Potato meal

INTRODUCTION

Now a day's pig farming is one of the fastest growing agribusiness of the world. The success of pig farming is greatly dependent upon the continuous and assured supply of good quality feeds at competitive price. Rapid body weight gain with balanced and cheap ration in minimum time is the main aim of pig production. Researchers have been making efforts to optimize the cost of production by utilizing cheaper and readily available energy and protein sources to be incorporated into the pig ration. Potato (*solanum tuberosum*) is a root crop grown in several Asian nations that can be used as an alternative source. Raw potatoes are sufficiently available at a very low price during harvesting season in December-January in Northern India. Some studies had shown that corn can be replaced by potato (*solanum tuberosum* L.) in the diet to some extent (Felix D'mello *et al.* 1975). Chemical composition of potato on dry matter basis for swine is approximately 15% dry matter (DM), 9.2% crude protein (CP), 0.4% ether extract (EE), 2.9% crude fibre (CF) and 5.4% total ash. Digestible energy (DE) of maize *i.e.* 3700 (DE, kcal/kg) which is almost similar to potato i.e 3630 (DE, kcal/kg), so it may be replaced with potato meal as an energy source. So it will be advantageous to feed raw potato to animals which are excellent source of nutrients. Moreover, their utilization can also reduce the cost of feeding, giving higher profits to farmers. So this study was carried out for nutritional evaluation of potato meal as pigs feed.

MATERIALS AND METHODS

Potatoes were purchased from local market and washed with tap water to remove dirt. After rinsing with water, potatoes were mashed by using grinding machine. Mashed potatoes were put on roof top for sun drying. Later on, sun dried potatoes were grinded to make powder. The samples of different feed ingredients along with potatoes were analysed for proximate composition (AOAC, 2007). A total of 12, growing pigs of both sexes were procured from Piggery Farm of Department of Livestock Production Management, GADVASU, Ludhiana. Pigs were reared at GADVASU pig farm under standard conditions. Three experimental rations were formulated as per NRC (1998) specifications (Table 1).

Experimental trial was continued for 60 days. Total 12 pigs were weighed individually on first day of experiment and distributed into 3 groups having 4 pigs in

Table 1. Ingredient composition of experimental diets (parts/100 parts)

Ingredients (kg/100 kg)	T_1	T_2	T_3
Maize	45	37	29
Potato meal	0	8	16
Wheat bran	10.14	11	13.8
Rice polish	20	19	16
Soybean Meal	12	6	5
Groundnut extraction	10	15.51	15.8
Mineral mixture	2	2	2
Methionine (g)	0.16	0.09	0
Salt (g)	0.5	0.5	0.5
Additives* (g)	0.2	0.2	0.2
Total	100	100	100

Each 100 kg ration contained:*Additives include Vitam. AB_2D_3K 15g, vitam B complex 20 g, Vitam. B_{12} preparation 25 g, toxin binder 50 g, probiotic 100 g

each treatment. Sex ratio was maintained. The pigs were divided under the treatments as: T_1-Control (without potato meal), T_2 - with potato meal, replacing 25% of crude protein contributed by maize, T_3 - with potato meal, replacing 50% of crude protein contributed by maize.

Digestion trials for seven days were conducted at the end of experiment to study the effect of potato meal supplementation on the apparent nutrient digestibility. Three pigs from each treatment with comparable body weight were selected for digestion trial. After a four-day adaption period, pigs were fed with known quantity of feed in the morning and evening for the next three days. On the fourth day, the residual feed was removed and weighed to record the actual feed consumption for each replicate. Three day total collection method of faeces was used to determine the digestibility of nutrients for various proximate parameters. The blood samples were collected at the end of experiment, *i.e.* on 60th day from external jugular vein, serum harvested and stored at -20°C till further analysis. Serum samples were analyzed for SGOT, SGPT, cholesterol, triglycerides and glucose using commercial kit (Transasia (ERBA) Biochemistry test kits) following manufacturer's instruction.

Collected data were analyzed using ANOVA in SAS (version 9.3). The treatment means were compared by Duncan's Multiple Ranged Test (Duncan,

1995) and significance was declared at 5% level of significance ($P \leq 0.05$).

RESULTS AND DISCUSSION

Perusal of the growth parameters data (1-30 days) presented in Table 2 revealed that initial body weight (IBW) of pigs in all the three treatments were similar. The gain in body weight (GIW) was highest ($P \leq 0.05$) in control where no potato meal was included in the diet. The lowest ($P \leq 0.05$) gain in body weight was observed at the highest level of potato meal inclusion. Similar trend of linear decrease in average daily gain (ADG) was observed as the potato meal increased in the pig ration. Higher body weight gain and non-significant difference in feed consumption affected the feed conversion ratio (FCR). Best ($P \leq 0.05$) FCR was observed in the control and poorest ($P \leq 0.05$) FCR was observed in group T_3 containing highest level of potato meal. Similar results were found in the study of Bocian *et al.* (2017) which reported that feeding potatoes without being steamed produced better weight gain and feed intake as compared to the pigs fed ration containing steamed potatoes. However, results of experiment conducted by Tusnio *et al.* (2011) did not show any significant changes in GIW, ADG and feed intake with different level of incorporation of potato protein concentrate.

Data pertaining to the growth performance of

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Table 2. Effect of potato meal on growth performance in growing pigs

Period (Age)	Parameter	T_1	T_2	T_3	S.E.M	P-value
(1-30 days of experiment)	Initial body weight.	16.25	16.5	16.25	0.288	0.999
	Final body weight.	22.00	21.10	20.58	0.410	0.394
	Gain in weight.	5.475[a]	4.600[b]	4.050[c]	0.198	0.001
	Average daily gain.	0.1825[a]	0.1533[b]	0.1350[c]	0.006	0.001
	Average feed intake	0.534	0.528	0.524	0.009	0.931
	Feed conversion ratio	2.928[c]	3.444[b]	3.885[a]	0.120	0.000
(31-60 days of experiment)	Initial body weight.	22.00	21.10	20.57	0.410	0.394
	Final body weight	29.12[a]	26.97[ab]	25.65[b]	0.643	0.067
	Gain in weight	7.12[a]	5.87[b]	5.07[c]	0.278	0.000
	Average daily gain.	0.23[a]	0.19[b]	0.17[c]	0.009	0.000
	Average feed intake.	0.76	0.72	0.69	0.152	0.152
	Feed conversion. ratio.	3.23[c]	3.68[b]	4.10[a]	0.109	0.000
Overall (1-60 days of experiment)	Initial body weight	16.25	16.5	16.25	0.288	0.999
	Final body weight	29.12[a]	26.97[ab]	25.65[b]	0.643	0.067
	Gain in weight	12.60[a]	10.47[b]	9.12[c]	0.474	0.001
	Average daily gain	0.21[a.]	0.17[b]	0.15[c]	0.008	0.001
	Average feed intake	0.62	0.60	0.58	0.011	0.526
	Feed.conversion ratio	2.96[c]	3.44[b]	3.87[a]	0.113	0.001

[a,b,c] Mean values with different superscripts in a row differ significantly (P≤0.05)

pigs during the second month (31-60 days) of the experiment is presented in Table 2. The final body weight at the end of second month of experiment was found to be lowest (P≤0.05) in T_3 group where potato meal was supplemented replacing 50% crude protein of maize. The FBW was maximum (P≤0.05) in the control group where potato meal was not added. FBW in group T_2 was intermediate between control and T_3 group. The gain in body weight (GIW) was maximum in control group and it reduced (P≤0.05) linearly as the level of incorporation of potato meal increased from 0 to 50%. At the highest amount of potato meal inclusion, lowest (P≤0.05) gain in body weight was observed. Similar trend was observed in average daily gain. Weight gain ultimately was reflected in the FCR. Accordingly, best (P≤0.05) FCR was observed where gain in body weight was better. The FCR deteriorated (P≤0.05) as the level of potato meal increased. The results are in accordance

Table 3. Apparent digestibility (%) of nutrients in growing pigs supplemented with different level of potato

Parameter	T_1	T_2	T_3	S.E.M	P value
Dry matter	77.15[a]	74.49[b]	71.61[c]	0.80	0.001
Organic matter	78.82[a]	77.76[b]	76.60[c]	0.34	0.003
Crude protein	75.39[a]	72.90[b]	70.57[c]	0.70	0.000
Crude fibre	35.11[a]	34.42[ab]	33.19[b]	0.35	0.049
Ether extract	74.68	74.43	74.82	0.28	0.885
Calcium	42.88	42.55	42.44	0.14	0.497
Phosphorus	38.80	38.22	38.61	0.27	0.746

[a,b,c] Mean values with different superscripts in a row differ significantly (P≤0.05)

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Table 4. Blood parameters in growing pigs supplemented with different level of potato during two months

Parameter	T_1	T_2	T_3	S.E.M	P value
Glucose (mg/dl)	104.00	104.00	110.33	2.82	0.637
SGPT (u/l)	44.66	44.00	42.33	1.09	0.727
SGOT (u/l)	51.67	53.00	53.33	1.95	0.950
Cholesterol (mg/dl)	86.00	86.33	87.33	1.56	0.952
Triglyceride (mg/dl)	60.67	55.33	68.33	3.81	0.431

with Bocian *et al.* (2017) who reported lower weight gain and feed intake in group fed steamed potato. However contradictory results were found in the study of Xue *et al.* (2011) in which sows fed with 5% fermented potato pulp had better weight gain compared to control.

The findings of overall growth parameters during 0-60 days are shown in Table 2 which reveals that the gain in body weight (GIW) was highest (P≤0.05) in control group where no potato meal was included in the diet. With every addition of potato meal, GIW reduced (P≤0.05). With the addition of potato meal to the pig ration, a similar trend of linear decline in average daily gain (ADG) was seen. Higher body weight gain and non-significant difference in feed consumption affected the feed conversion ratio (FCR). Best (P≤0.05) FCR was observed in control group and poorest (P≤0.05) FCR was observed in group T_3, at the highest level of potato meal inclusion. Rahnema and Borton *et al.* (2000) found that group fed with varying levels of potato chip scraps had lower FCR than the control group. However, Tusnio *et al.* (2011) reported that groups fed potato protein concentrate had a higher FCR than the

control group.

The data presented in Table 3 suggested that apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and crude fibre was significantly (P<0.05) higher in control group as compared to T_2 and T_3 groups. Similar results were found in study of Tusnio *et al.* (2011) in which the pigs fed with diet without potato protein concentrate (PPC) have shown significantly higher CP digestibility as compared to those fed PPC, but fat digestibility was higher with potato feeding. The digestibility of ether extract (EE), calcium and phosphorus were similar among the groups. Similarly, Heo *et al.* (2014) did not show any significant changes in dry matter (DM), crude protein (CP), calcium (Ca) and phosphorus (P) digestibility.

Data on blood parameters *i.e.*, glucose, SGPT, SGOT, cholesterol, triglyceride are presented in Table 4. No significant difference was observed between the control (T_1) group and the group fed with potatoes, *i.e* T_2 and T_3. Similar result were found in the study of Singh *et al.* (2005) in which rats were fed on control, and addition of 5% or 10% potato peel powder in other 2 treatments for four weeks. The results showed that

Table 5. Effect of supplementing different levels of potato meal on economics of pig feeding

Variables	Cost Economics		
	T_1	T_2	T_3
Feed cost / Kg	24.30	23.66	23.66
Total feed consumed (60days)	37.2	36	34.8
Total Feed Cost (₹)	903.96	851.76	823
Total weight gain (Kg)	12.60	10.47	9.12
Price/Kg of live weight (₹)	120	120	120
Total income (₹)	1512	1256.4	1094.4
Net profit (₹)	608.04	404	271

triglyceride, total cholesterol and blood glucose were similar among the groups. Xue *et al.* (2011) also observed non-significant differences on plasma glucose with addition of 5% fermented potato pulp in the diet of lactating sows. However, Tusnio *et al.* (2011) reported increase in level of triglyceride with potato protein diet as compared to control diet.

As presented in Table 5, the total cost of feed including all the ingredients and additives in T_1 was ₹ 2430 per quintal of feed whereas feed cost of T_2 and T_3 was ₹ 2366 per q. The same cost in both the treatments was due to higher quantity of oil in T_3 to balance the energy requirement. The amount of feed consumed during 60 days period was highest in T_1 followed by T_2 than T_3. Since feed intake was less in the groups in which potato meal was added leading to lower total cost of feed to rear pigs for 60 days in treatment groups and was more in T_1 group. Total weight gain was significantly (P≤0.05) higher in T_1 than T_2 and T_3 and was lowest (P≤0.05) in T_3. The selling price per kg body weight was Rs 120. Highest body weight in T_1 (control diet) where potato meal was not supplemented in the diet fetched highest income. The T_3 treatment, in which 50 percent of the crude protein of maize was replaced with potato meal, yielded the lowest income.

CONCLUSION

It was observed that as the amount of potato meal supplementation increased, the gain in body weight and the digestibility of nutrients decreased. Further research aiming at incorporation of potato meal at lower level is warranted.

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Effects of Supplementation of Aloe vera *(Aloe barbadensis)* and Tulsi *(Ocimum sanctum)* as Feed Additives on Performance of Broiler Chickens

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ABSTRACT

A study was carried out to investigate the efficacy of dietary supplementation of Aloe vera (*Aloe barbadensis*) and Tulsi (*Ocimum sanctum*) powder as a feed additive on growth performance, feed consumption, and feed conversion efficiency of broilers. The experiment was conducted in a completely randomized design with a total of 240-day-old broiler chicks (Vencobb) randomly assigned to four treatment groups with four replicates having 15 birds in each replicate for a period of five weeks. Birds were offered the maize-soybean based basal diet. The treatment groups consisted of control group (T_1) fed only basal diet without additive, T_2 supplemented with 0.5% Aloe vera powder, T_3 supplemented with 0.5 % Tulsi powder and T_4 supplemented with 0.25 % Aloe vera powder + 0.25 % Tulsi powder in the diet. No significant effect on feed intake was observed among different groups due to supplementation of feed additives. The final body weight and body weight gain were significantly ($P<0.01$) higher in groups supplemented with either Aloe vera, Tulsi or both as compared to control. Feed conversion ratio in the T_2 group was significantly ($P<0.05$) improved in comparison to other groups. Dressing % was not significantly different among the groups. It was concluded that the use of polyherbal supplement in the form of Aloe vera and Tulsi as feed additives at a level of 0.5 % and 0.25%, and a combination of both enhanced the overall performance of broiler chicken.

Key words: Aloe vera, Broilers, Feed additives, Growth performance, Tulsi

INTRODUCTION

Poultry is one of the rapidly developing sectors of Indian agriculture today, with an annual growth rate of 11.4 per cent, India is the fourth largest producer of chicken in the world with 3.9 million metric tons after US, China and Brazil (USDA, 2015) and it is approaching 4.2 MMT. Poultry production, particularly broiler production is the quickest way to increase the availability of high quality protein for human consumption (Karnani *et al.,* 2019). The broiler industry has grown due to consumer demand for affordable poultry meat. Poultry feed industry is facing increased pressure from consumers to reduce the use of antibiotic growth promoters (AGPs) in poultry diets. Plant extracts and various phytobiotic that originate from leaves, roots, tubers or fruits of herbs, spices and other plants have shown to be excellent growth enhancers in poultry (Steiner *et al.,* 2013; Wallace *et al.,* 2010).

In the present research, two medicinal plants Tulsi and Aloe vera were tested as feed additives in the poultry diet. Major ingredients of Aloe vera include anthraquinones, saccharides, vitamins, enzymes, and low-molecular-weight compounds (Choi and Chung, 2003) which give Aloe vera its anti-inflammatory, immunomodulatory, wound-healing, anti-viral, anti-fungal, anti-tumor, anti-diabetic, and anti-oxidant effects (Christaki and Florou-Paneri, 2010).

Ocimum sanctum (Tulsi) is reported to possess anti-infertility, anticancer, antibacterial (Joshi *et al.,* 2009), antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antioxidant (Subramanian *et al.,* 2005), antispasmodic, analgesic, anti-ulcerogenic and ulcer healing properties, adaptogenic (Singh *et al.,* 2010) and diaphoretic actions (Mondal *et al.,* 2009). It was hypothesized that these two herbal ingredients would improve performance of broilers either alone or in combination. Therefore, the present experiment was planned to study the effect of

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dietary Aloe vera and Tulsi powder on the performance of broiler chickens.

MATERIALS AND METHODS

The present experiment was carried out at Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur. Two hundred and forty day-old, unsexed, apparently healthy broiler chicks of cobb-400 strain were individually weighed (almost similar average weight) and randomly divided into four groups with four replicates having 15 chicks in each replicate by completely randomized design. The required quantities of the feed ingredients and feed additives such as Aloe vera and Tulsi for formulations of experimental diets were procured from the local market of Jaipur, Rajasthan. The control group T_1 was fed basal maize soybean based diet (Feed without additives), T_2: 0.5% Aloe vera powder, T_3: 0.5% Tulsi powder and T_4: 0.25% Aloe vera and 0.25% Tulsi powder. The broiler pre starter, starter and finisher rations were formulated as per the ICAR, 2013. The feed was offered *ad libtum* to each group throughout the experimental period and group-wise feed consumption was recorded at weekly intervals.

The body weight of the experimental broilers was recorded at beginning of the experiment and thereafter at weekly interval to assess the body weight change and the growth pattern. The weighing of the birds was done in the early hours of the day before feeding, using an electronic scale. Live weight gain at weekly interval was calculated from difference in body weight attained between the two consecutive weeks. Feed conversion ratio (FCR) was calculated by dividing the cumulative feed intake by body weight gain of chicks for every week. In order to take into account the feed efficiency as well as the growth rate, an index (Performance Index (PI)) was obtained for each treatment by dividing the average weight gained by the feed conversation ratio. Protein efficiency ratio (PER) was calculated by dividing the weight gain by protein consumed. A nitrogen balance study was conducted using 12 chicks from each group for 5 days at the end of feeding trial. During this period the twelve birds from each treatment

were transferred to separate pens and polythene sheet of appropriate size was spread over the ground for collection of mixed excreta in each group. The chicks were offered a weighed amount of experimental ration at a fixed morning hour (9:30 AM) every day during the trial period. The mixed droppings were collected at the end of every 24 hours and pooled to get the total excreta voided during the trial period. Daily feed intake was calculated after deducting the left over from the feed offered. Representative feed samples were drawn from the bulk, dried, ground and stored in sample bottles for analysis. The group wise aliquots from droppings after thorough mixing with the help of spatula were drawn for nitrogen estimation. For nitrogen estimation, samples in duplicate were preserved in 5 per cent sulphuric acid in wide-mouth glass stoppered bottles and kept in refrigerator. Dry matter determination of excreta was done with duplicate sample for each group by keeping the weighed excretal material in an oven at 85°C till constant weight was obtained. Proximate composition of the herbs, nitrogen content of feed and excreta were estimated by following the methods of AOAC (2005).

To study the effect of different treatments on dressing percentage, twelve representative birds from each group were sacrificed at the end of 5th week. The selected birds were weighed individually and allowed to fast for 12 hour to empty gut contents before sacrifice. The broilers were sacrificed as per standard procedure (Panda, 1995) by severing the occipito-atlantal joint and allowed to bleed completely. The birds were de-feathered in defeathering machine after scalding and carcasses were eviscerated to measures dressing percentage. Data obtained were analyzed using standard procedure (Snedecor and Cochran, 1989). Treatment means were separated using Duncan's multiple range tst (Duncan, 1955).

RESULTS AND DISCUSSION

Ingredient and nutrient compositions of the experimental diets are presented in Tables 1 and 2. All the diets provided adequate amount of crude protein and metabolizable energy to meet requirements (ICAR,

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Table 1. Ingredient and proximate composition of pre- starter, starter and finisher diets on dry matter basis

Ingredients	Pre-starter(%)	Starter (%)	Finisher (%)
Maize	55.1	58.02	62.2
Soyabean meal	37.2	34.01	30.1
Oil	3.0	3.0	3.0
Stone grit	1.65	1.60	1.85
Di-calcium phosphate	1.85	1.90	1.65
Salt	0.40	0.40	0.40
DL- Methionine	0.23	0.19	0.16
Lysine	0.19	0.14	0.12
Vitamin and mineral premix	0.5	0.5	0.5
Metabolizable energy (kcal/kg)	3015	3050	3095
Crude protein (%)	22.01	21.6	19.45

2013).The average total feed consumption in terms of g/bird in various treatment groups was found to be similar (Table 3). However, numerically highest total feed intake was found in T_3 group followed by T_4, T_1 and T_2 groups. No adverse effect of smell or taste of Aloe vera and Tulsi powder was found on the feed intake of broilers. Similarly, Hassanbeigy *et al.* (2004) found no significant effect on feed intake due to inclusion of Aloe vera at 0.6, 1.2, 1.8, 2.4 and 3 ml/l in drinking water. However, others research workers (Fallah, 2015; Darabighane *et al.,* 2017;Singh *et al.,* 2017) found increased feed intake when Aloe vera gel was added in the diet of broilers. On the other hand, other report (Eevuri and Putturu, 2013) indicated decreased feed intake due to dietary addition of turmeric, tulsi, amla and Aloe vera in broiler's diet. Yet other reports (Vasanthakumar *et al.,* 2013; Bhosale *et al.,* 2015; Hasan *et al.,* 2016) indicated no significant (P>0.05) effect of Tulsi on feed intake of broiler chicks.

Birds of treatment groups supplemented with Aloe vera and Tulsi powder and their combination attained significantly (P<0.01) higher body weight as compared to control. The highest total body weight was recorded in T_3 group followed by T_2 and T_4 groups. No significant difference was found among feed additives supplemented groups. These findings are in accordance with previous reports (Appusamy, 2012; Hassanbeigy *et al.,* 2012; Eevuri and Putturu, 2013; Singh *et al.,* 2014a; Fallah, 2015; Darabighane *et al.,* 2017; Singh *et al.,* 2017) which also found that the Aloe vera supplemented groups gained significantly higher final body weights than untreated groups. The significant improvement in body weight on account of supplementation of Tulsi was also reported earlier (Lanjewar *et al.,* 2008; Nath *et al.,* 2012; Khatun *et al.,* 2013; Alam *et al.,* 2015; Bhosale *et al.,* 2015 and Hasan *et al.,* 2016). The body weight gain was significantly (P<0.01) higher in groups having herbal feed

Table 2. Proximate composition of Aloe vera and Tulsi powder

Proximate Principle	Aloe vera powder	Tulsi powder
Dry matter (%)	91.62	93.00
on % dry matter basis		
Crude protein (%)	10.21	16.05
Ether extract (%)	1.76	2.36
Crude fibre (%)	10.52	15.29
Total ash (%)	17.45	14.83

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additives as compared to control group. Among herbs supplemented group highest body weight gain was found in T_3 group supplemented with Tulsi. Our results corroborate well with previous reports (Swati *et al.,* 2012; Bhosale *et al.,* 2015; Hasan *et al.,* 2016) which also observed significantly higher body weight gain of diet supplemented with Tulsi. Mmereole (2011) reported that the body weight gain was significantly higher in the birds fed diet that containing 1 per cent Aloe vera leaves powder than the birds fed control diet. Contrary to this, Singh *et al.* (2014b) reported that inclusion of 1, 1.5 and 2% whole leaf Aloe vera powder in diet of broilers had no significant effect on weight gain and final body weight.

Results revealed that birds of T_2 group showed significantly (P<0.05) improved FCR as compared to T_1 and T_4 groups, whereas no significant difference was found with T_3 group. Similarly, Namagirilakshmi, (2005) reported that supplementation of Aloe vera, probiotics and turmeric in chicken diet showed better feed efficiency. The results obtained are in accordance with finding of previous research workers (Raziq *et al.,* 2012; Shokraneh *et al.,* 2016) who reported better FCR in Aloe vera added groups. Results obtained in this study are also in agreement with other reports (Khatun *et al.,* 2013; Bhosale *et al.,* 2015) that reported improvement in FCR with the inclusion of Tulsi as a feed additive in the diet of broilers.

Performance index was significantly (P<0.05) higher in herbal products supplemented groups than in control group, there was no significant difference between Aloe vera and Tulsi supplemented groups. Srivastava *et al.* (2013) observed significant difference in performance index in broiler fed diet with herbal drugs. Karnani *et al.* (2018) found significantly higher performance index when feed was supplemented with curry leaves powder. The results obtained from this study are in agreement with the findings of Patel *et al.* (2014) who recorded similar effects on performance index due to supplementation of garlic in the poultry ration. The protein efficiency ratio was significantly (P<0.01) higher in T_2 and T_3 groups as compared to T_1 and T_4 groups. Results obtained in the study corroborate well with the findings of Karnani *et al.* (2108) who found significant improvement PER due to supplementation of curry leaves powder in broilers.

Nitrogen balance was significantly (P<0.01) higher in herbs supplemented group than control group. No significant difference was there between Aloe vera and Tulsi supplemented groups. However, Dwivedi (2013) recorded no effect of phytobiotic or herb supplementation on nitrogen balance. This could be due to species and form of herbs used.

No significant difference was found among the treatment groups due to supplementation of Aloe vera and Tulsi alone and their blend on dressed weight of carcass. Apparently, highest dressed weight was recorded in T_3 followed by T_2, T_4, and T_1. Sinurat *et al.* (2002) reported that supplementation of fresh Aloe vera

Table 3. Effect of Aloe vera and Tulsi powder on performance of broiler chickens

Particulars	Treatments				P- value
	T_1	T_2	T_3	T_4	
Avg. total feed intake (g)	3039±51	2995±80	3180±59	3131±30	NS
Final body weight (g)	1952[a]±10.7	2070[b]±36.7	2117[b]±30.6	2046[b]±17.9	**
Total body weight gain (g)	1912[a]±10.7	2023[b]±36.8	2074[b]±30.8	2005[b]±18	**
Feed conversion ratio	1.58[b]±0.02	1.47[a]±0.04	1.53[ab]±0.01	1.56[b]±0.018	*
Performance index	1290[a]±7.56	1482[b]±57.5	1433[b]±27.4	1389[ab]±36.0	*
Protein efficiency ratio	2.79[a]±0.03	3.10[c]±0.08	2.97[bc]±0.03	2.92[ab]±0.03	**
Dressing %	70.2±0.02	70.04±0.19	70.42±0.92	69.56±0.34	NS
Nitrogen balance	1.06[a]±0.239	1.92[b]±0.043	1.92[b]±0.20	1.86[b]±0.016	**

[a,b]Means superscripted with different letters within a column differ significantly from each other

gel (0.25 g/kg) and dry Aloe vera gel (0.25 and 1.0 g/kg) in broiler diet from 1 day old to 5 weeks of age showed no significant effect on carcass yield and internal organs. Mehala *et al.* (2009) also reported that dietary inclusion of Aloe vera in broiler diet has no significant effect in carcass quality. Contrary to this, Durrani *et al.* (2006) reported higher dressing percentage, breast, thigh and giblet weight in broilers fed diet contains 0.5% turmeric.

CONCLUSIONS

It was concluded that addition of 0.5% Aloe vera and 0.25 % Tulsi and blend of both (1:1) at 0.5% in the diet caused significant improvement in weekly weight gain and feed efficiency as compared to that of control group of broiler. Thus, polyherbal supplementation in the broiler rations may be useful for the safe, economical and efficient production of broiler and this formulation could be used as an alternative to commercial growth promoters.

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Moringa Oleifera Leaf Meal (MOLM) with Cocktail Enzyme Supplementation on Immunity, Serum biochemical and Antioxidant Activity of Layers

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ABSTRACT

The objective of the present study was to evaluate the effect of *Moringa oleifera* leaf meal (MOLM) on immunity status, antioxidant and serum biochemical parameters of layers. Two hundred, 34-week-old BV 300 White Leghorn layer birds were randomly allotted to 50 replicates with 4 birds in each replicate and these replicates were in turn allotted to 5 dietary groups: T_1 (Control) = Corn-soybean meal Basal diet (BD); T_2 = 5% MOLM in BD diet without enzyme supplementation; T_3 = 7.5% MOLM without enzyme supplementation; T_4 = 10% MOLM in BD diet without enzyme supplementation; T_5 = 10% MOLM with enzyme supplementation. The results revealed that the cell mediated immune response was significantly (P<0.01) higher in T_5 as compared to other treatments, whereas no significant difference was observed in the Humoral immune response among the birds fed diets with or without inclusion of moringa leaf meal. There was no significant difference was recorded in protein values among T_1, T_2 and T_5, however, lower protein values were observed in T_3 and T_4 but not significantly differ with T_1. There was no significant difference in albumin content among the treatments. Lower (P<0.01) cholesterol level was noticed in T_5 and higher (P<0.01) cholesterol level was observed in T_1. The calcium and phosphorus levels in serum were higher (P<0.01) in T_4 and T_5 as compared to other treatments, with the lowest (P<0.01) calcium and phosphorus levels recorded in T_1. The glutathione peroxidase activity was significantly higher (P<0.01) in the birds fed Moringa leaf meal diets as compared to the control. The lipid peroxidation and RBC catalase activity was inversely proportional to inclusion levels of MOLM in diets. Thus, 10% *Moringa oleifera* leaf meal with or without enzyme supplementation could be used for improving immunity and antioxidant status of layers.

Key words: Antioxidant activity, Cholesterol, Calcium, Immunity, Moringa leaf meal

INTRODUCTION

Moringa oleifera commonly referred to as the drumstick tree is a plant from the Moringaceae family and it is the most widely cultivated species of the genus Moringa. The tree is often called 'multipurpose' due to the fact that all parts including the leaves, pods, seeds, flowers, fruits and roots are edible (Orwa *et al.,* 2009). The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in Moringa leaves (Atawodi *et al.,* 2010). Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in Moringa leaves (Siddhuraju and Becker, 2003). Yang *et al.* (2006) and Jung *et al.* (2010) reportedd that *M.oleifera* was among the most promising species based on their high antioxidant activity, high contents of micro-nutrients and phytochemicals, processing

properties, ease of growing, and also on palatability, stability and shelf life of meat products. Akinola and Ovoter (2018) reported that *Moringa oleifera* leaf meal favoured the good cholesterol, HDL of the fresh eggs at levels of 0.5 and 1.0% inclusion in layers. Hossam *et al.* (2016) recorded increased antioxidant activity, immunity of chicks with Moringa leaf extract. Therefore, this study was designed to evaluate the effect of *Moringa oleifera* leaf meal on immunity status, antioxidant and serum biochemical parameters of layers.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Experimental Station, Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad. Two hundred, 34-week-old BV 300 White Leghorn layer birds were randomly allotted to 50

replicates with 4 birds in each replicate and these replicates were in turn allotted to 5 dietary groups. The birds were raised in cages under uniform management and fed the respective diets from 34th to 49th weeks of age. Birds were fed the experimental diet at 120 g/bird per day but this amount was adjusted with regard to the level of their production throughout the experimental periods (NRC, 1994). Leaf was harvested from young Moringa oleifera trees of about four years of age from forage farm under supervision of CVR food products. The harvested leaves from the trees were spread out on a concrete floor and allowed to dry for a period of three days under shade and aerated conditions, then run

through a hammer mill sieve with a size of five mm to produce the leaf meal.

Five treatment diets included T_1 (Control) = Corn-soybean meal Basal diet (BD); T_2= 5% *Moringa oleifera* leaf meal in BD without enzyme supplementation; T_3= 7.5% *Moringa oleifera* leaf meal in BD without enzyme supplementation; T_4= 10% *Moringa oleifera* leaf meal in BD without enzyme supplementation; T_5= 10% *Moringa oleifera* leaf meal in BD with enzyme supplementation was formulated to which MOLM substitutes SBM (Table 1). Cocktail of enzymes including (Cellulase- 1,50,255 CMCU/G, Phytase- 2,225 FYT/G, Beta mannanase- 2,00,000 IU/

Table 1. Proportion (%) of ingredients used for formulating experimental diets

Ingredients (%)	T_1	T_2	T_3	T_4	T_5
Maize	59.8	56.5	54.9	53.4	53.4
SBM	21.5	19.8	18.915	17.915	17.915
DORB	7	7	7	7	7
Moringa	0	5	7.5	10	10
Limestone	10	10	10	10	10
DL-Methionine (Degussa)	0.16	0.16	0.16	0.16	0.16
Salt	0.15	0.15	0.15	0.15	0.15
Vitamin Premix*(Venky s)	0.05	0.05	0.05	0.05	0.05
Trace mineral mixture**	0.1	0.1	0.1	0.1	0.1
Choline Chloride,75%	0.05	0.05	0.05	0.05	0.05
Toxin binder (Toximar)	0.05	0.05	0.05	0.05	0.05
Di-calcium phosphate	1.09	1.09	1.09	1.09	1.09
Enzyme (MAXIZYME EX)	0	0	0	0	0.025
L-Lysine HCl (Azinomoto)	0.08	0.08	0.08	0.08	0.08
Total	100	100	100	100	100
Calculated Nutrient composition*					
CP(%)	15	15	15	15	15
ME (kcal/kg DM)	2500	2500	2500	2500	2500
Calcium (%)	4	4	4	4	4
Available Phosphorus (%)	0.34	0.34	0.34	0.34	0.34
Lysine (%)	0.68	0.68	0.68	0.68	0.68
Methionine (%)	0.32	0.32	0.32	0.32	0.32
Sodium (%)	0.18	0.18	0.18	0.18	0.18
Chlorine (%)	0.22	0.22	0.22	0.22	0.22

*Vitamin premix provided per kg diet: Vitamin A 200000 IU, Vitamin B_2 25 mg, Vitamin D_3 3000IU, Vitamin K 2 mg.Riboflavin 25 mg, Vitamin B_1 1 mg, Vitamin B_6 2 mg, Vitamin B_{12} 40 mg and Niacin 15 mg; **Trace mineral provided per kg diet: Manganese 120 mg, Zinc 80 mg, Iron 25 mg, Copper 10 mg, Iodine 1 mg and Selenium 0.1 mg.

G, Protease- 7,00,115 IU/G, Alpha Amylase- 70,000 U/G, Lipase- 18,000 U/G, Xylanase- 2,80,000 IU/G, Arabinase- 2120 IU/G, Beta Galactosidase- 25,000 IU/G) was added in T_5. The Treatment diets were formulated to be iso-caloric and iso-nitrogenous, to meet the ME and CP requirement of laying hens according to NRC (1994).

Layers were vaccinated against ND by subcutaneous route at 47 wks of age with Lassota vaccine (Indovax Pvt., Ltd., Hyderabad, India). After 14 days, blood was collected and serum was separated. Subsequently, micro-hemagglutination activity of serum was estimated and the antibody titers (log_2) measured following the standard procedure (Wegmann and Smithies., 1966). A phytohemagglutinin-P (PHA-P) injection assay (Cheng and Lamont 1988) was used to evaluate *in vivo* T-cell-mediated immune response of laying hens. At the end of experiment, blood sample were collected aseptically from wing vein (one bird from each replicate) in vacutainers and kept in incubator at room temperature for serum collection. The serum was used for estimation of cholesterol, total protein, albumen, globulin and antioxidant activity by using spectrophotometer with commercially available kits (Coral Clinical Systems, Goa).

Data were analyzed for mean, standard errors and analysis of variance as per method of Snedecor and Cochran (1989) and comparison of means were done using Duncan (1955) using software of Statistical Package for Social Sciences (SPSS) 15.0 version and significance was considered at P<0.05.

RESULTS AND DISCUSSION

There was no significant difference observed in the humoral immune response among the birds fed diets with or without inclusion of Moringa leaf meal (Table 2). However, the cell mediated immune response was significantly (P<0.01) higher in T_5 when compared to other treatments and control. Moringa leaves contain compounds such as protein, flavonoids, tannin, *etc.* and these compounds are efficiently utilized by the enzymes in T_5 which might have contributed to give better immune response. More immunity levels were observed in birds fed MOLM based diets may be attributed to antimicrobial and antioxidant properties of Moringa leaves (Ebenebe *et al.,* 2012; Hassan *et al.,* 2016). Melesse *et al.* (2013) reported that *Moringa oleifera* leaves have a beneficial effect in enhancing the immune responses. Eze *et al.* (2013) reported increase in total and differential cell numbers and haemagglutination inhibition (HI) titre in the chicken fed *Moringa oleifera* against Newcastle disease (ND). Chollom *et al.* (2012) demonstrated *M. oleifera* seed extract have nutritional value as well as strong antiviral activity against NDV *in-ovo*. Hossam *et al.* (2016) found that, Moringa leaf extract acts as antibacterial, growth promoter,

Table 2. Effect of dietary inclusion of *Moringa oleifera* leaf meal on immune parameters in layers

Diet	HI	CMI
T_1	6.30	159.8[b]
T_2	6.70	163.8[b]
T_3	6.90	164.2[b]
T_4	6.90	169.3[b]
T_5	7.10	223.7[a]
P-Value	0.330	0.000
N	10	10
SEM	0.125	4.994

[a,b] Means with different superscripts in a column differ significantly (P<0.05); T_1= Corn-soybean meal Basal Diet (BD) ; T_2= 5% *Moringa oleifera* leaf meal in BD without enzyme supplementation ; T_3= 7.5% *Moringa oleifera* leaf meal in BD without enzyme supplementation ; T_4= 10% *Moringa oleifera* leaf meal in BD without enzyme supplementation ; T_5= 10% *Moringa oleifera* leaf meal in BD with enzyme supplementation. P value = probability value; N = number of replicates (10 birds in each replicate); SEM= Standard Error Mean. HI= Humoral immunity, CMI=Cell Mediated Immunity.

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antioxidant and have beneficial effect on immunity and hemato-biochemical parameters. Moringa leaves are rich in Vitamin A which might have improved the function of immune system. Similarly, increased immunity *Moringa oleifera* leaf meal was also reported by Divya *et al.* (2015).

Dietary inclusion of *Moringa oleifera* leaf meal in layer diets had shown a significant effect in serum biochemical profile except in albumin (Table 3). Lower cholesterol levels and higher calcium and phosphorus in serum were noted in birds fed Moringa leaf meal diets. However, there was an increase in the total protein level in serum at 5 % MOLM and then decrease as the MOLM content increased and again it was hiked at 10% MOLM with enzyme supplementation. The increase in the protein level in 5% MOLM fed birds might be due to increase in digestibity. As MOLM was increased, the protein digestibility was decreased, might be due to higher fibre level (10.4%) in Moringa leaf meal. Whereas, the increased protein in 10% with enzymes supplementation was due to action of protease enzyme. Total serum protein has been reported as an indication of the protein retained in the animal body (Esonu *et al.,* 2001). The relatively greater total serum protein content of birds receiving dietary MOLM might be an indication of the good protein and/or quality of the leaf meal. The result of the total protein of the present study tallied with the report of Hassan *et al.* (2016) who reported difference in total protein of the blood in

broilers that were fed the same leaf meal.

Fahey *et al.* (2005) stated that Moringa is a good source of phytochemicals (natural plant chemicals) such as glucosinolates, alkaloids and isothiocyanates that provides cholesterol lowering activities. The present study had shown the lower cholesterol activity with the increase of the Moringa leaf meal in the diets. It supported the findings of Ghasi *et al.* (2000), Nabila *et al.* (2015) and Yassmine *et al.* (2017) and Olugbemi *et al.* (2010) that moringa leaf has hypocholesterolemic properties. Dey and Parthasarathi (2013) noted the decrease in total cholesterol as the MOLM increased in the diet. The cholesterol reducing action was also reported by Ashong and Brown (2011), may be attributed to a bioactive phytoconstituent alpha sitosterol, a plant sterol with a structure similar to cholesterol present in Moringa leaves (Ghasi *et al.,* 2000). Moringa leaves powder of the present study was having 10 % crude fibre and increase in fiber may also be responsible for less absorption of cholesterol from the intestinal tract of the birds.

The serum calcium and phosphorus levels were higher in T_4 and T_5 since Moringa leaf meal is the rich source of calcium and phosphorus. The proximate composition of the present study had shown calcium of 2.5% and phosphorus of 0.36% which might attribute to the increase of calcium and phosphorus levels in serum as compared to other treatments.

The glutathione peroxidase activity was

Table 3. Effect of dietary inclusion of *Moringa oleifera* leaf meal on serum biochemical parameters in layers

Diet	Protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Phosphorus (mg/dl)	Calcium (mg/dl)
T_1	4.630[ab]	1.967	176.8[a]	5.224[c]	16.13[c]
T_2	5.007[a]	1.634	140.5[b]	5.352[bc]	18.74[b]
T_3	4.325[b]	1.791	133.7[b]	5.662[b]	19.21[b]
T_4	4.212[b]	1.505	134.9[b]	6.230[a]	20.17[a]
T_5	5.103[a]	1.491	112.0[c]	6.301[a]	20.29[a]
P-Value	0.022	0.114	0.001	0.001	0.001
N	10	10	10	10	10
SEM	0.108	0.067	3.569	0.087	0.230

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Table 4. Effect of dietary inclusion of *Moringa oleifera* leaf meal on antioxidant parameters in layers

Diet	GPx	LPO	RBC Catalase
T_1	800[b]	4.662[a]	82.00[a]
T_2	1381[a]	4.424[a]	77.40[a]
T_3	1599[a]	3.453[b]	77.35[a]
T_4	1198[a]	2.567[c]	55.44[b]
T_5	1553[a]	1.623[d]	47.76[b]
P-Value	0.001	0.000	0.000
N	10	10	10
SEM	72.74	0.168	3.151

significantly higher (P<0.01) in the birds fed Moringa leaf meal included diets as compared to the control (Table 4). Lipid peroxidase enzyme activity was significantly higher (P<0.01) for T_1 and T_2, and the least (P<0.01) activity was noticed in T_5. There was a decreasing trend in the lipid peroxidation from T_1 to T_5 with increase in Moringa levels in the diet. Catalase activity was significantly higher (P<0.01) in T_1, T_2 and T_3 as compared to T_4 and T_5. The results of the current study regarding glutathione peroxidase activity are similar to the findings of Wei *et al.* (2016) who noted the increase of glutathione peroxidase activity in the Moringa leaf meal included diets. Abdulaziz *et al.* (2015) reported that the phenols and flavonoids of Moringa leaf meal play an important role to cure and even prevent oxidative damages caused by free radicals. The increased glutathione peroxidase activity in Moringa leaf meal included diets as compared to control diet might be due to the presence of flavonoid groups such as quercetin and kaempferol and their antioxidant activity is higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in Moringa leaves (Siddhuraju and Becker, 2003). In the present study, the RBC catalase activity was more in control birds that implies presence of more free radicals. The phenols and flavonoids of Moringa leaf meal prevented the oxidative damages caused by free radicals and decreased the stress of animals, which was noticed in 10% *Moringa oleifera* leaf meal included diets. The antioxidant activity of phenols was mainly due to their redox properties which played an important

role in neutralizing free radicals, quenching singlet and triplet oxygen molecule (Gupta and Abu Ghannam, 2010).

The decrease in lipid peroxidation indicates role of *M. oleifera* leaves as an antioxidant. lipid peroxidation is a process involved in degradation of structural components of cell membranes and reactive oxygen species (ROS) accumulation is known to induce lipid peroxidation and oxidative stress. The influence of MOLM diets on lipid peroxidation and serum antioxidant enzymes in present study are in congruence with Rama Rao *et al.* (2013) and Habibi *et al.* (2014) in broilers. The antioxidant potential of MOLM is attributed to presence of phytochemicals such as, carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics.

CONCLUSION

It can be concluded that supplementation of 10% *Moringa oleifera* leaf meal with enzyme supplementation increased the immune status, antioxidant activity, serum protein, calcium and phosphorus levels and lowered the serum cholesterol levels of layers. Thus, 10% *Moringa oleifera* leaf meal with or without enzyme supplementation could be safely included for improving immunity and antioxidant status of layers.

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Effect of *Lactobacillus* in Broiler Chicken Fed Diets Enriched with ω-3 Fatty Acids

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ABSTRACT

A research was conducted to study the effect of *Lactobacillus* supplementation in broiler chicken fed diet enriched with ω-3 fatty acids. Two species of *Lactobacilli* were isolated from chicken gut and identified by *in vitro* sugar fermentation tests and by the gene sequencing of 16S RNA-23S RNA inter spacer region. The acid tolerance, bile tolerance and antioxidant activity test were done to determine the maximum probiotic potency by using probiotic score. *L. salivarius* with probiotic score of 170.94 compared to *L. agilis* with score of 129.14 was chosen for the feeding trial in broiler chicken. The freeze-dried *L. salivarius* exhibited 50% viability post freezing. In the feeding trial, the effect of *L. salivarius* at different level of supplementation *viz.,* T_1-without *L. salivarius* supplementation, T_2- supplemented with *L. salivarius* at 10^6cfu/kg, T_3- supplemented with *L. salivarius* at 10^9cfu/kg and T_4- supplemented with *L. salivarius* at 10^{12}cfu/kg in broiler chicken fed diet enriched with ω-3 fatty acid was studied. Group T_4 showed significant (P<0.05) increase in weight gain and improvement in feed conversion ratio (FCR). There was no significant (P>0.05) difference in the meat quality parameters among treatments. The T_1 showed significantly (P<0.05) increased level of linolenic acid and ω-3 fatty acids (0.60 ± 0.1 and 6.96 ± 0.99 g/100 g total fatty acid (FA), respectively). There was no significant (P>0.05) increase in linoleic acid, eicosa-pentaenoic acid (EPA), docosahexaenoic acid (DHA) and ω-6 fatty acids among the treatments. But the T_4 showed numerical increase in EPA level (1.52 g/100 g total FA). It is concluded that T_4 with dietary ω-3 to ω-6 fatty acid ratio of 1:3 and *L. salivarius* inclusion of 10^{12}cfu/kg feed had produced broiler meat with ω-3 to ω-6 fatty acid ratio of 1:2.17 and EPA level of 1.52 g/100 g Total FA.

Key words: *Lactobacillus*, Broiler chicken, ω-3 fatty acids, Eicosapentaenoic acid, Docosahexaenoic acid

INTRODUCTION

Chicken meat is fast becoming a solution to increasing meat demand because of its high protein content as well as lower cholesterol and fat content. The deficiency of ω-3 fatty acids as well as imbalance of ω-3: ω-6 ratio in the human diet is considered to be an important reason for the prevalence of certain degenerative diseases. A hen living in nature freely will have a good balance between ω-3 and ω-6 fatty acids as it selects its own feed. But the poultry feed used by the farmers contain higher amount of ω-6 fatty acids compared to ω-3 fatty acids. This will result in a high concentration of the ω-6 fatty acids and less EPA and DHA in chicken meat.

Probiotics, classified as zootechnical feed additives (European Commission, 2003), improve the gastro intestinal health and intestinal function through the live beneficial microorganisms (Fuller, 1989). In order to obtain the numerous health benefits of probiotics, a minimum recommended level of viable microorganisms (10^6cfu/g) is necessary during consumption (Samona and Robinson, 1994). Bacteria belonging to different lactic acid bacteria (LAB) are available as probiotic supplements, but *lactobacilli* are the most commonly used.

Salma *et al.* (2007) reported that dietary supplementation of probiotic bacteria could improve fatty acid profile in broiler chicken. Ringo *et al.* (1998) demonstrated that dietary lipids had an effect on gastrointestinal flora, in particular, on the level of lactic acid bacteria. Kankaanpaa *et al.* (2001) showed that poly unsaturated fatty acids (PUFA) altered bacterial adhesion sites on Caco-2 cells. It is suggested that dietary PUFA affect mucosal adhesion sites for gastrointestinal bacteria by modifying the composition of the intestinal wall. Thus, probiotic bacteria and PUFA

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mutually benefit each other. So, in this experiment are studied the effect of *Lactobacillus* on the growth performance and meat quality parameters of broiler chicken fed diet enriched with ω-3 fatty acids.

MATERIALS AND METHODS

Two strains of *Lactobacilli* were isolated from the mucosa of the gut of healthy 35 days old chicken and they were identified by sugar fermentation test (Lan *et al.,*2003) and confirmed by gene sequencing of 16S RNA-23S RNA inter spacer region. DNA was isolated from the culture as per the procedure of Pospiech and Neumann (1995). The Polymerase Chain Reaction (PCR) on synthesized DNA was carried out according to Seal *et al.* (1995). The 16S RNA-23S RNA interspacer region of the DNA was analyzed using gel extraction kit (Bio basic) as per the protocol given in the kit. Then the 16S RNA -23S RNA inter spacer region gene sequencing was done (Eurofin Pvt Ltd, Bangalore) on the eluted DNA and the sequences were compared with nonredundant nucleotides in the GenBank database using BLAST.

'Probiotics score' was considered as yardstick to identify the best species specific probiotics among probiotics organisms. 'Probiotics score' for chicken, among the isolated *Lactobacilli* species, was formed by considering the acid tolerance at pH 2, bile tolerance at 0.3 % bile acid in the MRS medium, and antioxidant ability of *Lactobacilli*.

The method of Khalil *et al.* (2007) was followed for acid tolerance test and bile tolerance test. Antioxidant activity was calculated by the reaction to thiobarbituric acid according to the methodology of Ohkawa *et al.* (1979). The experiments were conducted in triplicate.

The probiotics score was measured by assessing the cumulative points generated by each species for acid tolerance, bile tolerance at third hour of incubation and antioxidant ability of *Lactobacilli*. The cumulative points

Table 1. Sugar fermentation pattern of *Lactobacillus* species from the isolates of the chicken gut

Sugars	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Lactose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Salicin	-	-	-	-	+	+
Arabinose	-	-	-	-	-	-
Sorbitol	+$_{w*}$	+	+	+$_{w}$	-	-
Xylose	-	-	-	-	-	-
Maltose	+	+	+	+	+	+
Melibiose	-	+	+	+	+	+
Mannose	+	+	+	+	+	+
Rhamnose	-	+	+	+	+	+
Raffinose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Trehalose	-	-	-	-	+	+
Mannitol	-	-	-	-	+	+
Amygdalin	+	+	+	+	+	+
Esculin	-	-	-	-	+	+
Gluconate	-	-	-	-	-	-
Melezitose	-	-	-	-	-	-

*+$_{w \text{ Weak reaction}}$ + positive reaction – negative reaction

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were calculated by summing up the per cent value of individual species for each test and this per cent value for each test was calculated by dividing the result of the respective test for individual species by total of results obtained for all the species for that particular test and then multiplied by 100. Whichever organism resulted in highest cumulative points was considered to have highest probiotics score and hence the best species specific probiotics (SSP).

The identified best species specific probiotics was preserved through freeze drying until further use by following the procedure adopted to freeze dry species specific probiotics (Pascaul *et al.,* 1999). The species specific probiotics in powder form in the vial was sealed with hand operated aluminium capper and stored at 4ºC until use. The freeze dried product containing species specific probiotics was evaluated for viable cell count by pour plate technique prior to usage.

Sugar fermentation test (Lan *et al.,* 2003) with 1 % peptone water was performed to identify the species of the isolated pure culture of the lactic acid bacteria. The experiment was conducted in triplicate. The results of sugar fermentation test were compared with Bergey's table (Kandler and Weiss, 1986) to identify the *Lactobacilli* species.

To assess the effect of *L. salivarius* on broiler chicken fed diet enriched with ω-3 fatty acids, a feeding trial was conducted for 35 days. The feeding trial had four experimental groups including control group (T_1) (without *L. salivarius*) and three treatment groups with 10^6cfu/kg feed (T_2), 10^9cfu/kg feed (T_3) and 10^{12}cfu/kg feed (T_4) of *L. salivarius*. The composition of broiler starter (0-21 days) diet is shown in Table 4. Similarly, ingredient composition of broiler finisher (22-35 days) diet is shown in Table 5. The experimental diet was formulated as per BIS (2007) specifications.

In the *in vivo* trial, 120 numbers of day-old broiler chicks (COBB-400) belonging to a single hatch were purchased from a commercial farm. Three replicates of 10 birds were maintained for each treatment. The management practices adopted were as per the standards and were uniform for all the treatment groups. The birds were fed *ad libitum* quantity of their respective experimental diet in separate feed troughs. Clean drinking water was provided *ad libitum*. The birds were weighed individually once in a week in a calibrated balance to document their weight gains. The body weight was gender corrected. The feed conversion ratio of individual birds was calculated at 35 days by dividing the total feed consumed (kg) per bird with its respective

Table 2. Acid tolerance test, bile tolerance test and antioxidant capacity of *Lactobacillus* species (Mean[*] ± SE)

TEST	HOURS	*Lactobacillus salivarius*		*Lactobacillus agilis*	
		Pre- freeze drying	Post- freeze drying	Pre- freeze drying	Post- freeze drying
Acid tolerance Test	1st	0.155[b]±0.002	0.154[b]±0.001	0.132[a]±0.001	0.130[a]±0.002
(Optical density of	2nd	0.156[b]±0.003	0.156[b]±0.004	0.135[a]±0.002	0.132[a]±0.012
MRS medium at	3rd	0.186[b]±0.004	0.186[b]±0.013	0.152[a]±0.002	0.150[a]±0.011
hourly interval)	4th	0.193[b]±0.006	0.191[b]±0.004	0.164[a]±0.001	0.157[a]±0.001
Bile tolerance test	1st	0.212[b]±0.011	0.202[b]±0.001	0.201[a]±0.002	0.171[a]±0.004
(Optical density of	2nd	0.226[b]±0.003	0.212[b]±0.014	0.101[a]±0.002	0.101[a]±0.011
MRS medium at	3rd	0.188[b]±0.004	0.153[b]±0.006	0.100[a]±0.012	0.094[a]±0.014
hourly interval)	4th	0.152[b]±0.007	0.126[b]±0.012	0.089[a]±0.002	0.071[a]±0.006
Antioxidant activity Malondialdehyde concentration (nmol/ml)		0.352[a]±0.03	0.453[a]±0.03	0.410[b]±0.04	0.501[b]±0.04

[a,b]Means with different superscripts in a column differ (P≤0.05) [*]Mean of 3 observations

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Table 3. Percent viability and concentration of *L. salivarius* before and after lyophilization

Characteristics	Concentrations of *L. salivarius* (cfu)/ml		
	10^6	10^9	10^{12}
Viability before lyophilisation (cfu/ml)	100×10^6	32×10^9	8.3×10^{12}
Viability after lyophilisation (cfu/ml)	50×10^6	17.33×10^9	4.33×10^{12}
% Viability	50.00	51.00	50.00
Product yield after lyophilisation (g/ml)	0.67	0.60	0.50
Concentration of *L. salivarius* in lyophilized powder (cfu/g)	75×10^6	29×10^9	9×10^{12}

cumulative body weight gain (kg).

The pH, water holding capacity (WHC), tyrosine value and thiobarbituric acid conent of breast meat sample from 6 birds randomly selected from each treatment were done on the day of slaughter, as well as on 1st, 3rd and 5th day after slaughter. The pH of the meat sample was measured using a digital pH meter (Digisun Electronic System, Model: 2001) by following the procedure of Trout *et al.* (1992). The water holding capacity of the meat was calculated by following the protocol outlined by Grau and Hamm (1957). The Tyrosine value of the meat was calculated by a modified method of Pearson (1968) as described by Strange *et al.* (1977). The Thiobarbituric acid content of the meat sample was calculated by a method of Strange *et al.,* (1977). Shear force value of meat sample was recorded by using Warner Bratzler Shear Press as per Bratzler (1954). At the time of slaughter, 1 cm

Table 4. Ingredient composition (g/kg) of broiler starter (0-21 days) diet

Ingredients	Treatment groups			
	T_1 (Control)	T_2 (10^6cfu/kg feed)	T_3 (10^9cfu/kg feed)	T_4 (10^{12} cfu/kg feed)
Maize	531.5	531.5	531.5	531.5
Soya bean meal	390.0	390.0	390.0	390.0
Palm oil	30.0	30.0	30.0	30.0
Linseed oil	5.0	5.0	5.0	5.0
Sardine oil	5.0	5.0	5.0	5.0
Dicalcium phosphate	18.0	18.0	18.0	18.0
Calcite	11.5	11.5	11.5	11.5
Vitamin-Mineral mixture*	2.0	2.0	2.0	2.0
L-Lysine	0.3	0.3	0.3	0.3
DL-Methionine	1.7	1.7	1.7	1.7
Common Salt	5.0	5.0	5.0	5.0
Calculated Values				
ME (kcal/kg)	3102	3102	3102	3102
Crude protein (%)	22	22	22	22
Lysine (%)	1.2	1.2	1.2	1.2
Methionine (%)	0.5	0.5	0.5	0.5

*Provided per kilogram of diet: Vitamin A 10000 IU, Vitamin D_3 3000 IU, Vitamin E 40 IU, Vitamin K_3 1.5 mg Vitamin B_{12} 0.01 mg, Biotin 0.15 mg, Choline 500 mg, Folic acid 1 mg, Niacin 40 mg, Pantothenic acid 15 mg, Pyridoxine 5.5 mg, Riboflavin 6.5 mg, Thiamine 2.5 mg, Copper 12 mg, Iodine 1.6 mg, Iron 80 mg, Manganese 100 mg, Zinc 80 mg.

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thickness breast muscle was collected from 30 broiler carcasses with 2 birds selected randomly from each replicate contributing 6 birds per treatment and kept at – 20°C till measurement. Sensory evaluation was assessed by subjecting six meat samples from each treatment to a sensory scores of colour, odour and overall acceptability by trained and semi trained panel drawn from the Department of Livestock Products Technology (Meat Science), Madras Veterinary College, on a 9-point Hedonic scale of a standard score card. Data obtained were subjected to analysis of variance (ANOVA) using the package of SPSS (version 17.0) When significant differences (P<0.05) was detected, multiple range test was used to separate the mean values (Duncan, 1955).

RESULTS AND DISCUSSION

The results of *in vitro* sugar fermentation test to identify *Lactobacillus* species from the isolates derived from chicken gut are presented in Table 1.The results
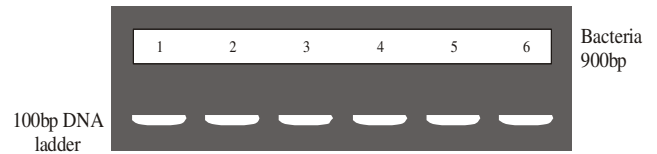
Plate 1. Agarose gel electrophoresis of the isolates obtained from the chicken gut

revealed that sorbitol was not fermented by isolates 5 and 6 alone. Further, trehalose was fermented by isolates 5 and 6 alone similarly isolates 5 and 6 fermented mannitol. These fermentation features of isolates 5 and 6 differentiated them from isolates 1,2,3 and 4. The isolates were subjected to 16S-23S RNA interspacer region and further confirmed at *Lactobacillus* species level by sequencing. The isolates 1,2,3 and 4 were identified as *L. salivarius* and isolates 5 and 6 were *L. agilis*. The PCR products were observed in 1.5 % agarose gel electrophoresis and presented in plate 1.

The gene sequence obtained from the isolates were then analysed with GenBank sequences using

Table 5. Ingredient composition (g/kg) of broiler finisher (22-35 days) diet

Ingredients	Treatment groups			
	T_1 (Control)	T_2 (10^6cfu/kg feed)	T_3 (10^9cfu/kg feed)	T_4 (10^{12} cfu/kg feed)
Maize	580.5	580.5	580.5	580.5
Soya bean meal	331.7	331.7	331.7	331.7
Palm oil	38.0	38.0	38.0	38.0
Linseed oil	7.0	7.0	7.0	7.0
Sardine oil	5.0	5.0	5.0	5.0
Dicalcium phosphate	18.0	18.0	18.0	18.0
Calcite	11.5	11.5	11.5	11.5
Vitamin-mineral mixture*	2.0	2.0	2.0	2.0
L-Lysine	0	0	0	0
DL-Methionine	1.3	1.3	1.3	1.3
Common Salt	5.0	5.0	5.0	5.0
Calculated Values				
ME (kcal/kg)	3208	3208	3208	3208
Crude protein (%)	20	20	20	20
Lysine (%)	1.0	1.0	1.0	1.0
Methionine (%)	0.45	0.45	0.45	0.45

*Provided per kilogram of diet: Vitamin A 10000 IU, Vitamin D_3 3000 IU, Vitamin E 40 IU, Vitamin K_3 1.5 mg Vitamin B_{12} 0.01 mg, Biotin 0.15 mg, Choline 500 mg, Folic acid 1 mg, Niacin 40 mg, Pantothenic acid 15 mg, Pyridoxine 5.5 mg, Riboflavin 6.5 mg, Thiamine 2.5 mg, Copper 12 mg, Iodine 1.6 mg, Iron 80 mg, Manganese 100 mg, Zinc 80 mg.

BLAST to check for homology. Based on sequencing data, *L. salivarius* and *L. agilis* were identified. The BLAST analysis revealed 99 % homology with respective reference strain available in GenBank.

The results of the acid tolerance test, bile tolerance test and antioxidant activity of the *Lactobacilli* is presented in the Table 2. *L. salivarius* exhibited highest (P<0.05) acid tolerance and bile tolerance and significantly (P<0.05) lower malondi-aldehyde concentration when compared to the *L. agilis,* both pre-freezing and post- freezing. The *Lactobacillus* species, *L. salivarius* showed maximum probiotic score of 170.95, when compared to *L. agilis* with probiotic score of 129.14. Hence *L. salivarius* was chosen as the best probiotic and freeze dried to be used for the *in vivo* feeding trial. Gunasekaran (2008) showed that out of four species of Lactobacillus obtained from the chicken gut (*L. salivarius*, *L. acidophilus*, *L. crispatus*, *L. fermentum*). *L. salivarius* with 93.4/300 was having the maximum probiotic score. Ever since, Mitsuoka (1969) isolated *L. acidophilus*, *L. fermentum* and *L. salivarius* from the chicken gastrointestinal tract, many workers identified *L. salivarius*, *L. acidophilus* and *L. fermentum* as predominating *Lactobacilli* species in the chicken gut.

Probiotics specificity differs from species to species because of their biotypes (Mitsuoka, 1969). If probiotic bacteria of the species are exchanged to another species, they do not colonize the gut mutually (Tannock *et al.,* 1982). Colonization of the gut is best achieved if the probiotic organism is derived from the same species of animal (Gibson and Fuller, 2000). Hence, in this study, probiotic bacterial isolates were derived from the gut of broiler chicken to evolve species specific probiotics.

Data on the viability percentage of freeze dried *L. salivarius* before and after freeze drying, for different inclusion levels to be used for *in vivo* feeding trial are presented in Table 3. After freeze drying the *L. salivarius* was evaluated for viability percentage which was found to be 50 to 51 %. Palmfeldt and Hahn-Hagerdal (2000) showed that *Lactobacillus reuteri* cultivated at pH 6 showed viability of 50 % irrespective of harvest time once it entered the stationary phase.

The viability percentage of a freeze dried bacteria depends upon many factors including the protectant used as well as pH. Palmfeldt and Hahn-Hagerdal (2000) showed that at pH 5 the culture of *L. reuteri* showed 80 % survivability at 2.5 hours after entering the stationary phase. Zayed and Roos (2004) showed that when trehalose and sucrose were added in addition to skim milk it gave a survival rate of 83–85 % immediately after freeze-drying, and enhanced stability during subsequent storage. Jalali *et al.* (2012) showed that maximum survival rate of 72-76 % for *L. paracasei* subsp. *tolerance* and *L. delbrueckii* subsp. *bulgaricus* with media containing 6 % skim milk, 8 %

Table 6. Effect of *L. salivarius*on weekly weight gain and cumulative FCR in chicken fed ω- 3 enriched diets (Mean* ± SE)

Treatment (ω-3 : ω-6) 1 : 3+ *L. salivarius*(cfu/ kg feed)	Weekly weight gain (g) [NS]					Cumulative	
	Week 1	Week 2	Week 3	Week 4	Week 5	WG	FCR[NS]
T_1 (Control)	108.70±0.88	302.9±1.8	350.6[a]±1.02	402.3[a]±1.12	421[a]±2.10	1585.5[a]±6.1	1.874[b]±0.02
T_2 (*L. salivarius* 10^6)	110.70±0.9	302.6±2.02	351.6[a]±2.07	401.4[a]±1.03	424.9[a]±2.13	1591.2[a]±7.3	1.864[b]±0.04
T_3 (*L. salivarius* 10^9)	113.2±1.5	304.2±1.9	354.5[a]±1.01	405[a]±1.05	427.2[a]±1.19	1604.1[a]±5.7	1.844[b]±0.05
T_4 (*L. salivarius* 10^{12})	115.2±1.2	309.1±2.08	379.2[b]±1.09	458.8[b]±1.19	511.2[b]±1.87	1773.5[b]±5.8	1.748[a]±0.11

*Mean of 30 observations; [a, b] means with different superscripts in a column differ (P≤0.05); NS – Not significant (P>0.05); WG - weight gain; FCR - feed conversion ratio

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trehalose and 4 % sodium ascorbate.

The freeze dried *L. salivarius* had count of 75×10^6cfu/g, 29×10^9cfu/g and 9×10^{12}/g as assessed by viable cell count by pour plate technique. In order to obtain 10^6, 10^9 and 10^{12}cfu/kg feed in broiler feed, the freeze dried product were included at the rate of 0.013g, 0.034g and 0.11g/kg feed, respectively. Gunasekaran (2008) produced freeze dried *L. salivarius* with 10^{13}cfu/g.

Data pertaining to the effect of *L. salivarius* on weekly weight gain and feed conversion ratio is presented in Table 6. The treatment T_4 with *L. salivarius* inclusion at 10^{12} cfu/kg feed had significantly (P<0.05) higher weekly weight gain at third, fourth and fifth week (379.2 ± 1.09, 458.8 ± 1.19 and 511.2 ± 1.87 g, respectively) and had significant (P<0.05) improved feed efficiency (1.748± 0.11). The T_4 group also showed significant (P<0.05) increase in cumulative weight gain

(1773.5 ± 5.8). The improvements in body weight and feed to gain ratio of broilers fed *Lactobacillus* supplement were probably due to the *Lactobacillus* spp. used in the supplement. It has been suggested that to obtain the best effects from *Lactobacillus* as a growth promotant, the bacteria used must be able to survive the gastrointestinal tract so that their beneficial functions could be performed (Jin *et al.,* 1996). The *Lactobacillus* spp. used in the present study are resistant to the bile and acidic conditions as shown by the results of acid tolerance test and bile tolerance test. This may be the reason for their positive influence on the weight gain and feed conversion ratio. Peng *et al.*(2016) showed that when the basal diet was mixed with *L. plantarum* at the dose rate of 2×10^9cfu/kg the average daily weight gain and feed conversion ratio improved (P<0.05) in broiler chicken.

Data pertaining to the effect of *L. salivarius* on

Table 7. Effect of *L. salivarius* on the fatty acid (FA) concentrations (g/100 g total FA) of broiler chicken breast meat (Mean* ± SE)

Fatty acid	Dietary concentration of *L. salivarius* (cfu/kg feed)			
	T_1 (Control)	T_2 (10^6)	T_3 (10^9)	T_4 (10^{12})
Myristic acid (C14:0) [NS]	0.60±0.03	0.56±0.07	0.60±0.04	0.47±0.16
Palmitic acid (C16:0)	23.95[a]±0.27	23.47[a]±0.43	25.51[b]±0.35	23.77[a]±0.51
Stearic acid (C18:0) [NS]	12.01±0.37	12.12±0.78	11.30±0.40	12.05±0.23
Arachidic acid (C20:0)	0.6[a]±0.03	0.69[ab]±0.06	0.77[b]±0.06	0.67[ab]±0.05
Behenic acid (C22:0) [NS]	6.99±0.44	7.35±0.42	6.28±1.1	7.92±0.46
Oleic acid (C18:1 ω -9) [NS]	23.66±1.06	24.88±1.06	23.94±0.42	23.57±0.87
Palmitoleic acid (C16:1 ω -7)[NS]	1.05±0.22	1.46±0.26	1.09±0.07	1.03±0.18
Linoleic acid (C18:2 ω -6) [NS]	14.92±0.73	16.05±0.25	15.56±0.33	14.84±0.39
Linolenic acid (C18:3 ω -3)	0.60[b]±0.1	0.50[ab]±0.05	0.51[ab]±0.03	0.38[a]±0.04
EPA (C20:5 ω -3) [NS]	1.43±0.05	1.50±0.11	1.36±0.06	1.52±0.08
DHA (C22:6 ω -3) [NS]	4.91±0.16	4.33±0.16	4.7±0.15	4.91±0.20
Others	9.26[b]±0.61	7.10[a]±0.68	8.38[ab]±0.18	8.87[b]±0.35
SFA [NS]	43.15±1.01	43.19±0.81	44.46±0.48	4.88±0.54
MUFA	24.71[a]±0.83	26.34[b]±0.72	25.03[ab]±0.61	24.6[a]±0.72
ω 3	6.96[b]±0.19	6.32[a]±0.13	6.40[a]±0.13	6.8[ab]±0.15
ω 6 [NS]	14.92±0.73	16.05±0.25	15.73±0.33	14.84±0.39
PUFA [NS]	21.88±0.14	22.37±0.44	22.13±0.53	21.64±0.13
ω -3 to ω -6 ratio	1:2.1	1:2.56	1:2.44	1:2.17

*Mean of 6 observations ;[a,b,c,d]means with different superscripts in a column differ (P≤0.05) ; NS – Not significant (P>0.05)

the fatty acid profile of broiler chicken breast meat is presented in Table 7. There was no significant (P>0.05) difference in linoleic acid, EPA, DHA and ω-6 fatty acids content among the groups. The T_1 groups showed significantly (P<0.05) higher level of linolenic acid and ω-3 FA (fatty acids) (0.60 ± 0.1 and 6.96 ± 0.99g/100 g of total FA, respectively). The short chain fatty acids did not differ significantly among treatments which ranged from 43.15±1.01 to 44.88± 0.54 g/100 g of total FA.

The T_2 group showed significantly (P<0.05) higher MUFA percentage (26.34 g/100 g of total FA) as compared to other groups. Present study did not result

in any significant increase in EPA and DHA level among groups, however, *L. salivarius* supplementation at 10^{12}cfu resulted in numerical increase in EPA level (1.52 g/100 g of total FA) as compared to the control (1.43 g/100 g of total FA) which is 6.29 % more than that of control. Similarly, dose rate of *L. salivarius* did not influence the EPA and DHA level among *L. salivarius* supplemented treatments.

There was also no significant difference in ω-3 to ω-6 ratio between T_1 and T_4. The ω-3 to ω-6 ratio was significantly increased at L. *salivarius* supplementation at 10^6 and 10^9 cfu/kg feed when compared to control and *L. salivarius* supplementation

Table 8. Effect of *L.salivarius* on the meat quality parameters of broiler chicken fed diet enriched with ω 3 fatty acids (Mean* ± SE)

Meat quality parameters	Days	Treatments			
		T_1	T_2	T_3	T_4
pH	0[NS]	6.15±0.03	6.15±0.02	6.12±0.01	6.15±0.02
	1[NS]	5.78±0.02	5.77±0.01	5.78±0.01	5.77±0.01
	3[NS]	6.08±0.01	6.08±0.01	6.07±0.01	6.07±0.01
	5[NS]	6.18±0.01	6.18±0.02	6.17±0.01	6.17±0.01
WHC %	0[NS]	66.62±1.9	66.67±1.4	67.26±2.4	67.24±1.8
	1[NS]	62.89±1.0	62.14±1.1	62.88±2.1	62.61±1.4
	3[NS]	60.57±2.9	60.63±1.4	59.92±2.5	59.95±2.8
	5[NS]	58.18±1.5	58.81±1.2	57.78±1.1	57.86±1.1
Tyrosine value(mg/ 100 g meat)	0[NS]	13.49±1.04	13.33±0.95	12.88±1.02	13.57±1.02
	1[NS]	16.49±0.59	16.31±0.89	16.34±1.11	16.52±0.71
	3[NS]	18.27±0.85	18.46±1.17	17.98±0.95	18.04±0.84
	5[NS]	19.16±0.44	19.47±0.55	18.85±0.22	19.13±0.62
Thiobarbituric acid (Malonaldehyde,mg/kg)	0[NS]	0.051±0.001	0.050±0.001	0.048±0.001	0.047±0.001
	1[NS]	0.070±0.001	0.068±0.003	0.069±0.003	0.070±0.003
	3[NS]	0.109±0.002	0.109±0.003	0.109±0.002	0.107±0.002
	5[NS]	0.127±0.002	0.127±0.00	0.126±0.003	0.127±0.003
Shear force value (kg/cm²)[NS]		0.91±0.07	0.91±0.03	0.92±0.05	0.91±0.04
Sensory Characters	Appearance[NS]	6.60±0.37	7.00±0.21	7.33±0.23	7.33±0.45
	Flavour[NS]	6.00±0.61	7.00±0.21	7.00±0.26	7.33±0.37
	Juiciness[NS]	5.83±0.62	7.17±0.31	6.67±0.37	6.83±0.61
	Tenderness[NS]	6.33±0.54	7.33±0.33	7.33±0.37	7.17±0.60
	Overall acceptability[NS]	6.67±0.86	6.67±0.56	7.00±0.60	7.00±0.31

*Mean of 6 observations; NS – Not significant (P>0.05)

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at 10^{12}cfu/kg.

The *L. salivarius* supplementation showed non-significant difference (P>0.05) in the pH, WHC, tyrosine value, thiobarbituric acid value, shear force value and sensory characterstics of chicken meat between the treatments as shown in Table 8. Yang and Chen (1993) reported that pH value was 6.23 and increased by extended storage which is similar to the present study. Meng *et al.* (2010) observed increase in pH values in growing pigs when fed with combination of *Bacillus* and *Clostridium* in diets with high energy and crude protein content. Si *et al.* (2006) suggested that the diet is one of the main factors that modified the antimicrobial effect *in vitro*. They suggested that the different percentage of crude protein and energy levels will alter the influence of probiotics on meat quality by influencing their actions in GI tract.

CONCLUSION

Supplementation of *Lactobacillus salivarius* at the dose rate of 10^{12}cfu/kg to ω-3 fatty acids enriched diet will produce broiler chicken meat with ω-3: ω-6 ratio of 1:2.17, and increase weight gain and feed conversion ratio in broiler chicken.

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SHORT COMMUNICATION

Nutritional and Therapeutic Management of Ruminal Dysfunction in Dairy Cows Affected with Trypanosomosis

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ABSTRACT

It is well established that trypanosomosis as well as trypanocidal drugs elicit their adverse effects on rumen microflora and fauna, leading to severe depression of appetite in ruminants. Despite the fact, a very less attention has been paid to restore rumen functions using nutritional and therapeutic interventions during its treatment under field conditions. This report considered two dairy cows affected with *Trypanosoma evansi* that were treated by trypanocidal drug and exhibiting anorexia for more than five days. The supportive therapy included supplementing probiotics plus rumen stabilizing/conditioning agents (*i.e.*, ruminotorics and stomachics), which normalized the appetite by day three post therapy and the cows recovered uneventfully. Thus, the current findings reinforce to consider nutritional interventions to restore ruminal health when treating cows suffering from trypanosomosis under field conditions.

Key words: Anorexia, Cattle, Feed additives, Nutritional therapy, Trypanosomosis

The tick-borne hemoprotozoan parasites- namely *Trypanosoma* spp., *Babesia* spp. and *Theileria* spp.-cause a significant economic loss to livestock industry every year in India by adversely affecting health and productivity, besides causing mortality (Uilernberg, 1995; Maharana *et al.,* 2016; Parashar and Singla, 2019). Particularly, in trypanosomosis caused by *T. evansi,* the course of treatment is expensive and the prognosis is not always favourable, primarily due to the stage of disease and host immune competence. On top of it, treatment with trypanocidal drugs further exacerbate the ruminal microbial balance leading to depression of appetite (anorexia). If not addressed at proper time, the rumen microflora and fauna could be severely affected, resulting in complete anorexia and subsequently death (Radostits *et al.,* 2009; Rathore *et al.,* 2016; Senapati *et al.,* 2018). Hence, it becomes imperative to restore rumen functions as soon as possible by additional supportive therapy through a nutritional approach.

In Mangarajpur village of Odisha, the cattle population was predominated by local indigenous breeds and there was no reported incidence of hemo-parasitic diseases. However, recently in 2018, two dairy entrepreneurs started rearing crossbred cows. On seeing the remunerative economics of crossbred cows, villagers were inspired and purchased some cows from a village in Jagatsinghpur district with known incidence of hemo-parasitic disease. These cows showed the symptoms of trypanosomosis. After treatment with typanocidal drugs and supplementary regimen two of the cows became anorectic. In this field study, the supportive therapy protocol particularly involving nutritional supplements in dairy cows affected with trypanosomosis to reverse the ruminal dysfunctions was attempted.

Outbreaks of hemoparasitic diseases particularly trypanosomosis and theileriasis were reported in the village among crossbred cows along with two reported deaths. After treatment by the local veterinarian, some animals recovered, whereas two became anorexic with a characteristic rumen hypomotility. When presented, both the animals were diagnosed with trypanosomosis, appeared emaciated and treated with diminazene

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aceturate (1st case) or suramin (2nd case) along with fluid therapy, rumen buffer (Rumen FS Powder™) and supplementation of mineral-vitamin mixture. The animals showed recovery with near normal body temperature. However, the first animal showed severe depression in appetite and remained anorexic for 12 days. The feces had foul smell and turned brownish black to blackish with watery consistency after 11th day of developing anorexia. Furthermore, the animal could not drink more than 2-3 l of water per day. Although to a lesser extent than first, the second animal too exhibited off-feed condition for five days. Both the animals were supplemented with feed supplements, Floratone Forte™ (Sodium bicarbonate 660 mg, magnesium trisilicate 1000 mg, vitamin B$_1$ 145 mg, nicotinamide 165 mg, methionine 40 mg, copper sulphate 0.44 mg, ginger powder 44 mg, gentian powder 220 mg, dried yeast 700 mg, dextrose 500 mg, cobalt sulphate 0.88 mg, sodium phosphate 100 mg) at one bolus per day for three days along with Ecotas™ (*Saccharomyces cerevisiae* 25×10^9 CFU, *Lactobacillus* sp. 25×10^6 CFU, *Aspergillus oryzae* 25×10^6 CFU, fructo-oligosaccharide 250 mg, biotin 10 mg DL-methionine 1 g, zinc sulphate 200 mg, cobalt sulphate 40 mg, copper sulphate 100 mg) at two boluses daily for six days. Himalayan Batisa™ (10 g) and powdered jaggery (20 g) acted as vehicle to administer boluses orally. Furthermore, to stabilize the general body functions, improve immunity and reduce stress level, Restobal™ (a herbal performance enhancer and stress modulator) was drenched at the rate of 100 ml twice daily for two days followed by 100 ml once on the following day.

Mouth was washed with baking soda thrice daily along with rubbing with tamarind (*Tamarindus indica*) pulp and/or potassium permanganate solution on the gum. In addition, the feed supplements, K-Zyme™ (each 100 g containing cellulase 20.2 mg, xylanase 116 mg, pectinase 48.5 mg, phytase 50 mg, cobalt 50 mg, copper 200 mg, iron 100 mg, zinc 200 mg), was added at 30 g daily from day two post therapy along with 20 g common salt (NaCl) dissolved in the rice gruel and offered regularly for 10 days. As ticks were found in the shed, which act as vectors to transmit *Trypanosoma* hemoprotozoa, cypermethrin 100 EC was sprayed to the cracks and crevices of the shed. The animal was fed on locally available bamboo (*Bambusa* sp.) leaves when it gained appetite on the second day. Therapeutic evaluation was based on the estimation of hemoglobin (Hb), differential leukocyte count (DLC), prevailing anorexia and the presence of *T. evansi* in peripheral blood which were conducted at Animal Disease Research Institute, Nakhara, along with fecal consistency, color and odor by personal observation on daily basis. The values obtained from healthy cow examined for trypanosomosis and when found negative for the parasites was considered as control to compare hematological values.

On manual examination, the swollen prescapular lymph nodes became clearly palpable along with rough and dehydrated skin. Table 1 presents the data on hematological parameters of both the cows at 15-days prior to the start of the study and 7-days post therapy. The results revealed improvement with respect to Hb and DLC by 7 days post therapy with the aforesaid medication.

The first cow gained appetite after 12 h of

Table 1. Effect of treatment on blood parameters of the two dairy animals affected with trypanosomosis

Parameter	Before treatment		7th day after treatment		Control
	Cow-1	Cow-2	Cow-1	Cow-2	
Trypansoma evansi	+ve	+ve	+ve	-ve	-ve
Hemoglobin (g %)	4	6	6	8	10.5
Neutrophils (%)	48	46	41	35	35
Lymphocytes (%)	41	42	49	55	53
Monocytes (%)	6	6	5	6	6
Basophils (%)	1	1	1	0	1
Eosinophils (%)	4	5	4	4	5

supplementation and started consuming bamboo leaves, voluntarily drank water as well as rice gruel. The colour of feces also changed 32 h post therapy and the odour tended towards normal, indicating restoration of ruminal and gastro-intestinal functions. The second cow also recovered from anorexia within one-day post therapy, started nibbling and within three days began consuming the normal diet.

T. evansi infection decreased Hb level, increased neutrophil concentration and decreased lymphocyte count as compared with non-infected healthy cow (Pandya *et al.,* 2016). As the blood parasite infection resulted in severe blood loss, when treated with trypanocidal drugs, the stress in the animal becomes aggravated, resulting in side effects such as ruminal dysfunction and anorexia (Radostits *et al.,* 2009).

As summarized by different reports, diminazene aceturate is the most extensively used curative trypanocidal agent against trypanoomosis followed by isometamidium chloride, suramin and quinapyramine (Maharana *et al.,* 2016; Singh and Maharana, 2018). The curative or prophylactic effect of these drugs is much effective against the parasite. However, these trypanocidal drugs cause several side effects including oxidative stress affecting ruminal fermentation as well as body functions in animals (Rathore *et al.,* 2016; Hussain *et al.,* 2018; Senapati *et al.,* 2018). The rationale behind addition of herbal anti-stress and immune booster was to stabilize the body by removing metabolic stressors, which otherwise increase the body stress load causing anorexia. Furthermore, the therapy also included some beneficial microbes (probiotics) to stimulate rumen fermentation, ruminal enzymes, which might have also enhanced microbial fermentation of fibre (Mahesh *et al.,* 2021). Other feed additives including appetizers were used with the objective to improve intake, stabilize the rumen ecology and improve ruminal fermentation. Both the cows responded well to the above nutritional and therapeutic regimen.

The current study reiterates the significance of optimal rumen health that is achieved through nutritional means along with other supportive therapy when using trypanocidal drugs in dairy cows. This cost-effective and safe, yet highly crucial approach to reinstating ruminal functions through a combination of feed additives, could save dairy cows from succumbing to trypanosomosis in tropical countries.

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