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December, 2019 **Vol. 36** **#4**

CONTENTS

Ruminants

Non-Ruminants

Indian J. Anim. Nutr. 2019. 36 (4): 329-333
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Indian Journal of
Animal Nutrition

Effect of Feeding Straw Based Densified Complete Feed Blocks Containing Dhanwantharam Oil Residue and Tapioca Starch Waste on Body Weight, Dry Matter Intake, Feed Conversion Efficiency and Nutrient Digestibility in Crossbred Heifer Calves

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ABSTRACT

The study was carried out to evaluate the effect of feeding straw based densified complete feed blocks (DCFB) containing dhanwantharam oil residue and tapioca starch waste (TSW) on body weight (BW), dry matter intake (DMI), feed conversion efficiency (FCR) and nutrient digestibility in crossbred heifer calves. Eighteen healthy crossbred calves were divided into three groups of six each as uniformly as possible with regard to age, sex and body weight and each animal was allotted randomly to one of the three dietary treatments: T_1 (straw based densified complete feed block (DCFB), T_2 (DCFB containing 20% dhanwantharam oil residue) and T_3 (DCFB containing 20%TSW). Feeding trial lasted for 90 days. A digestion trial of five days duration was conducted to assess nutrient digestibility. Average daily gain (ADG), DMI and FCR did not differ in crossbred calves of different groups. The apparent digestibility of nutrients also did not differ among the groups. The cost of feeding in group fed with DCFB containing dhanwanthram oil residue was significantly reduced than control group fed with conventional feed blocks. Thus, feeding of straw based DCFB containing dhanwantharam oil residue and TSW are beneficial in terms of feed cost.

Key words: Densified complete feed blocks, Dhanwantharam oil residue, Nutrient digestibility, Tapioca starch waste

INDRODUCTION

The availability of fibrous crop residue especially that of cereal crop and coarse straw increased to 44.26 million tonnes in 2011-12 (NIANP, 2013). This provides a great opportunity to the livestock farmers to use these as source of roughage to feed their livestock. The storage and transportation of crop residues are highly unprofitable because of its low bulk density which prevents the usage of these residues. Hence the straws are left in the field or burnt which may cause environmental pollution (FAO, 2012). Densification of straws into blocks with concentrates and other nutrients increased the bulk density and nutritional quality (Preston and Leng 1984). Densified complete feed block (DCFB) comprised of proper ratio of roughage and concentrates along with other supplements like vitamins and minerals and made into a block. DCFB provides an opportunity for incorporating deficient nutrients and unconventional feed such as agro-industrial and ayurvedic pharmaceutical industry byproducts. Ayurvedic pharmaceutical industry by-product dhanwanthram oil residue and agro industrial byproduct TSW were found to be useful in improving growth in crossbred calves (Purushothaman, 2018 ;Seethal, 2018). The work on the incorporation of agro-industrial and ayurvedic pharmaceutical industry by-product in DCFB are scarce; hence, the present study was taken to assess the effect of feeding straw based DCFB containing dhanwantharam oil residue and tapioca starch waste on the performance of crossbred heifer calves.

MATERIALS AND MATHODS

Three complete rations were formulated with 13-15 % crude protein (CP) and 60 % total digestible nutrients (TDN). Straw was chopped, the concentrate mixture was prepared and 5 % corn-steep liquor

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(mixture containing water soluble extracts of corn soaked in water) was added which acted as a binder for block making. Then the chaffed straw and corn-steep liquor added, concentrate mixture were mixed thoroughly using horizontal mixer for about 5-10 minutes for uniform mixing. Finally, this mixture is fed to the block making machine (Real Tech Engineers, Coimbatore) and compressed to form DCFB at a pressure of 6000 psi for 30 sec. The prepared blocks were stored and used for feeding of experimental calves.

Eighteen healthy crossbred heifer calves of eight to twelve months of age were selected from University Livestock Farm and Fodder Research Development Scheme (ULF & FRDS), Mannuthy, Kerala, India. The calves were housed individually in well ventilated, clean and dry shed with facilities for feeding and watering. The calves were allocated into three groups of six each as uniformly as possible with regard to age, sex and body weight and each animal was allotted randomly to one of the three dietary treatments. The experimental design used was randomised block design. All the calves were dewormed before the commencement of the study. All the experimental calves were fed with straw based DCFB containing 60 % TDN and 13-15 % CP. Three experimental rations were T_1 - straw based DCFB (as control) T_2 - DCFB incorporating 20 % dhanwantharam oil residue and T_3 - DCFB incorporating 20 % TSW. Nutrients requirement of experimental animals were met by feeding as per the ICAR feeding standards (ICAR, 2013). Ad libium clean fresh drinking water was offered to all the animals. All the animals were maintained under identical conditions of feeding and management throughout the experimental period of 90 days.

Body weight of the animals was recorded at fortnightly intervals during the trial. Weighed quantities of DCFB were offered daily to all the experimental animals in the morning at 0900 h and afternoon at 1400 h. Residue left was collected manually and weighed, twice a day for analysing the moisture content and estimating daily DMI. A digestibility trial for a period of five days was carried out at the end of feeding trial. During digestion trial quantities of daily feed offered, residue left and feces voided were recorded. DM content of the feed offered as well as residue left was determined daily. The dung voided by each animal was collected quantitatively. The entire quantity of dung voided by each animal during the 24 h were weighed separately at 0800 h every day and representative samples (@1% of the total quantity voided) were taken after mixing thoroughly and stored in double lined polythene bags. The samples collected each day were stored in deep freezer (-20ºC) for further analysis. At the end of the trial, samples of dung collected for the five consecutive days from each animal were pooled, mixed and representative samples were taken after through mixing for chemical analysis. The moisture and CP content in faecal samples was estimated. The proximate composition of feed, fodder and dung were analyzed as per the standard procedure (AOAC, 2016).

Data obtained on the various parameters during the course of the experiment were analyzed statistically as per Snedecor and Cochran (1994) by analysis of variance (ANOVA) technique, using the software of Statistical Programme for Social Sciences (SPSS) version 24.0.

RESULTS AND DISCUSSION

Summarized data on mean initial and final BW, total weight gain and ADG for the heifer calves maintained on three treatments are illustrated in Table 1. Statistical analysis of the data revealed that there was no significance variation ($P>0.05$) in the total weight gain and ADG of heifer calves fed on various treatments.

Patil *et al.* (2017) also observed no significance variation in the body weight of crossbred calves fed with complete feed block (CFB) without mahua seed cake and CFB containing 5 % mahua seed cake and the values were 177.73 and 181.32 kg, respectively. The results obtained in the present study are similar to above observations. However, Sharma *et al.* (2010) reported that total body weight gain and ADG was insignificant in the crossbred female calves fed with complete feed in mash form, conventional ration and complete feed block form. On disagreement to present results,

Table 1. Performance of heifer fed on three different complete feed blocks

Parameters	Group			P-value
	T_1	T_2	T_3	
Growth performance				
Initial BW (kg)	135.33±8.49	135.63±13.05	132.47±18.28	0.984
Final body weight (kg)	196.50±9.36	201.30±18.25	191.77±23.26	0.932^{ns}
Total weight gain (kg)	61.17±3.14	65.67±6.78	59.30±5.25	0.687^{ns}
ADG (kg)	0.68±0.03	0.73±0.08	0.66±0.06	0.687^{ns}
Feed intake				
Total DMI (kg/animal)	425.50±23.06	436.84±44.13	419.94±54.65	0.960^{ns}
Average daily DMI (kg/animal)	4.65±0.25	4.72±0.46	4.56±0.58	0.971^{ns}
Average DMI/ 100kg BW	2.94±0.004	2.94±0.007	2.95±0.021	0.833^{ns}
FCE	7.03±0.47	6.73±0.41	6.97±0.38	0.872^{ns}

Mean values are based on six replicates with SE, non significant at P>0.05

Kulathunga *et al.* (2015) detected an escalation of the live weight gain in crossbred heifers fed with CFB. Similarly, Somasiri *et al.* (2010) also found that lactating dairy cattle fed with gliricidia leaf meal block showed greater live weight gain.

Statistically, there was no significant variations (P>0.05) in daily DMI and DMI per kg metabolic body size of heifer calves maintained on the three treatment groups. The data on DMI per 100 kg body weight of heifer calves revealed that during third fortnight T_1 and T_2 showed significantly higher (P<0.01) DMI percentage as compared to T_3. During fourth and sixth fortnight. T_1 showed significantly higher (P<0.01) DMI percentage as compared to treatment 2 and treatment 3.

Similar to the results of present study, Purushothaman (2018) also reported that the DMI was similar in the crossbred cattle maintained on TMRs containing dhanwantharam thailam residue and rape seed cake and the values obtained were 6.17and 5.92 kg, respectively. Yadav *et al.* (1990) reported that the daily DMI percentage was highest in adult buffalo bulls fed with ground paddy straw based feed block as compared to animals fed with raw wheat straw and paddy straw based feed block. Khan *et al.* (2017) also observed that DMI percentage was significantly higher in adult male goats fed with DCFB with condensed tannins as compared to DCFB without condensed tannins. On disagreement to this, Das *et al.* (2004) and Singh *et al.* (2007) observed higher level of DMI in crossbred calves

Table 2. Digestibility coefficients of nutrients (%) in heifer calves fed different complete feed blocks

Parameter	Dietary treatments			P-value
	T_1	T_2	T_3	
Dry matter	55.39±2.39	56.28±2.71	57.93±2.12	0.758^{ns}
Crude protein	58.03±1.10	58.37±2.40	57.97±2.18	0.988^{ns}
Crude fibre	50.88±2.39	52.17±2.88	56.87±1.69	0.203^{ns}
Ether extract	74.26±1.59	77.42±1.41	73.13±2.08	0.221^{ns}
Nitrogen free extract	59.98±2.76	58.20±3.01	62.20±2.52	0.603^{ns}
Neutral detergent fibre	31.88±3.42	42.25±3.46	38.44±3.71	0.144^{ns}
Acid detergent fibre	34.18±2.64	37.33±2.94	35.02±3.08	0.732^{ns}

Mean values are based on six replicates with SE; ns- non significant at P>0.05

fed with CFB and an increase in the DMI was also reported by Kulathunga *et al.* (2015) in crossbred cows fed with DCFB.

The digestibilities of nutrients of complete feed blocks are presented in Table 3. Statistically there was no significant variation (P>0.05) in the digestibility of nutrients in DCFB fed to heifer calves maintained on different treatments. Das *et al.* (2004) reported that there were no significant differences in the digestibility of DM, CP, CF, EE, NFE and ADF in complete feed mash and CFB fed crossbred calves. Chacko (2015) observed a similar DM, OM, CP, CF, NFE and NDF digestibility for complete feed in the lactating dairy cows.

On contrary to present results, Lailer *et al.* (2010) reported that DM, CF and NFE digestibility were significantly higher in wheat straw based CFB and bajra straw based CFB fed to Murrah male calves fed with conventional feed. Dwivedi and Pathak (2011) reported that digestibility of DM, OM, EE, NFE, NDF, CP and total digestible nutrient were significantly greater in block fed male buffaloes as compared to mash fed animals.

The mean FCE (Table 2) of experimental heifer calves fed on three complete feed blocks T_1, T_2 and T_3 were 7.03, 6.73 and 6.97, respectively. Statistically, no significant difference was observed in the cumulative FCE of heifer calves maintained on various treatments. In accordance with present results, Sharma *et al.* (2010) reported that there was no significant difference in the FCE in crossbred calves fed with complete feed in mash (7.47), block (7.59) or conventional feed form (7.93). Similarly, Kulathunga *et al.* (2015) found that there was no significant difference in the feed conversion efficiency in heifers fed with CFB prepared from different agricultural wastes such as coconut poonac, wheat flour and hybrid Napier grass. On contrary to this, Kumar *et al.* (2015) found that FCE increased by 16% in buffalo calves fed with fungal zoospore containing CFB. The costs of CFB per kg were ₹ 17.25, 13.89 and 16.42 for T_1, T_2 and T_3 respectively. The lower cost of CFB observed in T_2 might be due to inclusion of ayurvedic pharmaceutical industry by-product dhanwantharm oil residue which was obtained at free of cost. In accordance with these results, Seethal (2018) also had reported that the cost of feed will decrease with increased supplementation level of dhanvanthram oil residue in the feed of calves.

CONCLUSIONS

The cost of feeding in group fed with DCFB containing dhanwantharam oil residue was significantly reduced than control group fed with conventional feed blocks and hence can be recommended as an alternate cost-effective feed.

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Nutrient Intake, Digestibility, Rumen Parameters and Blood Metabolites of Kacang Goats Fed Silage of Forage Mixture Produced from Intercropping of Sorghum Differing in Planting Space with Butterfly Pea (*Clitoria ternatea*)

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ABSTRACT

An experiment was conducted to investigate the effect of feeding silage of sorghum-butterfly pea mixture (which was made of forage harvested from sorghum sown at differing planting space and intercropped with *Clitoria ternatea*) on intake, digestibility, rumen environment and blood metabolites of Kacang goats. The experiment was designed following a replicated latin square with three treatments and three periods. In each period, two goats were randomly fed one of the three silage mixture which was made of clitoria and sorghum mixture harvested from sorghum planting space of 20×20 cm^2 (S_{20}), 40×40 cm^2 (S_{40}) and 60×60 cm^2 (S_{60}). Data on feed intake, digestibility, ruminal pH, ammonia (NH_3) and volatile fatty acid (VFA) concentrations as well as blood glucose and total plasma protein were collected during the last week of each period which lasted for three weeks. Results showed that crude protein (CP) content were increased with increasing sorghum planting space as the proportion of clitoria in the forage mixture increased. Meanwhile, crude fiber (CF) were comparable among different silage. Dry matter intake (DMI) and digestibility were not different ($P>0.05$) in Kacang goats fed different silage. However, rumen NH_3 and VFA concentrations were significantly ($P<0.05$) lower in S_{20} compared to S_{40} and S_{60} groups. It is concluded that in order to produce good quality silage for Kacang goats there is no benefit of increasing sorghum planting space beyond 40×40 cm^2 when intercropped with *Clitoria ternatea*.

Key words: *Clitoria ternatea,* Intercropping, Kacang Goats, Silage, Sorghum

INTRODUCTION

There has been a growing interest of farmer in The Province of Nusa Tenggara Timur, Indonesia to utilize Sorghum (*Sorghum bicolor* L) fodder as ruminant feed to cope with feed scarcity during dry season. Compared to maize, sorghum is more drought tolerant capability and fodder production is also higher due to forage addition from second and third cut (Nullik, 2009). It is also palatable for ruminants when fed as fresh, hay or silage (Gupta *et al.,* 2000). Sorghum is commonly harvested at early generative stage and given to animals either fresh or in ensiled form. The quality of sorghum silage, however, is only sufficient for moderate level of daily gain. CP content of sorghum silage varies from 5.2% to 7.4% (Mahanta and Pachauri, 2005) with digestibility of 55-62% when harvested at milk stage (Owen, 1967). For higher level of animal production, it is therefore necessary to improve silage CP content by mixing with high-protein forages such as a variety of forage legumes during the ensiling process. Alternatively, sorghum can be sown inter-cropped with forage legumes, hence, it will produce a certain mixture of forage with higher quality of silage. In addition, forage legumes may provide additional soil nitrogen which improves forage production (Willey, 1990) and often nutritive quality of the companion plants (Mureithi and Thorpe, 1993). One of those forage legumes which have been reported to successfully thrive and producing appreciable amount of forage in intercropping system with grass, maize and sorghum in semi-arid area is butterfly pea (*Clitoria ternatea).* Mureithi and Thorpe (1993) reported that *C. ternatea* was the better legume to Napier grass inter-croppping compared to *Macroptilium atropupureus* and *Stylosanthes guyanensin* in coastal lowland of Kenya. This legume forage is also found to be well adapted to

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the local environment (Nullik, 2009; Jelantik *et al.,* 2015).

To obtain the expected silage quality from a clitoria-sorghum inter-cropping system, it is required to know the appropriate plant density for at least one of the species in the plant mixture. Different plant density obtained by planting certain forage species at certain planting space will determine the ratio of clitoria and sorghum biomass produced as it involves competition among the system component for light as well as for water and nutrients (Ali *et al.,* 2013). This further affects silage nutritive value which consequently influences nutrient intake, digestibility and the silage utilisation by goats. Scientific evidence on the effect of feeding silage made from forage mixture harvested from sorghum-clitoria intercropping system to meat type goats in the tropics is limited. Therefore, this study was designed to investigate the intake and nutrient utilization by Kacang goats fed silage made from sorghum clitoria inter-cropping system where sorghum was planted at different space.

MATERIALS AND METHODS

Eighteen 2×2 m^2 plots were prepared in vertisol soil during March 2018 and 1 kg cows manure compost were applied during land preparation. Clitoria seeds (*Clitoria ternatea* cv Millagra) were sown at planting space of 20×20 cm^2 in April 2018. Seeds were directly broadcasted and immediately watered. Two weeks after clitoria was sown and already germinating, sorghum was planted at different planting space, *i.e.* 20×20 cm^2, 40×40 cm^2 and 60×60 cm^2, respectively at six plots for each planting space randomly. Nitrogen fertilizer was applied at 120 g/plot or 300 kg/ha 20 days after planting. All plots were watered every 2 days and weeds were removed every two weeks.

Forages were harvested at early flowering stage *i.e.* at 60 days after planting. The harvested forage mixture was then wilted for 6 hours and thereafter chopped at 2-4 cm length before put in a plastic mini silo (50 kg each) and ensiled for four weeks. No additive was added during the ensiling process.

Six mature Kacang goats weighing 11.54 ± 1.15 kg were used in this experiment following a replicated 3×3 latin square design with three treatments and three periods of 21 days each. The animals were randomly allotted to one of the three experimental feeds, *i.e.* silage of forage produced form clitoria-sorghum inter-crops with different sorghum planting space: 20×20 cm (S_{20}), 40×40 cm (S_{40}) and 60×60 cm (S_{60}). All animals were also offered concentrate mixture containing ground maize, rice bran, coconut cake, coconut oil and premix. The nutrient content of concentrate mixture was 11.19% CP, 19.95% CF, 5.4% ether extract (EE), 57.74% nitrogen free extract (NFE) and 17.58 MJ gross energy (GE)/kg DM. Experimental goats had *ad libitum* access to clean water.

As much as 50 g of silage were sampled from every silo and about 10 g was sub-sampled for DM determination. The rest were sun-dried and pooled for each silage type every period. Silage was offered *ad libitum* to the animals twice a day at 0800 and 1600 h as soon as the concentrate mixture was completely consumed. Residues were collected before morning feeding, weighed and sampled for DM determination.

A digestion trial of five-day total fecal collection was conducted for determination of apparent digestibility of DM and nutrients at the last week of each period. Feces was collected before morning feeding. The collected feces were weighed and about 10% was sampled. Each fecal sample was sub-sampled for DM determination and the rest was sun-dried. The sun-dried fecal samples were then pooled at the end of the collection period for each animal and stored till further chemical analyses. Apparent dry matter digestibility was determined by the difference between dry matter or nutrients offered and dry matter or nutrients recovered in feces. Urine was also collected at the same period. The collected urine was weighed and sample was taken and frozen thereafter.

Rumen liquid was taken 2-4 hours after morning feeding by using a stomach tube from each animals at the last day of each period. The collected rumen liquid was strained with layers of nylon cloth followed by pH determination and thereafter quickly acidified using concentrated sulfuric acid to a pH below 3.0. The samples were then stored frozen. At the end of the experiment, the frozen rumen liquids were thawed

before analyses for ammonia and VFA concentration.

Blood samples were taken from jugular vein of each animals quickly after rumen fluid collection. Blood was collected using a venojeck connected to a vacuum glass bottle containing disodium EDTA as anticoagulant. Blood samples were then centrifuged at 3000 rpm for 15 minutes. Blood plasma was taken and stored frozen until analyses for glucose and total plasma protein.

Dry matter determination was conducted by drying in the ventilated oven at 105°C until constant weight. Proximate analyses were conducted following methodology of AOAC (2005). Ash content was determined by ashing feeds and fecal matter in a furnace at 550°C. CP content was determined by using Kjeldahl method and CF was determined after acid and alkali extractions. NH_3-N concentration was analyzed by micro-diffusion technique (Conway, 1957). Total VFA concentration was determined by steam distillation technique.

All data were analyzed using Proc. GLM (SPSS version 21). Significant difference between means was assigned at $P<0.05$.

RESULTS AND DISCUSSION

Biomass proportion of sorghum and clitoria as well as nutrient content of silage made from forage which was produced from a clitoria-sorghum inter-crops with different sorghum planting space is presented in Table 1. As expected, the biomass proportion of *C. ternatea* in the total fresh forage mix increased with higher sorghum planting space while sorghum biomass was reduced. Clitoria is known as a fast growing climbing forage legume (Gomez and Kalamani, 2003) that has been intercropped with maize (Jelantik *et al.,* 2016), grass (Mureithi and Thorpe, 1993; Ali *et al.,* 2013) and sorghum (Ayoub, 1985) with varying success. The biomass production of clitoria declines with narrower planting space of the companion plant due to the strong negative effect of shade on plant growth (Minson and Wilson, 1994). Ali *et al.* (2013) found that clitoria biomass production was suppressed when *C. ternatea* was grown intercropped with *Setaria splendida*. Similarly, Ayoub (1985) reported that biomass production of clitoria was reduced by 42% when grown inter-cropped with sorghum compared to monoculture. Some results, however, indicates that *C. ternatea* is one of shade-tolerant forage legumes (Congdon and Addison, 2003; Nicodemo *et al.,* 2015) with negligible effect on its biomass production when intercropped with dense planting space. Jelantik *et al.* (2016) reported that when harvested at 60 days the total biomass production was not reduced when intercropped with maize at different planting space, i.e 40×40 cm compared to 180×40 cm.

Silage CP content in the present experiment increased with increasing sorghum planting space due to increasing proportion of clitoria in the silage. Clitoria contains higher CP compared to sorghum. Ratnawati *et*

Table 1. Forage production and nutrient content of silage used in the experiment

Item	Sorghum planting space		
	S_{20}	S_{40}	S_{60}
Forage biomass proportion before ensiled, kg DM/kg			
Sorghum	0.652	0.590	0.462
Clitoria	0.348	0.410	0.538
Nutrient content (% DM basis)			
OM	80.76	80,06	79,73
CP	16.83	19,22	18,36
CF	31.51	30,54	31,21
EE	3.44	2,70	5,75
Nitrogen NFE	28.98	27,80	24,41
GE (kcal)	2.75	2,71	2,84

al. (2013) reported CP content varying 17.85-18.73% in Clitoria harvested at 120 days after planting. Hartutik *et al.* (2012) reported that CP content of *C. ternatea* grown monoculture and harvested at 90 days after planting was 18.38%. Sorghum harvested at 50 days contained 9.3% CP and declined to 5.1 % at 100 day after sowing (Saini, 2012). Ayub *et al.* (2004) also reported that CP content of green sorghum fodder was 9.29%. Depending on the ratio of the mixture, CP content of the mixture varied from 13.23% to 16.16 % in rice bean dominating forage mixture (*Ayub et al.,* 2004).

Unlike CP content, the silage CF content in the present experiment was comparable among different type of silage despite higher proportion of sorghum in the forage mixture with increasing planting space. Similar result was reported by Ayub *et al.* (2004) who found that CF content of sorghum rice-bean mixture varied between 30 to 34%, even when a larger variation in CF content was noted for rice-bean and sorghum grown alone, *i.e.* 29.5-36.5%, respectively. This was possibly due to the increasing sorghum fiber content with increasing plant density while that of clitoria remain unchanged. Aydemir and Kizilsimsek (2018) found that NDF and ADF of sorghum were increased in lower plant density but this did not occur with soybean when they were grown as intercrops.

Nutrient intake and digestibility of silage of forage mixture produced from sorghum-clitoria inter-crops with different sorghum planting space in Kacang goats is presented in Table 2. DMI varied narrowly between 35.3 to 38.2 g $LW^{0.75}$ day^{-1} or about 1.9-2.0% LW of Kacang goats weighing 10.06 to 13.45 kg. This level of DMI was lower than that reported earlier in goats consuming sorghum silage. Barros *et al.* (1986) reported DMI of goats consuming sorghum and clitoria silage were 65.9 and 77.2 g/kg $LW^{0.75}$, respectively. This low level of intake was unexpected

Table 2. Nutrient intake and digestibility in goats consuming sorghum-*C. ternatea* silage

Parameter	Treatments			SEM	P-value
	S_{20}	S_{40}	S_{60}		
Dry matter intake (g/day)					
DMI (% of BW)	2.068	1.915	2.018	0.112	0.634
DMI (g/kg $BW^{0.75}$)	38.12	35.30	37.18	2.132	0.651
Nutrient intake (g/day)					
OM	219.77	202.99	214.85	13.541	0.680
CP	40.98	40.95	41.82	2.34	0.981
CF	47.26	44.29	45.73	3.229	0.814
EE	13.70	10.27	14.75	1.091	0.046
NFE	106.74	96.44	101.63	6.65	0.572
GE (MJ/day)	37.40	33.81	36.83	2.357	0.277
Digestibility (%)					
DM	66.06	68.90	62.98	2.528	0.307
OM	68.42	70.69	64.79	2.360	0.262
CP	71.82	76.46	72.02	1.78	0.180
CF	35.73	46.60	35.53	4.596	0.211
EE	79.03a	70.89b	69.82b	2.127	0.025
NFE	75.84	74.14	69.58	2.741	0.156
Energy	73.87	74.84	70.27	1.960	0.277

Means within the same rows followed by a different alphabet differ significantly (P<0.05)

considering the diets offered to the animals were relatively of good quality. All silage used in the present experiment contained high crude protein, *i.e.* much higher than 6.2% at which the intake of ruminant animals started to fall (Minson, 1990). Potchoiba (1990) observed DMI between 3.6 to 3.9% BW in goats fed good quality feeds high in CP content.

There are a number of factors that may cause the low silage intake by Kacang goats in the present experiment. High fiber content in sorghum-based silage is often reported to limit intake. Ngongoni *et al.* (2008) explained the observed lower intake of sorghum based silage compared to maize-based silage as sorghum silage contained higher NDF as compared to maize silage. Nichols *et al.* (1998) found that DMI of dairy cows were reduced with increasing NDF content of sorghum silage. Cooke *et al.* (2008) also found high correlation between DMI and forage NDF content. Legumes often do not ensile well due to moisture content and high buffering capacity which can cause high effluent loss and unstable silage of high pH and such silage has low palatability (Titterton, 1997).

Nevertheless, the increasing DMI with increasing sorghum planting space observed in this experiment was expected due to the increasing proportion of clitoria biomass in the silage. As discussed before, the increase of clitoria in the silage improved protein content of the silage by 14.2%. With this increase, it would be expected that DMI is increased. Negesse *et al.* (2001) reported a 36% increase of DMI when dietary CP level was increased from 8% to 15.5%. Similarly, Jia *et al.* (1995) found increasing DMI with increasing dietary CP level. In addition, *C. ternatea* was reported to have higher intake compared to forage sorghum at any stage of maturity (Kawas *et al.,* 1985). The results of the present experiment, however, showed that DMI and nutrient intakes were not different (P>0.05) between goats offered different types of silage. This finding is contrary to other research finding where legumes were included during sorghum ensiling. Ngongoni *et al.* (2008) reported that the inclusion of 40% lablab (*Lab lab purpureus*) in sorghum silage increased DMI in sheep. One explanation is that dietary CP content of the control diet was optimum that provided NH_3 and/or amino acid or peptida for microbial population and activities, thus further increase in CP content of the diet did not result in any further improvement in intake. This phenomenon was for example reported by Negesse *et al.* (2001) who observed a significant increase of intake of Saanen kids when dietary CP was improved from 80 to 105 g/kg but no further increase was noted at higher dietary CP content. In our experiment, dietary CP content was above that level. Previously, Lu and Potchoiba (1990) observed a sharp 35.0% increase in DMI when dietary CP was increased from 11.2 to 12.7% compared to only 9.0% increase when dietary CP was increased from 12.7 to 15.1%.

Since the level of intake in this experiment was low in all treatments, the difference of CP content of silage used in the present experiment was expected to have more profound effect on the DM digestibility.

Table 3. Rumen and blood attributes in goats consuming Sorgum-*C. ternatea* silage

Parameters	Treatment			SEM	P-value
	S_{20}	S_{40}	S_{60}		
Rumen parameters					
pH	6.97	6.72	7.02	0.18	0.467
VFA (mmol/l)	104.18[a]	114.58[b]	121.81[b]	2.15	0.001
NH_3 (mmol/l)	10.13[a]	12.21[b]	11.07[ab]	0.39	0.017
Blood metabolites					
Total plasma protein (g/dl)	6.02	6.38	6.32	0.28	0.63
Glucose (mmol/dl)	115.29	113.18	112.86	3.789	0.835

Means within the same rows followed by a different alphabet differ significantly (P<0.05)

Results showed that the digestibility of nutrients did not differ (P>0.05) among treatments. This finding appears to corroborate well with some previous reports which indicate that *C. ternatea* had reasonably high digestibility due to its high CP content (Nasrullah *et al.,* 2003). Hartutik *et al.* (2012) reported *in vitro* DM digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of 65.95 and 66.28% for *C. ternatea* harvested at early flowering stage. Other researchers reported that IVDMD of *C. ternatea* varied between 59.9% (Ratnawati *et al.,* 2013) to 74.15% (Nasrullah *et al.,* 2003). Those recorded level of IVDMD was higher than that of sorghum. Hence, when there was an increase of biomass proportion of clitoria in the silage with higher sorghum planting space, it was rather expected that DM digestibility would be higher in S_{40} and S_{60} compared to S_{20}. The absence of increased digestibility with increasing clitoria proportion in the silage mixture may be caused by many different factors. One of these factors is the fermentative profile of the silage. Although CP content of silage with higher clitoria proportion due to higher sorghum planting space is higher, but most of the protein is degraded with improper ensiling process which ends up with high NH_3-containing silage which has lower digestibility (Nussio *et al.,* 2002). The other factor is that the content of water-soluble carbohydrates in sorghum was higher than in clitoria.

Rumen pH in this experiment varied between 6.72 and 7.02. This level is considered as favourable pH range which provides suitable environment for optimum ruminal fibre degradation (Van Soest, 1994). Similarly, NH_3 concentration in this experiment were well above the threshold level of 50 to 80 mg/l for optimal microbial growth and activities as suggested by Satter and Slyter (1974). Rumen pH did not differ (P>0.05) between goats consuming different type of silage, however, rumen liquid concentration of NH_3 and VFA were significantly lower (P<0.05) in S_{20}, *i.e.* in goats receiving silage made of forage with narrower sorghum planting space. Silage made from higher sorghum planting space (S_{40}) and (S_{60}) were comparable. Rumen pH is the balance between the acid production rate and the buffering capacity from saliva and to a lesser extent from weak base NH_3 produced in the rumen. It was therefore expected that when there was an increase VFA concentration in S_{40} and S_{60}, rumen pH would decline. However, since at the same time NH_3 concentration was also increased, it neutralized the effect.

Ruminal NH_3 concentration depends on CP content and its rate of degradation in the rumen. In this experiment, CP content of S_{60} was higher than S_{20}. However, the more determinant factors for ruminal NH_3 concentration is the extent of protein degradation in the rumen. In poor ensiling conditions, much of the protein is degraded into peptide, amino acids, NH_3 and amines. When consumed by the animals, these end products of ensilage will be quickly converted to NH_3 in the rumen which therefore gives rise of rumen NH_3 concentration. This probably explains for the increase of NH_3 concentration in goats consuming S_{60}.

Rumen VFA concentration was lower (P<0.05) in goats consuming silage made of a mixture of forage produced from clitoria-sorghum inter-cropping with narrow sorghum planting space compared to sparser planting space. But rumen VFA concentration was comparable between S_{40} and S_{60}. The increase indicated that there was a higher extent and rate of fermentation of OM in the rumen in S_{40} and S_{60} compared to S_{20}.

Nevertheless, rumen concentrations of both NH_3 and VFA were within the normal range reported earlier. Mc Donald *et al.* (2010) reported that rumen VFA concentration in ruminant typically varying between 70-150 mmol/l. Suryani *et al.* (2014) reported that goats consuming various forage and concentrate had VFA concentration of 40.45 mmol/l. Similarly, Naswantara (2006) noted a level of 71.81 mmol/l in goats consuming rice straw supplemented with different protein and energy sources. This indicates that silage of sorghum-clitoria mix in this experiment has reasonably high fermentation rate.

Blood glucose and total plasma protein were not different (P>0.05) in goats consuming different type of silage. However, the level of blood glucose was under normal level which is stated to be 50-80 mg/dl (Kaneko,

1997). Manu *et al.* (2007) reported blood glucose concentration varying between 51.00 to 94.80 mg/dl for Kacang goats in Timor savanas, Indonesia. The increase of glucose concentration in blood plasma in this experiment did not occur despite the increase of VFA concentration in the rumen fluid. In ruminant, glucose absorbed from carbohydrate digestion in the small intestine is generally low, only 3-8% of glucose synthesized in sheep (Preston and Leng, 1986). Hence, ruminants rely on the gluconeogenesis mechanism from propionate and amino acids to maintain blood glucose level. Major part (50-60%) of precursor for gluconeogenesis comes from propionate (Preston and Leng, 1986).

Total plasma protein in the present experiment. varied narrowly between 6.02 to 6.38 g/dl and no treatment effect (P>0.05) was found. This level was within the normal range reported by Mitruka and Rawnsley (1981), between 4.5-7.2 g/dl. Thus, it is apparent that the animal tends to maintain plasma protein concentration in a normal homeostatic level (Swenson and Reece, 1993).

CONCLUSIONS

It is concluded that CP content of the silage increased with increasing sorghum planting space as the proportion of clitoria in the mixture was increasing. Nutrient intake and digestibility, however, are not improved with increasing planting space although rumen NH_3 and VFA concentration was improved. It is therefore inferred that there is no benefit of increasing sorghum planting space beyond 40×40 cm^2 when sown together with *C. ternatea* to produce good quality silage for Kacang goats.

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Effect of Supplementing *Syzygium cumini* (Jamun) Fruit Shreds to Total Mixed Ration on the Performance of Lactating Crossbred Cows

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ABSTRACT

This study was taken up to assess the effect of supplementing *Syzygium cumini* (Jamun) fruit shreds on nutrient utilization, enteric methane (CH_4) emission and performance of the lactating crossbred cows (CBCs). Twelve lactating CBCs were allocated into two groups (n=6) and were fed a total mixed ration (TMR) containing concentrate mixture, green Berseem fodder (*Trifolium alexandrium*) and wheat straw in 40: 42: 18 ratio on DM basis supplemented with or without *S. cumini* @ 2% for 180 days. A 14 days digestion trial was conducted by indicator method using chromic oxide green as an external marker, before the termination of feeding trial. The enteric CH_4 production was assessed by sulfur hexafluoride (SF_6) tracer technique. *S. Cumini* fruit shreds are rich source of phenolics (21.78%), antioxidants (4.95%) and saponins (4.35%). Supplementation of diet with *S. cumini* fruit shreds did not affect intake and digestibility of nutrients. The CH_4 emission was mitigated ($P<0.05$) by 29% in CBCs fed on TMR supplemented with *S. cumini* fruit shreds. Supplementation of diet with *S. cumini* fruit shreds did not show any adverse effect on milk yield. It was concluded that CH_4 emission can be mitigated in lactating CBCs fed TMR supplemented with *S. cumini* fruit shreds without any adverse effect on the performance of lactating CBCs.

Keywords: Lactating crossbred cows, Methane, Milk yield, Nutrient utilization, *Syzygium cumini* fruit shreds

INTRODUCTION

India has the largest population of livestock in the world. Amongst the various livestock species, cattle population in India is 190.90 million and it contributes to around 37.28% of the total livestock population in the country (Anonymous, 2012). Being the highest milk producing nation, India contributes to 18.5% milk produced in the world (GOI, 2016). Despite the production potential of livestock, it also contributes directly or indirectly to climate change through the emissions of greenhouse gases such as carbon dioxide, CH_4 and nitrous oxide. Amongst various GHGs, CH_4 emission from enteric fermentation in ruminants contributes up to 63% (Herrero *et al.,* 2009). The enteric CH_4 production can be reduced considerably by improving the efficiency of nutrient utilization and the energy lost as gaseous products of digestion can be diverted to improve the productivity of the animals.

Various strategies can be used to mitigate CH_4 production which includes: i) Dietary inclusion of feed additives like organic acids, prebiotics, nitrates and sulphates, ii) Biological means by using yeast culture, bacteriocins and by enhancing acetogenesis, iii) Other strategies include animal breeding and selection and modification of rumen microbes genetically (Ingale *et al.,* 2013). Number of studies have revealed that the *in vitro* CH_4 production can be reduced significantly by supplementing the ration with plant secondary metabolites (PSM) like essential oils (Hundal *et al.,* 2016a), tannins (Hundal *et al.,* 2016b) or with herbal feed additives (HFAs) containing saponins (Wadhwa *et al.,* 2018; Singh *et al.,* 2018a). Further, supplementing the ration with HFAs containing saponins improved performance of growing cattle (Singh *et al.,* 2018b). Management strategies like genetic selection of efficient feed utilizers or high producing animals along with manipulation of diet (Bakshi and Wadhwa, 2009) can help in mitigating CH_4 emissions which would further help in increasing the efficiency in which animals use nutrients to produce milk or meat.

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We hypothesized that supplementation of *S. cumini* in lactating CBCs would improve their performance by reducing enteric CH_4 production. Hence, this study was conducted to assess the effect of supplementing Syzygium cumini (Jamun) fruit shreds on nutrient utilization, enteric methane (CH4) emission and performance of the lactating crossbred cows.

MATERIALS AND METHODS

The present study was conducted in the Department of Animal Nutrition and Livestock Dairy Farm, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, India. All experimental protocols were approved (GADVASU/2018/IAEC/14/09) and compliant with the guidelines established by Institutional Animal Ethics Committee constituted (IAEC) under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

The *S. cumini* (Jamun) fruit shreds (JFS) were procured from the Unati Cooperative Marketing-cum-Processing Society Ltd. Talwara, Hoshiarpur, Punjab and screened in triplicate for phenolics (Makkar *et al.,* 1993), flavonoids (Balbaa *et al.,* 1974) and saponins (Baccou *et al.,* 1977).Twelve lactating CBCs were randomly divided into two groups of six animals each. In control group, cows were fed on basal TMR. In the experimental groups, the control TMR were supplemented with *S. cumini* fruit @ 2.0% of DM intake. Milk samples were collected at monthly interval. Impact of HFAs on the nutrient digestion were assessed by conducting a 14 days digestion trial by using chromic oxide green as an external marker @ 0.2% of daily DM intake. Faecal samples were collected from the rectum by grab method. Spot urine samples were also collected individually and was preserved in glass bottles, which were kept in refrigerator till analysed for nitrogen content (AOAC, 2007). The amount of chromic oxide (mg %) in faeces was also calculated (Hill and Anderson, 1958). The faeces, urine and orts were weighed daily at 9.00 a.m. before feeding.

The dried and finely grounded feed, JFS, orts and faecal samples were analysed for DM, total ash, ether extract and nitrogen concentration (AOAC, 2000). Cell wall constituents were analysed by using methodology of Robertson *et al.* (1991). SF_6 trial was conducted in the 2nd and 5th month of feeding trial. CH_4 and SF_6 concentrations were determined by using gas chromatography. CH_4 emission rate was calculated as the product of the permeation tube emission rate and the ratio of CH_4 to SF_6 concentration in the sample. Samples were analyzed in duplicate. The gas chromatograph was fitted with a 3.3 m molecular sieve 5A column with inner diameter of 0.32 mm for SF_6 and a 1.5 m Porapak Q stainless steel column with inner diameter of 2 mm for CH_4. The injector, detector and oven temperature was maintained at 40, 220 and 50°C for SF_6 and 40, 100 and 52°C for CH_4 estimation, respectively. Nitrogen was used as a carrier gas, with flow rate of 30 ml/min both for SF_6 and CH_4 estimation. Prepared standards were used to standardize the gas chromatograph for SF_6 (39.2 ppt and 101.7 ppt) and CH_4 (10.4 ppm and 101.9 ppm, Scott-Marrin Inc., Riverside, CA, USA). Background CH_4 [(CH_4) b] was subtracted from CH_4 concentration in the canister [(CH_4) y]. The concentration of CH_4 was then calculated by using the equation of Johnson *et al.* (1994). Enteric CH_4 emission rate was calculated by using following equation:

$$QCH_4 = QSF_6 (CH_4) y - (CH_4) b / (SF_6)$$

Where, QCH_4 is the CH_4 emission rate (g/min), QSF_6 is the known release rate of SF_6 from permeation tube (g/min), (CH_4) b is the background CH_4, (CH_4) y is the CH_4 in sample in canister (g/m^3) and SF_6 is the SF_6 concentration of collected sample in canister (g/m^3).

Data were analyzed by using one way ANOVA and factorial design (Snedecor and Cochran 1994), by using SPSS (2009) version 16 and the significant differences in means were tested by Tukey's test.

Table 1. Chemical composition of TMR and *S. cumini* shreds (% DM basis)

Parameter	Ash	OM	CP	EE	NDF	ADF	Cellulose	ADL
TMR	7.60	92.40	14.82	3.10	47.20	27.00	10.85	5.30
S. cumini shreds	13.20	86.80	9.20	1.15	51.50	30.90	8.90	13.60

RESULTS AND DISCUSSION

The chemical composition of TMR and *S. cumini* shreds used during the experimental period is presented in Table 1. The active components present in *S. cumini* fruit shreds are presented in Table 2. Jamun seed contains various polyphenols such as ellagitannins, gallic acid, ellagic acid, *etc.*, and phytosterol such as β-sitosterol. Being rich in phytochemicals, it is a potential source of phenolics, flavonids, tannins *etc.*

Supplementation of *S. cumini* shreds in the diet did not affect the daily DM intake and digestibility of nutrients (Table 3). No information is available regarding the effect of Jamun supplementation on the performance of animals. Similar to the findings of the present study, supplementation of pomegranate peel extract (PPE) upto 800 ml/cow/day to dairy cows did not exert any effect on the intake of DM and apparent nutrients digestibility (Abarghuei *et al.,* 2013). Foiklang *et al.* (2016) studied the effect of grape pomace powder (GPP), mangosteen peel powder (MPP) and monensin on feed intake and nutrients digestibility in rumen fistulated steers. Results showed that GPP supplemented group had significantly higher (P<0.05) UTRS intake than those in other groups in terms of % BW and g/kg $BW^{0.75}$. However, total DM intake was not significantly different among treatments (P>0.05).

Effect of supplementation of diet with *S. cumini* shreds on enteric CH_4 emission was assessed by SF_6 tracer technique. The results of the present study revealed that CH_4 emission (g/d, g/DMI, g/kg DOMI) in *S. cumini* fruit shred supplemented group was decreased (P<0.05) by 27% (Table 4). The findings of the present study was in accordance with the results of Hundal *et al.* (2019), who observed that enteric CH_4 emission (g/kg DMI) was reduced (P<0.05) by 25.3% with supplementation of diet with PPE and by 27.7% when diet was supplemented with *T. undulata* extract in comparison to the animals fed control diet. CH_4 emission per liter of milk showed a decreasing trend but due to less number of observations, the effect was non-significant. The gross energy (GE) intake was same in animals fed control diet or diet supplemented with *S. cumini*. However, the loss of energy (MJ/d) from animals was less (P<0.05) by 27%, while loss of energy as percent of GE was less (P<0.05) by 28% in animals fed diet supplemented with *S. cumini*. CH_4 emission (g/d) was observed to be higher when assessed after 5 months of feeding, however, CH_4 emission per liter of milk remained same during the experiment period. GE intake and loss of energy (MJ/d) was observed to be higher when estimated at the end of the experiment period (5 month), however loss of CH_4 % GE remained similar at 1 or 5 month of feeding trial.

Supplementation of diet with *S. cumini* shreds did not affect the milk yield of CBCs, irrespective of duration of feeding (Table 5). However, Anantasook *et al.* (2014) found that supplementation with RPM and RPO to diets of cows increased milk yield.

CONCLUSIONS

From the results it can be concluded that supplementation of *S. cumini* fruit shred reduced enteric CH_4 emission without impairing nutrient utilization and performance of lactating CBCs.

Table 2. Active components in *S. cumini* shreds

Active component	% DM basis
Total phenolics	21.78
Leucocyanidin (CT)	0.32
Saponins	4.35
Vitamin C	2.65
DPPH	2.00
Flavonoids	0.30

Table 3. Effect of *S. cumini* fruit shred supplementation on feed intake and nutrient digestibility

Parameters	Group		SEM	P-Value
	Control	*S. cumini*		
Dry matter intake, kg/d	14.83	14.88	0.113	0.134
Digestibility of nutrients, %				
Dry matter	66.08	71.66	1.514	0.660
Organic matter	70.98	71.99	1.408	0.547
Crude protein	78.98	78.76	1.065	0.601
Ether extract	73.86	72.12	1.351	0.326
Neutral detergent fibre	63.92	64.73	1.763	0.691
Acid detergent fibre	56.59	58.19	2.095	0.625
Cellulose	64.96	66.19	1.700	0.543

Table 4. Effect of supplementing *S. cumini* fruit shred on enteric CH_4 production

Parameter	Group		SEM	P-Value	Period, months		SEM	P-Value
	Control	*S. cumini*			1	5		
CH_4, g/d	272.17^b	197.97^a	13.44	0.008	215.29^a	254.86^b	9.126	0.023
CH_4, g/kg DMI	21.21^b	15.54^a	0.473	0.002	18.88	17.88	0.853	0.324
CH_4,g/kg DOMI	32.68^b	23.68^a	0.727	0.003	28.95	27.41	1.314	0.324
CH_4,g/l milk	21.79	16.41	2.735	0.214	14.79	23.41	1.485	0.100
GE intake, MJ/d	208.33	209.66	19.305	0.950	185.54^a	232.45^b	6.597	0.006
Loss of energy, MJ/d	15.19^b	11.04^a	0.165	0.010	12.02^a	14.22^b	0.509	0.023
% Loss of GE	7.33^b	5.26^a	0.75	0.003	6.47	6.12	0.294	0.324

$^{a, b}$Mean with different superscripts in a row differ significantly (P<0.05)

Table 5. Effect of *S. cumini* fruit shred on milk yield

Parameters	Group		SEM	Period, month					SEM
	Control	*S. cumini*		1	2	3	4	5	
Milk yield, kg/d	14.04	14.09	0.18	14.16^c	12.97^b	16.22^e	15.41^d	11.58^a	0.28

^{a,b,c,d}Mean with different superscripts in a row differ significantly (P<0.01)

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Comparative Evaluation of Oat Hay and Silage Based Rations on Nutrient Utilization and Methane Emissions in Murrah Buffaloes

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ABSTRACT

In the present study oat hay and silage were compared for their methane (CH_4) production and nutrient utilization potential in Murrah buffaloes. Sixteen dry buffaloes (566.5± 12.5 kg live weight) were selected from Livestock Research Centre of the National Dairy Research Institute, Karnal. Animals were distributed randomly into two groups (T_1 and T_2) based on body weight. Animals in group T_1 and T_2 were offered oat hay and oat silage, respectively along with supplementation of mineral mixture and salt. After feeding for a duration of 60 days, a digestibility trial was conducted followed by methane emission measurement by SF_6 tracer technique. Average daily DM intake was similar in both the groups, whereas, fibre (NDF, ADF) intake was significantly ($P<0.05$) higher in hay fed group. Digestibility of nutrients (DM, OM, EE, NDF and ADF) except CP was not affected by hay or silage; CP digestibility was higher (60.59%) on silage feeding as compared to hay (58.28%) feeding. Feeding of hay resulted in more ($P<0.05$) enteric CH_4 emissions (341.35 l/d) than silage feeding (317.86 l/d). Total CH_4 production was reduced by 6.86% on oat silage than hay. Higher ($P<0.05$) percent of ME was lost in methane in group T_1 (16.41) as compared to T_2 (13.97). Thus, Oat could be stored as silage than hay for lowering methane emission from Murrah buffaloes.

Key words: Hay, Murrah buffaloes, Methane emission, Oat silage

INTRODUCTION

Scarcity of feed and fodder is the common problem faced by livestock owners of India. The problem is aggravated further by non-uniform availability of fodder throughout the year, as growing fodder availability is chiefly dependent upon the climate. There is surplus availability of green fodder during flush season, whereas, during extreme seasons majority of Indian farmers get less or even no green fodder. The excess fodder produced, stored either as hay or silage, fed to animals during scarcity period. The previous one is dehydrated product, whereas the latter one is an anaerobically fermented high moisture product. The above aspect may affect the rate of utilization of either of the stored forages in the rumen. The rate of utilization of the fodder can be best estimated from their intake level and nutrient digestibility. Utilizable crude protein (Zhao and Lebzien 2000) gives the idea of the protein entering the duodenum for digestion. Methane is a major greenhouse gas produced especially from fodder digestion in ruminant livestock which not only contribute to environmental pollution, but also results in substantial loss (2-12%) of gross energy intake which could have been otherwise utilized for productive purposes. Buffalo and cattle contribute about 39 and 53.1% of total livestock methane production in India (Singhal *et al.,* 2005). Considering the fact that environmental issues can no longer be ignored while designing feeding regimes for large ruminants, it was hypothesized that comparative nutritive value and methane mitigating potential would vary according to methods of conservation of forage. The specific objective of this experiment was to compare nutrient utilization and methane emissions in Murrah buffaloes fed either oat hay or silage diets.

MATERIAL AND METHODS

The fodders were evaluated for their chemical composition and *in vitro* utilizable crude protein (Zhao

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and Lebzien 2000) content before start of the animal experiment. The experiment was conducted in the experimental shed of the National Dairy Research Institute, Karnal, India (latitude 29.43°N & longitude 77.2°E). Environmental temperature during the study ranged from 27 to 31 °C, and humidity ranged from 16 to 18 %. All the experimental procedures were approved by Institutional Animal Ethics Committee of NDRI (IAEC/21/14 dated 04.01.2014).

Sixteen dry Murrah buffaloes (566.5 ± 12.5 kg body weight) were selected from Livestock Research Centre of National Dairy Research Institute, Karnal and distributed randomly into two groups based on body weight. Animals were housed in Dairy Cattle Nutrition experimental shed and given an acclimatization period of 10 days. Animals in group 1 were given hay and those of group 2 were fed silage for 60 days. In both the groups mineral mixture and salt were supplemented. A digestibility trial of 7 days duration was conducted to evaluate the nutrient digestibility. Methane emission was measured by SF_6 tracer technique (Johnson *et al.,* 1994). A permeation tube containing SF_6, standardised for steady release rate (0.0035 g/day) over a period of 6 weeks, was placed in the rumen 2 days prior to the experiment. The animals were fitted with halter with capillary tubing (SS Capillary tubing 0.0635" X 0.004") in such a way that the gas collection inlet was close to nose. The eructed gas from nose and mouth was collected in an evacuated canister. Five collections each of 24-h duration were made for each animal. Methane and SF_6 concentrations in the collected sample were quantified using a gas chromatograph (Nucon-5700) fitted with flame ionization and electron capture detectors (FID and ECD), respectively. The data obtained were subjected to the statistical analysis as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Detailed chemical composition of oat silage and oat hay is presented in Table1. A relatively higher content of CP was observed in oat silage (11.97%) as compared to oat hay (9.20. The NDF (56.17 vs 43.16%) and ADF (47.40 vs 32.35%) content in oat hay were higher than the oat silage.

Average daily dry matter intake (DMI kg/day)

Table 1. Chemical composition of oat hay and oat silage fed to buffaloes

Parameter	Oat hay	Oat silage
DM (%)	87.00±0.72	26.72±0.80
	% DM	
OM	91.10±0.15	90.79±0.12
CP	9.21±0.22	11.97±0.09
EE	1.82±0.02	1.97±0.01
NDF	56.17±0.41	47.40±0.52
ADF	43.16±0.27	32.35±0.31
Ash	8.90±0.19	9.21±0.29
NDICP	1.3±0.07	2.1±0.09
ADICP	0.6±0.03	1.0±0.06
Hemicellulose	13.02±0.69	15.05±0.43
Cellulose	40.53±0.23	41.11±0.31
ADL	8.29±0.65	6.01±0.39
TDN %	54.61±0.61	56.83±0.72
DE (MJ/kg)	10.07±0.03	10.48±0.12
ME (MJ/kg)	8.29±0.05	8.71±0.01
uCP	10.01±0.49	10.32±0.97

Table 2. Effect of feeding oat hay or oat silage on intake and digestibility of nutrients in dry buffaloes

Parameter	Oat hay group (T_1)	Oat silage group (T_2)
BW, kg	566±21.29	567±29.65
DM intake, kg	8.30±0.19	8.34±0.20
DMI intake, (kg/100kg BW)	1.46±0.01	1.47±0.01
DMI intake, $W^{0.75}$	71.07±0.66	71.55±0.67
CP intake, kg	0.82^{b}±0.04	1.06^{a}±0.07
CP intake, g/kg$W^{0.75}$	6.54^{b}±0.06	8.73^{a}±0.08
EE intake, kg/d	0.15±0.03	0.16±0.01
NDF intake, kg/d	4.66^{a}±0.04	3.95^{b}±0.21
ADF intake, kg/d	3.58^{a}±0.03	2.70^{b}±0.02
Digestibility coefficient (%)		
DM	68.87±2.19	69.09±1.76
OM	70.44±3.33	69.51±1.88
CP	58.28^{b}±1.78	60.59^{a}±2.72
EE	80.18±2.92	82.99±3.11
NDF	64.50±2.22	67.99±1.89
ADF	61.31±1.41	61.46±1.12

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

was similar (P>0.05) in both the groups (T_1,8.30 and T_2, 8.34 kg/d). Neutral detergent fibre and acid detergent fiber intake was significantly (P<0.05) higher in T_1 than T_2, while the CP intake was significantly (P<0.05) higher in T_2 than T_1 group. Wallsten *et al.* (2009) reported increasing intake of oat silage DM with increasing plant maturity when fed to heifers. The increase was from 1.6 kg/100 kg LW at early milk stage to 2.0 kg/100 kg LW at dough stage. In the present study silage DMI averaged 1.47 kg/100 kg BW for buffaloes.

Data pertaining to the nutrient digestibility of buffaloes fed on oat hay or silage are presented in Table 2. The CP digestibility (%) was significantly (P<0.05) higher in T_2 than T_1, whereas digestibility coefficients of all other nutrients were similar between the groups. The DM and CP digestibility coefficients were 68.87 and 69.09; 58.28 and 60.59% in T_1 and T_2, respectively. Silage protein usually serve as rumen degradable protein (RDP) as most of the proteins are broken down during fermentation to non-protein

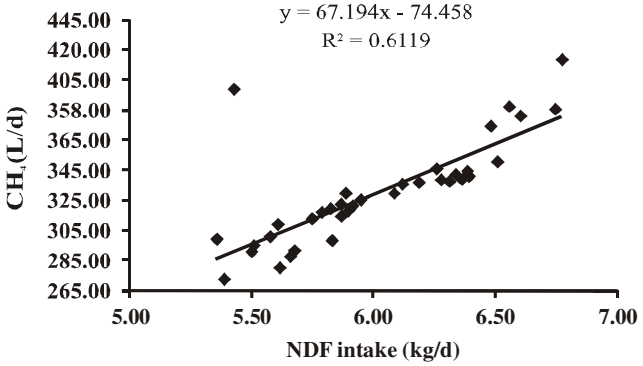

Fig 1. Relationship between neutral detergent fibre intake (NDFI) and methane emissions in buffaloes

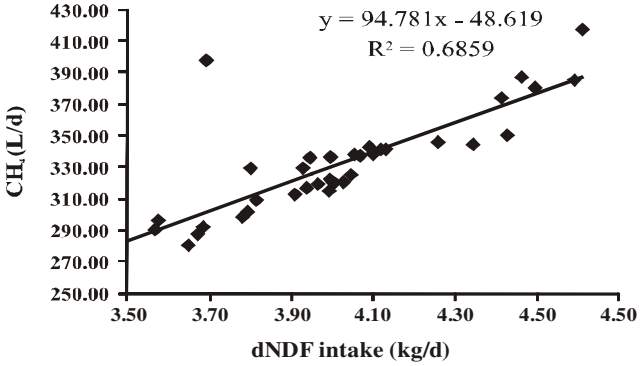

Fig 2. Relationship between digestible neutral detergent fibre intake (dNDFI) and methane emissions in buffaloes

nitrogen, like ammonia nitrogen, (Papadopoulos and Mckersie, 1983; Wilkinson, 2005). In the present study *In vitro* utilizable CP were found to be similar in both the groups.

The energy intake and loss of energy in the form of methane from buffaloes fed on oat hay or silage is presented in Table 3. The energy intake in terms of gross energy (GE) and digestible energy (DE) was similar between the groups. The overall methane production was significantly lower (P<0.05) in oat silage fed group than oat hay fed group. The higher methane emission (g/kg DM, MJ/kg DM and g/kg NDF intake) was in T_1 (24.36, 1.36 and 35.68) as compared to T_2 (21.79, 1.21 and 34.52). Percentage of DE and ME lost in methane was 13.51, 16.41% and 11.61, 13.97% in T_1 and T_2, respectively. The difference in energy loss was analysed to be significant (P<0.05). Methane loss (MJ/d) was 6.86% lower in dry buffaloes fed on oat silage instead of oat hay-based diets. Methane release in buffalo in relation to NDF intake and digestible NDF intake is depicted in fig. 1 and 2, respectively. Significant correlation was observed between methane emissions and NDF intake (R^2 = 0.61, P<0.05). Sundstol (1981) observed a reduction in loss of gross energy in the form of methane when forages were ensiled than when dried. This could be due to reduced rate of carbohydrate fermentation in the rumen with silage as extensive fermentation already occurred during ensiling. Similar findings to present study were reported on comparison of alfalfa hay and silage (Benchaar *et al.,* 2001). They used a mechanistic model approach to assess a 33% depression in total CH_4 production (Mcal/d) on alfalfa silage rather than alfalfa hay. Kirkpatrick and Steen (1999) observed no differences in gross energy loss (%) as methane when forages were conserved either as silage or by freezing directly after harvesting. Silage based diets when fed to Merino lambs recorded reduced loss of energy via methane, urine and faeces (Beukes, 2013). Martin *et al.* (2008) observed less methane production on DM intake basis on corn silage based diet as compared to hay diet in two successive experiments. Martin *et al.* (2010) reviewed methane mitigation strategies to uncover that methane production tend to be lowered when forages were ensiled than when they were dried. Many studies suggest that methane yield in cattle respond in a quadratic manner when pasture forage was substituted with maize silage (Blaxter and Wainman 1964; Arndt *et al.,* 2010). Blaxter and Clapperton (1965) found that both feed intake and digestibility were important factors affecting CH_4 yield

Table 3. Methane emissions and energy loss in buffaloes fed oat hay or oat silage

Parameter	Oat hay group (T_1)	Oat silage group (T_2)
DMI, kg/d	9.00±0.26	9.37±0.19
GEI, MJ/d	162.00±1.68	168.66±2.79
DEI, MJ/d	90.63±1.57	98.20±4.85
MEI, MJ/d	74.61^b±1.31	81.61^a±1.57
CH_4, l/d	341.35^a±3.44	317.86^b±6.28
CH_4, g/d	219.27^a±2.21	204.18^b±4.03
CH_4, g/kg DMI	24.36^a±0.88	21.79^b±0.82
CH_4, g/kg NDFI	35.68^a±0.22	34.52^b±0.37
CH_4, MJ/d	12.24^a±0.12	11.40^b±0.23
CH_4, MJ/kg DM	1.36^a±0.05	1.21^b±0.02
CH_4, MJ/kg DDM	1.77^a±0.13	1.65^b±0.03
CH_4 loss as %GE	7.56^a±0.05	6.76^b±0.08
CH_4 loss as % DE	13.51^a±0.07	11.61^b±0.14
CH_4 loss as %ME	16.41^a±0.10	13.97^b±0.19

^{a,b}Means bearing different superscripts in a row differ significantly (*P<0.05)

(% of GEI) and that at the maintenance level of feeding CH_4 yield increased with higher digestibility of the diet. McCaughey *et al*. (1997, 1999) reported that feed intake rather than digestibility was the major determinant of CH_4 production. In the present study, where buffaloes were fed close to maintenance, neither feed intake nor feed digestibility was related to CH_4 yield (% of GEI), whereas, CH_4 production (g/d) was directly related to NDF intake and digestible NDF intake. A similar relationship was found in small ruminants (sheep and alpaca) fed forages ad libitum indoors or grazed (Pinares-Patiño *et al.,* 2003). Moe and Tyrrell (1980) also advocated the concept of CH_4 production as a function of the amount of cell walls digested in the rumen. Singh *et al.* (2011) attributed the variation in CH_4 production from dry roughages to significant difference in the NDF, ADF, carbohydrate and protein fractions. Woodward *et al.* (2001) found CH_4 production of 26.9 and 35.1 g/kg DMI in lactating dairy cows fed on silages made from *Lotus corniculatus* and pasture, respectively.

CONCLUSIONS

Enteric CH_4 emissions (l/d) was significantly higher ($P<0.05$) in oat hay fed group (341.35) than oat silage fed group (317.86). Total CH_4 production was reduced by 6.86% in dry buffaloes fed on oat silage instead of oat hay, whilst nutrient intake and digestibility values remained unaltered. Thus, oat fodder could be better preserved as silage than hay for lowering methane emission from Murrah buffaloes.

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Effect of Depotash Vinasse on Rumen Fermentation Kinetics *in vitro*

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ABSTRACT

Vinasse is a by-product of distilleries which may be used in animal feeding otherwise it is a pollutant of water and soil. An *in vitro* experiment was conducted to observe the effect of molasses and depotash vinasse on rumen fermentation parameters such as *in vitro* dry matter digestibility (DMD), organic matter digestibility (OMD), ammonia nitrogen (NH_3-N), partition factor (PF) and microbial biomass production (MBP). Concentrate mixture, pellet with molasses (Pellet M) and pellet with depotash vinasse (pellet DPV) were prepared with same ingredients while pellet M contained 8% molasses and pellet DPV contained 8% depotash vinasse (as at this level binding was optimum) with little change of ingredients, chemical composition was similar. The substrate for the *in vitro* experiment comprised of concentrate mixture/ pellet M/, pellet DPV individually and TMRs with concentrate/ pellets, oat green and wheat straw in different proportions (40:40:20). The results revealed no noticeable changes due to addition of depotash vinasse and molasses pellets (upto 8%) on rumen fermentation parameters. DM, OM and crude protein (CP) digestibility was similar in all the groups. MBP and NH_3-N was also similar in all the groups. It was concluded that depotash vinasse can be used in pellet making and subsequent feeding to animals without any harmful effect on rumen fermentation parameters.

Keywords: Composition and *In vitro* digestibility, Depotash vinasse, Rumen fermentation

INTRODUCTION

Vinasse is a by-product from industrial production of yeasts, alcohol, citric acid or other substances by fermentation of molasses (Fernandez *et al.,* 2009). Vinasse is produced mostly from raw materials *viz*, corn, wheat, rice, potatoes, sugar beets, sugarcane and sweet sorghum. It is also called as condensed molasses solubles (CMS). It is a light-brown to dark brown liquid which contains water (93%), organic solids and minerals (7%) that is condensed to 60% (on wet basis) and is variable depending on source, batch and location *etc.* though, for production of each liter of ethanol, 12-15 liter of water is used which cannot be recycled (Christofoletti *et al.,* 2013). The vinasse have generally been disposed directly to the flowing water that affects water quality, subsequently aquatic life, thus, a threat to ecosystem. As an alternate to control the water pollution, significant proportion of vinasse have been disposed to agriculture land (Nataraj *et al.,* 2006). Vinasse has less energy, high ash content. It is especially rich in sulphur (S) and potassium (K), than molasses. Vinasse may be used in feeding of cattle and sheep because its crude protein consists mainly of non-protein nitrogen (NPN) compounds such as free amino acids and betaine. In cattle and poultry feed, vinasse may be used as binder and dust reducer rather than as a protein replacer. Use of vinasse beyond 5% level as a replacement for molasses in ruminant diet results reduced voluntary feed intake, weight gain and feed conversion efficiency (Campos *et al.,* 2014). Possible reason for poor performance of animal on vinasse containing diet is due to its high level of S. High S content in vinasse reduces selenium (Se) and copper (Cu) absorption and responsible for excess production of hydrogen sulphide (H_2S) in rumen. High K reduced magnesium (Mg) absorption which in turn leads to hypomagnesaemia. However, depotash vinasse, (after removal of maximum potassium) kind of new product with minerals, low CP with binding properties, though not exploited yet in India or elsewhere. Moreover, number of rumen protozoa and the amount of ruminal ammonia nitrogen in dairy cattle and sheep were also reported to be increased due to vinasse in diet (Sugoro *et al.,* 2016). Under these circumstances, depotash

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vinasse have been used for preparing feed pellet and total mixed ration and tested for *in vitro* rumen fermentation and nutrient digestibility.

MATERIALS AND METHODS

Samples of molasses were collected from Sagbara, Gujarat and depotash vinasse was collected from Demo plant at Kemrej, Gujarat. Physical characteristics of depotash vinasse and molasses were observed visually and pH by digital pH meter. The cattle feed pellets were manufactured at Nand Gopala Feeds, Sagbara, Gujarat. Ingredients used for pellets preparation were maize grain, mustard cake, ground nut cake, wheat bran, mineral mixture and salt. Molasses and depotash vinasse were added at the level of 8% in each formulation by little changing the composition (Table 1). Eight percent level of molasses and depotash vinasse was selected because at this level binding and pelleting were optimum. The chemical composition *viz.*, moisture, CP, total ash, acid insoluble ash (AIA) of depotash vinasse, molasses, concentrate mix and pellets were analyzed by following protocol of AOAC (2007) and mineral composition *viz.*, calcium (Ca), Mg, K, sodium (Na), Cu, cobalt (Co), zinc (Zn) and manganese (Mn) were analyzed by using Atomic Absorption Spectrophotometer (AAS; Hitachi Z-5000, Polarized Zeeman) (Table 2).

TMR was prepared by mixing of concentrate mixture, and pellets with green oats and wheat straw in 40:40:20 ratios. A total of 6 groups were there *i.e.* concentrate mixture, pellet with molasses, pellet with depotash vinasse individually and TMR with concentrate mixture: green oats: wheat straw (40:40:20), TMR with molasses containing pellet: green oats: wheat straw (40:40:20) and TMR with depotash vinasse containing pellet: green oats: wheat straw (40:40:20). Samples of TMRs in triplicate were used to observe the gas production and nutrient digestibility (Menke and Steingass, 1988).

The generated data were analyzed by one way ANOVA using IBM SPSS version 20 to observe the treatment effect on fermentation kinetics.

RESULTS AND DISCUSSION

Colour of the molasses was light brown while that of depotash vinasse was dark brown. Odour of the molasses was sweetish while it was mild sweetish for depotash vinasse. pH of the samples varied between 4.5-5.1. These parameters indicated that depotash vinasse was visually similar to the molasses. Chemical composition of depotash vinasse and molasses is depicted in Table 2. DM of depotash vinasse was around 76.36% and in molasses it was 60.85%. The OM varied from 85-90% in both the samples. The CP content was higher in depotash vinasse than molasses (7.70 *vs.* 5.36%). Total ash content was higher in depotash vinasse than molasses due to higher mineral content. Acid insoluble ash was 1.5 to 2.01% in both the samples. Calcium level was higher in DPV and low in the molasses varied from 0.4–0.8% in DPV and 0.2–0.4% in molasses. The concentration of potassium low in depotash vinasse as well as in molasses (3.3-3.9%). The concentration of sodium was higher in DPV, varied from 0.1–0.3% and low in molasses at

Table 1. Ingredient composition of concentrate mixture and pellets (% DM basis)

Ingredient	Concentrate mix	Molasses pellets	Vinasse pellets
Maize	40	35	35
Ground nut cake	10	12	12
Mustard oil cake	20	21	21
Wheat bran	27	21	21
Molasses	-	8	-
De-potash vinasse	-	-	8
Mineral mixture	2	2	2
Common salt	1	1	1

Table 2. Chemical composition of depotash vinasse and molasses (% DM basis or as mentioned)

Parameter	Depotash vinasse	Molasses
DM	76.36	60.85
CP	7.70	5.36
Total ash	16.49	8.66
AIA	2.01	1.52
S	2.05	0.67
Ca	1.20	0.33
Mg	2.80	0.19
K	1.80	3.26
Na	0.26	0.07
Cu (ppm)	45.26	35.08
Co (ppm)	19.21	17.00
Zn (ppm)	8.62	9.16
Mn (ppm)	55.51	35.21

around 0.1%. The concentration of zinc varied from 8.2–9.6 ppm in DPV and 9.6–10.4 ppm in molasses, whereas spent wash had high zinc concentration around 112 ppm. The iron concentration was high in DPV, varied from 92–585 ppm and low in molasses around 33–154 ppm. The value of manganese was 55 and 35 ppm which was higher in DPV followed by in molasses group, respectively. The finding of present study was

Table 3. Chemical Composition of concentrate mixture and pellets samples

Parameter	Concentrate	Pellet (M)*	Pellet (V)**
Chemical composition (% DM)			
DM	90.4±0.27	89.3±0.18	88.7±0.15
OM	89.5±0.18	90.6±0.11	89.5±0.05
CP	20.6±0.15	19.3±0.12	21.7±0.17
EE	4.47±0.19	4.83±0.02	4.75±0.05
NDF	24.1±0.17	26.7±0.05	25.7±0.09
ADF	12.1±0.25	13.7±0.05	12.3±0.05
CF	6.15±0.24	5.60±0.02	4.99±0.05
AIA	2.15±0.05	2.35±0.05	1.07±0.01
	10.5±0.16	9.40±0.03	10.6±0.05
Mineral composition (% or as mentioned)			
Ca	0.61±0.01	0.71±0.01	0.67±0.04
K	2.12±0.17	2.35±0.05	2.35±0.05
Na	1.84±0.24	0.99±0.02	1.04±0.02
Mg	0.81±4.42	0.84±6.12	0.79±6.22
Cu (ppm)	29.42±2.74	28.5±3.52	27.08±2.11
Zn (ppm)	42.1±8.32	41.55±1.54	45.3±1.22
Fe (ppm)	210.1±8.41	221.5±14.5	206.2±6.58
Mn (ppm)	69.5±2.52	66.3±1.45	63.55±2.09
Durability Index (%)	-	95.71%	96.74%

Table 4. Effect of molasses and depotash vinasse on fermentation kinetics

Parameter	Group						P-Value
	Concentrate	Pellets (M)	Pellets (V)	TMR Conc	TMR (M)	TMR (V)	
Net gas(ml/24h)	35.07±6.40	34.47±1.56	36.63±1.79	33.88±1.35	35.93±1.09	32.81±0.23	0.935
Gas (ml/kg DM digested)	175.35±32	172.34±7.79	183.13±8.94	169.41±6.77	179.67±5.47	164.04±1.16	0.935
IVDMD (%)	61.52±.66	62.19±2.0	62.32±0.64	62.89±2.0	62.79±2.4	60.65±0.60	0.907
TOMD (%)	65.16±2.21	65.68±0.04	68.69±1.15	63.36±2.3	66.10±5.9	61.84±0.62	0.643
PF	3.49±0.52	3.45±0.15	3.37±0.12	3.48±0.04	3.36±0.21	3.45±0.08	0.997
MBP (mg)	39.91±10.3	40.98±3.40	40.57±2.0	41.10±0.86	39.38±8.69	38.97±1.92	1
NH_3-N mg/dl)	6.40±0.50	6.00±0.20	5.50±1.5	6.00±0.10	5.50±0.30	5.10±0.20	0.774

Pellets (M), - molasses pellets; Pellets (V), - depotash vinasse pellets; TMR Conc, total mixed ration with concentrate mixture; TMR (M), - total mixed ration with molasses pellets; TMR (V), - total mixed ration with depotash vinasse pellets.

similar to that of Khan *et al.* (2006), they found similar chemical composition of cane sugar molasses. Brito *et al.* (2015) reported that molasses had a similar DM, CP, and Ca. The present findings are in line with the findings of Maneerat *et al.* (2015) and Zali *et al.* (2017). The difference in the chemical composition of molasses and vinasse may be due to nature of raw material used for alcohol making, their inclusion level, and the type of channels they pass through, and batch to batch variation, application of fertilizer during crop production.

Chemical composition of concentrate mixture and pellets prepared from molasses (PM) and depotash vinasse (PV) have been presented in Table 3. The value of DM, OM, CP, ether extract and NDF level were alike in concentrate mixture and both the pellets. Ca level was 0.6 to 0.7% in the feeds. Mineral concentration, including the trace minerals was comparable in all the groups.

Results of the *in vitro* experiment have been shown in Table 4. The net gas production and total gas production were similar in concentrate, pellets of molasses, pellets of vinasse and TMR with concentrate, with molasses pellets and with depotash vinasse pellets. It was similar statistically, though there were little variations among the groups. The *in vitro* dry matter digestibility (IVDMD) was similar with the average value around 62%. The total OM digestibility (TOMD) was also similar in all the groups with the average value of 66%. PF and MBP were not different among the samples of different groups. NH_3-N was also similar among the samples, but higher with concentrate/ pellets individually as it was not utilized in optimum quantity by the microbes.

The results are similar with the findings of Mavimbela and Ryssen (2001) as they observed that the NH_3-N concentrations in ruminal fluid did not differ between the treatment groups. The results are conflicting with Iranmehr *et al.* (2011) who found that the ruminal NH_3-N and population of protozoa in rumen fluids were increased in all treatment groups. In the present experiment no such result observed.

CONCLUSIONS

Depotash vinasse, a by-product of distillery industry was evaluated for its chemical and mineral composition and found comparable to molasses. Its incorporation in pellet and incubation with rumen liquor individually and with TMR indicated no adverse effect on rumen fermentation characteristics and digestibility of nutrients, hence, can be used in cattle feed at 8% in concentrate mixture. Though, before incorporation in the dairy ration, it must be validated by *in vivo* experiments.

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The Effects of Dietary ω -3 and ω -6 Fatty Acids on Nutrient Utilization and Growth Performance in Sahiwal Heifers

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ABSTRACT

This study was conducted to evaluate the effect of dietary supplementation with ω -3 and ω-6 fatty acid rich oil on nutrient utilization, growth performance and pubertal age in Sahiwal heifers. Eighteen healthy Sahiwal heifers of average 18.33±1.14 month's age and 194±4.16 kg body weights were randomly assigned to 3 treatment groups *viz* T_1, T_2 and T_3 with 6 animals in each group. Heifers were individually fed diets of chopped wheat straw, green fodder and concentrate mixture to meet nutrient requirement as per ICAR (2013). The concentrate mixtures fed to the heifers under treatment group T_2 and T_3 were supplemented with ω-6 fatty acid rich soybean oil and ω-3 fatty acid rich linseed oils @ 3.5 percent, respectively. To make the rations iso-caloric, palm oil @ 3.5 percent was supplemented in the concentrate mixture of heifers in control group (T_1). All the concentrate mixtures were isonitrogenous. Average daily DMI was significantly (P<0.05) higher in heifers fed linseed oil supplemented concentrate mixture as compared to others. Total body weight gain and ADG was significantly (P<0.05) higher T_3 than T_2 and control heifers. The OMD was significantly (P<0.05) improved in groups fed ω-6 and ω-3 fatty acid rich oils as compared to control. The CP, DCP and TDN percent; and TDN intake did not differ among different dietary treatments. Feeding of linseed oil resulted in significant (P<0.05) reduction in the estrous age of Sahiwal heifers. The total dry matter intake up to first estrous was reduced by 32.12 and 12.77 percent in T_3 and T_2, respectively, as compared to control (T_1). Similarly, total concentrate mixture intake up to attainment of heifers' pubertal age in ω-3 rich linseed oil and ω-6 rich soybean oil fed group reduced by 38.82 and 16.11 percent, respectively, over control diet. The study deduced that ω-3 fatty acid supplementation reduced pubertal age of Sahiwal heifers.

Keywords: ω-3 and ω-6 fatty acids, Growth, Nutrient utilization, Pubertal age, Sahiwal heifers

INTRODUCTION

Livestock rearing is an important source of employment, income and livelihood of rural population of India. Demand of milk is ever increasing over time. For maximum output from commercial dairy farming sound rearing approach of heifers so as to replace the older and unproductive animals through culling play a significant role. However, heifer production is considered to be the most expensive part of dairy farm operation because it needs more inputs for a longer period with no immediate returns. Proper planning for replacement heifers affects success and longevity of a dairy herd. For successful dairy farming, a complete package of heifer rearing is imperative to exploit the performance potential at minimum expense. As reported by Razzaque *et al.* (2010) the input in dairy industry in term of feed cost represents around 84% of the total input costs for heifer production. Heifers must reach 30 to 40% of their mature body weight in order to reach puberty. Adequate nutrient supply to the animal is paramount to development of heifers. Age at puberty is an important production trait; and hormones, nutrition, and genotype play key roles in attainment of puberty (Bellows and Hall, 1996).

High levels of grains in the concentrate mixture can adversely affect DMI, rumen fermentation and fiber digestibility. High level of dietary energy intake influences reproductive performance of animals, as reported by Barth *et al.* (2008) in young bulls through direct effect on the age at puberty and growth of scrotal circumference. Fat supplements can be used @ 20 to 30 g/kg DMI (Palmquist, 1988) without

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negatively affecting fibre digestibility.

The ω-6 and ω-3 FAs as well as their different dietary ratios can affect fermentation and nutrient digestibility in rumen (Zhang *et al.* 2008). Traditionally, lipids were added to the diet of bovine to increase the energy density and alleviate the negative energy balance during early lactation. But, recent studies have unveiled many beneficial effects of lipids other than providing energy, especially the one rich in polyunsaturated fatty acids. Some groups of FA are considered essential to mammals because mammalian cells are unable to synthesize them. Juchem (2007) indicated that between 70 and 85% of the PUFAs get biohydrogenated in the rumen when cows are fed with unprotected oils. Despite extensive biohydrogenation of unsaturated FA, feeding increasing amounts of ω-6 and ω-3 FA alters tissue composition and influences cellular function and animal performance and reproductive functions (Silvestre *et al.,* 2011). Therefore, it is clear that despite limitations in delivery of specific amounts of polyunsaturated fatty acid for absorption, altering the FA composition of the diet is capable of influencing animal performance. Hence, this experiment was conducted to study effect feeding ω-3 and ω-6 fatty acid rich oil on nutrient digestibility and growth performance of sahiwal heifers.

MATERIALS AND METHODS

The experiment was performed after taking due permission from Institutional Animal Ethics Committee, CPCSEA, New Delhi. The experiment was conducted at cattle farm, Department of Animal Genetics and Breeding, LUVAS, Hisar, using 18 Sahiwal heifers with no overt clinical sickness. The heifers averaged 194±4.16 kg in body weight and 18.33±1.14 months in age. The animals were assigned to 3 groups *viz.* T_1, T_2 and T_3 of 6 animals each following completely randomized design. Animals were dewormed against internal parasites and dipped against ecto-parasites before the start of the experiment following standard protocol. Routine vaccination against Hemorrhagic Septicemia and Foot and Mouth disease was done. All experimental animals were housed in feeding stalls having arrangements for individual feeding and watering. The animals under different dietary treatments were maintained under similar husbandry practices. All the animals were fed isonitrogenous and isoenergetic diets containing weighed quantity of green fodder and concentrate mixture; and wheat straw was fed free of choice. The concentrate mixture fed to heifers in groups T_2 and T_3 was supplemented with soybean oil as ω-6 PUFA source and linseed oil as ω-3 PUFA source, respectively @ 3.5% (3.63 kg per 100 kg). To make the diets isocaloric, palm oil was added in the concentrate mixture of control (T_1). The diets were fed twice daily at 8:00 AM and 4:00 PM. The proximate compositions of feed ingredients, fodder, wheat straw and the different concentrate mixtures were analyzed according to methods of AOAC (2005) and cell wall fractions were determined as per the procedure of Van Soest *et al.* (1991).

Amount of feed offered, residues left and body weight were measured at fortnightly interval for two consecutive days. A digestion trial of 7 days collection period was conducted during which quantitative collection of total feces voided on 24 hourly bases was made to determine the nutrients digestibility of nutrients. Feed cost was calculated according to the price of different feedstuff during the experiment at local market. Data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994) following procedures for Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

Dry matter and crude protein content of T_1, T_2 and T_3 were 93.16, 92.92 and 93.24 percent; and 21.25, 20.98 and 21.14 percent, respectively, indicating that concentrate mixtures were iso-nitrogenous. Ether extract and nitrogen free extract content were 5.32, 5.18 and 5.57; and 56.55, 55.22 and 55.03 percent in concentrate mixture of groups T_1, T_2 and T_3, respectively which revealed that all the concentrate mixtures were isocaloric. Accordingly, TDN intake and TDN content of the diets were also similar. The crude protein content of wheat straw and green fodder were 2.45 and 10.45 percent, respectively. Neutral detergent

fibre (NDF) and acid detergent fibre (ADF) contents in concentrate mixture of T_1, T_2 and T_3 were 20.20, 20.86 and 19.63 percent; and 14.30, 14.50 and 14.35, respectively. The NDF and ADF content of wheat straw and green fodder were 75.82 and 50.37 percent; and 43.5 and 33.96 percent, respectively.

Data pertaining to intake and digestibility of nutrients are presented in Table 2. Intake of DM, CP, EE, CF, NFE, NDF and ADF was similar among the groups. The lack of significant effects on intake of these nutrients agrees with results of Muller *et al.* (2004) who showed that ω-3 and ω-6 fatty acids supplementation in crossbred heifers resulted in no differences (P>0.05) in intake of DM, CP, and NDF and ADF. Fiorentini *et al.* (2013) also found no effect of adding soybean oil to the diets of crossbred heifers on nutrient intakes.

In the present investigation PUFA supplementation increased (P<0.05) the organic matter digestibility in T_2, and T_3 groups by 4.01 and 6.20 percent over that of control group. This corroborates well with the earlier reports by Kumar and Thakur (2007). Linseed oil supplementation increased the intake of digestible OM significantly (P<0.05) in T_3 (3160.35g). Linseed as well as soybean oil, both increased the digestible EE intake significantly (P<0.05) which might be due to the numerically higher d igestibility coefficient of EE in PUFA fed heifers. In the same way, Len *et al.* (2016) reported that dietary supplementation of fat sources rich in PUFA has

Table 1. Ingredient (kg/100 kg) and chemical composition (% DM basis) of different concentrate mixtures

Attributes	Concentrate mixtures			Roughages	
	T_1	T_2	T_3	Maize fodder	Wheat straw
Ingredient Composition					
Maize	30	30	30	-	-
Groundnut cake	25	25	25	-	-
Mustard cake	10	10	10	-	-
Wheat bran	32	32	32	-	-
Mineral mixture	2	2	2	-	-
Common salt	1	1	1	-	-
Total, kg	100	100	100	-	-
Palm oil, kg	3.63	-	-	-	-
Soybean oil, kg	-	3.63	-	-	-
Linseed oil, kg	-	-	3.63	-	-
Chemical composition					
DM (%)	93.16	92.92	93.24	25.11	94.43
OM	91.42	91.17	91.03	89.63	90.73
Crude protein	21.25	20.98	21.14	10.45	2.45
Crude fibre	8.30	9.79	9.29	26.40	36.79
Ether extract	5.32	5.18	5.57	4.31	2.87
NFE	56.55	55.22	55.03	48.47	47.62
Total ash	8.58	8.83	8.97	10.37	9.27
NDF	20.20	20.86	19.63	70.69	80.01
ADF	14.30	14.50	14.35	34.92	52.46

positive effects in the improvement of digestibility of EE and OM in Murrah buffalo calves. The CP% and TDN% of the different TMR (Table 3) were not affected due to PUFA addition. Similarly, DCP content was also statistically similar in all the rations. No noteworthy changes were seen in intake of CP, DCP and TDN among the different groups. CP intake varied (P>0.05) from 674.76 g/day in control to 685.40 g/day in T_3. DCP intake was 480.46, 491.81 and 507.44 g/day in T_1, T_2 and T_3, respectively. Dietary PUFA supplementation increased the daily TDN intake from 3.251 kg/day in control to 3..352 kg/d in soybean and 3.491 kg/day in linseed oil fed heifers.

The overall mean values of daily total dry matter intake were 6.01, 6.25 and 6.67 kg in treatment groups T_1 (palm oil), T_2 (soybean oil) and T_3 (linseed oil), respectively (Table 4). The statistical analysis of the data revealed that daily dry matter intake was significantly (P<0.05) higher in heifers fed linseed oil supplemented concentrate mixture as compared to palm oil or soybean oil supplemented groups. The higher value in linseed oil fed group might be due to comparatively more body weight gain in that group. Similar to this study, Childs *et al.* (2008c) also reported higher overall feed intake in fish oil supplemented crossbred heifers as source of ω-3 fatty acids compared to control.

Table 2. Intake and apparent digestibility of nutrients in heifers under different dietary treatment groups

Attribute		Treatments		
		T_1	T_2	T_3
DM	DM Intake, kg/d	5.00±0.03	4.97±0.18	5.14±0.06
	% digestibility	66.58±0.85	67.59±1.74	67.51±2.55
	DDM Intake, kg/d	3.33±0.04	3.38±0.20	3.47±0.14
OM	OM Intake, g/d	4434.84±66.31	4441.13±179.12	4419.25±148.62
	% digestibility	65.38^{b}±0.67	69.39^{a}±1.22	71.58^{a}±0.60
	DOM Intake, g/d	2898.15^{b}±33.04	3071.52^{ab}±75.32	3160.35^{a}±91.20
CP	CP Intake, g/d	674.76±1.27	674.00±6.68	685.40±1.93
	% digestibility	71.20±0.65	72.91±1.31	74.04±1.96
	DCP Intake, g/d	480.46±4.88	491.81±13.23	507.44±13.52
EE	EE Intake, g/d	244.76±5.83	246.32±0.12	248.83±0.62
	% digestibility	71.00±1.88	74.57±0.83	74.85±1.70
	DEE Intake, g/d	173.32^{b}±2.44	183.67^{a}±2.06	186.26^{a}±4.44
CF	CF Intake, g/d	1101.02±15.60	1106.59±53.07	1194.16±4.53
	% digestibility	58.28±2.23	58.53±2.39	57.43±3.15
	DCF Intake, g/dd	641.76±26.31	653.94±56.57	686.33±39.28
NFE	NFE Intake, g/d	2468.22±21.95	2505.14±89.31	2609.70±10.72
	% digestibility	70.41±1.68	71.34±1.28	71.93±2.75
	DNFE Intake, g/d	1738.28±48.66	1792.40±93.52	1878.45±79.31
NDF	NDF Intake, g/d	3059.06±18.63	3121.16±128.03	3107.97±87.51
	% digestibility	60.32±1.93	59.51±2.27	58.93±2.45
	DNDF Intake, g/d	1846.22±65.46	1871.60±145.41	1828.63±82.05
ADF	ADF Intake, g/d	1639.13±26.65	1600.62±70.32	1691.70±11.55
	% digestibility	53.25±2.69	52.58±2.87	51.77±4.34
	DADF Intake, g/d	874.99±54.66	851.15±81.67	877.06±77.09

The mean values bearing different superscripts in a row differ significantly (P<0.05)

Table 3. Mean nutrient intake and nutritive value of experimental ration under different dietary treatments

Attributes		Treatments		
		T_1	T_2	T_3
CP	CP%	11.65±0.02	11.64±0.12	11.84±0.03
	Intake, g/day	674.76±1.27	674.00±6.68	685.40±1.93
DCP	DCP%	8.30±0.08	8.49±0.23	8.76±0.23
	Intake, g/day	480.46±4.88	491.81±13.23	507.44±13.52
TDN	TDN%	55.01±1.53	56.70±3.26	59.49±2.63
	Intake, kg/day	3.251±0.08	3.352±0.16	3.491±0.13

There was no significant effect on DMI per 100 kg body weight by supplementing palm oil or soybean oil or linseed oil in concentrate mixture of Sahiwal heifers. Similar trend was observed when dry matter intake was calculated in terms of dry matter intake per kg metabolic body size, which did not differ significantly

Table 4. Mean dry matter intake, growth performance and economics of feeding different diets (kg/day, DM basis) in experimental heifers

Attributes	Treatments		
	T_1	T_2	T_3
DMI, kg/d	6.01^{b}±0.12	6.25^{b}±0.11	6.67^{a}±0.14
DMI, %BW	2.54±0.02	2.53±0.03	2.54±0.02
DMI, g/kg $W^{0.75}$	98.99±1.19	100.58±1.29	102.53±1.17
Total DMI, kg	901.53^{b}±18.17	937.62^{b}±16.66	999.98^{a}±20.89
Av. roughage intake, kg	3.31^{b}±0.09	3.56^{b}±0.08	3.96^{a}±0.12
Av. CM intake, kg	2.70±0.07	2.69±0.07	2.70±0.07
Total roughage intake, kg	496.28^{b}±14.19	533.42^{b}±12.02	594.39^{a}±17.27
Total CM intake, kg	405.25±10.61	404.20±10.58	405.59±10.62
Initial BW	185.67±9.30	196.17±4.38	201.16±6.79
Final BW	268.33^{b}±7.11	283.00^{b}±6.42	310.33^{a}±7.79
Total gain, kg	82.67^{b}±3.21	86.83^{b}±6.75	109.17^{a}±7.26
ADG, g	551^{b}±21	579^{b}±45	728^{a}±48
FCE	0.09±0.01	0.09±0.01	0.11±0.01
Days taken to 1st estrous	131.33^{a}±10.31	110.17^{ab}±13.79	80.33^{b}±13.66
Pubertal age, days	713.00^{a}±29	665.33^{ab}±26	623.67^{b}±20
DMI till 1st estrous, kg	789.31±61.95	688.54±86.16 (-12.77%)	535.82±91.09 (-32.12%)
Roughage intake till 1st estrous, kg	434.71±34.12	392.19±49.07	318.12±54.08
CM intake till 1st estrous, kg	354.67^{a}±27.83	297.50^{ab}±37.14 (-16.11)	217.00^{b}±36.86 (-38.82)
CM cost, ₹/kg	24.53	25.51	28.13
Cost of CM feeding till 1st estrous, ₹	8698.34±682.68	7587.95±949.47 (-1110.39, -12.77%)	6101.40±1037.26 (-2596.94, -29.86%)

The mean values bearing different superscripts in a row differ significantly (P<0.05)

in different treatment groups. The results of the study are in agreement of earlier finding of Dirandeh *et al.* (2014) who reported that average dry matter intake was not affected by dietary inclusion of roasted soybean and linseed as a source of (P<0.05) ω -6 and ω -3 fatty acid, respectively, in the diets of lactating cattle.

Statistical analysis of the data revealed that total body weight gain during experimental period was significantly (P<0.05) higher in heifers fed linseed oil (ω-3 rich oil) supplemented concentrate mixture as compared to soybean oil (ω-6 rich oil) and palm oil supplemented heifers(Table 4). Similar response with respect to average daily gain (ADG) was also noted. The results corroborate with findings of Childs *et al.* (2008) who found that on feeding of graded level of fish oil as source of ω-3 fatty acids significantly increased (P<0.05) the daily body weight gain of crossbred heifers as compared to non-supplemental groups of heifers. Kumar and Thakur (2007) also confirmed that bypass fat supplementation with varying degree of unsaturation increased growth rate in female buffalo calves. The present findings are also in agreement with the report Hill *et al.* (2011) who reported, ADG and efficiency of calves were improved by feeding ω-3 fatty acid rich feed as compared to control diets.

The results of the study revealed that numerical values of FCE improved upon supplementation of ω-3 rich FA oil, however, the difference was statistically non-significant. In earlier report, Garcia *et al.* (2014) revealed that increasing mean intake of linoleic acid from approximately 4.6 to 11.0 g/day during the first 60 day of life increased average daily gain (0.50 *vs.* 0.45 kg/d) without a change in dry matter intake, thus improving feed efficiency.

The cost of feeding during experimental period and up to attainment of first estrous in Sahiwal heifers under different dietary treatments have been depicted in Table 4. The results of the study revealed that daily dry matter intake and total dry matter intake during 150 days of experimental period was significantly (P<0.05) higher in the animals fed concentrate mixture containing ω-3 fatty acid rich oil (T_3) as compared to ω-6 (T_2) and saturated fatty acid (T_1) rich oils. However, the experimental heifers fed soybean oil added concentrate mixture did not show any significant effect of dry matter intake over control group. The mean number of days taken from initiation of experiment to first estrous sign was 131.33, 110.17 and 80.33 days in T_1, T_2 and T_3, respectively, indicating that supplementation of linseed oil has significantly (P<0.05) reduced the days for first sign of heat over soybean and saturated fatty acid oils.

The findings of present investigation revealed that supplementation of ω-3 rich linseed oil in the concentrate mixture of Sahiwal heifers resulted in significant (P<0.05) reduction in the pubertal age of heifers (623.67 days) as compared to ω-6 rich soybean oil supplemented group (665.33 days) and control group (713 days) despite the fact that all the diets were isocalric and iso-nitrogenous. Tran *et al.* (2016) reported that feeding of ω-3 PUFA enriched diet reduced pubertal age and improved both fresh and post-thawing semen quality in male buffalo. The total dry matter intake up to first estrous was reduced by 32.12 and 12.77 percent in T_3 and T_2, respectively, as compared to control (T_1). Similarly, total concentrate mixture intake up to attainment of heifers' pubertal age in ω-3 rich linseed oil and ω-6 rich soybean oil fed group was reduced by 38.82 and 16.11 percent, respectively, over control diet. Hence, the perusal of data on feed cost demonstrated that there was a net saving of ₹ 2596.94 and 1110.39 in Sahiwal heifers by feeding PUFA rich linseed and soybean oil, respectively, from the age 18.33 months to pubertal age.

CONCLUSION

The results of the present study inferred that feeding ω-3 and ω-6 fatty acid rich oil to Sahiwal heifers leads to improved organic matter digestibility and increased intake of digestible ether extract. In addition to that, a diet enriched with source of ω-3 FA such as linseed oil might be a candidate diet to be fed to pre-pubertal heifers to increase the average daily gain; and decrease pubertal age and the cost of feeding.

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A Newly Developed Mixture of Herbal Plants and Spices Enriched with Special Extracts and Essential Oils Enhances Feed Utilisation, Growth Performance and Lowers Harmful Caecal Bacteria in Rabbits

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ABSTRACT

This study was conducted to evaluate the effect of feeding a mixture of herbal plants and spices enriched with special extracts and essential oils (phytogenic additives mixture) on feed intake, feed utilisation, blood chemistry, slaughter characteristics and caecal bacteria in rabbits. Ninety weaned unsexed V-Line rabbits, weighing 668.7±8.2 g and aged 30±1 day, were randomly allocated to three groups of 30 rabbits each. Animal were fed either a basal diet without additives (Control) or supplemented with 0.5 ml (LA) or 1 ml (HA) of the additives mixture per litre of drinking water. Additives did not affect feed intake; however, the LA rabbits showed better (P<0.001) feed conversion ratio as compared to the other treatments. The LA treatment increased (P<0.01) final body weight and average daily gain as compared to the control and HA treatments. Both the LA and HA treatments decreased (P<0.05) blood cholesterol, triglycerides and low-density lipoproteins; however, the LA treatment increased (P<0.05) high-density lipoproteins and total antioxidant capacity and decreased malondialdehyde (P<0.05) relative to the control. Treatments did not affect (P>0.05) carcass characteristics. The LA treatment followed by HA treatment increased (P<0.001) beneficial *Lactobacillus* spp. bacteria and decreased the concentrations of coliform bacteria and *E. coli* compared with the control. It is concluded that phytogenic additives mixture enhanced feed conversion, daily gain and positively altered caecal bacteria profile best response being observed at the lower dose of the phytogenic additives mixture as compared to the higher dose.

Key words: Blood metabolic profile, Carcass characteristics, Pathogenic bacteria, Phytogenic additives mixture, Rabbits, Weight gain

INTRODUCTION

The acute shortage of meat supply compels livestock farmers to improve feed utilization, health status and meat production from their animals. This shortage can be bridged by the farming of highly prolific animals with short production cycles, such as rabbits. Rabbits have high fertility rates and fast growth rates, making them good sources of meat. Rabbit meat is highly digestible, tasty, low in fat and cholesterol contents, rich polyunsaturated fatty acids content with higher nutritional advantage (ω-6/ω-3 ratio =5.9) and low in calorie content as compared to other meats (Combes, 2004; Dalle Zotte, 2002).

Feed additives such as herbal plants and seeds have been used in ruminants *(*Kholif *et al.,* 2017; Morsy *et al.,* 2018) and recently in the diets of rabbits (Cervantes-Valencia *et al.,* 2015; Gado *et al.,* 2016; Hafsa *et al.,* 2016) to enhance feed conversion and health. Essential oils, spices and herbs have been successfully used in animal feed to increase the oxidative stability of meat and reduced total mesophilic aerobes, *Pseudomonas* spp. and *Enterobacteriaceae* (Fortier *et al.,* 2012; Koné *et al.,* 2016; Soultos *et al.,* 2009). Combining herbal plants, spices, special extracts and essential oils (phytogenic additives mixture) has attracted an increased attention from rabbit producers due to its notable antioxidative, antibacterial and antifungal activities and ability to enhance feed flavour, palatability, voluntary feed intake and weight gain (Zeng *et al.,* 2015).Studies on single components of herbal

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plants, spices, special extracts or essential oils have showed positive effects on feed utilization, animal health and live performance of rabbits (Celia *et al.,* 2016). Benlemlih *et al.* (2014) reported that mortality was reduced in growing rabbits fed diet supplemented with 0.5 g fennel and thyme essential oil/kg diet. Furthermore, a diet supplemented with 0.5% fennel seed increased the digestibility of organic matter, crude fibre and ether extract, and final weight and body weight gain (Omer *et al.,* 2013).

Plant extracts, essential oils and herbal plants possess antimicrobial (Lambert *et al.,* 2001) and antioxidant (Sarac *et al.,* 2007) activities, and had been evaluated in broiler chickens (Lewis *et al.* 2003) and rabbits (Celia *et al.,* 2016; Placha *et al.,* 2013), with mixed results on performance and animal health. During the period around weaning, young rabbits are threatened by frequently-occurring enteric diseases. In order to control such problem there is extensive use of antibiotics (Bovera *et al.,* 2010b, 2010a).Considering the harmful effects of antibiotics, there is an increasing interest among animal nutritionist to explore new and safe antimicrobial agents that can suitably replace antibiotics and effectively prevent and/or overcome infections, and improve welfare and health of the animals. Keeping these points in mind, the present experiment was conducted to study the effect of different doses of a phytogenic feed additive mixture (herbal plants, spices enriched with special extracts and essential oils) in the drinking water on feed conversion, blood metabolites, carcass characteristics and caecal bacterial profile of growing rabbits.

MATERIALS AND METHODS

This experiment was conducted at the Nucleus Rabbit Breeding Unit of the Poultry Research Centre, Poultry Production Department, Faculty of Agriculture, Alexandria University, Egypt during spring season.The area has a climate with winter rains and an annual average rainfall of 22 mm and mean annual temperature ranges between 14 and 32°C. Rabbits care and procedures were conducted under established approved standards of the Animal and Fish Production Department, Faculty of Agriculture, Alexandria University (Egypt).

A newly developed mixture (Aromix® 2336, Masa Egypt Company, Alexandria, Egypt) of natural finely ground herbs and spices enriched with special extracts and essential oils, was evaluated that contained (per litre):2.5 g carvacol, 0.75 g thymol, 17.8 g menthol and 50 g propylene glycol dissolved in 1 l of distilled water.

Ninety weaned unsexed V-Line rabbits, aged 30±1 day and with a bodyweight of 668.7 ± 7.7 g, were used in the study The total duration of the experiment was 63 days. During the first 2 weeks of the experiment, rabbits were housed in 5 galvanized wire pens (6 rabbits per pen) provided with feeders and automatic waterer system. They were there after housed in 10 galvanized wire pens (3 rabbits per pen) during the rest of the experiment. Each pen was 35 cm high × 40 cm width × 50 length. Pens were the experimental units for the intake and performance studies. All rabbits were kept under the same managerial, hygienic and environmental conditions in rooms with standard air conditioning maintaining 20-25°C temperature, 55-65% relative humidity and 14-16 h of lighting. Rabbits were treated subcutaneously with a coccidiostat (Sulphadimidine sodium BP solution Dimi-Vet® injection; Square Pharmaceuticals, Dhaka, Bangladesh) once at the beginning of the experiment at 1 ml/rabbit.

Rabbits of the control treatment were fed on a basal diet as a Control diet (Table 1). Other rabbits were supplemented with 0.5 ml (LA) or 1 ml of phytogenic additives mixture (HA) per litre drinking water. Feed was provided in two equal meals at 08:00 and 17:00 h daily. Droppings from the cages on the floor were collected and removed daily. Body weight and feed intake of rabbits were recorded weekly, while mortality rate was recorded daily. Feed conversion was calculated as the ratio of the feed intake (kg) to weight gain (kg).

At the end of the experiment, 10 rabbits were sampled and about 3 ml blood from each rabbit was taken at 08:00 – 09:00 h from the marginal ear vein into non- heparinized tube. Blood samples were centrifuged for 15 minutes on 4000 ×g, serum harvested and stored

in a deep freezer at -20°C until biochemical analysis using specific kits (Stanbio Laboratory, Boerne, TX, USA) according to manufacturers' recommendations. Serum samples were analysed for total proteins, albumin, total lipids, triglyceride, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphate (ALP), uric acid, creatinine, calcium, inorganic phosphorus, immunoglobulin (IgG and IgM), glucose, triiodothyronine (T_3), thyroxin (T_4), total antioxidant capacity (TAC) and activity of malondialdehyde (MDA).

At the end of the experimental period (9 weeks of age), ten rabbits from each group (one from each pen) were randomly taken, fasted for 12 hours, weighed individually, slaughtered followed by exsanguinations (Cheeke, 1987). After complete exsanguinations, the carcass was weighed again and the difference between the pre-slaughter weight and weight after exsanguinations was considered as the blood weight. The slaughtered rabbits were skinned, the skin weight recorded, and the carcass eviscerated. The stomach, intestine, liver, heart, lung, and periscapular and perirenal fat were separated and weighed. The remaining part was considered as hot carcass. The following carcass traits were determined: dressing percentage (weight of hot eviscerated carcass including liver, heart, abdominal fat and head divided by the live body weight); organs weight as a percentage of live body weight (liver, heart, lung, pancreas, kidney, thyroid and adrenal glands, abdominal and shoulder fat, full stomach, full intestine and caecum). Slaughtering and dissection were carried out according to World Rabbit Science Association (WRSA) recommendations (Blasco and Ouhayoun, 1993). Hot carcasses were chilled at 4°C for 24 h. Intestine, caecum and carcass lengths were also measured.

Total number of caecal bacteria (total gut bacilli and lactobacilli populations) was counted according to American Public Health Association (APHA, 1960). The isolation of coliforms and *Escherichia coli* from the caecum was completed using the method described by the Food and Drug Administration (1984). Dilution factor is a reciprocal value of dilution exponent. Such value is expressed as CFU/g (Colony Forming Units), (Barnes and Impey, 1970). Incubation was done at 30°C for 2-7 days. Microbiological contents of the caeca from 6 rabbits/ treatment were collected. The numbers of anaerobic bacteria, lactose entero-bacteria and coliform bacteria, were counted on appropriate selective and non-selective agar plates. The colony counts were expressed in $\log_{10}$ CFU related to 1 g of sample. The pH of caecal content was determined with a glass electrode pH meter.

Except for bacterial counts and mortality data, data collected were analysed using the GLM procedure (SAS Inst. Inc. Cary, NC, USA) for a completely randomized design. The treatment (Control, LA or HA) was the only source of variation (fixed effect). For the intake and performance studies, pen within each treatment was the experimental unit, whereas for the carcass characteristics and caecal bacterial count study, rabbit within each treatment was the experimental unit. Measurements (body weight changes, feed intake and conversion, and mortality) recorded daily or weekly were analysed as a repeated measure. Bacterial count values were log transformed and analysed using the PROCGLIMMIX of SAS with log of bacterial counts as the response variables and diet (Control, LA or HA) as the factor explanatory variable, assuming that random residual variance followed a Normal distribution. When treatment effects were significant, the means were compared using Duncan's Multiple Range Test. Polynomial (linear and quadratic) contrasts (adjusted for the equal spacing of treatments) were used to examine dose responses to increasing doses of additives mixture and for comparison between control *vs*. the average of additives mixture treatments. Significance was declared when $P<0.05$.

RESULTS AND DISCUSSION

Treatment LA showed greater ($P=0.001$) final body weight and average daily gain ($P<0.001$) compared with HA and control (Table 2). With no difference for feed intake ($P>0.05$), the LA rabbits showed better feed conversion ratio ($P<0.001$) compared with the other

treatments. The treatments LA and HA decreased mortality rate as compared to control. LA and HA treatments decreased serum cholesterol (P=0.002), triglycerides (P=0.023) and LDL cholesterol (P=0.032) (Table 3). The LA treatment increased HDL cholesterol (P=0.047) and TAC (P=0.049) and decreased MDA (P=0.041).

Additive doses did not affect pH of caecal content, carcass weight, relative organ weights, cecum length and carcass length (Table 4).The LA treatment showed greatest (linear and quadratic effects, P<0.001) beneficial effect with respect to *Lactobacillus* spp. count followed by the HA treatment while the control had the least count (Table 5). Conversely, coliform bacteria and *E. coli* counts were lowest for the LA, followed by the HA and highest for the control (linear and quadratic effects, P<0.001).

Table 1. Ingredient and chemical composition of the basal diet fed to rabbits

Ingredient	g/kg DM
Alfalfa hay	240
Wheat bran	217
Soybean meal	180
Ground barley	160
Wheat straw	80
Yellow corn	70
Molasses	30
Di-calcium phosphate	12
Salt	4
Vitamins and minerals mixture[1]	3
Limestone	2
Methionine	1.5
Lysine	0.5
Chemical analysis	(g/kg DM)
Dry matter	917
Organic matter	902
Crude protein	177
Crude fibre	144
Ether extract	27
Nitrogen free extract	554
Digestible energy (kcal/kg)[2]	2584

[1]Each 1 kg contains: 13340 IU vitamin A, 2680 IU vitamin D_3, 10 IU vitamin E, 2.68 mg vitamin K, 10.68 mg calcium pantothenate, 0.022 mg vitamin B_{12}, 0.668 mg folic acid, 400 mg choline chloride, 26.68 mg chlorotetracycline, manganese 133.34 mg, 66.68 mg iron, 53.34 mg zinc, 3.2 mg copper, 1.86 mg iodine, 0.268 mg cobalt, 0.108 mg selenium; [2]DE Calculated according to Schneider and Flatt (1975).

The present study aimed to explore the effects of two levels of a phytogenic feed additive in drinking water of rabbits on feed intake and conversion, growth performance, carcass characteristics and some cecum microflora. Due to scarcity of available data on effect of phytogenic additives mixture on rabbits, comparison was done with studies that used different essential oils, spices and herbs either in rabbits or broiler chickens.

The insignificant differences among the treatments for the initial body weights reveal effective random distribution and homogeneity of the experimental rabbits at the beginning of the experiment. The low and high doses of the phytogenic additives mixture increased daily gain by about 13 and 6%, respectively, compared with the control. The increased daily gain and final body weight reveal enhanced feed utilization which may be partly explained by the increased nutrient absorption (Zeng *et al.,* 2015) and improved secretions of saliva, bile, mucus and enzyme activity (Platel and Srinivasan, 1996, 2000; Jang *et al.,* 2004; Jamroz *et al.,* 2005; Muhl and Liebert, 2007).In addition, the observed decreased numbers of pathogenic bacteria in the cecum may improve the ability of epithelial cells to regenerate villus and thus enhance intestinal absorptive capacity (Mourão *et al.,* 2006), resulting in improved daily gains. Similarly, the improved population of the beneficial lactic acid bacteria (*Lactobacillus* sp) possibly enhanced the gut function and health status of the additives mixture administered rabbits which might have improved feed utilisation and weight gains as a result of the substantial reduction in digestive disorders of the rabbits (Celia *et al.,* 2016). Simitzis (2017) reported that phenolic compounds in additives mixture renders antimicrobial activity against several microorganisms. Due to their hydrophobic characteristics they alter the permeability of the cytoplasmic membrane to hydrogen ions and potassium, and deplete the intracellular ATP pool leading to the disruption of essential cellular processes (Costa *et al.,* 2013). These results corroborate well with

the caecal microbial profile discussed later. Stein and Kil (2006) showed that the hydrophobic constituents of essential oils disintegrate the outer membrane of pathogenic bacteria (e.g., *E.coli*).

Phytogenic additives mixture did not affect feed intake; however, the low dose of the additives mixture increased feed conversion, revealing unaffected palatability and enhanced feed efficiency of the diets with the additives. The unchanged feed intake was not expected since essential oils, herbs and spices are often used to improve flavour and, indirectly, palatability of feeds (Zeng *et al.,* 2015). Supplementation of phytogenic additives (essential oils) and herb extracts improved feed conversion ratio (Jamroz *et al.,* 2005). In consonance with the current findings, Celia *et al.* (2016) observed unaffected feed intake and enhanced feed conversion ratio with the feeding of herbal formulation containing a mixture of essential oils, herbs, spices and extracts. In addition, Hong *et al.* (2012) reported that the addition of essential oil improved feed efficiency in broilers. In the present experiment, the lower dose of the phytogenic additives mixture was more effective than the higher dose, suggesting that 0.5 ml additives mixture as the optimum level for the young rabbits. It appears that the higher dose additives mixture contained relatively high amount of phenolic compounds which depressed performance relative to the lower dose. Nevertheless, the phenolic compounds concentration was not as high as to induce toxicity or negatively affect the performance of rabbits. This conjecture was confirmed by the superior performance of the HA rabbits relative to the control rabbits. Zeng *et al.* (2015) earlier attributed relatively low performance of animals on high dose of phytogenic additives to the concentration of phenolic compounds.

The LA and HA treatments decreased mortality rate by 60 and 50%, respectively. This effect was expected since the health status of the rabbits supplemented with the additives mixture was affected positively. As discussed later, the additives increased the count of the beneficial *Lactobacillus* spp. with a concurrent decrease in the number of the pathogenic bacterial. The presence of pathogenic bacteria causes digestive disturbances that are often responsible for high morbidity and mortality of young rabbits after weaning and economic losses in rabbit farms (Bovera *et al.,* 2010a; Licois, 2004).The higher mortality of rabbits on high dose of additives mixture relative to the low dose further confirms the efficacy of the low dose. It appears that the high dose increased the concentration of active substances which mildly affected the rabbits but the effect was not pronounced as to raise mortality rate above that of the control rabbits. As previously stated, the low additive dose was more effective than the high dose in enhancing rabbit performance and reducing mortality. Nevertheless, the HA was superior to the control (no additive supplementation). The better gain and feed utilisation efficiency and lower mortality

Table 2. Growth performance, feed intake and feed efficiency of rabbits fed different treatments (Mean± SEM)

	Treatment[1]			P-Values				
	Control	LA	HA	T	W	T×W	L	Q
Initial BW (g)	672±14	669±14	665±14	0.938	-	-	0.723	0.966
Final BW (g)	1840^{a}±25.9	1985^{b}±23.7	1908^{b}±23.7	0.001	-	-	0.059	0.001
ADG, (g/d)	33.4^{c}±0.70	37.7^{a}±0.68	35.5^{b}±0.69	<0.001	<0.001	<0.001	0.039	0.001
FI (g/d)	112.2±1.60	111.3±1.55	109.9±1.57	0.603	<0.001	<0.001	0.318	0.918
FCR	3.42^{a}±0.05	3.00^{b}±0.051	3.38^{a}±0.052	<0.001	<0.001	<0.001	0.639	<0.001
Mortality (%)	16.7^{a}±0.33	6.7^{c}±0.21	10.0^{b}±0.31	<0.001	<0.001	<0.001	0.043	0.004

Means in the same row with different superscripts differ, P<0.05; [1] Rabbits were fed either a basal diet without additives (Control) or supplemented with 0.5 ml (LA) or 1 ml (HA) of the additives mixture per litre of drinking water; T, treatment; W, week; T×W, treatment x week interaction; L, linear effect; Q, quadratic effect; ADG, average daily gain; FI, feed intake; FCR, feed conversion ratio

of HA than the control indicate that the concentrations of the phenolic compounds and other active substances in this treatment were below intoxicating levels.

Blood metabolic profiles have been used for diagnosis and prognosis of diseases in animals, and are also useful to assess the welfare condition of animals or to understand if some changes in a diet can affect animal physiology (Olafadehan, 2011). The concentrations of total protein, albumin, globulin, creatinine and uric acid were within the ranges for healthy rabbits (Ajayi and Raji, 2012). Abd-El-Hady (2005) noted unchanged serum total protein, albumin and globulin in rabbits fed on a diet supplemented with a mixture of natural finely ground herbs and spices enriched with special extracts and essential oils at 300 and 400 g/ton feed. In addition, the non-significant change in concentrations of immunoglobulins of the LA and HA rabbits are indicative unaffected immune defences. The additives mixture did not affect glucose, T_3 and T_4 hormones concentrations; however, Abd-El-Hady (2005) observed that feeding of phytogenic additives mixture increased blood glucose and T_3 hormone concentrations. Glucose concentrations observed in this study were within the range indicated

Table 3. Blood measurements of rabbits fed different doses of additives mixture

	Treatment[1]			SEM	P-Values		
	Control	LA	HA		T	L	Q
Total protein (mg/dl)	6.54	6.85	6.76	0.191	0.493	0.408	0.395
Albumin (mg/dl)	3.59	3.74	3.93	0.149	0.288	0.119	0.875
Globulin (mg/dl)	2.94	3.12	2.83	0.099	0.136	0.426	0.067
Glucose (mg/dl)	128	138	130	10.1	0.749	0.846	0.467
Total lipids (mg/dl)	229	184	182	11.8	0.013	0.008	0.160
Cholesterol (mg/dl)	107^{a}	88.8^{b}	93.7^{b}	3.31	0.002	0.008	0.008
Triglycerides (mg/dl)	137^{a}	111^{b}	117^{b}	6.5	0.023	0.045	0.048
HDL (mg/dl)	51.9^{b}	65.7^{a}	57.4^{ab}	3.7	0.047	0.314	0.023
LDL (mg/dl)	35.4^{a}	30.5^{b}	29.6^{b}	1.59	0.032	0.015	0.303
AST (U/l)	23.8	19.5	21.1	1.83	0.265	0.303	0.205
ALT (U/l)	35.0	33.1	32.4	2.28	0.706	0.426	0.822
ALP (mg/dl)	97.7	106.4	104.4	4.06	0.304	0.256	0.295
Creatinine (mg/dl)	0.94	0.70	0.75	0.052	0.006	0.012	0.027
Uric acid (mg/dl)	3.43	3.09	3.27	0.317	0.757	0.728	0.514
Calcium (mg/dl)	9.82	11.0	10.6	0.46	0.217	0.219	0.209
Phosphorus (mg/dl)	4.04	4.28	4.11	0.250	0.791	0.843	0.516
Immunoglobulin G (mg/dl)	57.2	61.3	59.7	4.79	0.832	0.710	0.636
Immunoglobulin M (mg/dl)	72.4	78.4	77.7	3.25	0.374	0.261	0.403
Triiodothyronine (T_3; ng/ml)	1.22	1.54	1.44	0.105	0.116	0.162	0.118
Thyroxine (T_4; ng/ml)	33.1	36.8	35.9	1.51	0.210	0.197	0.222
T_4/T_3	27.8	24.5	25.9	1.31	0.222	0.312	0.157
Malondialdehyde (nmol/ml)	137^{a}	129^{b}	131^{ab}	2.51	0.041	0.087	0.093
TAC (ìmol/l)	1.49^{b}	1.89^{a}	1.76^{ab}	0.121	0.049	0.127	0.089

Means in the same row with different superscripts differ, P<0.05; [1]The rabbits were fed either a basal diet without additives (Control) or supplemented with 0.5 ml (LA) or 1 ml (HA) of the additives mixture per litre of drinking water. T, treatment effect; L, linear effect, Q, quadratic effect; HDL, high-density lipoproteins; LDL, low-density lipoproteins; AST, aspartate aminotransferase; ALT, alanine aminotransferase, ALP; alkaline phosphatase; TAC, total antioxidant capacity

for healthy rabbits (Özkan *et al.,* 2012). Liver enzymes (AST, ALT and ALP) are conventionally used for diagnosing domestic animal hepatic damage. The unchanged values suggest that no damage occurred in the liver. The reported concentrations fell within reference ranges for healthy rabbits (Ajayi and Raji, 2012).

Both of LA and HA treatments decreased cholesterol, triglycerides and LDL cholesterol, suggesting beneficial modulatory influence of phytogenic feed additive on cholesterol metabolism and turnover. These effects support the cardiovascular protective influence of the phytogenic feed additives supplementation. The active components and phenolics in the additives have the ability to affect lipid metabolism in animal tissues by increasing the antioxidative enzymes activity and preventing the production of reactive oxygen species and off-flavours derived from the peroxidation of polyunsaturated fatty acids (Miguel, 2010).Moreover, lowering of the cholesterol and LDL cholesterol concentrations could possibly be associated with cellular cholesterol biosynthesis (Fuhrman *et al.,* 2000) and decrease in intestinal absorption of cholesterol resulting in an increase in faecal excretion of neutral lipids (Purohit and Vyas, 2006).Phytogenics (polyphenolics and flavonoids) have the ability to inhibit the hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, a key regulatory enzyme in cholesterol synthesis, (Lee *et al.,* 2004). Elshater *et al.*(2009) reported that reduction of

Table 4. Carcass characteristics of rabbits fed different doses of additives mixture

	Treatment[1]			SEM	P-Values		
	Control	LA	HA	T	L	Q	
Caecal pH value	6.33	6.29	6.32	0.020	0.469	0.731	0.244
Carcass weight (g)	1073	1122	1085	23.8	0.350	0.733	0.165
Relative carcass weight	57.3	58.5	57.5	0.95	0.668	0.927	0.380
Dressing out percentage	63.4	64.8	64.1	1.03	0.631	0.639	0.409
Liver percentage	3.88	4.19	4.14	0.242	0.638	0.468	0.551
Heart percentage	0.33	0.35	0.41	0.025	0.091	0.038	0.498
Lung percentage	0.72	0.70	0.76	0.055	0.680	0.567	0.512
Spleen percentage	0.12	0.10	0.13	0.013	0.510	0.624	0.298
Pancreas percentage	1.03	1.04	1.21	0.059	0.081	0.047	0.280
Kidney percentage	1.03	1.04	1.21	0.059	0.081	0.047	0.280
Thymus gland percentage	0.10	0.10	0.08	0.009	0.337	0.416	0.220
Thyroid gland percentage	0.009	0.008	0.008	0.0006	0.994	0.921	0.973
Adrenal gland percentage	0.008	0.009	0.009	0.0010	0.554	0.415	0.481
Abdominal fat percentage	0.90	0.75	0.74	0.099	0.457	0.276	0.550
Shoulder fat percentage	0.31	0.28	0.25	0.048	0.683	0.391	0.955
Full stomach percentage	4.89	4.67	4.80	0.260	0.837	0.807	0.593
Full intestine percentage	7.29	8.03	7.63	0.331	0.316	0.470	0.183
Full cecum percentage	5.88	6.76	6.68	0.509	0.418	0.281	0.450
Intestine length (Cm)	379	386	388	6.2	0.601	0.348	0.739
Cecum length (Cm)	46.8	48.0	48.7	1.32	0.619	0.341	0.879
Carcass length (Cm)	33.7	33.5	32.8	0.55	0.518	0.276	0.762

Means in the same row with different superscripts differ, P<0.05; T, treatment effect; L, linear effect, Q, quadratic effect; [1]The rabbits were fed either a basal diet without additives (Control) or supplemented with 0.5 ml (LA) or 1 ml (HA) of the additives mixture per litre of drinking water

cellular cholesterol biosynthesis is associated with increased activity of the LDL receptor, leading to enhanced removal of LDL from blood and thus causing a decreased serum or plasma cholesterol concentration.

The LA treatment increased HDL cholesterol and TAC, and decreased MDA because the additives mixture contains phenolic compounds that elicit antioxidant action by scavenging reactive oxygen species, enhancing the cellular antioxidant enzyme (e.g., superoxide dismutase, catalase and glutathione peroxidase) and increasing glutathione in the cells (Borek, 2001). The MDA is an indicator of cell membrane injury and its concentration depends on lipid peroxidation (El-Gogary *et al.,* 2018). Increasing HDL cholesterol reveals reduced atherogenic risk by virtue of increased reverse cholesterol transport from peripheral organs to liver (Hermansen *et al.,* 2003). The antioxidative activity of some phenolics components in feed additives mixture has been attributed to scavenging of superoxide anion and hydroxyl radicals (Elkirdasy *et al.,* 2015). Although the phytogenic additives mixture altered some blood metabolic profiles, the occurrence of all the blood parameters within the normal physiological ranges ruled out the possibility of any intoxication, especially with the high dose. Therefore, the results indicate the safety of the additives mixture doses used in the current study.

The weak effects of additives on carcass weight, relative organs weights, cecum length, carcass length are in agreement with previous studies in which feeding of phytogenic additives did not affect carcass characteristics and relative organ weights (Cho *et al.,* 2014; Hong *et al.,* 2012; Kirkpinar *et al.,* 2011) or meat pH value (Cho *et al.,* 2014). Abdel-Wareth *et al.* (2018) observed that supplementation of rabbits with thyme essential oil did not affect the percentage of head, liver, heart, lungs and kidneys of the chilled carcass as well as the percentage of fore, mid and hind parts.

The LA and HA treatments increased the beneficial (*Lactobacillus* spp.) bacteria counts by about 12 and 6%, respectively. It is well documented that essential oil, herbs and phytogenic extracts suppress harmful microorganisms and stimulate beneficial microbes such as *Lactobacillus* spp. (Simitzis, 2017). *Lactobacillus* spp. is a normal member of the rabbit intestinal microflora, with a positive effect on regular gut function (Carabaño *et al.,* 2010; Celia *et al.,* 2016). Therefore, increasing the number of *Lactobacillus* spp. could play a role in preventing the mortality of the rabbits. *Lactobacilli* bacteria activate the intestinal immune system and increase the resistance to diseases through the release of low-molecular weight peptides which induce immune activation (Muir *et al.,* 2000). Moreover, increasing the count of *Lactobacilli* bacteria contributes to the colonization resistance against pathogenic microbes by modifying the receptors used by them (Adil and Magray, 2012; Rinttila and Apajalahti, 2013).

As compared to control, the LA and HA treatments decreased coliform bacteria and *Escherichia coli* counts by about 49 and 33%, and 28 and 23%, respectively. Placha *et al.* (2013) observed decreased number of coliforms in the caecum of rabbits fed a diet supplemented with 0.5 g/kg DM of thyme essential oil. Celia *et al.* (2016) observed reduced

Table 5. Caecal bacteria of rabbits (log_{10} cfu/g) fed different doses of additives mixture

	Treatment[1]			SEM	P-Values		
	Control	LA	HA	T	L	Q	
Lactobacillus spp.	7.80[c]	8.74[a]	8.25[b]	0.067	<0.001	<0.001	<0.001
Coliform bacteria count	7.54[a]	3.88[c]	5.02[b]	0.099	<0.001	<0.001	<0.001
Escherichia coli	6.41[a]	4.61[c]	4.91[b]	0.082	<0.001	<0.001	<0.001

Means in the same row with different superscripts differ, P<0.05; T, treatment effect; L, linear effect, Q, quadratic effect; [1]The rabbits were fed either a basal diet without additives (Control) or supplemented with 0.5 ml (LA) or 1 ml (HA) of the additives mixture per litre of drinking water

bacterial diversity in the caecum of rabbits fed a diet supplemented with herbal formulation containing a mixture of essential oils, herbs, spices and extracts. A reduction in the number of pathogenic bacteria changes the microbial ecology in favour of beneficial species in the intestine (Michiels *et al.,* 2009), resulting in improved ability of epithelial cells to regenerate villus and thus enhances intestinal absorptive capacity (Mourão *et al.,* 2006).Such effects reveal the antimicrobial activity of additives mixture to control pathogenic bacteria. Phenolic compounds in essential oils, herbs and extracts display antimicrobial action against *Escherichia coli* (Jang *et al.,* 2007) and many other pathogens, and prevent their adhesion, colonization and proliferation in the gut of broilers (Simitzis, 2017). Essential oils, phytogenic extracts and aromatic plants are well known to exert antibacterial, antifungal and antiviral activity. The pathogenic bacteria are normal inhabitants of the intestinal tract of rabbits and can inflict digestive disturbances that are often responsible for high morbidity and mortality of young rabbits after weaning, causing economic losses in rabbit farms.

Decreasing pathogens such as *E. coli* contributes to healthy microbial metabolites, improves intestinal integrity and ensures protection against enteric disease (Baker *et al.,* 2012; Oviedo-Rondón *et al.,* 2006; Placha *et al.,* 2013; Tiihonen *et al.,* 2010). Phenolic compounds of essential oils, extracts and herbal plants cause structural and functional damage to cytoplasmic membranes of harmful bacteria (Soultos *et al.,* 2009). Bozin *et al.* (2006) showed the *in vitro* antibacterial activity of carvacol and thymol (the main components of oregano essential oil) on multi-resistant strains of *E. coli*. The mechanisms of reduction of such pathogenic bacteria in the intestinal tract after administration of phytogenic additives have not been rigorously studied up till date. However, the hypothesis that plant extracts/essential oils inhibit target cells in the membrane and deplete the transmembrane potential and/ or the pH gradient which result in the leakage of cellular materials and destruction of bacterial cells has been confirmed (Cleveland *et al.,* 2001; Szabóová *et al.,* 2012).

CONCLUSIONS

The results of the present experiment demonstrate that supplementation of phytogenic additive mixture enhanced feed conversion, daily gain and positively altered caecal bacterial profile of rabbit. Better response was obtained at lower dose (0.5 ml/l drinking water) as compared compared to dose (1 ml/l drinking water) of supplementation. Thus, it was concluded that supplementation of phytogenic feed additive at 1 ml/l drinking water would be beneficial to improve performance and gut health of rabbits. Additional studies, involving *in vitro* and *in vivo* evaluations, are recommended to investigate different levels of the additives mixture on the performance of rabbits at different stages of growth.

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A Nursery Feed for Indian Major Carps (*Labeo rohita*) and its Evaluation in Farmer's Field

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ABSTRACT

Realizing the importance of carp feed for its nursery phase, the Fish Nutrition and Physiology Division of ICAR- Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar has been working since last three years and has developed a nursery feed for carp which ensured better survival, growth and palatability. The feed was prepared from maize, soybean meal, rice bran, groundnut oil cake, fish meal, oil, minerals and vitamin mixture by extrusion technology and then crumbled into suitable size for feeding the carp fry. The prepared feed was evaluated through demonstration experiments in different locations of Odisha in Government fish farm and farmer's ponds following standard protocols. The evaluation was done in terms of growth, feed efficiency and survivability with existing feed commonly used by the farmers of the region. Result of this experiment showed that, the fish fed with developed nursery feed had 1.5 to 2 times higher growth performance, 25% higher survivability and better feed efficiency compared to the feed which is used by most of the farmers of this region.

Keywords*:* Carps, Extrusion, FCR, Growth performance, Nursery feed

INTRODUCTION

Nursery phase is one of most critical phase in the production of stockable seed materials in carp culture. Generally, it is observed that the recovery of spawn to fry and fry to fingerling is around 25-30% and 40-50%, respectively in farmers' practice. Nutrition plays a vital role in growth and survival of the carp in nursery rearing. Non-availability of quality feed is one of the major reasons for the poor survival and growth making the nursery rearing of carp less remunerative. Realizing the importance of carp feed for its nursery phase, the Fish Nutrition and Physiology Division of ICAR-CIFA, Bhubaneswar has been working since last three years and has developed a nursery feed (Carp starter) for carp which ensures better survival and growth.

Extrusion technology is gaining importance for production of fish feed for Indian Major Carp. Extrusion process improves nutrient digestibility, palatability, pellet durability, water stability and pellet storage life (Barrows and Hardy, 2000). It also increases *in vivo* digestibility of dry matter (DM) and energy of feed in rainbow trout *Oncorhynchus mykiss* (Cheng and Hardy, 2003). Good quality feed containing til oil cake could be produced through extrusion technology by maintaining extrusion temp of 130°C and moisture of 20% (Das *et al.,* 2018). The nursery feed for carp (Carp starter) was produced from maize, soybean meal, rice bran, groundnut oil cake, fish meal, oil, minerals and vitamin mixture by extrusion and then crumbled into suitable size for feeding the fry. This feed is nutritious and highly palatable. It has been tested at indoor facilities and demonstrated multi-locationally in Government fish farm and farmer's pond. In this experiment, Carp starter produced through extrusion technology was evaluated in terms of growth, feed efficiency and survivability with existing feed commonly used by the farmers of the region.

MATERIALS AND METHODS

A fish feed for carp fry was formulated using high quality locally available feed ingredients *i.e.* maize, soyabean meal, groundnut oil cake (GNOC), fish meal and de-oiled rice bran (DORB) with supplementation of minerals and vitamin mixture. The ingredients were pulverised, mixed, extruded and then dried. The extruded and dried feed was then ground into suitable size, packaged and stored for feeding the fish (Fig. 1). The

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Fig. 1. Carp Starter after preparation for carp fry

feeds were produced in the feed mill facilities of ICAR-CIFA, Bhubaneswar, India with extrusion temperatures of 130°C and moisture of 20% maintaining constant pressure (10 kg/cm^2). After production of extruded feed, it was packaged for easy transportation to the experimental site. The feed was then analysed for chemical characteristics to ascertain the quality of the feed (Table 1). Developed nursery feed was then compared with a feed (control feed) which is normally used by most of the farmers of this region. A control feed was also prepared after mixing GNOC and rice bran in 1: 1 ratio and then grinded properly into powder form as it is the standard practices of feeding carp fry in many places of India including Odisha.

The chemical parameters *i.e.* DM, crude protein (CP), ether extract (EE), crude fibre (CF), nitrogen free extract (NFE) and total ash were analysed for quality evaluation (AOAC, 2012). DM was estimated by oven drying the samples at 105°C till a constant weight and CP content was calculated by estimating nitrogen content by micro-Kjeldahl method and multiplying with a factor 6.25. EE was determined by solvent extraction with petroleum ether, boiling point 40–60°C, for 10–12 h. Total ash content was determined by incinerating the sample at 650 °C for 6 h and CF was determined by acid digestion (1.25%) followed by alkali digestion (1.25%).

Demonstration experiment was simultaneously conducted in five location of Odisha *i.e.* state fishery farm, Keonjhar; College of Fisheries, Rangailunda (OUAT), Berhampur; KVK, OUAT, Ranital, Bhadrak; ICAR-CIFA, Bhubaneswar and Sahu Fish Farm, Subarnapur, Gop, Puri.

Pond preparation was done in all the five places for demonstration experiment. Before stocking the fish, all the ponds were dried and bleaching powder (30% active chlorine) was applied at 300 kg ha^{-1} to eradicate the unwanted predatory and weed fishes. Lime was applied at 1000 kg ha^{-1}. After 7 days of lime application, the water was filled up to 1.0 m depth and ponds were fertilized with raw cow dung, urea and bleaching powder at 3.0 ton ha^{-1}, 40.0 kg ha^{-1} and 60.0 kg ha^{-1}, respectively (ICAR 2009).

Table 1. Chemical characteristics of nursery feed (Carp Starter)

Parameters	% on DM basis
Moisture	7.5
Crude Protein (CP)	35.58
Crude Fibre (CF)	4.62
Total Ash	10.74
Ether Extract (EE)	6.10
Nitrogen Free Extract (NFE)	42.96
Energy (kcal/g)	3.61

Rohu fry (average weight 0.5 gm) were either procured from CIFA Farm, Bhubaneswar, India or purchased from the local market for stocking in the respective pond sites. Carp fry were stocked randomly into triplicate ponds (each 130×60 ft) for both control and treatment following a completely randomized design and were stocked @ 1 lakh/ acre. The fish of control ponds were fed with GNOC and rice bran (1:1 ratio) as it is the standard practices of rearing fry in many places of India including Odisha. The fish of treatment ponds were fed with carp starter to compare the performance with fish of control ponds. Both

Fig. 2. Rohu fry after feeding with Carp Starter for one month

treatment and control fish were fed at the rate of 10% of body weight (BW) for 2 weeks followed by 5 % of BW for another 2 weeks. Sampling of fish was done in fortnightly interval to regulate the feeding requirement and to estimate the average BW and biomass of fish in each pond (Fig. 2). The experiment was continued for one month.

The data of the experiment were statistically analyzed by using computer software (GraphPad PRISM 2007).

RESULTS AND DISCUSSION

The chemical characteristics of nursery feed (Carp Starter) used in the experiment is presented in Table 1. The feed contained 35.58% protein, 6.10% fat and 4.62 % CF and size of feed particles varied from 0.4 to 0.5 mm. The chemical characteristics showed that, it is a very good feed for carp fry.

The growth performance of *L. rohita* fry fed with different test diets in different locations of Odisha like Keonjhar, Bhadrak, Bhubaneswar, Berhampore and Gop

Table 2. Growth performance of L. rohita fry fed with different diets in Odisha

Locality	Parameters	Control	Carp Starter	P value	SEM
Keonjhar	Initial weight (g)	0.30	0.30	-	-
	Final weight (g)	3.36 ^{a}	4.72 ^{b}	0.0001	0.3069
	Weight gain (g)	3.06 ^{a}	4.42 ^{b}	0.0001	0.3069
	SGR (% day^{-1})	8.77 ^{a}	12.27 ^{b}	<0.0001	0.7877
	FCR (Apparent)	2.46 ^{a}	1.26 ^{b}	0.0002	0.2717
	Survival (%)	63 ^{a}	87 ^{b}	0.0114	5.888
Bhadrak	Initial weight (g/fish)	0.75	0.75	-	-
	Final weight (g/fish)	2.53 ^{a}	6.00 ^{b}	0.0042	0.8188
	Weight gain (g/fish)	1.78 ^{a}	5.25 ^{b}	0.0042	0.8188
	Survival (%)	53.0 ^{a}	82.0 ^{b}	0.0056	6.913
	FCR (Apparent)	2.10 ^{a}	1.69 ^{b}	0.0371	0.1094
Bhubaneswar	Initial weight (g/fish)	0.93	0.93	-	-
	Final weight (g/fish)	5.07 ^{a}	6.12 ^{b}	0.0135	0.2598
	Weight gain (g/fish)	4.14 ^{a}	5.19 ^{b}	0.0135	0.2598
	SGR (% day $^{-1}$)	4.8 ^{a}	5.3 ^{b}	0.0376	0.1335
	Survival (%)	80 ^{a}	95 ^{b}	0.0086	2.422
	FCR (Apparent)	1.57 ^{a}	1.24 ^{b}	0.0014	0.076
Berhampore	Initial weight (g/fish)	0.7	0.7	-	-
	Final weight (g/fish)	1.45 ^{a}	3.68 ^{b}	0.0001	0.5008
	Weight gain (g/fish)	0.75 ^{a}	2.98 ^{b}	0.0001	0.5008
	SGR (% day -1)	2.43	5.53	0.0376	0.1335
	Survival (%)	75 ^{a}	94 ^{b}	0.0004	4.394
	FCR (Apparent)	3.43 ^{a}	1.5 ^{b}	0.0001	0.4349
Gop	Initial weight (g/fish)	0.5	0.5	-	-
	Final weight (g/fish)	3.37 ^{a}	5.60 ^{b}	0.0001	0.5016
	Weight gain (g/fish)	2.87 ^{a}	5.10 ^{b}	0.0001	0.5016
	SGR (% day^{-1})	6.36	8.05	0.0376	0.1335
	Survival (%)	71 ^{a}	95 ^{b}	0.0001	5.241
	FCR (Apparent)	2.45 ^{a}	1.5 ^{b}	0.0004	0.2045

Means with different superscripts in a row differ significantly (P<0.01).

is presented in table 2. The initial average weight of fry was 0.63 g (varied from 0.3 g to 0.93 g) in both control and treatment groups and attained 3.15 g (varied from 1.45 g to 5.07 g) in control group and 5.22 g (varied from 3.68 g to 6.12 g) in treatment groups after 30 days of feeding trial. The weight gain in all the feeding experiments were significantly higher in treatment groups compared to control groups. The specific growth ratio (SGR) were also significantly higher in treatment group compared to control group of fish and is indicated in table. Similarly, feed conversion ratio (FCR) of fish fed on carp starter was significantly superior ($P<0.05$) compared to fish fed on control feed. The growth performance of fish during the experiment showed that the growth rate of fish in Keonjhar, Bhadrak, Bhubaneswar, Berhampore and Gop was higher than control group of fish fed with ground nut oilcake and rice bran. There was an average increase of 1.5 to 2 times of weight gain fed with carp starter compared to control feed.

Nursery feed (carp starter) was developed by extrusion process in the feed mill of ICAR-CIFA, higher feed utilization by extrusion process may be responsible for improved growth and survival of carp fry (Das *et al.,* 2018). Extrusion is the process in which the feed material is pushed through the barrel by means of screws of different configurations and pressed through the die at the end of barrel. The basic concept of extrusion process is high temperature, short time, whereby the high temperature is a direct result of friction or pre-conditioning and steam injection or a combination of both (Levic, 2010). Extrusion technology has been used in the feed industry for almost one century (Hardy and Barrows, 2000) and it has become popular for aqua feed production (FMT, 2005) as extruded pelleted feed reduces the feed wastage and improve the over all growth performane of fish.

The survival percentage of fish fed on carp starter was higher compared to fish fed on control feed. The survival percentage in control group of fish was 68 percentage (53 -80 percent) where as survivability in treatment group of fish was 90.6 percent (82 - 95 percent). Survivability of carp fry is an important parameters as generally, it is observed in the farmers practice that recovery of spawn to fry and fry to fingerling is around 25-30% and 40-50%, respectively. Nutrition plays a vital role on growth and survival in nursery rearing of the carp. Non-availability of quality feed is one of the reasons for the poor survival and growth making the nursery rearing of carp less remunerative. Though planktons are available in natural rearing process, it is not sufficient to meet nutritional requirement of all nursery carps. In our experiment, survivability of fish in different experimental stations varied from 82 to 95 percent with an average of 90.6 percent. Availability of nutrition rich carp starter along with natural planktons are prime factors for improving survival of carp fry.

Carp starter as used in this experiment contained many feed ingredients like maize, soyabean meal, groundnut oil cake (GNOC), fish meal and de-oiled rice bran (DORB). Supplementation of many feed ingredients in the production of fish feed improve the keeping quality of feed compared to two or three feed ingredients as has been observed by Das *et al.* (2016). There may be balancing of amino acids like methionine, lysine and arginine in carp starter produced from soyabean meal, ground nut cake and fish meal compared to feed of control group. This might be due to better utilization of protein in Carp starter compared to control feed. It has been reported that the feed prepared by using more than one protein sources always resulted in better growth of fish due to proper balancing of amino acids (Djissou *et al.,* 2016; Gaylord *et al.,* 2017). This showed that, carp starter had better growth performance as compared to control feed. The palatability of feed was higher in carp starter compared to control feed as observed during feeding time. Use of fish meal/fish oil in carp starter might have increased palatability of feed as observed by many workers. This indicated that, carp starter is a very high quality feed and may be provided to carp fry for higher growth performance and survivability.

CONCLUSION

Result of this experiment showed that, the fish fed with developed nursery feed had 1.5 to 2 times higher growth performance, 25% higher survivability and better feed efficiency compared to the feed which is used by most of the farmers of this region. Thus, the carp starter developed at Fish Nutrition and Physiology Division of ICAR- Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar could be used at farm level as an efficient nursery feed for carp which would ensure better survival and growth.

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Effects of Feeding Graded Levels of Distillers Dried Grains with Soluble (DDGS) With or Without Supplementation of Multi-enzymes on Blood Bio-Chemical Constituents of Indigenous Chicken

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ABSTRACT

An experiment was conducted using distillers dried grains with soluble (DDGS) at 0, 10 and 20% levels with or without enzymes supplementation in the diets of indigenous chicken to study the effect on different blood bio-chemicals parameters. A total of one hundred and eighty (21 days old) indigenous chicks were divided into six groups *viz.* T_1, T_2, T_3, T_4, T_5 and T_6 containing 30 chicks in each group. The birds of T_1 (control) and T_2 groups were offered the standard chick, grower and layer feeds as per BIS, 2007 without and with multi-enzymes (Xzyme), respectively. The birds of T_3, T_4, T_5 and T_6 groups were fed the rations containing 10% DDGS without and with enzymes and 20% DDGS without and with enzymes, respectively. The feeding trial was conducted for a period of 13 fortnights. The average levels of different blood parameters, estimated at the end of the experiment, *viz.* haemoglobin, glucose, serum protein, lipid, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium and phosphorus ranged from 9.96±0.20 to 10.91±0.24 g/dl, 185.08±0.54 to 188.67±1.86 mg/dl, 3.05±0.17 to 3.32±0.09 g/dl, 72.45±0.29 to 72.96±0.49 mg/dl, 130.91±0.59 to 133.71±2.07 mg/dl, 71.66±0.44 to 72.28±0.40 U/L, 11.42±0.32 to 11.93±0.12 U/L, 10.16±0.44 to 10.81±0.29 mg/dl and 4.93±0.11 to 5.94±0.14 mg/dl, respectively. Non-significant ($P>0.05$) differences were observed with respect to average values of different blood parameters among the treatment groups except for blood inorganic phosphorus. Serum concentration of inorganic phosphorus was significantly ($P<0.05$) lower in T_1 as compared to other groups. It can be concluded that the incorporation of DDGS up to 20% level in the diets of indigenous chicken did not have any adverse effect on the blood bio-chemical constituents of experimental birds.

Key words: Blood bio-chemical, Distillers dried grains with soluble (DDGS), Enzyme, Indigenous chicken

INTRODUCTION

The maize and soybean meal are the major conventional sources of energy and protein, respectively, in poultry feeds. These ingredients are becoming scarce day by day due to their increased demand in livestock and poultry sector. Further, due to its use for production of biofuel ethanol, availability of maize for use in poultry sector may be challenging. So, utilization of non-conventional feed resources in the poultry feeding is indispensable. The replacement of costlier traditional ingredients with cheaper non-conventional ingredients is probably the most viable proposition to address this situation. Distillers dried grains with soluble (DDGS), a co-product of ethanol production process, has been identified as one of the promising non-conventional feed resource that can be used in the poultry ration as energy and protein source. In the recent years there is an escalation of DDGS production and its quality has also seen a positive change when derived from new generation ethanol plants (Panda *et al.,* 2016). It is a good source of energy, protein, exogenous amino acids, B-group vitamins and minerals, including phosphorus (Koreleski and Swiatkiewicz, 2006; Thaker and Widyaratne, 2007; Min *et al.,* 2008). The DDGS is also better source of fibre, protein and fat than cereal grains (Koreleski and Swiatkiewicz, 2008). However, DDGS contains considerable amount of non-starch polysaccharides (NSP) (Ward *et al.,* 2008), which restrict its extensive use in poultry ration. In this regard, exogenous enzymes are able to offer nutritional benefits by hydrolyzing NSP (Cost *et al.,* 2008). The use of appropriate enzymes to hydrolyze these compounds can increase the nutritional value of DDGS and enables greater inclusion in poultry diets (Juanpere

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at al., 2005). In view of the above facts, the present study was undertaken to investigate the effect of dietary incorporation of distillers dried grains with solubles (DDGS) at different levels with or without multi-enzyme supplementation on the blood bio-chemical constituents of indigenous chicken.

MATERIALS AND METHODS

All procedures followed in this experiment was approved by Institutional Animal Ethics Committee, Assam Agricultural University, Khanapara, Guwahati (770/ac/CPCSEA/FVSc/U/IAEC/17-18/479 dated: 09.08.2017). Following a completely randomized design, a total of one hundred and eighty numbers of 21 days old indigenous chicks were randomly distributed into six groups *viz.* T_1, T_2, T_3, T_4, T_5 and T_6 containing 30 chicks with 3 replicates of 10 chicks in each group. The birds were reared under deep litter system of management throughout the experimental period following standard and uniform managemental practices and in similar environmental condition (temperature 17-28°C, humidity 80-92% and rainfall 0-14 mm). The birds of T_1 group (control) were offered the standard chick, grower and layer feeds as per BIS, 2007 (Table 1). The birds of T_2 group were fed with the same standard chick, grower and layer feeds as per BIS, 2007 with supplementation of multi-enzyme (Xzyme). Maize DDGS was incorporated at 10% level in the rations of T_3 and T_4 groups, whereas, the rations of T_4 group was additionally supplemented with Xzyme. In the same way, the birds of T_5 and T_6 groups were fed with rations containing 20% DDGS without and with enzymes, respectively. The feeding trial was conducted for a period of 182 days (13 fortnights) using chick feeds for first 3 fortnights (0-42 days), grower feeds for next 7 fortnights (43-140 days) and layer feeds for last 3 fortnights (141-182 days). Weighed quantities of feeds were offered thrice daily at 6.30 AM, 12.30 PM and 5.00 PM to the birds of different treatment groups ensuring *ad libitum* feeding throughout the study period. During the last week of the experiment, blood was collected from four numbers of birds from each experimental group at 9.30 AM, after three hours of post feeding. Blood samples were collected aseptically from brachial vein of birds in two different tubes, one part in clean sterilized tube containing EDTA for haemoglobin analysis and another part in clean sterilized glass tubes and kept in slanted position at room temperature for serum collection. The samples were then centrifuged at 3000 rpm for 5 minutes, serum harvested and transferred to 2 ml Eppendorf tubes which were then stored at -20°C for estimation of other blood biochemical parameters.

The blood haemoglobin and glucose as well as other blood bio-chemical constituents (serum total protein, cholesterol, lipids, AST, ALT, calcium and inorganic phosphorous) were analyzed by using commercial kits following particular protocols as per the manufacturers' guidelines. The statistical analyses of the experimental data were carried out according to the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Total feed consumption per bird for the last fortnight (13^{th}) and entire period of experiment were 109.25 and 11653, 110 and 11665, 108.75 and 11664, 111.50 and 11669, 110.25 and 11739, and 111.25 and 11748 g for groups T_1, T_2, T_3, T_4, T_5 and T_6, respectively. Feed intakes (g/bird) were found to be comparable among the birds. Other research workers (Thacker and Widyaratne, 2007; Youssef *et al.* 2013; Hassan and Aqil, 2015) also reported no significant difference in feed consumption among different treatment groups with DDGS inclusion levels up to 10-15%. In this experiment, maximum and minimum total consumption was recorded in T_6 and T_1 groups, respectively. Similar trend was also observed by Pineda *et al.* (2008) who reported that the higher levels of incorporation of DDGS in the layer ration increased feed intake.

No significant ($P>0.05$) difference was observed in Hb. levels of experimental birds among various treatment groups (Table 2). However, a non-significant ($P>0.05$) increase in blood Hb. levels in birds fed DDGS supplemented with multi-enzyme was observed, which might be due to the positive effects of enzymes as well

Table 1: Composition of chick, grower and layer rations

Ingredients (%)	Rations																	
	Chick						Grower						Layer					
	T_1	T_2	T_3	T_4	T_5	T_6	T_1	T_2	T_3	T_4	T_5	T_6	T_1	T_2	T_3	T_4	T_5	T_6
Maize	40.40	40.40	34.00	34.00	29.32	29.32	40.40	40.40	32.26	32.26	29.32	29.32	43.18	43.01	34.21	34.21	32.27	32.27
Soybean meal	15.50	15.50	10.50	10.50	5.00	5.00	15.50	15.50	10.00	10.00	5.00	5.00	25.00	25.10	19.85	19.85	14.30	14.20
Rice polish	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	7.00	7.00	8.00	8.00
Distillers dried grains with soluble	00	00	10.00	10.00	20.00	20.00	00	00	10.00	10.00	20.00	20.00	00	00	10.00	10.00	20.00	20.00
Di-calcium phosphate	1.30	1.30	1.20	1.20	1.00	1.00	1.30	1.30	1.20	1.20	1.00	1.00	1.30	1.30	1.20	1.20	1.00	1.00
Limestone powder	1.70	1.70	2.00	2.00	2.00	2.00	1.70	1.70	2.00	2.00	2.00	2.00	7.00	7.00	7.20	7.20	7.30	7.30
Methionine	0.10	0.10	0.07	0.07	0.08	0.08	0.10	0.10	0.07	0.07	0.08	0.08	0.10	0.10	0.07	0.07	0.08	0.08
Lysine	0.00	0.00	0.05	0.05	0.18	0.18	0.00	0.00	0.05	0.05	0.18	0.18	0.00	0.00	0.05	0.05	0.18	0.18
Salt	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Minpremix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
BrokenRice	7.05	7.05	10.00	10.00	10.00	10.0	7.05	7.05	10.00	10.00	10.00	10.00	6.00	6.50	10.0	10.0	6.00	6.00
De-oiled rice bran	28.53	28.53	26.76	26.76	27.00	27.0	28.53	28.53	29.00	29.00	27.00	27.00	12.00	11.50	10.00	10.00	10.45	10.50
Enzymes	00	0.05	00	0.05	00	0.05	00	0.05	00	0.05	00	0.05	00	0.05	00	0.05	0.00	0.05
Crude Protein (%)	19.25	19.37	19.33	19.41	19.22	19.33	15.49	15.53	15.54	15.61	15.45	15.53	17.55	17.65	17.51	17.53	17.47	17.48
ME[2] (kcal/kg)	2798	2798	2793	2793	2800	2800	2527	2527	2503	2503	2539	2539	2580	2580	2600	2600	2604	2604

[1]Mineral mixture contains (per 1.2 kg): Calcium- 255 g, Phosphorous- 127.5 g, Magnesium- 6 g, Manganese- 1.5 g, Iron- 1.5 g, Iodine- 325 mg, Copper- 4.2 g, zinc-9.6 g, Cobalt- 150 mg, Sulphur- 7.2 g, Potassium- 100 mg, Sodium- 6mg, Selenium- 10 mg, Vitamin A- 700000 IU, Vitamin D_3- 70000 IU, Vitamin E- 250 mg, Nicotinamide- 1000 mg & Chromium- 78 mg.; Composition of multi-enzyme (Xzyme: Each kg of Xzyme premix contains: Lactic Acid Bacillus-30,000 million spores, Saccharomyces Cervisiae- 100 billion CFU, Amylase- 29,000 IU, Betaglucanase-4,05,000 IU, Phytase- 44,500 IU, Lipase- 31,000 IU, Protease- 7,40,000 IU, Cellulase- 5,500 IU, Pectinase -1,01,000 IU and Hemicellulase- 25,000 IU; [2](Metabolizable Energy) Calculated value

as higher iron contents of DDGS. Similarly, Gupta *et al.* (2017) reported that the inclusion of DDGS at 5, 7.5 and 10% levels enhanced the Hb level as compared to control. further improvement in Hb level was observed when DDGS was supplemented with multi-enzyme.

The mean concentration of blood glucose were 188.67±1.86, 188.40±1.96, 187.20±1.61, 187.61±1.37, 185.08±0.54 and 185.79±0.30 mg/dl in T_1, T_2, T_3, T_4, T_5 and T_6 groups, respectively. The mean values for blood glucose levels in birds of different groups were comparable though some numerical differences existed among the values. Findings of Jiang *et al.* (2013) was in the same line with the present study who reported that dietary inclusion of rice DDGS up to 25% did not affect the plasma content of glucose in juvenile red seabream. However, Gupta *et al.* (2017) reported that glucose level decreased significantly in rice DDGS supplemented groups. In this study, the non-significant (P>0.05) decrease in blood glucose levels in DDGS added groups might be due to lower starch and higher fibre contents in the rations of various treatment groups.

The highest (3.32±0.09 g/dl) mean total serum protein was recorded in the birds of T_6 group while the lowest (3.05±0.17) value for same was recorded in T_1 group (Table 2). No significant (P>0.05) difference was observed in total serum protein values of experimental birds among the treatment groups. Bor-Ling *et al.* (2011) also reported that feeding of corn DDGS at different levels (0, 6, 12 and 18) did not influence the serum protein in laying hen. Numerically increased total serum values were recorded in T_2, T_4 and T_6 groups, where enzymes were supplemented, in comparison to T_1, T_3 and T_5 groups. Osmana *et al.* (2011) conducted a feeding trial in Hubbard broiler chicks using DDGS and reported higher plasma protein values in birds fed 20% DDGS along with enzymes supplementation. In this study, the non-significant (P>0.05) increase of serum total protein levels in enzyme treated groups might be due to the effect of protease present in the multi-enzyme used in the experiment.

There was no significant (P>0.05) difference in blood lipid values of experimental birds among the treatment groups (Table 2). Similarly, Bor-Ling *et al.* (2011) reported that feeding of corn DDGS at different levels (0, 6, 12 and 18) did not influence the serum triglycerides of laying hens. Findings of Jiang *et al.* (2013) are also in the same line with the present study, who reported that dietary inclusion of rice DDGS up to 25% did not affect the plasma content of triglycerides in juvenile red seabream. No significant (P>0.05) difference was observed in blood cholesterol values of experimental birds among different treatment groups (Table 2). Jiang *et al.* (2013) also reported that dietary inclusion of rice DDGS up to 25% did not affect the plasma content of cholesterol in juvenile red seabream. Activity of AST and ALT were similar among the groups (Table 2). The present findings corroborate well with that of Ghazalah *et al.* (2011), who reported substitution

Table 2. Mean (±SE) blood parameters in experimental birds of different treatment groups

Parameters	Treatments groups						P value
	T_1	T_2	T_3	T_4	T_5	T_6	
Haemoglobin (g/dl)	9.96±0.20	10.40±0.16	10.65±0.27	10.74±0.36	10.85±0.22	10.91±0.24	0.118446
Glucose (mg/dl)	188.67±1.86	188.40±1.96	187.20±1.61	187.61±1.37	185.08±0.54	185.79±0.30	0.439116
Total serum protein (g/dl)	3.05±0.17	3.20±0.13	3.18±0.09	3.25±0.10	3.21±0.10	3.32±0.09	0.714567
Total lipids (mg/dl)	72.51±0.08	72.45±0.29	72.96±0.49	72.84±0.19	72.78±0.30	72.64±0.09	0.771293
Cholesterol (mg/dl)	133.71±2.07	131.85±2.28	132.61±1.84	131.71±1.04	131.90±0.97	130.91±0.59	0.869824
AST (U/L)	72.28±0.40	72.09±0.04	72.00±0.12	71.66±0.44	72.11±0.25	71.97±0.43	0.831994
ALT (U/L)	11.79±0.33	11.75±0.10	11.93±0.12	11.42±0.32	11.69±0.17	11.67±0.28	0.776553
Serum calcium (mg/dl)	10.59±0.36	10.81±0.29	10.37±0.31	10.53±0.41	10.16±0.44	10.63±0.34	0.851216
Serum phosphorous (mg/dl)	4.93^{a}±0.11	5.62^{b}±0.11	5.56^{b}±0.22	5.75^{b}±0.17	5.69^{b}±0.18	5.94^{b}±0.14	0.009138

Means bearing the same superscript within the row do not differ significantly (P<0.01)

of soybean meals with DDGS at four levels (0, 25, 50 or 75%) and supplementation of Avizyme 1500 at two levels (0 or 0.075%) did not cause any change in the activity of these enzymes.

The mean blood ALT levels of experimental birds of different treatment groups ranged from 11.42±0.32 to 11.93±0.12 U/L. There was no significant (P>0.05) difference in blood ALT values of experimental birds among various treatment groups. The results of the present study was in agreement with the findings of Ghazalah *et al.* (2011), who reported from a feeding trial in Bovans Brown layers by substituting soybean meals with DDGS at four levels of 0 or 25 or 50 or 75% and adding Avizyme 1500 at two levels (0 or 0.075%) that there was no significant (P>0.05) difference among the groups for ALT levels.

The mean serum concentration of calcium of experimental birds of different treatment groups ranged from 10.16±0.44 to 10.81±0.29 mg/dl. No significant (P>0.05) difference was observed in average serum calcium values of experimental birds among the treatment groups. The overall values of blood calcium in all the treatment groups were found to be on higher side which might be due to the fact that the birds of all the groups were in egg laying stage at the time of collection of blood. The results of the present study were in agreement with the findings of Ghazalah *et al.* (2011), who reported that there was no significant difference among the birds for calcium levels in Bovans Brown layers. Hack *et al.* (2015) also noticed that serum calcium was not altered when hens were fed with diets containing DDGS.

The serum inorganic phosphorus values were found to be maximum (5.94±0.14 mg/dl) in T_6 and minimum (4.93±0.11 mg/dl) in T_1 groups (Table 2). Statistically significant (P<0.05) difference was observed in serum phosphorus levels between T_1 (control group) and all other treatment groups. There was increased levels of serum phosphorus in all the DDGS as well enzyme supplemented groups in comparison to control group, which corroborated well with the findings of Ali *et al.* (2006), who reported that the addition of enzyme preparation to laying hen diet increased plasma phosphorous level. Dorra *et al.* (2013) also observed significant differences in plasma phosphorous level with different inclusion level of corn DDGS. In the present study the significant difference in blood inorganic phosphorus levels between T_1 and rest of the treatment groups might be due to presence of higher amount of available phosphorus in DDGS and supplementation of phytase in the diet which are in concurrence with the observations of earlier researchers.

CONCLUSION

Based upon the results of this experiment, it is evident that the incorporation of DDGS up to 20% level in the diets of indigenous chicken did not have any adverse effect on the blood bio-chemical constituents of experimental birds. Therefore, it can be concluded that DDGS can be used as a cheap source of protein and energy in the rations of indigenous chicken for economical and profitable poultry production.

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Effect of Inclusion of Black Pepper Powder as Natural Feed Additive on the Performance of Japanese quail

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ABSTRACT

This study was conducted to determine the effect of inclusion of black pepper (BP) powder in the diet on nutrient digestibility, production performance, and carcass characteristics of Japanese quail. One hundred and fifty, day-old quail chicks were randomly allotted into five experimental groups, each with 3 replicates of 10 chicks and were offered respective diets for 5 weeks. BP was ground and was included at 0% (T_1; Control), 0.25 (T_2), 0.50 (T_3), 0.75 (T_4) and 1.0 (T_5) percent levels in iso-caloric and iso-nitrogenous broiler quail diets to meet the nutrient requirements. Results indicated increased body weight gain ($P<0.05$), decreased overall feed intake ($P<0.01$) and improved feed conversion ratio (FCR) ($P<0.01$) with inclusion of BP at 1.0% in the diet as compared to the control.There was a significant increase ($P<0.01$) in the digestibility of gross nutrients and fibre fractions with incorporation of BP at 1.0% in the diet as compared to the control group. Similarly, incorporation of BP at 1.0% level in the diet had increased the carcass yield, ready to cook yield ($P<0.05$), dressing percentage and meat to bone ratio ($P<0.01$) in quails as compared to the control. However, incorporation of BP upto 1.0% level in the diet had no effect ($P>0.05$) on feed cost/kg gain. Thus, it is concluded that BP can be included at 1.0% level in quail diets as natural feed additive without any adverse effects.

Keywords: Black pepper, Carcass characteristics, Nutrient digestibility, Production performance, Quail

INTRODUCTION

Dietary additives have an important role in animal production as growth and health promoters. Black pepper (BP) is one such alternative growth promoter. Black pepper (*Piper nigrum*) is a well-known medicinal plant and has many therapeutic effects like anti-ache effect (Moorthy *et al.,* 2009), anti-oxidant and anti-bacterial effects (Gulcin, 2005). It is a good source of dietary protein and fatty acids for consumption by human and animals. The active principle, piperine present in BP has hepatoprotective and gastroprotective properties and can increase absorption of selenium, vitamin B-complex, β- carotene, curcumin and other nutrients (Khalaf *et al.,* 2008). BP as an alternative to antibiotic growth promoters has been recommended for feeding broilers (Ghaedi *et al.,* 2013; Valiollahi *et al.,* 2013) while some other report observed no significant improvement in body weight gain and FCR (Puvaca *et al.,* 2014).

Quail is considered a prolific meat and egg producer with dual production capacity and thus attained a valuable status in commercial enterprises. They are popular for their high protein (26%) and less fatty (3%) meat (Shinde *et al.,* 2014). However, studies on the feeding value of BP in Japanese quails are very limited. Hence, the present investigation was conducted to study the effect of BP powder inclusion at varying levels in the diet on growth performance, nutrient digestibility, carcass characteristics and cost economics in Japanese quails.

MATERIALS AND METHODS

One hundred fifty, day-old Japanese quail chicks were weighed individually (mean BW, 7.42 ± 0.38 g), wing banded and randomly divided in to 5 equal groups of 3 replicates each with 10 chicks/replicate. The experiment was carried out for 5 weeks in a completely randomized design (CRD). During the experiment, BP seed was ground and included at 0% (T_1; control), 0.25 (T_2), 0.50 (T_3), 0.75 (T_4) and 1.0 (T_5) percent levels in iso-caloric and iso-nitrogenous quail diets (Table 1) to meet the nutrient requirements (NRC, 1994). All the chicks were housed in battery brooders under uniform management conditions. Feed and water were provided *ad libitum*. The feed offered and feed left over was

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Table 1. Ingredient (%) and chemical composition (% DM basis) of quail diets

Constituent	Groups					Cost/kg (₹)
	T_1	T_2	T_3	T_4	T_5	
Maize	47.5	47.5	47.5	47.0	47.0	16.00
De-oiled rice bran	7.5	7.25	7.0	7.25	7.0	13.50
Soybean meal	36.5	36.5	36.5	36.5	36.5	38.00
Fish meal	5.00	5.00	5.00	5.00	5.00	30.00
Black pepper	0.00	0.25	0.50	0.75	1.00	550
Oil	0.50	0.50	0.50	0.50	0.50	20.00
Di-calcium phosphate	1.00	1.00	1.00	1.00	1.00	26.00
Shell grit	1.00	1.00	1.00	1.00	1.00	0.00
Salt	0.25	0.25	0.25	0.25	0.25	3.00
Trace min mix	0.15	0.15	0.15	0.15	0.15	240.0
Feed additives	0.60	0.60	0.60	0.60	0.60	1116.00
Total	100	100	100	100	100	
ME* Kcal/kg	2899.56	2902.39	2905.21	2902.29	2905.11	
Crude Protein (%)	23.98	23.99	23.99	24.00	24.00	
Feed cost/100 kg (₹)	2616	2750	2885	3017	3152	

*Calculated Value

weighed daily, to quantify the feed utilized. The data for growth rate was recorded at weekly intervals.

During the last 3 days of the trial, faeces were collected of birds from all the 5 treatments which were previously fasted for 12 hours to empty the bird gut to mark the beginning and end of faeces collection. At the end of the collection period, the faecal samples collected from each treatment per day were pooled, ground and thoroughly mixed to obtain a homogenous mixture. Samples of feed and excreta samples were analyzed for proximate principles (AOAC, 2007). The retention of a nutrient was calculated by applying the following formula:

Nutrient retention (%) =

$$\frac{\text{Nutrient intake - nutrient excreted}}{\text{Nutrient intake}} \times 100$$

At the end of 5th week, two birds per replicate and thus six birds per treatment were randomly selected, weighed and slaughtered. The data on dressing percentage, carcass yield, meat bone ratio, ready-to-cook yield and per cent weight of heart, liver, gizzard and giblet were recorded. All the data were analyzed statistically (SPSS, version 17) as per Snedecor and Cochran (1993) and comparison of means was done using Duncan's multiple range tests (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

The ingredient composition of quail diets formulated by incorporating BP at varying levels was shown in Table 1. The diets were iso-nitrogenous and iso-caloric with a protein: energy ratio of 1:120. Animal fat was added to the diets to make up the energy deficit. The CP (12.87%) content of BP reported in the present study was higher than the value (9.3%) reported by Bolanle *et al.* (2014) and lower than the value (25.45%) reported by Mohammed *et al.* (2013).

Body weight gain, feed intake and FCR under different dietary treatments are presented in Table 2. The mean body weight gain was higher ($P<0.05$) in quails fed diets containing BP at 0.5,0.75 and 1.0 % level as compared to the control group, but inclusion at 0.25% level in feed did not show any significant effect ($P>0.05$) on body weight gain over control group. The increased body weight gains observed in quails may be attributed to the stimulating effect of BP on the digestive system thus leading to improved body weight gain. Further, the level of BP used reflects the high activity of piperine citrate included in the diet which may have affected the

Table 2. Effect of dietary inclusion of black pepper on performance and feed cost

Parameter	Groups					Level of significance
	T_1	T_2	T_3	T_4	T_5	
Body weight gain (g)	187.73^{a}±3.57	195.04^{ab}±3.52	198.53^{b}±3.48	202.36^{b}±3.41	205.36^{b}±3.70	P<0.05
Overall feed Intake (g)	726.08^{e}±7.07	701.11^{d}±3.37	686.09^{c}±3.45	659.60^{b}±3.73	639.62^{a}±1.15	P<0.01
Feed conversion Ratio	3.90^{c}±0.07	3.63^{b}±0.07	3.46^{b}±0.07	3.26^{a}±0.06	3.11^{a}±0.07	P<0.01
Feed cost / kg gain (₹)	102.02±1.80	99.825±1.90	99.82±1.80	98.35±2.08	98.03±0.86	NS

^{abcde}Means with different superscripts in a row differ significantly

flow of digestive juices across the stomach (Al-Kassie *et al.,* 2011).The results of the present study corroborated with earlier workers who also reported increased body weight gain in quails (El-Tazi *et al.,* 2014; Valiollahi *et al.,* 2014) with incorporation of BP powder in the diet. In contradiction to the present findings, Ndelekwute *et al.* (2015) reported that the final live weight gain was improved (P<0.05) in broilers fed BP at 0.25% in diet while there was a negative effect when fed at 0.75 and 1.0% level. However, Puvaca *et al.* (2014) reported that feeding BP upto 1.0% level in the diet had no effect (P>0.05) on body weight gain in broilers. Feed intake for overall growth period (0-5 weeks) was lower (P<0.01) in quails fed diets incorporated with BP at 0.25, 0.5 and 0.75 % and 1.0% as compared to the control group. Herbal feed additives inhibit the growth and colonization of various pathogenic microorganisms including *E. coli* in the intestinal tract of chicken due to their antimicrobial activity (Galib *et al.,* 2010). When the number of harmful bacteria in the intestinal tract is low, more nutrients are absorbed by the birds. As a result, the birds gain higher weight with lower feed consumption. Another reason for decreased feed intake with the addition of black pepper in the diet might be due to strong and spicy flavour that could reduce the palatability of the feed (Windisch *et al.,* 2008).Similarly, decreased feed intake upon feeding diets containing BP powder were reported by Ndelekwute *et al.* (2015), Ghaedi *et al.* (2013) and Tripathi *et al.* (2013) in broiler chicken. In contrast, increased feed intake upon feeding diets containing BP were also reported earlier (Al-Kassie *et al.,* 2011; Shahverdi *et al.,* 2013; El-Tazi *et al.,* 2014). However, Moorthy *et al.* (2009), Akbarian *et al.* (2012) and Valiollahi *et al.* (2014) reported that feeding BP in broiler diet had no effect (P>0.05) on feed intake.

The FCR improved significantly (P<0.01) with increased level of inclusion of black pepper up to 1.0% level in the diet of quails as compared to those fed control diet. The improved feed conversion ratio observed in quails fed diets containing BP may be attributed to the presence of piperine citrate which probably enhances the secretion of digestive juices in the stomach leading to greater efficiency in utilization of feed resulting in enhanced growth (Al-Kassie *et al.,* 2011). These results corroborated with the findings of

Table 3. Effect of dietary inclusion of black pepper on nutrient digestibility

Parameter	Group					Level of significance
	T_1	T_2	T_3	T_4	T_5	
Dry matter	65.23^{a}±0.08	66.26^{b}±0.40	67.27^{c}±0.55	68.93^{d}±0.38	69.16^{e}±0.90	P<0.01
Organic matter	59.68^{a}±0.33	61.10^{b}±0.20	62.24^{c}±0.10	63.42^{d}±0.09	64.91^{e}±0.24	P<0.01
Crude protein	55.58^{a}±0.67	57.86^{b}±0.18	58.04^{c}±0.29	60.36^{d}±0.42	61.86^{e}±0.33	P<0.01
Crude fibre	47.06^{a}±0.22	49.98^{b}±1.5	50.71^{bc}±1.31	51.63^{bc}±1.91	52.83^{d}±0.39	P<0.01
Ether extract	49.87^{a}±2.33	50.82^{b}±2.19	55.58^{b}±0.49	60.39^{c}±2.12	62.07^{d}±1.15	P<0.01
NFE	50.00^{a}±0.45	51.66^{b}±0.11	52.97^{c}±0.16	54.10^{d}±0.19	55.07^{d}±0.16	P<0.01

^{abcde}Means with different superscripts in a row differ significantly

Table 4. Effect of dietary inclusion of black pepper on carcass characteristics

Parameter	Group					Level of significance
	T_1	T_2	T_3	T_4	T_5	
Carcass yield (g)	118.33^{a}±2.7	123.83^{ab}±2.3	126.17^{b}±2.8	129.17^{b}±2.4	129.67^{b}±1.3	P<0.05
Dressing (%)	58.16^{a}±1.15	59.59^{b}±0.92	60.30^{b}±1.6	60.55^{b}±2.8	60.79^{b}±1.0	P<0.01
Ready to cook yield (g)	109.17^{a}±1.6	113.33^{ab}±2.1	115.50^{bc}±2.1	119.33^{c}±1.5	120.67^{c}±1.8	P<0.05
Meat: bone ratio	3.67^{a}±0.02	4.19^{b}±0.00	4.61^{c}±0.01	5.20^{d}±0.00	5.45^{e}±0.02	P<0.01
Heart (%)	1.50±0.22	1.52±0.22	1.54±0.22	1.67±0.21	1.90±0.16	NS
Liver (%)	4.00±0.40	3.83±0.30	3.67±0.40	3.49±0.20	2.83±0.40	NS
Gizzard (%)	3.83±0.30	4.00±0.02	4.17±0.30	4.33±0.20	4.76±0.20	NS
Giblet (%)	9.33±0.30	9.35±0.20	9.38±0.30	9.39±0.30	9.49±0.80	NS

^{abcde}Means with different superscripts in a row differ significantly

other researchers (Al-Kassie *et al.,* 2011; Ghaedi *et al.,* 2013; Shahverdi *et al.,* 2013; Tripathi *et al.,* 2013; El-Tazi *et al.,* 2014; Valiollahi *et al.,* 2014). In contradiction, Ndelekwute *et al.* (2015) reported poor feed conversion ratio in broilers upon feeding BP in the diet.

Incorporation of BP up to 1.0% level in the diet of quails resulted in increased (P<0.01) digestibility of DM, OM, CP, CF, EE and NFE (%) as compared to the control group (Table 3).The improvement in the digestibility of proximate constituents observed with the addition of BP in the diet might be attributed to the ability of BP to induce saliva secretion, hydrochloric acid and mucus production (Srinivasan *et al.,* 2007). On the other hand, Jang *et al.* (2004) attributed better nutrient digestion to antimicrobial property of the essential oil in BP. In line with the present findings, Ndelekwute *et al.* (2015) also reported increased (P<0.05) digestibility of DM, CP, EE and CF with inclusion of BP upto 1.0% in the diet of broilers.

Data results pertaining to carcass characteristics are presented in Table 4. Inclusion of BP up to1.0% level in the diet of quails resulted in increased carcass yield, ready to cook yield (P<0.05), dressing (%) and meat to bone ratio (P<0.01) as compared to the control. The increase in the carcass yield in quails observed in the present study may be attributed to the presence of essential oils in BP. Similarly, increased carcass yield (Ghaedi *et al.,* 2013; Shahverdi*et al.,* 2013) and dressing per cent(Al-Kassie *et al.,* 2011; El-Tazi *et al.,* 2014) were also reported earlier. However, Moorthy *et al.* (2009) reported that feeding BP powder at 0.2 % level in the diet had no effect (P>0.05) on dressed weight and ready to cook yield of broilers. Inclusion of BP up to 1.0% level in the diet had no effect (P>0.05) on per cent weight of heart, liver, gizzard and giblets in quails, which is in line with the findings of earlier workers (Moorthy *et al.,* 2009; Nath *et al.,* 2012; El-Tazi *et al.,* 2014).

The present study indicated that the feed cost/kg gain decreased by ₹ 2.19 in T_2, ₹ 2.20 in T_3, ₹ 2.67 in T_4 and ₹ 3.99 in T_5 group of quails fed diets containing BP at varying levels as compared to the control group (Table 2). Similarly, El-Tazi (2014) reported that inclusion of a mixture of red pepper and BP upto 1.0% level in broiler diets resulted in higher net profit/kg meat.

CONCLUSIONS

The results of the present study indicated that incorporation of BP powder at 1.0 % level in the diet of quails had improved the performance of quails as evidenced from increased body weight gains, improved FCR and increased digestibility of nutrients in comparison to the control group.Thus, it is concluded that black pepper can be incorporated up to 1.0% level in the diet of quails without any adverse effect.

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Chemical Evaluation and Nutrient Digestibility of Shrimp Waste Meal in Broilers

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ABSTRACT

This experiment was conducted to study the nutrient utilization in broilers fed shrimp waste meal (SWM) at various levels supplemented with or without amino acids as a substitute for fish meal in broiler diets. The percent dry matter, crude protein, ether extract, crude fibre, total ash, nitrogen free extract of shrimp waste meal (SWM) were 94.17, 50.5, 8.2, 15.2, 19.1, and 7.0, respectively. The calcium and phosphorous content of the SWM was 6.0, and 1.2 %, respectively. The percent lysine and methionine were 1.66, and 0.88, respectively. Metabolizable energy content of SWM was calculated as 1515 kcal/kg diet. The experimental diets in pre-starter phase were prepared by replacing fish meal protein of the basal diet with the shrimp waste meal protein at 20% level (T_2), 30% level (T_3); and T_4, T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. In starter and finisher phases, five experimental diets were prepared by replacing fish meal protein of the basal diet with the shrimp waste meal protein at 50% level (T_2), 100% level (T_3); and T_4, T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. The basal diet T_1 was used as control containing maize, SBM, DORB and 10 % fish meal. All diets were iso-nitrogenous and iso-caloric. Metabolism trials were conducted to study the digestibility of nutrients during starter (0-28 days) and finisher (29-42 days) phases. The nutrient digestibility of DM and CF was found to be non-significant among treatments during starter and finisher phase. However, during starter and finisher phases CP and EE digestibilities were significantly ($P<0.01$) higher in birds fed T_4 diet when compared to birds fed other diets (T_1, T_2, T_3 and T_5). The results of the present study conclude that the protein from fishmeal can be safely substituted up to 30% with the SWM protein in pre-starter and up to 50% in starter and finisher broiler diets along with the supplementation of synthetic lysine and methionine.

Key words: Amino acids, Broilers, Chemical composition, Fish meal, Nutrient digestibility, Shrimp waste meal

INTRODUCTION

Escalating cost of poultry feed has promoted the use of alternative feed sources, especially agro-industrial by-products. To avoid the problem of disposal of agro-industrial wastes, which pose threat to the environment, can be better utilized as potential feed resources. Shrimp waste meal is one of such unconventional protein source that has the potential of being an alternative source of protein in broiler rations, partially or totally replacing conventional protein sources like fish meal. Shrimp waste meal is the dried and milled waste of the shrimp industry which consists of heads, shells and appendages of shrimp (Ingweye *et al.,* 2008). Shrimp waste contains high CP content and reasonably good balance of essential amino acids (Ngoan *et al.,* 2000a). It is particularly rich in lysine which makes it an ideal supplement for cereals (Fanimo *et al.,* 1996). It is palatable and has a pleasant aroma. This study was therefore designed to provide an insight into the chemical composition and the nutrient utilization potential of diets containing various levels of SWM with or without amino acids supplementation.

MATERIALS AND METHODS

Shrimp waste meal used in the preparation of experimental diets was procured from Om Sai Aqua Industry, Venkateswarapuram, Nellore district. Feed ingredients like maize, soybean meal, fish meal and de-oiled rice bran (DORB) for preparation of experimental diets were procured from the local market. Representative samples of feed ingredients were analyzed for their proximate composition (AOAC, 2005). The ME values for all feed ingredients except fish meal

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was estimated using equation suggested by NRC (1994), while for fish meal the ME value was derived as per equation suggested by Leeson and Summers (2008). Representative samples of feed ingredients and broiler diets were analyzed for calcium and phosphorous as per the method of Talapatra *et al.* (1940).

Three hundred and seventy-five, day old commercial broiler chicks were distributed randomly to five treatments with three replicates of twenty five birds each. Experimental diets were formulated for pre-starter (0-14 days), starter (15-28 days) and finisher (29-42 days) phases. The experimental diets in pre-starter phase were prepared by replacing fish meal protein of the basal diet with the SWM protein at 20% level (T_2), 30% level (T_3); and T_4, T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. In starter and finisher phases five experimental diets were prepared by replacing fish meal protein of the basal diet with the SWM protein at 50% level (T_2), 100% level (T_3) and T_4, T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. The basal diet T_1 was used as control containing maize, SBM, DORB and 10 % fish meal.

All the five diets during each phase were made iso-nitrogenous and iso-caloric and other ingredients like palm oil, L-Lysine and DL-Methionine were also used to meet the dietary concentration for energy, protein, lysine and methionine as per ICAR (2013) specifications for broiler diets. The CF and AIA content of the basal diets was within the permissible limits of BIS (1992). The synthetic lysine and methionine were added to the T_4 and T_5 diets as the SWM was having these amino acids in lesser amounts as compared to FM.

Metabolism trials were conducted during the starter and finisher phases of the biological trial. Two birds from each replicate, thus a total of six birds per treatment were kept separately in six metabolic cages. Birds in the cages were fed with the respective experimental diets consecutively for 3 days and the total feed offered was weighed and recorded for each cage. Similarly feces voided and feed left over in each cage was carefully collected, weighed and recorded. The representative samples of experimental diets offered and fecal samples from each cage were collected separately and analyzed for dry matter, crude protein, ether extract and crude fiber as per AOAC (2005). All the data obtained in this experiment were subjected to analysis of variance (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The proximate composition of shrimp waste meal revealed that it contained 94.17% DM, on a dry matter basis it contained 50.5 % CP, 8.2 % EE, 15.2 % CF, 19.1 % TA, 7.0 % NFE, 6.0% Ca, 1.2 % P, 1.6 % lysine, 0.88% methionine and 1515 kcal ME /kg diet. The nutrient content (%DM) of feed ingredients, ingredient composition and nutrient content (%DM) of experimental diets are presented in the Tables 1,2,3 and 4.

The CP content of SWM in the present study was 50.5 % which is similar to the values reported by Rosenfeld *et al.* (1997). On the contrary lower, CP values were reported by Okonkwo *et al.* (2012) and Mahata *et al.* (2008). The EE, CF, TA values of SWM used in the present study were higher than those reported by Rosenfeld *et al.* (1997). This variation among different studies may be due to the difference in shrimp species (Ngoan *et al.,* 2000; Heu *et al.,* 2003), source of shrimp meal (head/shell) (Meyers, 1986) and/or processing method, as these can affect the nutritional values of SWM. Shrimp waste meal made from heads contains more CP and EE, less CF and TA than shrimp shells (Meyers *et al.,* 1973).

During starter phase and finisher phases no significant differences were noticed regarding the digestibility of DM and CF among treatments (Table 5 and 6). These findings were on line with Khempaka *et al.* (2011) who reported that DM, OM and ash digestibility values and nitrogen retention did not change significantly when SWM was added in the ration at or below 15% level. Ojewola and Annah (2006) also reported non-significant differences regarding fat, ash, crude fibre digestibility and nitrogen retention among treatment groups fed with SWM.

During starter and finisher phases the digestibility

Table 1. Chemical composition of feed ingredients used in the experiment

Constituents	Ingredients				
	Maize	Soybean meal	Fish meal	De-oiled rice Bran	Shrimp waste meal
Dry matter (%)	90.2	90.8	91.17	93.6	94.17
			%DM		
Crude protein	10.5	45.00	55.00	13.0	50.5
Ether extract	1.50	1.02	2.5	0.80	8.20
Crude fibre	6.7	9.46	2.4	16.8	15.2
Nitrogen free extract	75	36.28	19.6	53.9	7.0
Total ash	6.30	8.24	20.50	15.5	19.1
Calcium	0.01	0.20	6.50	0.06	6.0
Phosphorus	0.13	0.3	3.50	0.80	1.2
Lysine*	0.2	1.28	3.5	0.51	1.66
Methionine*	0.2	0.33	1.19	0.29	0.80
ME (k.cal /kg)**	3300	2300	2200	1400	1515

*Calculated as per Panda *et al.* (1984); **Calculated as per Leeson and Summer (2008).

of CP and EE was significantly (P<0.01) higher in birds fed T_4 ration (50% SWM protein + amino acids) and lower values were observed with the rations in which fish meal protein was completely substituted with SWM protein. Similar findings were reported by Mahata *et al.* (2008) that there was a significant decrease in the

Table 2. Ingredient composition (%) and chemical composition (%) of broiler pre-starter diets

Ingredients	T_1	T_2	T_3	T_4	T_5
Maize	58	57.9	58	57.9	58
Soybean meal	20.55	20.4	20.3	20.34	20.21
Fish meal	10	8	7	8	7.0
Shrimp waste meal	0	2.18	3.27	2.18	3.27
De-oiled rice bran	5	5	4.83	5	4.83
Palm oil	3.7	3.77	3.85	3.77	3.85
Mineral mixture*	2	2.0	2.0	2.0	2.0
DL-methionine	0.2	0.2	0.2	0.21	0.21
L-lysine	0.55	0.55	0.55	0.6	0.63
Feed additives**	+	+	+	+	+
Total	100	100	100	100	10
Chemical composition					
CP (%)	22.1	22.05	22.0	22.08	22.04
ME (kcal/kg)	3002	2998	2999	2997	3000
Lysine (%)	1.18	1.15	1.13	1.20	1.19
Methionine (%)	0.51	0.51	0.50	0.52	0.51
Ca (%)	1.01	1.02	1.02	1.02	1.02
Available P (%)	0.7	0.66	0.63	0.66	0.63
Cost (₹/kg)	28.7	28.96	28.89	29.04	29.04

*Contained Ca, 25; P, 15; NaCl, 2.5; Fe, 0.35% and Cu, 100; Mn, 200; Co, 50; I, 100 ppm; **All diets contained Meriplex® – FDS @ 10g/100 kg : (Each gram contains: Vit-B_1, 8mg; Vit-B_6, 16 mg Vit-B_{12}, 80µg; Vit-E_{50}, 80 mg; Niacin, 120 mg; Folic acid, 8 mg; Calcium D Pantothenate, 80 mg, Merivite®-AB_2D_3K@10g/100kg :(Each gram contains: Vit-A 82,500 IU, Vit-B_2 52mg, Vit-D_3 1200 IU, Vit-K 10mg, Calcium 166 mg, Phosphate 395 mg) and Cosmodot @ 50g/100 kg: (3-5, Dinitro-O-Toluamide:25 percent W/W)

Table 3. Composition of broiler starter and finisher diets

Ingredient	T_1		T_2		T_3		T_4		T_5	
	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher
Ingredient composition (%)										
Maize	59	65	60.25	65	59.51	64.81	60.25	65	59.77	65.24
Soybean meal	19.3	14	19.5	14.2	19.8	14.7	19.39	14.06	19.3	14
Fish meal	10	10	5	5	0	0	5	5	0	0
Shrimp	0	0	5.45	5.45	10.89	10.89	5.45	5.45	10.89	10.89
De-oiled rice bran	5	4.8	3	3.65	2.5	2.5	3.0	3.65	2.5	2.5
Palm oil	4.1	3.8	4.2	4.3	4.7	4.7	4.2	4.3	4.7	4.7
Mineral mixture*	2	2	2	2	2	2	2	2	2	2
DL-methionine	0.17	0.1	0.17	0.1	0.17	0.1	0.18	0.11	0.2	0.13
L-lysine	0.43	0.3	0.43	0.3	0.43	0.3	0.53	0.43	0.64	0.54
Feed additives**	+	+	+	+	+	+	+	+	+	+
Total	100	100	100	100	100	100	100	100	100	100
Chemical composition (%) DM Basis										
CP	21.5	19.55	21.49	19.5	21.5	19.5	21.5	19.6	21.5	19.5
ME (kcal/kg)	3049	3096	3049.3	3102	3048	3099	3047	3099	3049.2	3098
Lysine	1.09	0.92	0.99	0.83	0.99	0.74	1.01	0.93	1.01	0.92
Methionine	0.48	0.4	0.46	0.39	0.4	0.37	0.47	0.4	0.47	0.4
Ca	1.01	1.07	1.01	1.09	1.02	1.01	1.01	1.08	1.01	1.09
Available P	0.7	0.69	0.58	0.57	0.46	0.45	0.58	0.57	0.46	0.45
Cost (₹/kg)	28.39	26.39	28.30	26.53	28.49	26.71	28.53	26.82	29.06	27.56

*Contained Ca, 25; P, 15; NaCl, 2.5; Fe, 0.35% and Cu, 100; Mn, 200; Co, 50; I, 100 ppm; ** ll diets contained Meriplex® – FDS @ 10g/100 kg : (Each gram contains: Vit-B_1, 8mg; Vit-B_6, 16 mg Vit-B_{12}, 80µg; Vit-E_{50}, 80 mg; Niacin, 120 mg; Folic acid, 8 mg; Calcium D Pantothenate, 80 mg, Merivite®-AB_2D_3K@10g/100kg :(Each gram contains: Vit-A 82,500 IU, Vit-B_2 52mg, Vit-D_3 1200 IU, Vit-K 10mg, Calcium 166 mg, Phosphate 395 mg) and Cosmodot @ 50g/100 kg: (3-5, Dinitro-O-Toluamide:25 percent W/W)

Table 4. Chemical composition (%) of broiler basal experimental diets on dry matter basis

Nutrient	Basal diets		
	Pre-Starter	Starter	Finisher
Dry matter	90.1	90.36	91.06
Crude protein	22.04	21.50	19.57
Crude fat	5.37	5.12	5.18
Crude fibre	6.16	6.98	6.96
Total ash	10.40	10.43	10.56
Acid insoluble ash	2.08	2.15	2.16
Nitrogen free extract	56.03	55.97	57.73
Calcium	0.97	1.0	0.94
Phosphorus	0.57	0.55	0.56
Lysine*	1.20	1.09	0.97
Methionine*	0.52	0.48	0.47
ME (k.cal/kg)*	3000	3049	3099

*calculated values

Table 5. Effect of supplementation of shrimp waste meal on digestibility of nutrients during starter phase

Treatment	Digestibility %			
	DM^{NS}	CP**	EE**	CF^{NS}
T_1	66.70±0.32	68.23^{c}±0.10	79.02^{ab}±0.13	30.45±0.09
T_2	66.90±0.17	69.06^{b}±0.36	79.23^{a}±0.23	30.24±0.15
T_3	66.70±0.1	66.78^{d}±0.09	78.49^{b}±0.32	29.94±0.23
T_4	67.60±0.25	70.25^{a}±0.1	79.72^{a}±0.35	30.70±0.06
T_5	66.78±0.26	67.05^{d}±0.07	79.11^{ab}±0.12	30.15±0.28

^{abcd}Values in a column not sharing common superscripts differ significantly **(P<0.01), NS- Non-significant

Table 6. Effect of supplementation of shrimp waste meal on digestibility of nutrients during Finisher phase

Treatment	Digestibility %			
	DM^{NS}	CP**	EE**	CF^{NS}
T_1	70.77±0.2	68.58^{b}±0.22	77.9^{b}±0.21	31.49±0.02
T_2	70.64±0.04	68.99^{ab}±0.29	77.79^{b}±0.28	31.61±0.06
T_3	70.47±0.27	67.22^{c}±0.25	77.08^{c}±0.27	31.39±0.09
T_4	71.22±0.23	69.51^{a}±0.19	78.6^{a}±0.10	31.01±0.33
T_5	70.41±0.14	67.84^{c}±0.17	77.33^{bc}±0.21	31.19±0.25

^{abc}Values in a column not sharing common superscripts differ significantly **(P<0.01), NS- Non-significant

nitrogen retention when SWM was used beyond 8% level of inclusion in the diet. The enhanced nutrient digestibility in birds fed T_4 ration may be due to reduced number of pathogenic bacteria *Escherichia coli, Salmonella typhimurium* (Choi *et al.,* 1994; Le M ieux *et al.,* 2003; Wang *et al.,* 2003), increased immune function (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003), better secretion of digestive enzymes (Hou and Gao, 2001) and better absorption of nutrients (Wu, 1998; Huang *et al.,* 2005; Li *et al.,* 2007; Khambualai *et al.,* 2008, 2009).

The exoskeleton of the shrimp is composed mainly of chitin, an N-acetylated glucosamine polysaccharide that forms part of the protein complex, and is considered to have low digestibility when fed to animals (Austin *et al.,* 1981). Inclusion of SWM at higher levels in broiler diets caused impairment of digestion due to increase in chitin levels as it physically blocks the access of digestive enzymes to lipids and proteins, thus affecting the utilization of these nutrients (Castro *et al.,* 1989; Karasov, 1990).

CONCLUSION

Results indicates that shrimp waste meal can be utilized as a potential alternative protein source to fish meal in broiler diets. Along with the supplementation of synthetic lysine and methionine, the protein from FM can be safely substituted up to 30% with the SWM protein in pre-starter and up to 50% in starter and finisher broiler diets.

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Growth Performance, Carcass Traits and Economics of Kadaknath birds Rearing under Intensive Condition in Hot and Humid Climate

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ABSTRACT

This experiment was conducted to study the growth performance, carcass traits and economics of Kadaknath chicken reared under intensive managemental condition in hot and humid climate of Odisha. A total of 104 day-old straight run chicks of Kadaknath breed of chicken were taken for this experiment, divided into 4 replicates, each comprising of 26 chicks and reared in deep litter system. The weekly body weight, feed conversion ratio (FCR), and mortality of birds were recorded upto 20th weeks of age and carcass traits were studied at the end of the experiment. No significant difference of body weight gain (P>0.05) between male and female chicks was noticed upto 4th week, while males showed higher (P≤0.05) body weights gains from 5th to 20th week of age. The incidence of mortality recorded were 3.8% in the first week of age, whereas, no further mortality of birds was noticed during the rest of the experimental period. Males had higher (P< 0.01) slaughter weight, eviscerated weight and drumstick percentage, while females had higher (P<0.01) breast and giblet percentage. Breast muscle showed higher crude protein, total ash compared to thigh muscle. From the above study it is concluded that Kadaknath breed can profitably be reared under intensive system of management in hot and humid climatic condition.

Key words: Carcass trait, Economics, Kadaknath chicken, Intensive rearing, Growth

INTRODUCTION

Kadaknath is an important indigenous breed of poultry inhabiting vast areas of Western Madhya Pradesh, mainly the Jhabua and Dhar Districts and adjoining areas of Gujarat and Rajasthan. It tolerates extreme climatic conditions and thrives very well under minimal management inputs. It shows appreciable degree of resistance to diseases compared to other exotic breeds of fowl (Thakur *et al.,* 2006). Back yard poultry farming is a part and parcel of typical rural/tribal household. The meat and eggs are also reckoned to be a rich source of protein (Rao and Thomas 1984). The meat of the Kadaknath breed contains high percentage (25.47%) of protein and is believed to have aphrodisiac properties (Mohan *et al.,* 2008). Continual efforts are to be made for the improvement of this breed through effective healthcare, nutrition, management and breeding. However, most of the experiments that have been conducted on Kadaknath are under free range system of management. The present investigation was planned to study the growth performance and carcass traits of Kadaknath poultry birds reared intensive system in hot and humid climate of Odisha.

MATERIALS AND METHODS

The research work was carried out at the Central Poultry Development Organization (CPDO), Eastern region, Bhubaneswar from February to June. The maximum and minimum temperature and relative humidity were recorded daily and mean was calculated weekly. The weekly average maximum temperature ranged from 31.30 to 39.50°C, minimum temperature ranged from 17.80 to 28.10°C. Similarly, the weekly average maximum relative humidity ranged from 79.00 to 95.00 % and minimum ranged from 32.00 to 75.00 %. The experiment was conducted on 104 day-old straight run chicks of Kadaknath breed. Birds were divided into 4 replicates, each comprising of 26 chicks. All chicks were vaccinated with Marek's vaccine and

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wing banding was done at hatchery before transfer of chicks to the experimental shed. The experimental birds were reared under deep litter system and placed in the naturally ventilated house providing with 1 sq ft floor space per bird. Male and female birds were differentiated at 14th week by observing their comb pattern, then at 21 weeks of age birds were shifted to layer house. During the experiment, birds were offered weighed quantities of feed starting from day one till the end of the experiment as per the feeding practices carried out at CPDO, Bhubaneswar. All the birds were fed chick mash (0-8 weeks of age), grower mash (9-16 weeks of age) and breeder mash (17 week onwards). The weighed quantity of feed was offered daily in the morning and in the afternoon while fresh drinking water was supplied ad libitum. The composition of rations fed to the birds during different phases is presented in Table.1.

The body weight of birds was recorded individually at weekly intervals upto 20th weeks of age using a digital electronic balance. The birds were weighed in the morning before supplying feed. The weekly body weight gain at a given age was found out by deducting the body weight of the previous week from that of the current week. Daily feed given was weighed and recorded. Feed consumption was calculated by subtracting the residual feed at the end of each week from the total feed provided during that week to the birds in each replicate, from which cumulative feed consumption was calculated. Feed conversion ratio (FCR) was derived by dividing the feed consumed with weekly body weight gain. Mortality of chicks was recorded daily. The mortality of all the chicks of the replicate groups were added and expressed on weekly basis.

At the end of twenty weeks, 6 healthy birds (3 male and 3 female) were sacrificed for the carcass parameters. Before slaughter the selected birds were separated from the flock and fasted overnight but drinking water was provided *ad libitum* to facilitate proper bleeding and also to know their actual live weights. The birds were slaughtered by severing the jugular vein and

Table 1. Composition of rations provided to the birds

Feed ingredients (kg)	Chick mash (0-8 weeks)	Grower mash (9-16wks)	Breeder mash (17th week onwards)
Crushed yellow maize	45	45	45
Rice polish	22	25	15
Soya bean meal	30	19	27
De-oiled rice bran	-	8	6
Mineral Mixture	3	3	3
Shell grit	-	-	4
Total	100	100	100
Nutritive value (% DM basis)			
CP	20.15	16.20	17.95
ME (kcal/kg)*	2608	2515	2588
Ether extract	4.15	3.98	4.25
Crude fibre	4.65	8.50	5.25
Total ash	10.52	11.25	9.85
AIA	3.52	4.56	4.58
NFE*	53.25	51.25	52.82
Calcium	1.20	1.12	3.45
Lysine*	1.23	0.85	1.10
Methionine*	0.65	0.54	0.60

*Calculated value

carotid artery below the left ear lobe by a single incision and allowed to bleed for five minutes by holding the birds' heads down. After complete bleeding and cessation of movement the carcass weight was recorded. The carcass was then scalded at 55-58°C for 1 minute 30 seconds. This helps in easy removal of feathers and gives uniform skin colour along with good flesh quality.

The eviscerated carcass along with the edible offal was weighed and recorded as edible carcass yield. The total meat yield was calculated by subtracting the giblet weight (the weight of the heart without pericardium, liver without gall bladder and gizzard without the serous lining) from the weight of the edible carcass.

$$1.\ \text{Dressing}\ \% = \frac{\text{Dressed weight}}{\text{Live weight}} \times 100$$

$$2.\ \text{Giblet weight}\ \% = \frac{\text{Giblet weight}}{\text{Live weight}} \times 100$$

$$3.\ \text{Neck}\ \% = \frac{\text{Neck weight}}{\text{Eviscerated weight}} \times 100$$

$$4.\ \text{Wing}\ \% = \frac{\text{Wing weight}}{\text{Eviscerated weight}} \times 100$$

$$5.\ \text{Back}\ \% = \frac{\text{Back weight}}{\text{Eviscerated weight}} \times 100$$

$$6.\ \text{Breast}\ \% = \frac{\text{Breast weight}}{\text{Eviscerated weight}} \times 100$$

$$7.\ \text{Thigh}\ \% = \frac{\text{Thigh weight}}{\text{Eviscerated weight}} \times 100$$

$$8.\ \text{Drumstick}\ \% = \frac{\text{Drumstick weight}}{\text{Eviscerated weight}} \times 100$$

The proximate composition such as moisture, crude

Table. 2. Body weight, cumulative feed intake and cumulative FCR

Age (weeks)	Body weights (g)			Cumulative feed intake/ bird (g)	Cumulative body weight gain (g)	Cumulative FCR
	Male	Female	Combined			
1	57.00±1.47	53.08±1.13	53.89±0.71	100±0.32	31.95±0.17	3.13±0.01
2	105.40±1.61	94.63±2.08	96.87±1.28	220.00±0.70	74.93±0.53	2.93±0.02
3	155.90±2.62	138.16±3.34	141.93±2.04	350.00±0.72	119.99±0.78	2.91±0.01
4	212.80±4.57	182.026±4.94	188.43±3.16	494.0±0.50	166.49±1.20	2.96±0.04
5	278.00±6.84**	232.47±6.80	241.95±4.45	652.0±0.75	220.01±0.64	2.96±0.02
6	348.80±9.55**	293.55±8.12	305.06±5.42	826.0±1.04	283.66±0.39	2.91±0.03
7	416.40±11.56**	349.00±9.73	363.04±6.53	1022.0±0.91	341.10±2.14	2.99±0.04
8	506.40±15.96**	413.31±11.30	432.35±8.08	1232.0±0.75	410.41±1.78	3.00,0.05
9	591.50±18.02**	476.44±12.30	500.41±9.12	1460.0±1.29	478.47±1.98	3.05±0.06
10	698.30±18.58**	559.52±13.06	588.43±10.02	1702.0±1.43	566.49±2.06	3.00±0.04
11	787.00±16.82**	628.71±9.04	661.68±10.31	1962.0±1.82	639.74±2.43	3.06±0.12
12	905.70±19.02**	706.86±9.65	748.29±11.91	2236.0±1.38	726.35±1.54	3.07±0.03
13	922.80±18.32**	718.47±10.03	761.04±12.20	2490.0±2.21	739. 10±1.25	3.36±0.02
14	1028.40±20.45**	790.84±10.73	840.33±13.68	2776.0±1.32	818.39±2.87	3.40±0.04
15	1054.80±17.81**	821.50±11.48	870.10±13.78	3086.0±0.48	848.16±3.14	3.63±0.02
16	1143.80±21.42**	885.23±12.72	939.10±15.37	3368.0±1.88	917.16±2.16	3.67±0.03
17	1214.30±22.94**	941.42±13.76	998.27±16.42	3662.0±3.50	976.33±3.18	3.75±0.05
18	1274.20±23.95**	990.86±14.37	1049.89±17.09	3978.0±3.10	1027.95±3.36	3.86±0.06
19	1323.80±25.35**	1043.44±14.81	1101.85±17.33	4308.0±2.72	1079.91±2.74	3.98±0.04
20	1380.80±24.99**	1098.36±15.08	1157.20±17.51	4652.0±4.21	1135.26±3.48	4.10±0.07

**Mean differ significantly within the rows (P<0.01)

protein, ether extract and total ash content of the chicken meat from the breast and thigh muscles were measured according to the procedure of AOAC (1995). The data obtained from the study were statistically analysed according to Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The body weight of males birds were 1028.40 g and female were 790.84 g at 20th week (Table-2). It was observed that males have significantly (P<0.01) higher body weight than females from 5th week to 20th week. But no difference in body weight was found upto 4th week. This may be due to higher rate of cell multiplication in males as well as influence of androgenic hormones on the growth rate of males (Chandra *et al.,* 2004). In contrast to the present findings, Pathak *et al.* (2015) recorded higher body weights of Kadaknath at different ages *i.e.* from day old to 20 weeks under intensive system of management, but Jha *et al.* (2013) reported lower body weights for the same ages of Kadaknath. However, Thakur *et al.* (2006) found significantly lower body weights for the same genetic group under free range system. The higher body weight in intensive system might be due to the supplementation of balanced diet and other proper managemental care.

The body weight gains increased progressively from 1st week upto 20th week. The male chicks recorded significantly (P<0.01) higher cumulative body weight gain compared to female chicks from 5th week to 20th week. In this study, at the age of 20th week, the body weight was 1157.20 g which was comparable with the value of 1145.73 g for indigenous chickens reared under intensive system of management as reported by Doley *et al.* (2009). Thakur *et al.* (2006) reported that Kadaknath attain 886.12 g body weight at 20 weeks of age with an overall gain of 858.68 g during 0-20 weeks under field condition which was much lower than the present findings of 1135.26 g gain in body weight during the same age which could be attributed to better feeding and managemental practices adopted in the present study. Jha and Prasad (2013) reported that the body weight of Vanaraja, Gramapriya and Aseel at 20th week was 2013.39, 1574.31 and 1038. 75 g respectively. It was observed that the bodyweight of Kadaknath was comparable with Aseel birds.

Data pertaining to the weekly feed intake and cumulative feed intake of the birds are presented in Table 2. The cumulative feed intake per bird increased progressively. At the end of 20th week the cumulative feed intake of Kadaknath bird was 4652. 00 g and the

Table. 3. Carcass traits and percentage yield of different cut up parts

Parameter	Male (g)	Female (g)
Live body weight (g)	1150.33±25.78*	964.66±2.96
Dressing (%)	74.17±1.33	75.46±0.52
Eviscerated weight	812.66±24.53*	686.33±6.64
Heart (g)	3.66±0.33	3.66±0.33
Liver (g)	17.00±2.00	18.00±0.57
Gizzard (g)	19.66±1.33	20.00±0.57
Giblet (%)	3.50±0.22	4.31±0.08*
Neck (%)	8.88±0.26	7.91±0.1
Wing (%)	13.52±1.13	15.10±0.07
Breast (%)	23.16±0.11	24.86±0.06**
Back (%)	21.06±1.08	20.30±0.23
Thigh (%)	16.96±0.44	16.70±0.04
Drumstick (%)	16.32±0.08**	15.10±0.03

*/** Mean differ significantly within rows (P≤0.05, P≤0.01)

Table. 4. Meat quality traits

Parameters (%)	Thigh muscle	Breast muscle
Moisture	74.83±0.60	73.77±0.40
Crude protein	80.08±0.90	83.31±0.44
Ether extract	9.07±0.40	6.26±0.13
Crude fibre	0.57±0.01	0.58±0.02
Total ash	3.66±0.15	7.75±0.18
Acid insoluble ash	0.06±0.01	0.08±0.01

cumulative body weight gain was 1135.26 g. The Feed conversion ratio of the birds upto 4th, 8th, 12th, 16th and 20th week were recorded to be 2.96, 3.00, 3.07, 3.67 and 4.10, respectively. An FCR value of 5.24 at 40 weeks of age was reported by Jha *et al.* (2013) in Kadaknath birds maintained under deep litter system of management. The mean cumulative FCR was 3.07 at 12th weeks of age which was in agreement with the observation of Gupta and Mehta (2016). They also found the FCR of 3.18 at 12 weeks of age in Kadaknath birds kept in deep litter system of housing. Jha and Prasad (2013) also reported that the FCR (upto 40th week) of the three coloured breeds like Vanaraja, Gramapyiya and Aseel were 4.28, 3.85 and 5.47, respectively.

During the whole experimental period mortality rate of 3.8% was recorded during starter period (0-8 weeks) which was lower than 12.86% reported by Malik *et al.* (2009). On the other hand, the mortality of birds was higher under extensive system (17.13%) followed by those under semi-intensive system (15.28%) and intensive system (14.35%), but the differences were statistically non-significant in indigenous chicken as reported by Doley *et al.* (2009).

Data pertaining to mean carcass traits for the male and female birds are presented in Table 3. The live weight of males and females that was selected for carcass study was 1150.33 g and 964.66 g, respectively. Similarly the eviscerated weight was 812. 66 and 686.33 g in males and females, respectively. As the live weights of the male birds was higher than females at 20 week, the eviscerated weight was also significantly higher in male birds. The breast yield (%) of the female birds was higher whereas the drumstick % was higher in male birds. No difference was observed with respect to other cuts between the sex. Haunsi *et al.* (2013) reported lower dressing percent of 65-66% in Kadaknath and Aseel bird. But in the present experiment higher dressing % was observed. Other cut up parts percentage were similar as reported by Haunsi *et al.* (2013). Sharma and Naryankhedkar (2005) reported comparable carcass traits in the crosses of Kadaknath breeds.

From the proximate composition of thigh and breast muscle of male birds, it was found that the protein content of breast muscle (83.31%) was higher than thigh muscle (80.08%), whereas the ether extract of the thigh muscle was 9.07% as against 6.25% in breast muscle (Table 4). No difference was observed in moisture, crude fibre and acid insoluble ash content of both the muscle, but the total ash content of breast muscle was higher than the thigh muscle. Similar findings were also reported by Haunsi *et al.* (2013) who found higher protein value of 83.75 and 87.65 % for thigh and breast muscle, respectively from the meat of 20 week male Kadaknath bird. The economics of Kadaknath rearing in the intensive system was evaluated on 20 week taking the feed consumption and body weight gain (Table 5). From the dressing (%) the

Table. 5. Economics of production

Particulars	Values
Cumulative feed intake/bird (g)	4652
Cost of feed (₹/kg)	27.01
Feed cost/bird (₹)	125.60
Cumulative body weight gain/ bird (g)	1135.25
Feed cost/ kg live weight gain (₹)	110.63
Dressing (%)	74.81
Feed cost/kg of meat (₹)	129.41

feed cost per kg of meat was also evaluated. The cumulative FCR up to 20th week was 4.1. As the 20th week cumulative weight gain was 1135.25 g and feed intake was 4652 g, the feed cost per bird was ₹ 125.60 feed cost and per kg live weight gain was ₹ 110.63. Taking the dressing % of 74.81 (male birds) into account, the feed cost per kg of meat produced was ₹ 129.41.

CONCLUSION

From the above study it could be concluded that Kadaknath breed of chicken can be reared under intensive system of management in hot and humid climatic condition. From the economics of production it was found that the cost of production of per kg live weight gain was ₹ 110.63.

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Effect of Dietary Supplementation of Carrot Meal on Survival, Growth and Pigmentation of Freshwater Ornamental Fish, Koi Carp, *Cyprinus Carpio* (L.)

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ABSTRACT

The study was conducted to assess the efficacy of experimental diets supplemented with carrot meal (CM) @ 1, 3, 5 and 7 % with respect to pigmentation, survival and growth in freshwater ornamental fish koi carp, *Cyprnius carpio* (Linnaeus) for 120 days. Five diets including one control (CM0) without any supplemented carotenoid source and four supplemented experimental pelleted diets with 1% (CM1), 3% (CM3), 5% (CM5) and 7% (CM7) of powdered carrot meal were prepared. At the termination of the experiment, colouration studies indicated significantly higher ($P<0.05$) skin and muscle carotenoid (μg g^{-1} wet weight basis) content in fish fed with CM5 (4.87) followed by diets CM3 (3.28), CM7 (2.78), CM1 (1.95) and CM0 (1.24). RGB colour intensity based on digital photographs analysis was also found to be highest in CM5 followed by CM3, CM7, CM1 and CM0. Fish growth in terms of % NWG, PER and SGR was highest in CM5 (94.09%, 1.48, 0.55), with lowest values of NWG and SGR in CM7 (65.71% and 0.41), while PER in CM1 (0.75). Further, supplementation of CM @ 5% in Koi carp diet also improved the FCR (2.16) as compared to control (4.17). 100% survival of fish was recorded with diets supplemented with CM @ 3, 5 and 7 %, while it was 80 % in CM0 and CM1. During the experimental period, the water quality parameters did not vary significantly among control and treatments and remained in optimum range for fish growth. Hence, carrot meal can be supplemented in koi carp diet up to 5 % level both as colour enhancer as well as growth promoter under indoor rearing conditions.

Key words: Carotenoids, Carrot meal, Colour enhancement, Koi carp, Pigmentation

INTRODUCTION

Ornamental fish are nature's wonderful creation and ornamental fish keeping is the second most preferred hobby in the world and this sector plays a vital role in the international fish trade, having high export value (Jain *et al.,* 2016). The value of ornamental fish is determined by their unique body characteristics, good health and coloration. The beautiful and flamboyant coloration exhibited by ornamental fishes is due to the presence of carotenoid pigments along with overall nutrition. Therefore, there is a direct relationship between dietary carotenoids and pigmentation in fish (Halten *et al.,* 1997). Carotenoids are the main pigments, representing a class of over 600 natural lipid soluble pigments found in plants, algae, photosynthetic, some non-photosynthetic bacteria and animals, which are required for healthy growth, metabolism, photosynthesis and reproduction, besides colour development. Chemical composition of carotenoids include approximately 20% β-carotene, 30% γ-carotene and 30 % lycopene (oxygen free hydrocarbons) and 15% of oxygen containing carotenoids *i.e.* xanthophylls (Zeb, 2004). Fish contain various kinds of carotenoids, the proportion of which depends upon their physiological status and dietary conditions. Synthetic or natural carotenoids are required to be incorporated @ 50-400 mg kg^{-1} in diet of indoor reared fish to develop colour similar to fish feeding on live food like algae, tubifex, zooplankton, *etc.* (Boonyaratapalin and Lovell, 1977) under outdoor conditions.

For supply of carotenoids, various synthetic as well as natural sources (animal and plant) are available and are being developed for use in aquaculture for food as well as ornamental fishes. Synthetic processes results in production of only specific carotenoids like β-carotene or astaxanthin, whereas, natural sources are always a combination of several carotenoids

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like α-carotene, β-carotene, zeaxanthin, lutein, cryptoxanthin *etc.* (Gupta *et al.,* 2007). Moreover, synthetic carotenoids are expensive and their use in aqua feed is limited due to species specificity. Natural carotenoids of animal origin are mostly extracted from crustacean such as crayfish meal, shrimp meal, crab meal, *etc.* (Torrissen and Naevdal, 1984) and are rich source of astaxanthin. The major concerns with the production of animal-based carotenoid are limited supply due to declining catches of crustaceans along with high expenses, which leads to increase in cost of carotenoid incorporated aquaculture diets. Plant based natural carotenoids are mainly derived from flowers, fruits, vegetables, algae, yeast, *etc.* (Tsushima and Matsuno, 1998; Chapman, 2000), which are not only inexpensive, but also a potential source of mixed carotenoids.

Few studies have demonstrated the effect of natural carotenoids on the growth, survival, colour development and antioxidation responses in ornamental fishes. Among natural vegetable pigment source, carrot (family Apiaceae) is one of the important root vegetables rich in bioactive compounds *i.e.* carotenoids along with several other functional components having significant health promoting properties (Sharma *et al.,* 2012). The total carotenoid content in the edible portion of the carrot root range from 6-55 mg/100g (Simon and Wolff, 1987) with β-carotene (44-79 % of total carotenoids, 5.3 – 10 mg/100 g) as major component (Holland *et al.,* 1991) and is the single major source of β-carotene providing 17% of total vitamin A. Due to these properties, carrot alone or in combination with other natural antioxidants, is found to induce better growth, enhance colour intensity and improve health of ornamental fish in a natural way. In view of unique properties of carrot, the present study was designed to assess the efficacy of carrot (*Daucus carota*) meal on survival, growth and pigmentation of ornamental Koi, *C. carpio* (Linn.).

MATERIALS AND METHODS

The experimental fish, Koi carp, *C. carpio* L. were procured from ornamental fish culture unit of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The fishes were acclimatized under indoor conditions for one month with control diet. Ten uniformly sized fishes (av. length 2-4 cm, av. weight 1-2 g) were stocked in glass aquaria, having 50 liters of water with continuous oxygen supply. The experiment (in triplicate) was carried out for 120 days.

Four experimental supplemented pelleted diets were prepared by adding carotenoid source *i.e.*, carrot meal (CM) @ 1 % (CM1), 3 % (CM3), 5 % (CM5) and 7 % (CM7) in the basal diet (CM0) by adjusting the level of other feed ingredients (Table 1). Carrot was procured from market, washed, grated and dried under shade and grounded to prepare the powdered meal. All the feed ingredients were powdered, mixed thoroughly

Table 1. Composition (%) of experimental diets

Ingredients	Experimental diets				
	CM0(Control)	CM1	CM3	CM5	CM7
Soybean meal*	25	25	24.5	24	24
Groundnut meal*	25	25	24.5	24	23
Mustard meal*	25	25	24	24	23
Rice bran*	20	19	19	18	18
Vegetable oil	02	02	02	02	02
Vitamin-mineral mixture	02	02	02	02	02
Salt	01	01	01	01	01
Carrot meal	-	1	3	5	7

*Solvent extracted

and palletized. The proximate composition of feed ingredients and experimental diets (Table 2) was analyzed by following the methods of AOAC (2000). Carotenoid analysis of pigment source (carrot meal) was done by following method of Cyanotech (2002). Pelleted feed was provided to the fish @ 5 % fish body weight twice a day. Amount of feed was adjusted at every sampling (fortnightly interval) according to increase in fish weight.

Water quality parameters in terms of water temperature, pH, dissolved oxygen, total alkalinity, total hardness and ammonical nitrogen were measured at fortnightly intervals by following the methods of APHA (1991). Survival of the fish was calculated by subtracting the number of fishes harvested at the end of the experiment from the fish stocked at the initiation of the experiment.

Fish growth in terms of total body length and body weight was recorded at fortnightly intervals. At the end of the experiment, total length gain (TLG), per cent total length gain (%TLG), net weight gain (NWG), percent net weight gain (% NWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and condition factor were calculated as follows

TLG = Final total body length (cm)-initial total body length (cm)

%TLG = Final total body length (cm)-initial total body length (cm) /Initial total body length (cm) ×100

NWG = Average final body wt. (g) - Average initial body weight (g)

%NWG = Final body weight (g) - initial body weight (g) /initial body weight (g) × 100

SGR (% increase in weight /day) =

$$\frac{\ln \text{final body weight} - \ln \text{initial body weight}}{\text{Culture days}} \times 100$$

Where, ln = Natural logrithum

FCR = Feed given (g) / Weight gain (g)

PER = Weight gain (g) / Protein intake (g)

Condition factor (K) =

Body weight / (Body length)^3 ×100

Carotenoid analysis of fish muscle and skin was done by following method of Olson (1979) at initiation, middle and termination of the experiment. The digital photographs of experimental fish were taken at monthly intervals with a Sony full HD camera (DSC-HX 300). Colour of the fish skin was measured from opercular region, base of dorsal fin and caudal peduncle area of at least three fishes from each replicate. While taking the photographs all camera conditions were kept constant.

Table 2. Proximate composition (% DM basis), gross energy (kcal/g) and total carotenoid content (mg/ 100g) of carrot meal and carrot meal incorporated diets

Ingredients/ feed	Crude protein	Ether extract	Crude fibre	Ash	NFE	Gross energy (kcal/g)	Carotenoid content (mg/ 100g)
Rice bran*	21.94	1.46	10.86	7.00	58.72	3.77	-
Ground nut meal*	35.62	0.76	20.26	8.40	34.94	3.50	-
Mustard meal*	34.67	0.76	14.40	8.00	41.56	3.78	-
Soybean meal*	39.37	1.46	9.20	6.13	43.82	4.14	-
Carrot meal	17.33	1.33	10.33	8.50	59.03	3.50	16.45
CM0	31.93	1.04	13.20	7.60	46.46	3.79	-
CM1	31.20	1.06	13.06	6.95	47.73	3.81	0.152
CM3	31.38	1.08	13.04	7.12	47.38	3.81	0.431
CM5	31.20	1.06	12.93	7.16	47.65	3.81	0.670
CM7	30.85	1.00	12.81	7.11	48.23	3.80	0.987

*Solvent extracted

Photographs were analyzed in adobe photoshop (Hancz *et al.,* 2003; Tlusty, 2005) to compare these on the basis of RGB (Red, Green and Blue) values. To quantify the colour of the fish skin from digital photographs, CIE (Commission International de l'Eclairage) Lab is applied. Adobe Photoshop has a colour space called "Lab colour mode" which is based on CIE Lab. It consists of three parameters: L*, a* and b*. Where L* stands for luminosity (lightness ranging from 0 for black to 100 for white), channel a* is the balance between red / green and channel b* describes the balance between yellow/ blue.

RESULTS AND DISCUSSION

The study revealed that during the experimental period, water quality parameters (Table 3) such as temperature, pH, dissolved oxygen, total alkalinity, total hardness and ammonical-nitrogen did not vary significantly among the treatments and were within the recommended range for ornamental fish culture (Wurts and Durborow, 1992; OATA, 2008; Rinna *et al.,* 2013).

At the termination of the experiment, 100% survival of fish was recorded with diets supplemented with CM @ 3, 5 and 7 %, while it was 80 % in control (CM0) and CM1 (Table 4), indicating positive effect of carrot on survival of fish. According to Krinsky (1993),

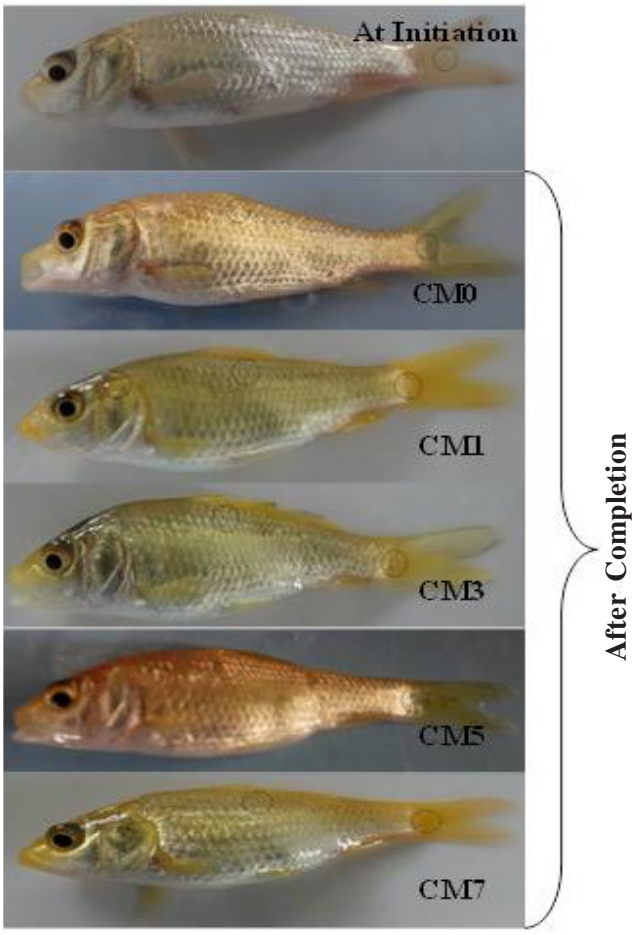

Fig. 1. Changes in skin colour of Koi carp, *C. carpio* L. before and after feeding

Table 3. Physico-chemical parameters of water in different treatments

Parameters	Diets				
	CM0(Control)	CM1	CM3	CM5	CM7
Water temperature (°C)	29.28^{a}±0.50	29.15^{a}±0.62	29.36^{a}±0.57	29.00^{a}±0.65	29.24^{a}±0.64
	(27.50-31.76)	(26.66-31.70)	(26.73-31.93)	(26.20-31.86)	(26.33-32)
pH	8.59^{a}±0.08	8.50^{a}±0.07	(8.24-8.75)	8.57^{a}±0.09	8.61^{a}±0.09
	(8.33-8.90)	(8.24-8.76)	8.51^{a}±0.07	(8.20-8.97)	(8.18-9.02)
Dissolved oxygen (mgl^{-1})	6.78^{a}±0.29	6.86^{a}±0.28	6.91^{a}±0.28	7.04^{a}±0.28	7.07±0.22
	(5.86-8.40)	(5.86-8.40)	(5.80-8.20)	(6.20-8.20)	(6.33-8.20)
Total alkalinity(mg $CaCO_3l^{-1}$)	211.14^{a}±12.2	203.33^{a}±9.98	214.67^{a}±11.79	214.22^{a}±10.92	219.44^{a}±14.1
	(140-254)	(154-254)	(160-256)	(150-257)	(148-277)
Total hardness(mg $CaCO_3l^{-1}$)	266.56^{a}±5.5	269.33^{a}±6.1	275.89^{a}±5.7	274.11^{a}±4.0	266.22^{a}±6.68
	(235-282)	(246-298)	(255-296)	(252-292)	(229-296)
NH_3-N (mgl^{-1})	0.185^{a}±0.020	0.179^{a}±0.022	0.159^{a}±0.027	0.188^{a}±0.024	0.171^{a}±0.024
	(0.116-0.308)	(0.063-0.251)	(0.045-0.260)	(0.049-0.268)	(0.0440.249)

Values are Mean ± S.E.; Values with different superscript (abcd) in a row differ significantly (P<0.05); values in parentheses indicate range

absorption of carotenoids enhances fish survival. In agreement to present study, Mirzaee *et al.* (2013) recorded colour enhancement in jewel cichlid (*Hemichromis bimaculatus*) with carrot as compared to red pepper. Likewise, Kop *et al.* (2010) reported carrot as an efficient carotenoid source in comparison to red pepper (*Capsicum annum*) for colour enhancement in cichlid (*Cichlasoma severum*). Comparison of carrot (*D. carota*) with three flower pigment sources *i.e.* marigold petal (*Tagestes erecta*), China rose petal (*Hibiscus rosasinensis*), rose petal (*Rosa cinensis*) also revealed that carrot resulted in significantly higher carotenoid content in fish skin/muscle (Ramamoorthy *et al.,* 2010). Yanar and Tekelioglu (1999) also suggested utilization of carrot as potential carotenoid source for colour development in gold fish, *Carassius auratus.*

At the end of culture period, maximum average final total body length (cm) was observed in CM1 followed by CM7, CM5 and CM0 and CM3, respectively and the differences among treatments were significant (P<0.05). TLG and % TLG were also maximum in CM1 and minimum in CM3 and the differences among different treatments were significant (P<0.05). The final body weight was highest in CM5 followed by CM1, CM0, CM3 and CM7 with significant differences. Likewise, NWG, %NWG and SGR was highest in CM5 and minimum in CM7. PER and CF were highest in CM5, while PER was lowest in CM0, and CF was lowest in CM1. Feed efficiency in terms of FCR also improved maximally in CM5 as compared to all other treatments and control.

Improved fish growth has been found in pigment supplemented diets as carotenoids are known to play a positive role in the metabolism of fish and might have improved nutrient utilization leading to improved growth (Amar *et al.,* 2001). In the present study also, it is clear that carotenoid do play a role in fish growth, as increasing level of CM (1-5%) resulted in positive effects on growth, in terms of increased weight gain and reduced FCR. Mirzaee *et al.* (2013) conducted comparative study to see the effect of carrot and red pepper (both providing 60 mg/kg of total pigment) on growth of jewel cichlid. Feeding of carrot resulted in

Table 4. Growth performance and of koi carp, *C. carpio* L. in different treatments

Parameters	Diets				
	CM0(Control)	CM1	CM3	CM5	CM7
% Survival	80	80	100	100	100
Final total body length (cm)	6.24^{bc}±0.20	6.48^{a}±0.19	6.13^{c}±0.21	6.24^{bc}±0.11	6.26^{bc}±0.06
TLG	0.70	0.96	0.62	0.82	0.67
	---	(+26.32%)	(-11.43%)	(+17.14%)	(-4.28%)
%TLG	12.63	17.39	11.25	14.49	11.98
Final body weight (g)	3.83^{cd}±0.44	4.18^{b}±0.26	3.62^{cd}±0.21	4.27^{a}±0.24	3.48^{cde}±0.16
NWG	1.60	1.96	1.44	2.07	1.38
		(+22.50 %)	(-10.00 %)	(+29.37 %)	(-13.75 %)
% NWG	71.74	88.28	66.05	94.09	65.71
FCR	4.17	3.40	3.32	2.16	3.46
PER	0.75	0.94	0.95	1.48	0.93
SGR	0.45	0.53	0.43	0.51	0.42
CF	1.57	1.53	1.57	1.75	1.41

Values are Mean ± S.E.; Values with differrent superscript (ab…d) in a row differ significantly (P< 0.05); Values in parentheses indicate % change over control; TLG = Total length gain; NWG = Net weight gain, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR= Specific growth rate, CF = Condition factor

significantly higher growth as compared to red pepper. Ramamoorthy *et al.* (2010) too showed enhanced growth of marine ornamental fish, *Amphiprion ocellaris* fed on four pigment sources (carrot, *D. carota;* marigold meal, *T. erecta;* China rose petal, *H. rosasinensis* and rose petal, *R. chinensis*) as compared to non-pigmented diet, where *D. carota* resulted in maximum growth rate as compared to other diets as well as control. Positive effects in terms of growth through carotenoid were also observed by Ahilan *et al.* (2008) in Gold fish, *C. auratus,* when amaranth and coriander were given @ 1 and 3 %, respectively. *Spirulina*, a rich source of carotrenoids, has also proved to be protein rich nutrient and resulted in significantly higher growth (@ 30 mg kg^{-1} of diet) in blue gourami, *Trichogaster trichopterus* (Rinna *et al.,* 2013) Similar to natural carotenoid sources, synthetic carotenoid source such as astaxanthin was also reported to influence fish and shellfish growth in a positive manner (Thongrod *et al.,* 1995). Liang *et al.* (2012) showed significant higher weight gain and specific growth rate along with significantly reduced FCR in red white koi carp with 150, 200 and 250 mg of astacin kg^{-1} of diet. Similarly, Nguyen *et al.* (2014) found that supplementation of astaxanthin @ 80 mg kg^{-1} in koi carp resulted in higher growth and feed utilization. However, few of the earlier reports suggested the role of carotenoids only on colour enhancement without having much effect on the fish growth (Kop *et al.,* 2010; Mirzaee *et al.,* 2013; Seyedi *et al* 2013).

The result of the present study revealed maximum colour enhancement in terms of carotenoid content at 5% incorporation level of carrot meal as compared to both low (1% and 3%) and high incorporation level (7%). The digital photographs also exhibited improved values for RGB along with increased a*and b* values in CM5. In the present study, carrot meal resulted in significantly increased carotenoid

Table 5. Colouration parameters in skin of Koi carp (*C. carpio* L.) in different treatments

Days	Particulars	Diets*				
		CM0	CM1	CM3	CM5	CM7
				Carotenoid content (µg/g)		
120		1.24^{e}±0.011	1.95^{d}±0.008 (57.25)	3.28^{b}±0.035 (164.52)	4.87^{a}±0.384 (292.74)	2.78^{c}±0.018 (124.19)
				RGB Values		
0	R	59.00^{a}±1.17	59.00^{a}±1.10	60.00^{a}±1.11	59.00^{a}±1.12	58.00^{a}±1.15
	G	51.00^{a}±1.21	50.00^{a}±1.12	52.00^{a}±1.15	52.00^{a}±1.14	51.00^{a}±1.16
	B	28.00^{a}±1.20	29.00^{a}±1.13	29.00^{a}±1.14	30.00^{a}±1.16	29.00^{a}±1.19
120	R	75.66^{d}±2.60	179.00^{ab}±5.77	187.66^{a}±3.17	189.00^{a}±9.81	150.66^{bc}±5.48
	G	66.66^{de}±0.33	166.00^{bc}±2.30	155.66^{cd}±3.17	185.00^{a}±0.57	$150.00c^{cd}$±4.61
	B	51.66^{d}±3.17	143.66^{bc}±1.45	174.66^{a}±7.79	179.00^{a}±1.73	167.00^{ab}±5.19
				Lab values		
0	L*	22.00^{a}±0.25	21.00^{a}±0.30	22.00^{a}±0.25	21.00^{a}±0.24	21.00^{a}±0.26
	a*	1.00^{a}±0.26	1.00^{a}±0.21	1.00^{a}±0.24	1.00^{a}±0.23	1.00^{a}±0.28
	b*	16.00^{a}±0.15	15.00^{a}±0.19	17.00^{a}±0.21	18.00^{a}±0.22	18.00^{a}±0.20
120	L*	31.00^{cd}±0.57	69.00^{ab}±1.15	64.66^{b}±0.88	62.00^{bc}±1.15	75.66^{a}±0.33
	a*	1.89^{de}±0.57	4.00^{bc}±0.00	3.66^{cd}±0.88	7.66^{a}±1.45	3.00^{cd}±0.57
	b*	15.00^{e}±2.00	34.00^{cd}±0.57	35.66^{bc}±0.33	39.00^{a}±2.00	34.66^{bc}±2.02

Values are Mean ± S.E.; Values with differrent superscript (ab…d) in row differ significantly (Pd" 0.05); Values in parentheses indicate % change over control

concentration at 5 % incorporation level, showing direct relationship with concentration of carotenoids on deposition of carotenoids. A positive relationship between feed dose and deposition of carotenoids in muscle of Atlantic salmon was also observed by Baker (2002). In agreement to present studies, Mirzaee *et al.* (2013) also recorded more colour enhancement in jewel cichlid (*H. bimaculatus*) with carrot as compared to red pepper. Likewise, Kop *et al.* (2010) also reported carrot (*D. carota*) as a more efficient carotenoid source in comparison to red pepper (*Capsicum annum*) for colour enhancement in cichlid (*C. severum*). Comparison of carrot with three flower pigment sources *i.e.* marigold petal (*T. erecta*), China rose petal (*H. rosasinensis*), rose petal (*R. cinensis*) also revealed that carrot resulted in significantly higher carotenoid content in fish skin/muscle (Ramamoorthy *et al.,* 2010). Yanar and Tekelioglu (1999) also suggested utilization of carrot as potential carotenoid source for colour development in gold fish, *C. auratus.*

Tlusty (2005) while feeding of American Lobster (*Homarus americanus*) also found the colouration values on RGB scale were proportional to amount of astaxanthin carotenoid in the diet. Liang *et al.* (2012) analyzed the red white ornamental koi carp by visual evaluation, photography as well as total carotenoid concentration after feeding with different levels of astacin. Red areas of skin, scale, head and fins showed marked increase in carotenoid content, when fed with 200 and 250 mg kg^{-1} of astacin. In addition, highest deposition of carotenoid was observed in red skin followed by scales, red head and fins. Sun *et al.* (2012) compared the effectiveness of synthetic and natural pigment sources in colouration of Japanese ornamental koi carp (*C. carpio).* Synthetic carotenoid *i.e.* carophyll red @ 1.5 g kg^{-1} and natural pigment source *i.e. Spirulina platensis* @ 75 g kg^{-1} of diet resulted in similar colour improvement. These studies clearly indicate the effectiveness of natural carotenoids similar to synthetic sources in colour enhancement of koi.

The results in terms of CIE colouration were in agreement with the findings of Yeider *et al.* (2014) in Zebra cichlid (*Maylandia estnerae*) with *spirulina* feeding, in which decreased L* values (lightness) along with significantly increased a* and b* values (red and yellow) were observed. Similarly, in the present study also, increased a* and b* values resulted in enhanced orange appearance of fish skin. These results are in accordance with the study of Nguyen *et al.* (2014) on koi carp, in which significantly higher values of a* and b* were reported, when fish were provided with synthetic astaxanthin @ 80 mg kg^{-1} of the diet. Skin pigmentation in terms of total carotenoids (0.9 µg/g) and L*a*b* values were significantly affected by paprika @ 16 % in the diet of pale chub (*Zacco platypus*). The skin lightness (L* values) significantly decreased with increased values of a* and b* in fish fed diets containing paprika (Lee *et al.,* 2010).

CONCLUSION

It can be concluded that the carrot meal has a positive effect on growth as well as colour enhancement of koi carp, *Cyprinus carpio* L. in indoor rearing conditions. Hence, carrot meal can be supplemented in koi carp diet at 5 % level both as colour enhancer and growth promoter.

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Effect of Dietary Incorporation of Short Chain and Medium Chain Fatty Acid on Feed Intake and Serum metabolites in Broiler Chickens

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ABSTRACT

An experiment was conducted to study the effect of incorporation of short chain and medium chain fatty acid (SCMCFA) on feed intake and serum parameters in broiler chickens. Three hundred one-day-old AP-95 strain broiler chicks were reared under uniform managemental conditions. The broiler chicks were individually weighed and distributed randomly into five treatments having four replicates with 15 birds per replicate. Birds were fed either a control diets (C), control plus probiotics (500 mg/kg; P), control plus SCMCFA (1000 mg/kg; F), control plus butyric acid supplement (500 mg/kg; B) or control plus SCMCFA + butyric acid (1000 mg/kg + 500 mg/kg; FB) during pre-starter, starter and finisher phases. The intake followed similar trend in all the groups during the six-week study period, except a significant decline in feed intake in the probiotic fed group during the fifth week. Blood glucose, cholesterol and serum triglycerides were similar among the groups. These results indicated that dietary supplementation of SCMCFA showed no adverse effect on intake, performance and serum metabolites of broiler chicken.

Keywords: Broilers, Feed intake, Serum chemistry, Short chain and medium chain fatty acid

INTRODUCTION

Emerging concerns on animal ethics, evolving customer demands and government regulations in India has resulted in changing the way of animal rearing, especially the use of antibiotics (Manyi-Loh *et al.,* 2018). An endless number of alternatives are being considered in order to achieve the same feed efficiency and growth rate that are accomplished with antibiotics (Phillips *et al.,* 2004; Den Hartog *et al.,* 2005). Among potential alternatives for antibiotic growth promoters (AGPs), short chain fatty acids (SCFAs) and medium chain fatty acids (MCFAs) act similarly like AGPs (Khatibjoo *et al.,* 2018). Short-chain fatty acids are saturated aliphatic organic acids that consist of one to six carbons, of which acetate (C_2), propionate (C_3), and butyrate (C_4) are found to be absorbed from both the small intestine and the ceca by passive transport (den Besten *et al.,* 2013). MCFAs are effective alternatives for antibiotics, due to their high antibacterial activity, and they enter the cell un-dissociated (Khatibjoo *et al.,* 2018). SCFA and MCFA have shown to have synergistic effect and may act by disrupting the cell wall membrane of the microorganisms helping the SCFCA to enter the cytoplasm where they act (Del Alamo *et al.,* 2007). Therefore, this experiment was carried out to determine the effect of SCFA and MCFA, in comparison with probiotic, butyric acid alone or SC&MCFA with butyric acid, on feed intake and serum parameters in broilers.

MATERIALS AND METHODS

The experiment was carried out at the experimental shed of College of Veterinary and Animal Sciences, Udgir, Maharashtra. Three hundred day-old straight run broiler chicks (AP-95) were purchased from a commercial broiler hatchery from Udgir, Maharashtra. The broilers were housed in identical sized pens, environmental temperature was set at 31°C for the first week and 28°C for the second week, which was further decreased to 22°C until the end of the experiment. The relative humidity was above 55% throughout the study. The basal diet was formulated to meet the nutrient requirements of broilers (BIS, 2007). Following a completely randomized design, broilers were allotted to five dietary treatments with four replicates of 15 birds each. The experimental diets consisted of control (C), control plus probiotics (500 mg/kg; P),

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control plus SCMCFA (1000 mg/kg; F), control plus butyric acid t (500 mg/kg; B) and control plus SCMCFA + butyric acid (1000 mg/kg + 500 mg/kg; FB). The supplements were fed during pre-starter, starter and finisher phase as mentioned in Table 1 and 2.

The birds were reared under uniform managemental condition following standard protocols for vaccination, and maintenance of temperature, ventilation and lighting. They were fed experimental diets from 1 to 42 day of age. Feed intake was recorded for each period and during the whole experiment (1–42 d of age). On 42nd day, eight birds from each dietary treatment were sacrificed and blood samples were collected, alalyzed for total glucose, serum cholesterol and serum triglycerides content using diagnostic kits. The data obtained were statistically analysed as per one-way ANOVA following method of Snedecor and Cochran (1994) and significant differences between them were tested by Duncan's New Multiple comparison Test according to methods described by SAS (1985). Significance was considered at P<0.05 levels.

RESULTS AND DISCUSSION

Data pertaining to the feed intake of the birds are presented in Table 3. Overall, feed intake was not significantly influenced by dietary treatments, except the probiotic supplemented group whose intake was significantly reduced in the 5th week. However, Duncan Multiple Range Test (multiple comparison test) revealed that the effect of supplementation did not differ significantly from each other except for the said week. The weekly feed intake of birds fed probiotic diet was comparable with that of 0.05% butyrate supplemented group. In all the treatments, the feed intake followed similar trend and supplementation of SCMCFA had similar intake as that of control. In the fatty acid supplemented groups, the feed intake was marginally improved as compared to other groups. Feed intake was unaltered in broilers fed diets supplemented with medium-chain fatty acids at 0, 1, 2 and 4 g/kg (Saeidi *et al.,* 2016). Khatibjoo *et al.* (2018) have also reported similar effect of treatments on feed intake during growing period of experiment. The inclusion of dietary

Table 1. Ingredient composition of experimental diets (%)

Ingredients (on DM basis)	Pre-starter (0-7 days)	Starter (8-21 days)	Finisher (21-42 days)
Maize	51.12	53.0	57.85
De-oiled soybean cake	42.00	39.5	33.44
Soybean oil, crude	3.00	4.0	5.00
Di-calcium phosphate	1.70	1.8	1.90
Limestone powder	1.00	0.8	0.80
Vitamin premix*	0.025	0.025	0.025
Salt	0.30	0.30	0.30
Methionine	0.17	0.20	0.21
Lysine	0.16	0.14	0.15
Trace mineral mixture** (TMM)	0.10	0.10	0.10
Coccidiostat	0.05	0.05	0.05
Choline	0.06	0.06	0.06
Toxin binder	0.10	0.10	0.10
Total	99.79	100.07	99.99

*Each kg of vitamin premix contains Vitamin A-22.5 million IU, Vitamin D_3-4.5 m IU, Vitamin E-60 g, Vitamin K_3-4 g, Vitamin B_1-4 g, Vitamin B_2-20 g, Niacin-60 g, calcium D-panthonate-30 g, Vitamin B_{12}-35 g, Folic acid-4 g, Biotin-0.2 g; **Each kg of TMM contains Mn-50 g, Zn-50 g, Fe-55 g, Copper-10 g, Iodine-1.25 g, Selenium-250 mg

Table 2. Nutrient composition of pre-starter, starter and finisher diets

Nutrients	Treatments*				
	C	P	B	F	FB
Pre-starter diet					
CP (%)	23.0	23.2	23.0	23.0	22.8
EE (%)	5.18	6.58	7.0	5.2	4.41
CF (%)	3.88	4.15	4.2	3.88	4.04
TA (%)	7.45	6.18	6.53	6.78	6.53
AIA (%)	1.6	1.63	1.67	1.62	1.87
ME (kcal)	3002	3006	3007	3005	3003
Starter diet					
CP (%)	22.0	21.8	21.9	21.9	21.9
EE (%)	7.04	6.98	7.5	7.4	7.67
CF (%)	3.97	3.92	3.9	3.94	3.95
TA (%)	7.08	6.8	6.9	6.5	6.4
AIA (%)	1.61	1.61	1.61	1.61	1.61
ME (kcal)	3107	3105	3108	3098	3097
Finisher diet					
CP (%)	20.0	20.1	19.0	19.9	20.0
EE (%)	8.41	8.6	8.7	8.6	8.7
CF (%)	3.2	3.4	3.5	3.4	3.3
TA (%)	6.1	6.0	6.2	6.1	6.3
AIA (%)	1.46	1.5	1.43	1.45	1.46
ME (kcal)	3230	3219	3220	3215	3219

*Treatments: T, control diet; P, probiotic-500 mg/kg; B, butyric acid-500 mg/kg; F, SCMFCA-1000 mg/kg; FB, SCMFCA 1000 mg + butyric acid- 500 mg

coconut oil as a source of medium-chain fatty acid by replacing 25, 50, 75 or 100 % of the soybean oil had no adverse effect on feed intake in broilers (Wang *et al.,* 2015). Supplementation of basal diet with 0.1, 0.15 or 0.2 % medium chain fatty acids for period 1-21 day of age, 0.15% for period 21-36 day of age and 0.1% for period 36-42 day of age did not affect feed intake (Mohammadzade *et al.,* 2013). Pinchasov and Jensen

Table 3. Effect of dietary supplementation of probiotics and SCMCFA on weekly feed intake (g)

Age in weeks	Treatments*					P-value
	C	P	B	F	FB	
1	129.7±7.45	129.1±2.83	134.6±6.60	119.9±2.33	111.6±7.43	0.080
2	409.0±38.89	359.4±19.70	410.0±18.78	432.1±35.70	337.4±6.95	0.120
3	569.3±54.91	563.0±46.25	585.8±7.11	574.8±14.2	579.6±26.53	0.980
4	900.6±25.73	892.4±9.69	948.5±18.44	957.8±18.77	872.0±43.97	0.140
5	1102±23.63^{ab}	1025±12.42^{a}	1076±13.01^{ab}	1145±20.52^{b}	1124±17.8^{ab}	0.002
6	874.4±35.11	876.5±19.75	909.6±54.19	900.3±13.3	877.1±4.56	0.88

^{a,b} Means bearing different superscripts within the same row are significantly different (P<0.05); *Treatments: T, control diet; P, probiotic-500 mg/kg; B, butyric acid-500 mg/kg; F, SCMFCA-1000 mg/kg; FB, SCMFCA 1000 mg + butyric acid- 500 mg

Table 4. Effect of dietary supplementation of probiotics and SCMCFA on serum profile

Parameters	Treatments*					P-value
	C	P	B	F	FB	
Blood glucose (mg/dl)	128±23.5	125±29.5	156±7.0	222±14.0	139±24.0	0.64
Serum cholesterol (mg/d)	163±12.0	139±15.0	149±11.0	178±8.0	176±34.0	0.55
Triglycerides (mg/dl)	97.5±1.5	80±18.0	70.5±1.5	130±13.5	133±27.5	0.12

*Treatments: T, control diet; P, probiotic-500 mg/kg; B, butyric acid-500 mg/kg; F, SCMFCA-1000 mg/kg; FB, SCMFCA 1000 mg + butyric acid- 500 mgg/kg; B, butyric acid-500 mg/kg; F, SCMFCA-1000 mg/kg; FB, SCMFCA 1000 mg + butyric acid- 500 mg

(1989) summarized that in case of well-nourished healthy chicks housed at a moderate stocking density and hygienic condition, dietary inclusion of MCFAs or SCFAs had no significant influence on the birds' performance. In an another study on MCFA, Miller *et al.* (2009) reported no differences with regard to feed intake, feed efficiency and weight gain in grower and finisher pigs when fed diets with 1, 3, or 6% of MCFA oil compared with diets containing the same amount of tallow, pig fat or corn oil. However, in our study birds were fed 1000 mg/kg of SCMCFA in feed and we did not observe any effect of supplementation on feed intake.

Serum concentration of glucose, cholesterol and triglycerides were similar among the groups (Table 4). In another study it was observed that the serum triglyceride concentrations was significantly increased in broilers with MCFA diet as compared to control (Baltic *et al.,* 2018). The present findings corroborate well with that Khatibjoo *et al.* (2018) who bserved no effects of supplemental dietary short-and medium-chain fatty acids on serum cholesterol and glucose of broiler chickens.

CONCLUSION

Our results suggest that combination of short chain and medium chain fatty acid supplementation in broiler diet to be beneficial and exert no adverse effect on feed intake and blood biochemistry.

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SHORT COMMUNICATION

Successful Management of Nitrite Poisoning in Crossbred Dairy Calves

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ABSTRACT

Nitrate/ nitrite poisoning in dairy cattle occurs when there is an abrupt change in diet from the introduction of a bulk feed source with high nitrate levels. Over fertilization of fodder with untreated manure and occurrence of drought season followed by a rainy season could result in higher level of accumulation of nitrate in plants. A sudden onset of illness was reported in eight crossbred dairy calves (between 8-11 months of age) at University Livestock Farm and one among the affected calf died after showing symptoms of discomfort, excessive salivation, tympany, dyspnoea, sternal recumbency progressing to lateral recumbency, pedalling movements and, stiffness of the limbs and neck. On clinical examination, the affected animals exhibited sialorrhoea, tympany, increased pulse and respiratory rates, laboured breathing, brownish discoloration of mucous membrane and loss of co-ordination. Microscopical examination of rumen fluid revealed normal protozoan motility. Blood colour was dark chocolate brown in all the affected animals and coagulation time was delayed. Animals had a history of *ad libitum* feeding with chopped lush green fodder grasses namely hybrid Napier and fodder maize that were harvested from cattle slurry irrigated fodder plots. Based on the history and clinical signs noticed, the case was presumptively diagnosed as nitrate/nitrite poisoning, later confirmed with diphenylamine test. All the animals were treated with intravenous administration of 1 per cent methylene blue @ 1-2 mg/kg BW. Subsequently the dairy calves showed an uneventful recovery from the condition.

Key words: Methylene blue, Nitrate, Nitrite, Sodium thiosulphate

Nitrate/ Nitrite poisoning is the one of the most widespread toxic condition in ruminants. Fodder crops namely maize, sorghum, brassicas, Italian rye grass, tama and green oats are considered as the principal accumulators of nitrate that could reach toxic levels for bovines. Accumulation of nitrate is more in rapid growing plants with reduced photosynthesis, over fertilization of the soil with excessive use of animal manure and poultry litter are the principle cause of nitrate accumulation in forages (Follet and Hatfield, 2001) and the accumulation is more in young and rapidly growing plants as compared to the mature forages (Haliburton and Edward, 1978).

The occurrence of a rainy season followed by a draught period, less adaptation of the ruminants to a nitrite rich diet and *ad libitum* feeding of bovines with nitrite rich plants have increased the risk of nitrate toxicity in ruminants (Gontigo *et al.,* 2017). Ruminants like cattle, goat and sheep are more prone to nitrate toxicity as the rumen bacteria reduce nitrate to a more toxic nitrite and when this conversion ratio is faster than the conversion of nitrite to ammonia, the excess nitrite that accumulates in the rumen is progressively absorbed into the blood stream. Nitrite in the blood reduces the oxygen carrying haemoglobin to a brownish black substance called methemoglobin that cannot carry oxygen and imparts the above colour to the blood (Osweiler 1996). Nitrite also causes vasodilatation that leads to a reduction in blood pressure and circulatory shock and foetal death in pregnant animals due to

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hypoxia. Affected animals show clinical signs when 20 per cent of haemoglobin is converted to methemoglobin and death results when it reaches to 60-80 per cent (Jones 1988). Clinical signs include chocolate brown discoloration of the mucous membranes, severe abdominal pain, ataxia, incordination, muscle tremor, weakness, severe dyspnoea, cyanosis, collapse and recumbency. In this paper we report a case of spontaneous occurrence of nitrate or nitrite intoxication in cross bred dairy heifers and its' successful management.

A sudden onset of illness was reported in eight crossbred dairy calves (8-11 months of age) in University Livestock Farm, Mannuthy and one among the affected calves died after showing symptoms like sudden onset of discomfort, excessive salivation, tympany, dyspnoea, sternal to lateral recumbency, pedalling movements and stiffness of the limbs and neck. It was reported that all calves were healthy and they were fed *ad libitum* chopped lush green fodder (hybrid Napier and fodder maize) harvested from cattle slurry irrigated fodder plots.

Detailed clinical examination was carried out on all the affected animals and observations were recorded. The affected animals exhibited sialorrhoea, ataxia, tachycardia, tachypnoea, dyspnoea, cyanotic mucous membrane and loss of balance. Microscopical examination of rumen fluid revealed normal protozoan motility. Blood colour was found to be dark chocolate brown in all affected animals.

Based on the feeding history and clinical signs noticed, the case was presumptively diagnosed as nitrite poisoning and treatment was carried out with 1 % methylene blue. The diphenylamine test was performed in grass samples by adding one drop of the reagent solution (0.5 g diphenylamine, 20 ml distilled water, and 80 ml concentrated sulfuric acid) to three drops of the plant extract on glass slides. The extract was obtained by manual pressure (Tokarnia *et al.,* 2012). The results of the diphenylamine test associated with clinical signs and epidemiological data helped to conclude the diagnosis of nitrate/nitrite intoxication. All the affected animals were treated with intravenous administration of 1% methylene blue @ 1-2 mg/kg BW. Subsequently the dairy calves showed an uneventful recovery from the condition.

Even though the chopped lush green grass was fed to animals in all the age group the intoxication occurred only in the heifers group, whose diet was almost exclusively composed of fodder that corresponded to approximately 60-80% of the total dry matter intake. Dairy cows, on the other hand received larger amounts of concentrate, and the calves received milk and ate a small amount of concentrates and grass. Hybrid Napier and maize fodders are good accumulator nitrates (Medeiros *et al.,* 2003). The condition was further aggravated the fact that the fodder were harvested from plots that were fertilized with large amount of untreated manure and pig slurry. Additionally, the site experienced a long period of drought

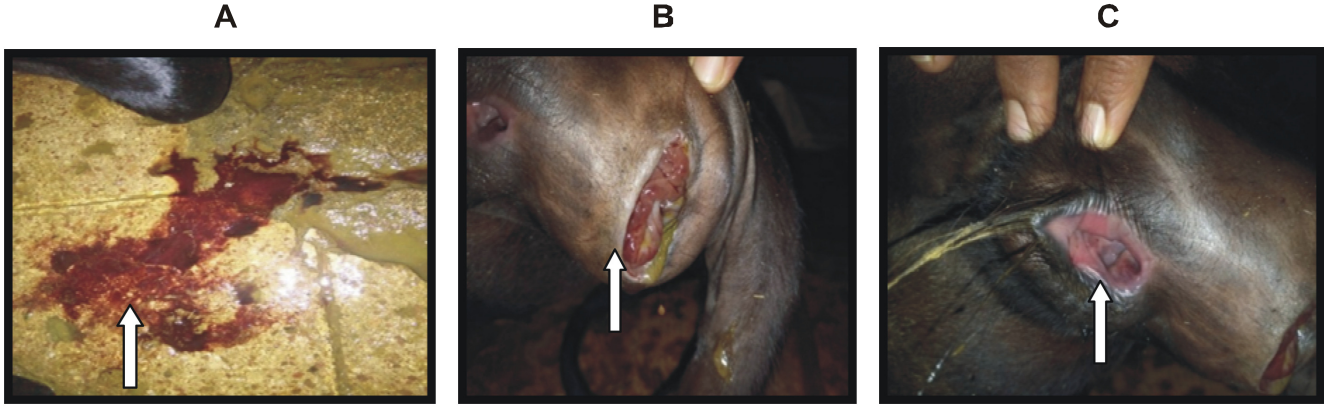

Fig. 1 Brownish discoloration of the blood (A), mucus membrane of vulva (B) and rectum (C)

without rain followed by high rainfall during the incident. All these factors might have contributed to the accumulation of nitrate in the fodders offered to the animals and bulk consumption of these fodder caused the intoxication.

In ruminants, nitrate/nitrite toxicity occurs when high nitrate levels in the feed overwhelm the animal's digestive system to the extent that the rate of conversion of nitrate to nitrite is faster than the conversion of nitrite to ammonia (Al Qudah, 2009). Microbes in the rumen reduce nitrate contents in the ingested plants in to nitrite, which is five to six times more toxic than nitrates (Rogers and Hope-Cawdery, 1980). Following its absorption into blood through rumen capillaries, nitrite oxidizes the haemoglobin to methaemoglobin which is unable to transport oxygen and also results in vasodilatation (Leng, 2008). These two predominant effects of nitrite lead to tissue hypoxia and circulatory collapse (Haymond *et al.,* 2005). The amount of nitrate ingested by the animal and methemoglobin formed influences the onset of clinical signs, disease progression and death (Jonk *et al.* 2013). The amount of methemoglobin formed in each animal depends on its nitrate recycling rates in the rumen and on the drop in nitrite levels, reflecting different levels of nitrate tolerance presented by the animals (Ozmen *et al.,* 2005).

The poisoned heifers exhibited a cyanotic or brownish discoloration of mucosa of conjunctiva, vaginal vestibule and rectum; dyspnoea, sialorrhoea, tympany, and progression to sternal recumbence and death. Similar findings were also reported by Medeiros *et al.* (2003) and Jonck *et al.* (2013). Among the intoxicated animals one heifer died after showing symptoms like stiffness in limbs, neck and jaw, tympany and pedalling movements. Necropsy was performed soon after the animal's death. A strong nitrous odour was noticed while opening the carcass. The blood was dark chocolate brown in colour with difficult coagulation, all the compartments of stomach were distended due to gas accumulation and a brownish discoloration was observed in lungs, kidneys and liver. These necropsy findings were in accordance with the report of Tokarnia *et al.* (2012) who noticed brownish discolouration of visceral organs in intoxicated animals. Chocolate-coloured and poorly clotted blood and intensely red coloured skeletal and cardiac musculature as observed in the present case were compatible with those described by Jonck *et al.* (2013).

The drug of choice for nitrate/nitrite poisoning is intravenous administration of 1% methylene blue at the dosage of 1 to 2 mg/kg body weight. Methylene blue acts by reducing Fe^{3+} to Fe^{2+}, and receiving an H^{+} from NADPH (becoming leukotolene blue) and donating it to methemoglobin, which is converted to haemoglobin. Since the time taken to metabolize the methylene blue is short, the poisoned animal shows recovery within minutes after its administration. The rapid response to the treatment is another form of diagnosis of the intoxication (Radostits *et al.,* 2007; Jonck *et al.,* 2013).

The clinical signs, necropsy findings, epidemiological data and the positive diphenylamine test confirmed the nitrate/nitrite intoxication. The sudden change in diet, with introduction of a bulky food source containing high nitrate levels, the over fertilization of grass with untreated manure, and the occurrence of a drought period followed by a rainy season were some of the causes of the intoxication. Intra venous administration of one percent methylene blue @ 1-2 mg/kg body weight resulted in uneventful recovery of the animals.

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SHORT COMMUNICATION

In Sacco Degradability Kinetics of Cumbu Napier (COBN-5) Fodder at Two Different Stages of Harvest

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ABSTRACT

In the present study, Cumbu Napier (COBN-5) fodder was evaluated for nutrient degradability *in sacco* using three rumen fistulated Murrah buffalo bulls (380 ± 9.36 kg BW) fed *ad libitum* COBN-5 fodder at two different stages (40 and 45 days) of harvest. The average *in sacco* disappearance values (%) of DM, CP, NDF and ADF were 80.49, 91.97, 74.11 and 73.79 after 72 h incubation in the rumen, respectively. The readily soluble fraction (a) for DM, CP, NDF and ADF was higher in the fodder harvested at 40 days, whereas, higher insoluble but degradable fraction (b) for DM, CP, NDF and ADF was reported in the fodder harvested at 45 days. Further, potentially degradable fraction, PD (a+b) for DM and CP was lower, and for NDF and ADF was higher in the fodder harvested at 40 days. The effective degradability (%) of DM, CP, NDF and ADF was higher in the fodder harvested at 40 days than in 45 days. Results indicated that COBN-5 fodder can be effectively included in the rations of livestock harvested at 40 days for effective utilization.

Keywords: COBN-5 fodder, Degradation kinetics, *In sacco* degradability, Stage of harvest

The ruminant sector provides a significant proportion of self-employment opportunities and forms a basis for supplementary income to most sections of India's agrarian society. Forages usually constitute the major portion of the ruminant feeds and high quality nutritious green fodder is required to exploit the productive potential of dairy animals. With the increase in industrialization, ever-increasing population pressure of human beings, there is a little chance of having good arable land available for cultivation of green fodder. To meet the current level of livestock production, cultivation of perennial fodders which can yield higher biomass per unit area with early harvesting period is the immediate solution.

A number of breeding programmes came into force for evolving best quality green fodder. Hybrid Napier varieties proved to be promising fodders for their high yielding capability and usage as forage for livestock (Woodard and Prine, 1991). Cumbu Napier (COBN-5) fodder is a newly developed hybrid Napier variety by Tamil Nadu Agricultural University (TNAU) at Coimbatore and released for commercial cultivation in 2013 (Babu *et al.,* 2014). The yield data from research station trials showed a higher fodder yield in COBN-5 (3260 q/ha/yr) compared to CO-3 (2960 q/ha/yr). A key measure of the nutritive value of feedstuffs is digestibility, either *in vitro* or *in vivo* (Minson, 1990). Data on chemical composition, ruminal degradation and their relationships determine the economic value of forage (Weiss, 1994). The time of harvest also effects the chemical composition and nutrient degradability of forage inside the rumen. It is very important to know the proportion of dry matter or protein or fibre that is degraded in the rumen in certain times (Topps, 1996). The *in sacco* degradability is the best available method for estimating digestibility similar to *in vitro* disappearance and it is easy to perform (Weiss 1994). Therefore, the present study was undertaken to ascertain the *in sacco* degradability of DM, CP, NDF and ADF in COBN-5 fodder at two different stages of harvest.

Cumbu Napier (COBN-5) fodder was procured from the cultivated fields of Livestock Farm Complex (LFC), NTR College of Veterinary Science,

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Gannavaram. First cut was done after 75 days of plantation. Subsequently, the fodder was harvested on 30, 35, 40, 45 and 50 days in the second cut and is used for nutritional evaluation. Chemical composition (AOAC, 2007) and forage fibre constituents (Van Soest *et al.,* 1991) of the fodder were evaluated. Further, *in sacco* degradability of DM, CP, NDF and ADF was estimated in the rumen (Orskov and Mc Donald, 1979) using COBN-5 fodder harvested at 40 and 45 days.

Three adult rumen fistulated Murrah buffalo bulls (380 ± 9.36 kg BW) maintained on COBN-5 fodder were used to determine the *in sacco* degradability of DM, CP, NDF and ADF in COBN-5 fodder harvested at 40 and 45 days. The COBN-5 fodder was chaffed, dried and ground in a Wiley mill (2 mm size mesh screen) for incubation in the rumen. The pre-weighed nylon bags (13.5 cm x 7.5 cm), containing 5 g of feed were incubated for 0, 3, 6, 12, 24, 48 and 72 h in the ventral sac of rumen. The bags containing samples for 0 h were washed without incubation in rumen. The bags with feed samples incubated in the rumen were drawn in a sequential removal method (Osuji *et al.,* 1993). After removal, the bags were washed under slow running tap water by rubbing between fingers and the thumb for ten minutes, oven dried at 70°C for 48 h to constant weight and DM loss during the incubation was calculated. The CP, NDF and ADF content in the residue was analyzed to determine their respective degradabilities. From the degradability data obtained at different intervals, the constant a, b and c from the expression $P=a+b\ (1-e^{-ct})$ were obtained. Where, P is the degradability at time t, the constant, a is the intercept or instantly degradable fraction, b is the potentially degradable and c is the degradation rate or rate constant. The effective nutrient degradability (%) of crop residues, conventional and complete rations were calculated by time measurements and fitted values in NEWAY programme (Mc Donald, 1981) using a computer, assuming an outflow rate (K) of 0.05%/hr. The data was analyzed statistically (Snedecor and Cochran, 1994) using SPSS 17.0 version.

The chemical composition and cell wall constituents of COBN-5 fodder harvested at different days is presented in Table 1. Data revealed that the

Table 1. Chemical composition (% DM basis) of COBN-5 fodder at different days of harvest

Nutrient	Days of harvest					
	30	35	40	45	50	Mean ± SE
Dry matter	16.68^{a}	17.02^{a}	17.42^{ab}	18.03^{b}	20.54^{c}	17.94±1.09
Organic matter	82.49^{a}	84.27^{b}	86.05^{c}	87.20^{cd}	88.17^{d}	85.64±1.61
Total ash	17.51^{a}	15.73^{b}	13.95^{c}	12.80^{d}	11.83^{e}	14.36±1.61
Crude protein	14.00^{a}	13.41^{b}	13.02^{c}	12.70^{d}	12.10^{e}	13.05±0.51
Ether extract	3.27^{a}	2.90^{b}	2.82^{b}	2.72^{c}	2.41^{d}	2.82±0.22
Crude fibre	19.24^{a}	23.07^{b}	27.96^{c}	30.50^{d}	34.69^{e}	27.09±4.30
Nitrogen free extract	45.98^{a}	44.89^{b}	42.25^{c}	41.28^{d}	38.97^{e}	42.67±1.99
Neutral detergent fibre	62.00^{a}	65.00^{b}	67.06^{c}	69.10^{d}	73.38^{e}	67.31±3.03
Acid detergent fibre	38.29^{a}	39.26^{b}	40.20^{c}	41.64^{d}	44.80^{e}	40.84±2.41
Hemi-cellulose	23.71^{a}	25.74^{b}	26.86^{c}	27.46^{d}	28.58^{e}	26.47±0.44
Cellulose	30.94^{a}	32.61^{b}	34.25^{c}	36.70^{d}	40.87^{e}	35.07±2.97
Acid detergent lignin	4.97^{a}	5.31^{b}	5.68^{c}	6.13^{d}	6.54^{e}	5.73±2.74
Silica	2.95^{a}	3.17^{b}	3.42^{c}	3.69^{d}	3.82^{e}	3.41±0.25
Calcium (%)	0.74^{a}	0.81^{a}	1.15^{b}	0.48^{b}	0.47^{c}	0.73±0.20
Phosphorus (%)	0.68^{a}	0.51^{a}	0.75^{a}	0.45^{b}	0.44^{b}	0.57±0.10

Each value is a mean of 3 observations; ^{a,b,c,d,e}Values in the rows bearing different superscripts differ significantly (P<0.05)

protein content was higher, while NDF, ADF and lignin were lower in Cumbu Napier fodder at 40 days of harvest as compared to 45 days. Forage age is the most influential factor on animal response parameters when forages form the main source of diet for ruminants. The fodder harvested at 40 and 45 days is studied *in sacco* by incubating in the rumen upto 72 h. The average *in sacco* disappearance values (%) and degradation kinetics of DM, CP, NDF and ADF in COBN-5 fodder is presented in Tables 2 and 3.

Data revealed that as the period of incubation extended from 0 to 72 h in the rumen, the *in sacco* disappearance (%) values of DM, CP, NDF and ADF increased progressively and higher values were observed in the fodder harvested at 40 days compared to that harvested at 45 days with non-significant differences. The rapidly soluble fraction (a) was higher, while the insoluble but degradable fraction (b), potentially degradable fraction (a + b) and rate constant/h (c) were lower in DM in the fodder harvested at 40 days (Table 2). Further, ED (0.05%$^{-h}$) of DM was higher in the fodder harvested at 40 days. Similar findings were reported by other research workers (Abdul Razak *et al.,* 1996; Tessema and Baars. 2004) in Napier grass. The rapidly soluble fraction 'a' and ED (0.05%$^{-h}$) of CP was higher in fodder harvested at 40 days, while the insoluble but degradable fraction 'b' was higher in the fodder harvested at 45 days (Table 2). Further, decreases in protein content was caused by an increase in stem proportion (Gutteridge and Shelton, 1994), which had a lower protein percentage than leaves, and there will be a reduction in leaf and stem fraction with age. Muia *et al.* (2001) reported similar values for fractions 'b' and 'c' but lower values for 'a' and ED of CP in Napier fodder compared to the values in present study. The higher fraction 'a' and lower 'b' fraction in the present study indicates presence of more soluble nitrogen content as compared to other Napier varieties. The effective degradability of CP was higher than the other Napier grass variety suggesting high degradable nitrogen content (Kabi *et al.,* 2005) in COBN-5 fodder. Higher fraction 'a', (a+b) and ED (0.05%$^{-h}$) of NDF were reported in the fodder harvested at 40 days compared to fodder harvested at 45 days. The lower

Table 2. Average *in sacco* disappearance (%) and degradation kinetics of DM and CP in COBN-5 fodder at 40 and 45 days of harvest

Parameter	DM			CP		
Days of harvest	**40**	**45**	**Mean ± SE**	**40**	**45**	**Mean ± SE**
Incubation period (h)						
0	38.37	37.04	37.70±0.67	55.18^{a}	53.47^{b}	54.33±0.86
3	40.47	39.20	39.83±0.64	62.99^{a}	60.49^{b}	61.74±1.25
6	48.00	46.97	47.49±0.52	69.74^{a}	67.84^{b}	68.79±0.95
12	57.23	51.87	54.55±2.68	73.85^{a}	71.73^{b}	72.79±1.06
24	70.05	67.49	68.77±1.28	79.05^{a}	75.41^{b}	77.23±1.82
48	77.46	72.55	75.05±2.45	88.73^{a}	86.12^{b}	87.43±1.31
72	82.54	78.44	80.49±2.05	92.10^{a}	91.84^{b}	91.97±0.13
Degradation Kinetics						
a	38.37	37.04	37.70±0.67	55.18^{a}	53.47^{b}	54.33±0.86
b	43.98	50.27	47.12±3.15	39.33^{a}	44.56^{b}	41.95±2.62
c	0.056	0.024	0.04±0.02	0.036^{a}	0.024^{b}	0.030±0.01
PD (a+b)	82.35	87.31	84.83±2.48	94.51^{a}	98.03^{b}	96.27±1.76
ED (0.05%/h)	58.50	54.20	56.35±2.15	76.10^{a}	74.80^{b}	75.45±0.65

Each value is a mean of 3 observations; ^{a,b}Values in the rows bearing different superscripts differ significantly P<0.05

Table 3. Average *in sacco* disappearance (%) and degradation kinetics of NDF and ADF in COBN-5 fodder at 40 and 45 days of harvest

Parameter	NDF			ADF		
Days of harvest	**40**	**45**	**Mean ± SE**	**40**	**45**	**Mean ± SE**
Incubation period (h)						
0	25.34[a]	18.98[b]	22.16±3.18	19.11[a]	14.46[b]	16.79±2.32
3	30.16[a]	24.36[b]	27.26±2.90	25.39[a]	19.14[b]	22.27±3.13
6	36.20[a]	32.05[b]	34.13±2.08	33.77[a]	26.74[b]	30.26±3.52
12	44.98[a]	42.64[b]	43.81±1.17	44.69[a]	38.46[b]	41.58±3.11
24	63.13[a]	53.78[b]	58.46±4.68	61.01[a]	52.96[b]	56.99±4.02
48	71.54[a]	62.08[b]	66.81±4.73	71.42[a]	63.24[b]	67.33±4.09
72	76.54[a]	71.68[b]	74.11±2.43	76.52[a]	71.06[b]	73.79±2.73
Degradation Kinetics						
a	25.34[a]	18.98[b]	22.16±3.18	19.11[a]	14.46[b]	16.79±2.32
b	52.41[a]	52.48[a]	52.45±0.04	58.11[a]	57.05[b]	57.58±0.53
c	0.0501[a]	0.0449[b]	0.048±0.001	0.0530[a]	0.0478[b]	0.05±0.002
PD (a+b)	77.75[a]	71.46[b]	74.61±3.15	77.22[a]	71.51[b]	74.37±2.86
ED (0.05%/h)	48.90[a]	43.10[b]	46.00±2.90a	46.74[a]	39.99[b]	43.37±3.38

Each value is a mean of 3 observations; [a,b]Values in the rows bearing different superscripts differ significantly P<0.05

ED (%) of NDF in the fodder harvested at 45 days might be due to higher cellulose and acid detergent lignin content (Table 3). The potentially degradable fraction of elephant grass was reported to be lower than the value in the present study (Gallardo *et al.,* 2010) in Napier grass. The rapidly soluble fraction (a), insoluble but degradable fraction (b) and PD (a+b) of ADF were higher in the fodder harvested at 40 days. Further, the effective degradability of ADF was higher in the fodder harvested at 40 days (Table 3). Lower potential degradability values were reported for elephant grass than the values observed in the present study (Gallardo *et al.,* 2010; Singh *et al.,* 1992). Decreased ADF degradability in late cut forages, may be attributed to presence of higher lignified fibre. Further, as forage matures its content of less digestible nutrients decreases (Bosch et al, 1992).

It was concluded that the COBN-5 fodder was better utilized in terms of *in sacco* nutrient degradability harvested at 40 days as compared to 45 days. This, will help to use the fodder for animal feeding at early stages of harvest, minimizing the cost of cultivation practices. Hence, the fodder can be better utilized by including it in the rations of livestock starting at 40 days of harvest for economical feeding.

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(www.indianjournals.com; www.ansi.org.in; http://epubs.icar.org.in/ejournal/index.php/IJAN)

March, 2019 **Vol. 36** **#1**

CONTENTS

INDIAN JOURNAL OF ANIMAL NUTRITION

(www.indianjournals.com; www.ansi.org.in; http://epubs.icar.org.in/ejournal/index.php/IJAN)

June, 2019 **Vol. 36** **#2**

CONTENTS

Review

Ruminants

INDIAN JOURNAL OF ANIMAL NUTRITION
(www.indianjournals.com; www.ansi.org.in; http://epubs.icar.org.in/ejournal/index.php/IJAN)
September, 2019 **Vol. 36** **#3**

CONTENTS

INDIAN JOURNAL OF ANIMAL NUTRITION
(www.indianjournals.com; www.ansi.org.in; http://epubs.icar.org.in/ejournal/index.php/IJAN)

December, 2019 **Vol. 36** **#4**

CONTENTS

Ruminants

Non-Ruminants

Author Index

Subject Index

List of referees during 2019

1. Dr. A. Sahoo, Avikanagar, Rajasthan
2. Dr. Amrish Tyagi, NDRI, Karnal, Haryana
3. Dr. Anand Pathak, Sher-e-Kashmir, University of Agricultural Sciences & Technology, Jammu
4. Dr. Anju Kala, IVRI, Izatnagar, Uttar Pradesh
5. Dr. Arun Panda, Directorate of Poultry Research
6. Dr. Asit Das, NDRI, Karnal, Haryana
7. Dr. Avijit Dey, CIRB, Hisar, Haryana
8. Dr. Avishek Biswas, CARI, Izatnagar, Bareilly
9. Dr. B. Prakash, ICAR-DPR, Hyderabad
10. Dr. Chander Datt, NDRI, Karnal, Haryana
11. Dr. Daisy Wadhwa, CSKHPKV, Palampur
12. Dr. Debashis Roy, DUVASU, Mathura, Uttar Pradesh
13. Dr. Debasis De, CIBA, West Bengal,
14. Dr. G.H. Pailan, CIFE, Kolkata, West Bengal
15. Dr. Goutam Mondal, NDRI, Karnal, Haryana
16. Dr. Jaspal Hundal, GADVASU, Ludhiana
17. Dr. Keshab Barman, ICAR-NRC on Pig, Guwahati, Assam
18. Dr. Krushna Das, ICAR-CIFA, Bhubaneswar,
19. Dr. L.C. Chaudhary, IVRI, Izatnagar, Uttar Pradesh
20. Dr. M.K. Tripathi, CIRG, Makhdoom, Uttar Pradesh
21. Dr. M.S. Mahesh, Kemin Industries, Chennai
22. Dr. Madan Das, IGFRI, Jhansi
23. Dr. Madhu Datta, NDRI, Karnal, Haryana
24. Dr. Mahipal Choubey, Navsari Agricultural University, Navsari
25. Dr. Med Ram Verma, IVRI, Izatnagar, Uttar Pradesh
26. Dr. Muneendra Kumar, DUVASU, Mathura, Uttar Pradesh
27. Dr. N Dutta, ICAR-IVRI, Izatnagar, Uttar Pradesh
28. Dr. Niranjan Panda, Odisha University of Agriculture and Technology, Bhubaneswar
29. Dr. Nitin Tyagi, NDRI, Karnal, Haryana
30. Dr. P. Vasan, Namakkal, TANUVAS, Tamil Nadu
31. Dr. P.R. Pandya, Veterinary College, Anand, Gujarat
32. Dr. Pankaj Kumar Singh, Patna, Bihar
33. Dr. Parimal Sardar, CIFE, Kolkata
34. Dr. Prafulla Naik, ICAR-CARI, Bhubaneswar, Odisha
35. Dr. R.K. Dhuria, RAJUVAS, Bikaner, Rajasthan
36. Dr. Rajiv Chawla, NDDB, Anand, Gujarat
37. Dr. Raka Saxena, ICAR-NIAEPR, New Delhi
38. Dr. Rakesh Kumar, NDRI, Karnal, Haryana
39. Dr. Ram Singh, ICAR-CIRB, Hissar, Haryana
40. Dr. Raman Malik, NDRI, Karnal, Haryana
41. Dr. Ravindra Kumar, CIRG, Makhdoom, Uttar Pradesh
42. Dr. S.E. Jadhav, IVRI, Izatnagar, Uttar Pradesh
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INDIAN JOURNAL OF ANIMAL NUTRITION

(www.indianjournals.com; www.ansi.org.in; http://epubs.icar.org.in/ejournal/index.php/IJAN)

December, 2019 **Vol. 36** **#4**

CONTENTS

Ruminants

Non-Ruminants