ISSN 0970-3209
ISSN 2231-6744 (online)

MARCH 2019 | VOL. 36 | #1

INDIAN JOURNAL OF ANIMAL NUTRITION

I J A N

AN OFFICIAL PUBLICATION OF
ANIMAL NUTRITION SOCIETY OF INDIA
www.indianjournals.com | www.ansi.org.in
http://epubs.icar.org.in/ejournal/index.php/IJAN

Indian Journal of Animal Nutrition

Aims and Scope: The Indian Journal of Animal Nutrition (IJAN) publishes original papers on research in the fields of animal nutrition, feed technology, feed evaluation and conservation, physiological and biochemical aspects including microbiology, biotechnology, feed and fodders and other relevant areas related to livestock feeding in developing and tropical regions. Papers describing research on given areas for ruminants and non-ruminants are welcome. The Journal is a forum for presenting **peer reviewed** articles on basic and applied research. Submissions addressing the new frontiers of the subject including emerging areas of nutritional genomics, modelling and interface topics such as soil plant animal and ecosystem interrelationships are given priority. Reviews on specialized topics are also included. The IJAN is published quarterly since its inception in 1983 under the aegis of Animal Nutrition Society of India (ANSI) having about twelve hundred life members around the world. ANSI has instituted two awards to be given to two selected papers published in the Journal during the biennial conferences. For inquiry regarding status of article, the request may be sent to Chief Editor at **editoransi@gmail.com.** The articles may be sent on line using the hyperlink: **http://epubs.icar.org.in/ejournal/index.php/IJAN/about submissions#online Submissions** For any other details visit sites:- **www.indianjournals.com** and **www.ansi.org.in**

INDIAN JOURNAL OF ANIMAL NUTRITION

(A quarterly publication)

IJAN is supplied free of cost to members of ANSI

Life Membership of Animal Nutrition Society of India

Within India - ₹ 4,000/-

Student Membership - ₹ 2,500/- only

For Govt. and Public Institutions - ₹ 4,000/- only

For agent ₹ 6,000/- rebate 25% (postage extra)

For SAARC countries - US $ 100 (Postage extra)

For other countries - U S $ 150 (Postage extra)

Benefactor members for 10 years - ₹15,000/-

Sustaining members for 10 years - ₹ 15,000/-

Demand Draft may be sent in favour of Treasurer, Animal Nutrition Society of India.

The articles should be sent to Chief Editor using on line facility **(http://epubs.icar.org.in/ejournal index.php/IJAN/about/submissions#online Submissions).**

Chief Editor welcomes books for reviews; news items etc.

Change of Address: May be communicated to The Secretary as well as The Chief Editor.

BENEFACTOR MEMBERS

KAMDHENU FEEDS
An ISO 9001:2008 Company
7th,K.M,dehradun Road,
V & P.o Sadak Doodhlee, Saharanpur-u.p (247001)
Ph. : 0132-2765719, 2660289 M : 09412232419
Fax : 0132-2765719, email : kamdhenufeed@gmail.com
www.kamdhenufeeds.com

VOLAC INTERNATIONAL LTD.
Volac House, Orwell, Royston,
Hertfordshire, SG8

S. BALWANT SINGH MANDOR
Managing Director, Tara Feed Factory
Office - Village Gajjan Majra
Malerkotla, Distt. Sangrur, Punjab

BRINDAVAN PHOSPHATE PVT. LTD.
5/3, Ist Main Road, Jaymahal Extension
Bangalore-560 046

APPROPRIATE TECHNOLOGY INDIA
23, Yogaharam Society, Shreya Terka
Ambawadi, Ahmedabad 330 015

LABINDA INSTRUMENTS PVT. LTD.
201, Nand Chambers, LBS Marg
Vandna Cinema, Thane-400 602

AVITECH
GP-51, Sector-18, Udyog Vihar, Gurgaon
GUJARAT CO-OPERATIVE MILK
Marketing Fed Ltd.
Anand, Gujarat

POWER TECH
Aqua Cattle and Poultry Feed, Plant Manufacturers
900/1A GIDC, Makarpura, Baroda-390 010

KEMIN NUTRITIONAL TECH (Ind) Pvt. Ltd.
K3, 11th Cross Street, SIPCOT Industrial Complex
Gummidipundi-601 201

CREMACH DESIGNS PRODUCTS ENGINEERS
893/4 GIDC, Industrial Estate,
Makarpura, Baroda-390 010

TARNI CATTLE FEEDS
Budhram Oram Market, Kachery Road
Rourkela-769 012

DIRECTOR
KOYENCO FEEDS PVT. LTD.
Koyenco House, West Hill, Calicut-673 005, Kerala

MALWA FEEDS PVT. LTD.
Mfrs and Suppliers : Cattle Feed
6th Km Moga-Ludhiana, G.T. Road
Moga-142001 (Pb.)

New Life Members of ANSI

Shamsundar Nandkumar Jadhav
Ph.D. Scholar
Post-Jamsar, Tal- Jawhar-401603
Dist. Palghar, Maharashtra
Mobile : 9226374099/ 9420849407

Dr. Kavita A. Shende
Rathilayout Dhantoli (Near Maramay Mandir)
Katol-441302, Nagpur, Maharashtra, India

Dr. Umesh Kumar Prajapat
Naer Dauji Temle, Goswami Chowk,
Bikaner-334001, Rajasthan

Dr. Pallavi Sinha
c/o Srisitaram Prasad,
House No. 4D/9, Veer Kinwar Singh Path,
North SK Puri, Boring Road,
Patna-800013, Bihar, India

Gaurav Pratap Singh
Sutlej Hostel, NDRI, Karnal-132001
Haryana, India

Shimala Yadav
A-142, Near Alaknada Hostel,
NDRI, Karnal-132001, Haryana, India

Dr Avishek Biswas
Senior Scientist,
Avian Nutrition and Feed Technology Division,
ICAR-CARI, Izatnagar-243122, Uttar Pradesh, India

Dr. G. Subramanyeswar
Department of Animal Nutrition,
College of Veterinary and Animal Science,
Pookode, Wayanad- 673576.
E-mail: eeswar681@gmail.com
M: 9446233030

ACKNOWLEDGEMENT

Animal Nutrition Society of India puts on record its sincere thanks to Indian Council of Agricultural Research, New Delhi for grant of financial support for publication of IJAN

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

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

CONTENTS

Indian J. Anim. Nutr. 2019. 36 (1): 1-10
doi: 10.5958/2231-6744.2019.00001.X

Indian Journal of Animal Nutrition

REVIEW

Metagenomics and CAZymes in Rumen: A review

Anju Kala*, Devki Nandan Kamra, Lal Chandra Chaudhary and Neeta Agarwal
Animal Nutrition Division, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Uttar Pradesh, India

ABSTRACT

Rumen converts lignocellulsic biomass to high quality food by virtue of the diverse microbiota. The composition of rumen ecosystem is shaped up by various factors including diet, individuality, age, geographic region, postfeeding time *etc.* Earlier culture based techniques could study rumen microbes partially for the cultivable microbes. But, now it is known that about 90% of rumen microbes are unculturable and the knowledge that we have till now seems to be meager. With the advent of new technologies as next generation sequencing which study the whole ruminal ecosystem at one time contributed tremendously to our existing knowledge. Approaches like metagenomics, metatranscriptomics *etc.* have made it possible to study the structure and function of rumen microbes in their natural environment. The study of CAZymes revealed that there is an array of hydrolytic enzymes in rumen that perform the deconstruction of fibrous feed material. Also, these enzymes are not only contributed by well known microbes' *viz. Fibrobacter* and *Ruminococcus* but by a very diverse microbiota including *Roseburia, Porphryomonas, Balutia etc.* So, metagenomics has added to our knowledge in many spheres of rumen microbiology, its composition and interactions in rumen. But the problem in this field is that a robust database is not available to compare the data obtained. Much work is required in field of analysis of metagenomic database establishment.

Key words: CAZymes, Fiber, Metagenomics, Rrumen microbes

INTRODUCTION

Rumen is home to complex consortia of microbes and very less is known about the magnitude and direction of interaction of various components of this microbiome. Moreover, only 11% of rumen microbes appear to be culturable (Edward *et al.,* 2004) and those microbes which can be cultured have been studied as pure culture in isolation but in rumen these microbes act in dependent manner. Thus, holistic studies regarding interdependent components of rumen niche and how change in one component affects the other components and finally rumen fermentation needs to be evaluated. There has been a considerable addition to our knowledge in terms of diversity and to some extent functioning of rumen microbiome.

Also, these techniques are biased towards the laboratory cultivable microbes. High throughput techniques like metagenomics provides greater depth of sequencing which produces useful and extensive coverage of the microbial diversity allowing us not only to identify the prevalent 'core' members of the community but also 'rare' community members that could be associated with feeding practices (Shanks *et al.,* 2011). Metagenomics addresses the collective genetic structure and functional composition of microbial community without the bias or necessity for culturing its individual inhabitants (Galbraith *et al.,* 2004).

Rumen metagenomics follows two approaches namely functional metagenomics and computational methagenomics (Puniya *et al.,* 2015). The microbial genes responsible for regulating enzyme production in rumen are expressed in response to various diets and other environmental conditions producing corresponding enzyme. Functional metagenomics include study of collective genome of a microbial community by expressing it in a foreign host (Ekkers *et al.,* 2012) which includes cloning of genetic diversity from rumen in vectors as plasmids, fosmids, cosmids and bacterial artificial chromosome etc followed by expressing it in foreign host as *E. coli* and characterization of desired functional activities in expression libraries using various strategies (Simon and Daniel, 2009). Mining of unexplored and untapped fibre degrading enzymes through this approach may lead to

*Corresponding author: E-mail: anjukalavet2002@gmail.com

revolutions in animal husbandry and industrial sector. Numerous efforts have been made to isolate fibre digesting enzymes of glycosyl hydrolase families as GH3, 5, 8, 10, 13, 43 and 48 (Gruninger *et al.,* 2014; Lee *et al.,* 2012a; Pope *et al.,* 2012).

The current methods are less sensitive in expressing target genes in foreign host and throughput of these screening methods is low (Puniya *et al.,* 2015). But like all new approaches, this approach has many possibilities yet to be explored. The second approach *i.e*. computational metagenomics has made it possible to have deeper insights into structure and functional setup of rumen microbiome which were unachievable earlier. It includes SSU (single sub unit) rRNA approach (16S or 18S) and whole genome shotgun (WGS) approach which provides chance to understand protein range, its metabolic significance and taxonomical assignments of rumen microbes. This will increase our understanding of the structural and functional order of rumen microbes.But these approaches are in infancy and there are many limitations with theses approaches. The major one being that SSU of rumen origin is underrepresented in public database in SSU rRNA approach and inability to assign taxonomy to novel sequences.Whereas in WGS (whole genome sequence) approach and sequences generated by NGS technologies have platform biases, high error rates and short read lengths.

Metagenomics does not reveal how this genetic information is actually expressed in rumen. To study how these genes are expressed and regulated, a new field 'metatranscriptomics' is being explored which aims at studying expression of genes through abundance of mRNA transcripts. Metatranscriptomics gives insight into gene expression and regulation at mRNA level, metaproteomics studies the product *i.e.,* proteins for which these genes are encoded. A step further to this is study of all metabolites of feed and rumen origin (metabolomics) to decipher various metabolic pathways and their interdependence within rumen and how they are affected by varying composition of ruminal consortia. An integrated approach including all 'omics' may give holistic insight into structure and function of rumen microbiome, one of the most complex ecosystem. This review is just touching the tip of the iceberg of this vast knowledge.

METAGENOMIC COGNIZANCE OF RUMEN MICROBIOME

Due to the amazing and unique property of transforming lignocellulosic feed into useful products for human consumption, ruminants, rumen and its microbes have been subject of study since long.

Insights in developing rumen

Lot of research work has done on how rumen microbes are established in rumen since birth and later with development of rumen. Our basic understanding is that rumen is inhabited with microbes within first 24 h of birth (McCann *et al.,* 2014). Bacteria including fibrolytic and methanogens are the first to occupy rumen within first week of life followed by protozoa (Morvan *et al.,* 1994). Earlier studies observed establishment of methanogens within 3-4 days of life (Fonty *et al.,* 1989) but recent studies (Gagen *et al.,* 2012) showed that methanogens can be detected in ovine rumen at 17 h of birth. A detailed study of microbial diversity and functionality in calves (14 and 42 dold) fed similar diet showed that more than 24 prokaryotic phyla and 22 eukaryotic phyla and 8298 Pfam protein families highlighting the diversity richness in pre ruminant calves (Li *et al.,*2011). Further, a total of 156 and 120 genera were found in 14 and 42 d old calves, respectively. Interestingly, rumen of younger calves has more heterogenous rumen microbiome and harbors a greater number of microbial genera than older calves. It might be due to the fact that as rumen develops, opportunistic microbes are eliminated and a certain consortia is developed which favors microbes aiding in fibre degradation.

Jami *et al.* (2013) revealed that *Ruminococcus albus* is detectable at one day of age while another fibrolytic species *F. Succinogenes* developed much later. Presence of fibrolytic microbes much before introduction of solid diet has been observed earlier (Li *et al.,* 2012). The developmental stage of rumen appears to be one of major determinants of microbial setup in rumen and the rumen microbiome becomes more

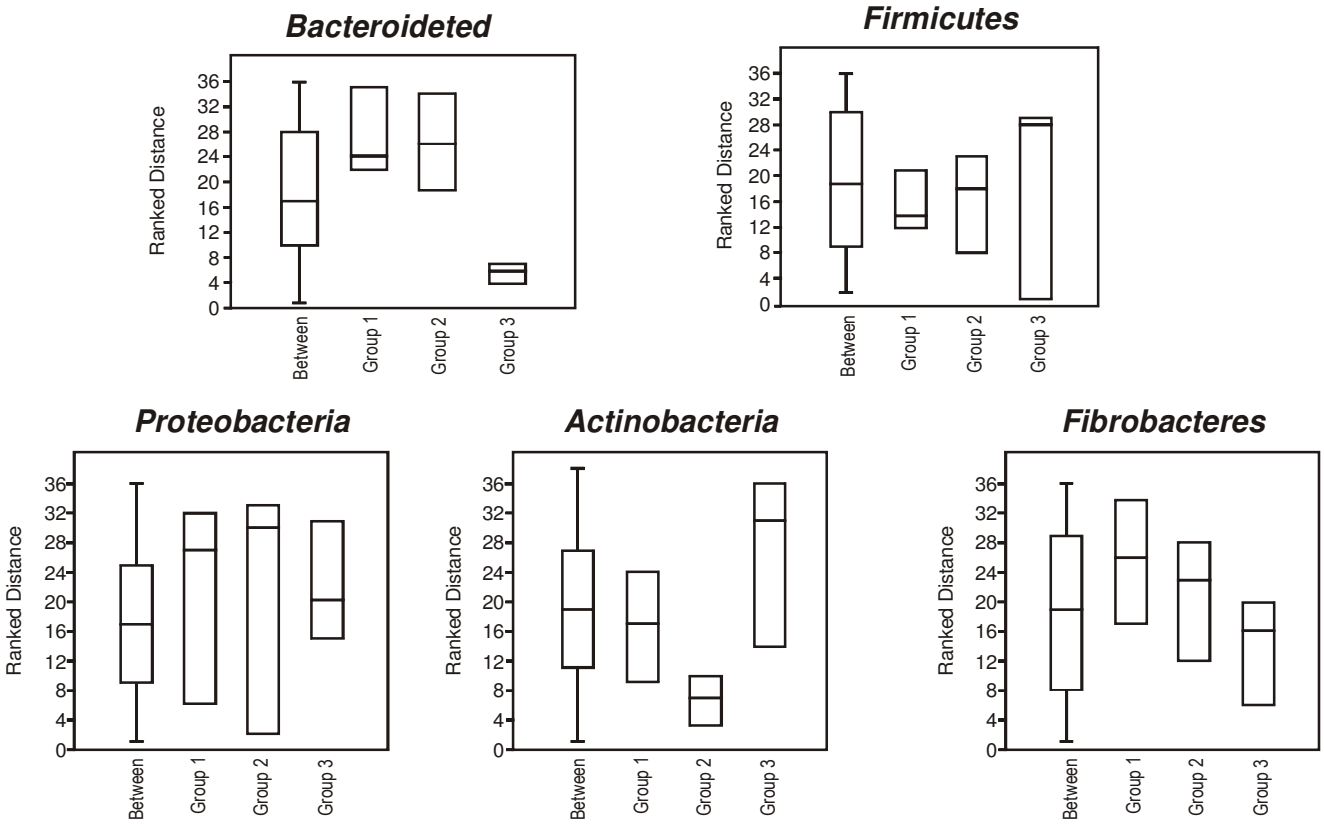

Fig.1. Box plot indicating mean of ranked distances for most abundant five rumen phyla in buffaloes fed three levels of TDN. Group 1, 2 and 3 indicate 70, 85 and 100 % TDN fed groups. Boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentile) and the horizontal line defines the median (Source: Kala *et al.,* 2017a).

homologous with increased diversity as the age of the calf advanced.

Revealations from nutritional interventions

Pioneer studies on rumen metagenomics (Brulc *et al.,* 2009) using next generation sequencing (NGS) indicated that individuality of animal may be dominating over other factors (as diet, environment) in establishing rumen microbial composition. In above study fibre adherent portion microbiome in which enrichment (66 *vs.* 8% abundance) in *Gamma proteobacteria* at the expense of *Firmicutes* and *Bacteroidetes* was observed in third animal as compared to other two animals. It appeared that third animal had not adapted to experimental diet, which may be due to individuality of animal. Similar observation were made by Kala *et al.* (2017) wherein buffaloes fed varying TDN diets showed great variation in rumen microbial abundance which might be attributed to individuality of buffaloes in group fed similar diet.

There are many factors (diet, individuality of animal, type and time of sample, processing of sample, sequencing technologiy *etc.*) which need to be considered during metagenomic studies.

Distribution of microbes in rumen

Metagenomics of rumen microbes has provided the opportunity of studying rumen ecosystem in its enteriety considering all the cultivable and non-cultivable microbes. In rumen, bacterias are the most abundant domain followed by eukaryota, archea virus and phages (Kala *et al.,* 2017a). Baceriodetes and firmicutes have been reported to be the most abundant phyla in the rumen of beef cattle, Sika deer and buffaloes irrespective of diet or treatment (Durso *et al.,* 2011; Mao *et al.,* 2015; Nardi *et al.,* 2016; Kala *et al.,* 2017a). In buffalo rumen, Bacteroidetes and firmicutes followed by proteobacteria, actinobacteria and fibrobacteres

were predominant bacterial phyla (Fig. 1) all the experimental animals except two animals, one from 85% TDN group and another from 100% TDN group where fibrobacteres were higher than actinobacteria (Kala *et al.,* 2017a) which may be individual animal variation. PCA plots for diversity analysis at phylum level revealed that buffaloes supplemented 85% TDN diet had the most diverse rumen microbiome followed by 100 and 70 % TDN in diet (Fig 1) and that some of the phyla were common to all the experimental animals.

At the level of genera, *Prevotella* is reported to be the most dominant bacteria followed by *Bacteroides* and *Clostridium* (Singh *et al.,* 2012; Kala *et al.,* 2016b). Many studies observed similarity in predominance of the major genera in bovine rumen (Li *et al.,* 2014; Mao *et al.,* 2015; Kala *et al.,* 2017a) irrespective of different dietary interventions. Most of metagenomic studies have reported a considerable proportion of rumen microbes to be unidentified or lesser known. Kala (2017) also observed that about 50% sequences of metatranscriptomic libraries of buffalo rumen were devoted to sequences with less than 1% abundance, unknown or unassigned sequences and were termed as others but this cluster of 'others' might have microbes of importance in rumen function and need to be explored further.

Abundance of microbes in rumen could be classified with *Firmicutes* and *Bacteroidetes* forming major chunk of rumen bacterial population. Kala (2017) reported that distribution of rumen phyla showed shift in abundance with respect to three different levels of TDN (70, 85 and 100%) in diet, showing highest diversity for 85 % TDN group (Fig. 2). However, Menezes *et al.* (2011) reported that the phylum separation was more evident for sample type (solid and liquid portions) rather than separation between diets (Menezes *et al.,* 2011). They observed that *Fibrobacteres* and *Spirochaetes* were more prevalent in solid phase whereas *Actinobacteria* was more abundant in liquid phase. The overall dissimilarity was higher for solid and liquid fractions (14.9%) rather than diets (10.5%). They revealed that bacteria and archea were affected by both diets and rumen phase (solid or liquid). Fouts *et al.*

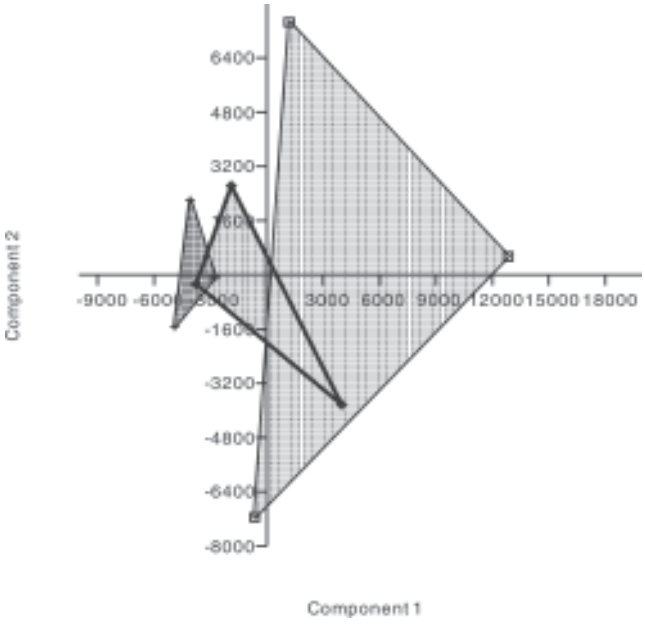

Fig. 2. PCA plots representing diversity shifts at phylum level in buffalo rumen with supplementation of TDN levels. The experimental groups are represented by colors red (70 % TDN), purple (85 % TDN) and green (100% TDN).

(2012) reported that 80% of species levels OTU were dominated by *Clostridales, Bacteridales, Erysipelotrichales* and unclassified TM7 in cows fed similar diets. *Prevotella* and *Taneralla* were abundant in liquid portion while *Butyrivibrio* and *Balutia* were higher in solid portion but no statistical difference was observed in solid and liquid portions. Analysis of cross domain correlations suggested far greater interdependent rumen microbial diversity. Kittelmann *et al. (*2011) showed rhatcommunity compositions of ciliate population in cattle were observed to be influenced by diet whereas diet effects in deer and sheep were weaker than the animal-to-animal variation. Further. it was revealed that New Zealand cattle contained A-type communities with most sequences closely related to those of the genera *Polyplastron* and *Ostracodinium* whereas deer and sheep harboured B-type communities, with the majority of sequences belonging to the genera *Epidinium* and *Eudiplodinium* (Kittelmann *et al.,* 2011).

It is still not very clear as to what affects the

composition of rumen microbiome in ruminants but diet and individuality of animals seem to play major role along with other factors as species, geographic region, age of animal *etc.* Methanogenic archaea in the rumen have not been very well documented and not much information was available as compared to other anaerobic habitats making rumen environment highly selective. In buffaloes, we found about 22 genera along with some unidentified groups thus unfolding the richness of rumen methanogen community structure upto some extent (Kala *et al.,* 2017c). Domain archea is further divided to phylum, Crenarchaeota, Euryarchaeota, Thaumarchaeota, Nanoarchaeota. Most of the methanogenic archea belonged to Euryarcheot including *Methanobrevibacter* which has been reported to be the most abundant methanogen in rumen (Zhu *et al.,* 2017; Kala *et al.,* 2017a) and is responsible for majority of methane production in ruminants. The enteric methane production was said to be positively correlated to abundance of rumen methanogens (Ross *et al.,* 2013; Wallace *et al.,* 2015) but some researchers reported that enteric methane production is dependent on transcription of methanogenic pathways (Shi *et al.,* 2014) or composition of methanogenic consortium in rumen ((Danielsson *et al.,* 2017).

Earlier, *Fibrobacter* and *Ruminococcus* were known to be most abundant rumen microbes (Kala *et al.,* 2017b) and their role in fiber degradation is well known but their low abundance in metagenomic studies (Kala *et al.,* 2017a; Lim *et al.,* 2013) pointed that there are other rumen bacteria playing crucial role in fiber degradation. Stevenson and Weimer (2007) also reported that the key fibrolytic bacteria *viz.*, *R. flavefaciens, R. albus* and *F. succinogenes* represented only ~2% of ruminal bacterial 16S rRNA. In our studies also, microbes like *Paludibacter, Blautia, Porphyromonas* and *Roseburia* contributed sizably to the CAZymes involved in fiber digestion. Many studied have reported *Bacteroides* as one of the most abundant bacteria in buffalo rumen after *Prevotella*. Naas *et al.* (2014) reported that *Bactreoides* has celluloytic activity of and the presence of polysaccharide utilizing loci (PUL, a system of lignocellulosic feed utilization other than extracellular and cell bound cellulosomes) in their genome further strengthens the presence of an alternate mechanism of fiber utilization in rumen.

Core rumen microbiome

The rumen microbiome is responsive to diet, developmental stages, genetics and environmental factors which may alter rumen microbial composition substantially. But it may be possible that a set of core genus/OTU is present across all ruminant species which is involved in basic rumen functioning. This may be said as core microbiome which may deepen our understanding of basic structure and function of rumen microbiome. The core rumen microbiome of bovine rumen microbiome, developing as well as mature was reported to consist of 8 phyla, 11 classes and 15 families (Wu *et al.,* 2012). These 8 phyla included Bacteroides, Firmicutes, Proteobacteria, Spirochaetes, Fibrobacteres, Verrucomicrobia, Synergistetes and Actinobacteria in descending order of relative abundance. A comparative study of microbial community composition of bovine, caprine and ovine rumen showed that a total of 22 phyla and 94 families were detected in rumen microbiome of cattle, goat and sheep (Kim *et al.,* 2011) with mean number of phyla per animal rumen 16.8, 11.8 and 16.9 for cattle, goat and sheep, respectively. Moreover, the relative abundance of the core microbiome varied as per species of ruminants. The author has also observed 46 and 70 phyla common to nine metatranscriptomic libraries in two different experiments in buffaloes fed different TDN (Fig. 3) or blend of essential oils (Kala, 2017).

Metanalysis of rumen microbial diversity of 16S rRNA gene sequences of rumen origin deposited in RDP database identified 19 phyla and, 5271 and 942 OTUs for rumen bacteria and archea, respectively. Of these 19 phyla, Firmicutes, Bacteriodetes and Proteobacteria were most predominant.

Rumen plasmidome

Till now, focus of rumen microbiology has been on more known rumen bacteria, archea and fungi but now it is being diverted to other less studied components as 'plasmidome' and 'virome' of rumen. These small components are major factors deciding

adaptation of rumen microbiome during changing conditions of rumen and they should be taken to consideration when studying a complex system as rumen. Plasmids have not been studied much till now because copy numbers of plasmids are very low per bacteria and it is difficult to differentiate them from chromosomal DNA. Brown Kav *et al.* (2013) reported a method to enrich rumen plasmid DNA. Plasmids may affect the representation of important phyla in rumen. *Proteobacteria* account for 20% of rumen plasmid sequences whereas its abundance appears to be significantly lower (approx. 5%) as per 16 S approach (Brown Kav *et al.,*2012).These plasmidome share similarities with rumen metagenome and display higher functional representation of cell wall and capsule, cofactors and vitamins *etc.* This hints at possible lateral gene transfer between rumen microbes (Puniya *et al.,* 2015).

Rumen virome

Due to lack of conserved protein and genes as 16S rRNA, rumen viral diversity has not been studied much. In a recent study (Ross *et al.,* 2013), 14 putative viral sequences over 30 kbp are identified. Rumen virome of cows housed together and fed similar diets displayed similar taxonomical virome profiles than non -cohabitated animals indicating rumen viromes might be functionally conserved. This also infers that rumen virome are related with transfer of antibiotic resistance, control bacteria population dynamics; regulate protein metabolism and animal nutrition. These factors may finally affect the resilinence of rumen microbes to alteration of rumen environment and host immunity.

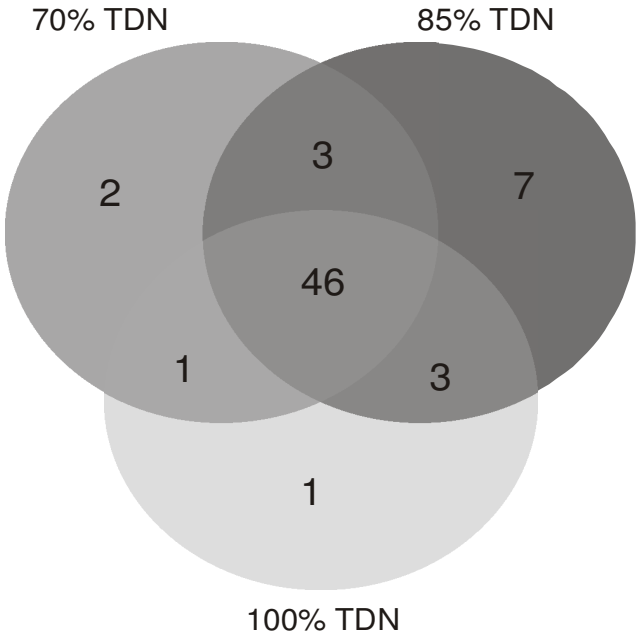

Fig. 3. Venn plot showing shared and unique phyla in rumen of buffaloes fed 70, 85 and 100 % TDN. All taxa present within each group are plotted (As per Kala *et al.,* 2017a).

Carbohydrate active enzyme profile

Rumen microbes produce a vast array of hydrolytic enzymes which are responsible for deconstruction of structural plant polysaccharides. Activity of hydrolytic enzymes in rumen is documented (Agarwal *et al.,* 2000, Patra *et al.,* 2010) to some extent. Functional metagenomics has been used to identify majority of these enzymes of biotechnological interest using specific substrates. This approach was pioneered by Ferrer *et al.* (2005) who detected 9 endoglucanases, 12 esterases and 1 cyclodextrinase from a dairy cow rumen metagenomic library. Functional properties of complex ecosystem as rumen needs to be understood as closely related microbes may have different functions and metabolic characteristics (Achenbach and Coates, 2000; von Mering *et al.,* 2007). Arumugam *et al.* (2011) suggested that functional biomarkers are more robust than phylogenetic biomarkers for identifying enterotypes associated with host phenotype characteristics. In agreement to this, we also found in our studies that CAZymes which are considered important for fiber utilization in rumen (GH2, 3, 5, 94) had a larger contribution from rumen microbes of lesser known origin. Microbes as *Paludibacter, Roseburia, Balutia* and *Porphyromonas* were some of the microbes contributiong sizably to CAZymes in buffalo rumen (Kala *et al.,* 2017a).

The CAZyme database gives information regarding the gene sequences encoding for carbohydrate utilizing enzymes mainly glycoside hydrolases (GHs) which hydrolyze the glycosidic bonds of polysaccharides, carbohydrate esterases (CEs) and break the ester bonds between lignin and carbohydrates,

cellulose binding module (CBMs) involved in enzyme substrate binding, pectin lyase related pectinolysis, glucosyl transferase involved in transfer of glucosidic bonds (Kala *et al.,* 2016). Earlier, it was not possible to mine this Carbohydrate Active Enzyme (CAZymes) profile of rumen at such greater depths but with advent of NGS and meta-transcriptomics, it became possible to identify putative CAZymes of rumen origin. Functional screening and sequencing of fosmid libraries from Svalbard reindeer rumen revealed CMCase positive clones affiliated to the abundant SRM-1 clade that lacked known endoglucanases inferring the possibility of novel cellulolytic mechanisms that are yet to be characterised. This approach also identified *Bacteroidetes*-affiliated GH5-linked PULs (Pope *et al.,* 2012). Similar cellulose linked PULs were found in the cow and buffalo rumen and macropod foregut microbiomes indicating that these structures might be involved in adaptation to growth on cellulose by *Bacteroidetes* species. The solid and liquid mass of rumen together form heterogenous rumen content harbouring microbes producing CAZymes (Hobson, 1997) and the whole rumen content forms ideal sample for mining CAZymes.

Patel *et al.* (2014) found that the proportion of sequences showing > 90% identity to known CAZymes was very less which indicated the presence of novel enzymes which could be further explored for their activity. Among known CAZymes, only GH5 and 9 accounted for about 1.81% of all the glycoside hydrolases of cellulases representing families and GH 10, 11, 26 and 28 collectively represented a total of 4% of all hemicellulases GHs. In our study (Kala *et al.,* 2017a), the major contributors to CAZyme data were GH families like GH 2,3,5,9,29 and 39 (cellulase) and GH 43,51,78 (hemicellulase).Also, those GH families which are considered more important for fiber degradation sourced from a variety of rumen microbes indicating their source diversity. This diversity of source of enzyme might be a mechanism to counter situations when some particular species of rumen microbes are adversely affected in conditions like stress or diet change but the essential functions of rumen fermentation need to be carried using other microbial sources. This may answer why it is difficult to change rumen fermentation pattern by changing diet of experimental animals. The relative proportion of each class of the CAZymes increased with increase in the roughage proportion in the diet indicating enrichment of microbial for utilization of complex plant polysaccharides in rumen (Patel *et al.,* 2014).

The most abundant CAZymes in rumen metagenome are oligosaccharide degrading enzymes (Wang *et al.,* 2013) which include GH 2, 3, 10, 11,5, 43, 51, 95, 36 *etc.* in which GH2, 3, 43 are the most abundant family. Higher abundance of oligosaccharide degrading enzymes in rumen is quite expected as the hemicelluloses chain of dietary origin has variety of side chains and to break the side chains diverse enzymes will be required in rumen. A comparison of glycoside hydrolase and cellulosome functional genes revealed that in the rumen microbiome, initial colonization of fiber appears to be by organisms possessing enzymes that attack the easily available side chains of complex plant polysaccharides (families GH2 and GH3) and not the more recalcitrant main chains especially cellulose (GH5 and GH9). For example, the metagenomes displayed a diversity of enzymes that digest the side chains of cellulose, hemicelluloses and pectins and oligosaccharides but the number of enzymes devoted to the hydrolysis of the main chain of these polymers is very small (Brulc *et al.,* 2009). CBM also plays very crucial role in fiber utilization as CBM class helps in attachment of substrate and enzyme.Moreover CBM37 was observed to contribute solely from *R. albus* in rumen and *R. albus* is well known for fiber utilization (Ezer *et al.,* 2008). White *et al.* (2014) also reported that CBM37 binds to numerous types of polysaccharides and is associated with several enzymes including xylanase and esterase of plant cell wall catalytic modules.

CONCLUSIONS

Metagenomics has broadened horizons of our knowledge in revealing the lesser known facts of rumen microbiome. This approach is addressing some

long unanswered questions. The majority of rumen bacteria as observed through metagenomic studies are *Bacteroidetes, Firmicutes* and *Proteobacteria*. Among archea, *Euryarcheota* is major group. But in all the studies, a large number of sequences are unclassified which form major proportion of rumen microbiome. So, urgent focus is required on characterizing and classifying these unknown sequences. Further, rumen microbiome composition may be affected by species, genetics and individuality, type of sample (solid or liquid). Also, the presence of fiber degrading microbes in day old rumen indicates possibility of introducing fibrous diet at younger age. But further conformational studies are required. There could be alternate methods of fiber degradation in rumen as observed by PUL studies which might be explored further as tools for enhancing fiber degradation. There could be a possibility that the predominant and 'main' rumen microbes are not the only drivers of rumen functions but other 'rare' and less represented microbes might decide dimension and direction of rumen fermentation. Also, the CAZyme family studies indicated a diversity of microbes which contribute rumen enzymes, hence indicating involvement of other less represented microbes. Though metagenomics has added to our knowledge in many spheres of rumen microbiology but to put forth concrete facts for correlating rumen fermentation and rumen microbiome, more work is required in fields of analysis and database establishment.

REFERENCES

Achenbach, L.A. and Coates, J.D. 2000. Disparity between bacterial phylogeny and physiology –comparing 16S rRNA sequences to assess relationships can be a powerful tool, but its limitations need to be considered. *ASM News.* 66: 714-715.

Agarwal, N., Agarwal, I., Kamra, D.N. and Chaudhary, L.C. 2000. Diurnal variations in the activities of hydrolytic enzymes in different fractions of rumen contents of Murrah buffalo. *J. Appl. Anim. Res.* 18: 73-80.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T. and Mende, D.R. 2011. Enterotypes of the human gut microbiome. *Nature.* 473:174-180.

Brown Kav. A., Benhar, I. and Mizrahi, I. 2013. A method for purifying high quality and high yield plasmid DNA for metagenomic and deep sequencing approaches. *J. Microbiol. Methods* 95: 272-279.

Brown Kav, A., Sasson, G., Jami, E., Doron-Faigenboim, A., Benhar, I. and Mizrahi, I. 2012. Insights into the bovine rumen plasmidome. *PNAS.* 109: 452-457.

Brulc, J.M., Antonopoulos, D.A., Miller, M.E., Wilson, M.K., Yannarell, A.C. and Dinsdale, E.A. 2009. Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. In: "*Proc Natl. Acad. Sci. USA"*. 106: 1948-1953. http://dx.doi.org/10.1073/pnas.0806191105.

Danielsson, R., Dicksved, J., Sun, L., Gonda, H., Muller, B., Schnurer, A, and Bertilsson, J. 2017. Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. In: *Frontiers in Microbiol*. 8. 26.http,//doi.org/ 10.3389 fmicb.2017.00226.

de Menezes, A.B., Lewis, E., O2 Donovan, M., O2 Neill, B.F. and Clipson, N. 2011. Microbiome analysis of dairy cows fed pasture or total mixed ration diets. *FEMS Microbiol. Ecol.* 78: 256-265. doi: 10.1111/j.1574-6941.2011.01151.

Durso, L.M., Harhay, G.P., Smith, T.P., Bono, J.L., Desantis, T.Z., Harhay, D.M., Andersen, G.L., Keen, J.E., Laegreid, W.W. and Clawson, M.L. 2010. Animal-to-animal variation in fecal microbial diversity among beef cattle. *Appl Environ Microbiol*. 76: 4858-4862.

Edwards, E.J., McEwan, R.N., Travis, J.A. and Wallace, J.R. 2004. 16S rDNA library-based analysis of ruminal bacterial diversity. *Antonie Van Leeuwenhoek*. 86: 263-281.

Ekkers, DM, Cretoiu, M.S., Kielak, AM and Elasas J.D. 2012. The great screen anomaly- a new frontier in product discovery through functional metagenomics. *Appl. Microbiol. Biotechnol.* 93:1005-1020.

Ezer, A., Matalon, E., Jindou, S., Borovok, I. and Atamna, N. 2008. Cell surface enzyme attachment is mediated by family 37 carbohydrate-binding modules, unique to *Ruminococcus albus*. *J. Bacteriol.* 190: 8220-8222.

Ferrer, M., Golyshina, O.V., Chernikova, T.N., Khachane, A.N., Reyes-Duarte, D. and Santos, V.A.P.M.D. 2005. Novel hydrolase diversity retrieved from a metagenome library of bovine rumen microflora. *Environ. Microbiol.* 7: 1996-2010.

Fouts, D., Szpakowski, S., Purushe, J., Torralba, M. and Waterman, R. 2012. Next generation sequencing to define prokaryotic and fungal diversity in the bovine rumen. *PLoS one* 7: e48289. doi:10.1371/ journal.pone.0048289

Gagen, E.J., Mosoni, P., Denman, S.E., Al Jasim, R, Mcsweeny, C.S. and Forano. 2012.Methanogenic colonization does not significantly alter acetogn diversity in lambs isolated 17 h after birth and raised aseptically. *Microb. Ecol.* 64:628-640.

Galbraith, E. A., Antonopoulos, D. A. and White, B. A.2004. Suppressive substractive hybdidization as a tool for identifying genetic diversity in an environmental metagenome : the rumen as a model. *Environ. Microbiol.* 6: 928-937.

Gruninger, R.J., Gong, X., Forster, R.J. and McAllister, T.A. 2014. Biochemical and kinetic characterization of the multifunctional beta-glucosidase/beta-xylosidase/ alpha- arabinosidase Bgxa1. *Appl Microbiol. Biotechnol.* 98: 3003-3012.

Hobson, P.N. and Stewart, C.S. 1997. *Rumen Microbial Ecosystem.* (Dordecht: Springer).

Jami, E, Israel, A, Kotser, A. and Mizrahi, I. 2013.Exploring the bovine rumen bacterial community from birth to adulthood. *Int. Soc.Microb. Ecol.* 7:1069-1079.

Kala, A. 2017. *Effect of Diets and Essential Oils on Metagenomics of Rumen Microbes of Buffaloes.* Ph.D. thesis submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, India.

Kala, A., Kamra, D.N., Kumar, A., Agarwal, N., Chaudhary, L.C., Joshi, C.G. 2017a. Impact of levels of total digestible nutrients on microbiome, enzyme profile and degradation of feeds in buffalo rumen. *PLoS ONE.* 12 (2), e0172051.https,//doi.org/10.1371/journal.pone. 0172051.

Kala, A., Kamra, D.N., Agarwal, N. and Chaudhary, L.C. 2016b. Metatranscriptome of rumen of buffaloes fed diets with variable total digestible nutrients. *In: Proc. National Workshop on Metagenomics and Nutrigenomics for Research and Teaching in Animal Nutrition in India.* pp 103-105.

Kala, A., Kamra, D.N., Agarwal, N. and Chaudhary, L.C.2017b. Effect of a blend of essential oils on buffalo rumen microbial and enzyme profiles and *in vitro* feed fermentation. *Anim. Nutr. Feed Technol.* 17: 189-200. DOI: 10.5958/0974-181X.2017.00020.8

Kala, A., Kamra, D.N., Kumar, A., Agarwal, N., Chaudhary, L.C. and Joshi, C.G. 2017c. Evaluation of dietary TDN levels on rumen archeal and bacterial metagenomic profile of buffaloes. *In: Proc. XIII Annual Meeting of the Indian Society of Animal Genetics and Breeding & National Conference on "Challenges in Quantitative Genetics for Improvement of Indigenous Animal Genetic Resources (AnGR)"* ICAR-IVRI, Izatnagar, January 19-20, 2017.

Kala, A., Kamra, D.N., Kumar, A., Agarwal, N. and Chaudhary, L.C. 2016a.Effect of a blend of essential oils on metatranscriptome and CAZyme profile of buffalo rumen microbiome. *In: Proc. X Biennial ANA Conference*, Tirupati, November 9-11, 2016 pp 203.

Kim, M., Morrison, M. and Yu, Z.2011.Status of phylogenetic diversity census of ruminal microbes.*FEMS Microbiol. Ecol.* 76: 49-63.

Kittelmann, S. and Janssen, P.H. 2011. Characterization of rumen ciliate community composition in domestic sheep,deer,and cattle, feeding on varying diets,by means of PCR-DGGE and clone libraries. *FEMS Microbiol. Ecol.* 75: 468-481.

Lee, C.C., Kibblewhite, R.E., Wagschal, K., Li, R. and Orts, W.J. 2012. Isolation of alpha-glucuronidase enzyme from a rumen metagenomic library. *Protein J.* 31: 206-211.

Li, R.W., Connor, E.E., Li, C., Baldwin, V.I.R.L and Sparks, M.E. 2012. Characterization of the rumen microbiota of pre-ruminant calves using metagenomic tools. *Environ. Microbiol.* 14: 129-139. doi: 10.1111/j.1462-2920.2011.02543.

Li, Y., Ma, S., Zhang, X., Huang, S., Yang, H. and Zhao, F. 2014. Evaluation of bacterial and archaeal diversity in the rumen of Xiangxi yellow cattle (*Bos taurus*) fed *Miscanthus sinensis* or common mixed feedstuff. *Ann. Microbiol.* 64: 1385-1394.

Lim, S., Seo, J., Choi, H., Yoon, D., Nam, J. and Kim, H. 2013. Metagenome analysis of protein domain collocation within cellulase genes of goat rumen microbes. *Asian-Australas. J. Anim Sci.* 26: 1144-1151.

Mao, S., Zhang, M., Liu, J. and Zhu, W. 2 015 Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle, membership and potential function *Scientific Reports* 5, Article number, 16116. doi, 10.1038/srep16116.

McCann, J.C., Wickersham, T.A., Loor, J.J. 2014. High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. *Bioinf. Biol. Insights* 8:109-125.

Morvan, B., Dore, J,, Rieu-Lesme F., Foucat, L., Fonty, G. and Gouet, P.1994. Establishment of hydrogen - utilizing bacteria in rumen of new born lambs. *FEMS Microbiol. Lett.* 117: 249-256.

Naas, A. E., Mackenzie, A. K, Mravec, J., Schuckel, J., Wilates, W.G.T., Eijsink, V.G.H. and Pope, P. B. 2014. Do rumen Bacteroidetes utilize an alternative mechanism for cellulose degradation? *mBio.* 5: e01401-14.

Nardi, R.D., Marchesini, G., Shucong, L., Khafipour, E.K., Plaizier, J. C. and Gianesella, M., *et al*. 2016. Metagenomic analysis of rumen microbial population in dairy heifers fed a high grain diet supplemented with dicarboxylic acids or polyphenols. *BMC Veter. Res.* 12: 29. doi,10.1186/s12917-016-0653-4.

Newbold, C.J., Lassalas, B. and Jouany, J.P. 1995.The importance of methanogens associated with ciliate protozoa in ruminal methane production *in vitro*. *Lett. Appl. Microbiol.* 21: 230-234.

Patel, D. D., Patel, A. K., Parmar, N. R., Shah, T. M., Patel, J. B., Pandya, P. R. and Joshi, C.G. 2014.Microbial and Carbohydrate Active Enzyme profile of buffalo rumen metagenome and their alteration in response to variation in the diet. *Gene* 545: 88-94.

Patra, A.K., Kamra, D.N. and Agarwal, N. 2010. Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds *in vitro*. *J. Sci. Food Agri.* 90: 511-520.

Pope, P. B., Mackenzie, A. K., Gregor, I., Smith, W., Sundset, M. A. and McHardy, A. 2012. Metagenomics of the Svalbard reindeer rumen microbiome reveals abundance of polysaccharide utilization loci. *PLoS One.* 7:e38571. http://dx.doi.org/10.1371/journal.pone.0038571.

Puniya, A. K., Singh, R. and Kamra, D. N.2015.*Rumen Microbiology: From Evolution to Revolution* (e- book).

Ross, E.M., Petrovski, S., Moate, P.J. and Hayes, B.J. 2013. Metagenomics of rumen bacteriophage from thirteen lactating dairy cattle. *BMC Microbiol.* 13: 242 http://www.biomedcentral.com/1471-2180/13/242.

Singh, K.M., Ahir, V.B., Tripathi, A.K., Ramani, U.V., Sajnani, M., Koringa, P.G., Jakhesara, S., Pandya, P.R., Rank, D.N., Murty, D.S., Kothari, R.K. and Joshi, C.G. 2012. Metagenomic analysis of Surti buffalo (*Bubalus bubalis*) rumen, a preliminary study. *Mol.* Biol. Rep. 39: 4841-4848.

Kelty, O.C., Archibeque, C.A., Jenkins S., Newton, M. and McLellan, S. 2011. Community structures of fecal bacteria in cattle from different animal feeding operations. *Appl.and Environ. Microbiol.* 77: 2992-3001.

Shi, W., Moon, C.D., Leahy, S.C., Kang, D., Froula, J. and Kittelmann, S. 204. Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome. *Genome Res.* 24:1517-1525.

Simon, C. and Daniel, R. 2009. Achievements and new knowledge unraveled by metagenomic approaches. *Appl. Microbiol. Biotechnol*. 85: 265-276.

Stevenson, D.M. and Weimer, P.J. 2007. Dominance of *Prevotella* and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. *Appl. Microbiol. Biotechnol.* 75: 165-174.

von Mering, C., Hugenholtz, P., Raes, J., Tringe, S.G., Doerks, T, Jensen, L.J., Ward, N. and Bork, P. 2007. Quantitative phylogenetic assessment of microbial communities in diverse environments. *Sci.* 315: 1126-1130.

Wallace, R.J., Rooke, J.A., McKain, N., Duthie, C., Hyslop, J.J., Ross, D.W., Waterhouse, A., Watson, M. and Roehe, R. 2015. The rumen microbial metagenome associated with high methane production in cattle. BMC Genomics.16,839 DOI, 10.1186/s12864-015-2032-0.

Wang, L., Hatem, A., Catalyurek, U.V., Morrison, M. and Yu, Z. 2013. Metagenomic insights into the carbohydrate-active enzymes carried by the microorganisms adhering to solid digesta in the rumen of cows. *PLoS ONE.* 8: e78507.

White, B.A., Lamed, R., Edward, A. Bayer, E.A., Harry J. and Flint, H.J. 2014. Biomass utilization by gut microbiomes *Annu. Rev. Microbiol*. 68: 279-96.

Wu, S., Baldwin, R.L., Li, W., Li, C., Connor, E. E. and Li, R.W. 2012. The bacterial community composition of the bovine rumen detected using pyrosequencing of 16S rRNA gene. *Metagenomics* 1:e235571.

Zhu, Z., Noel, S.J., Difford, G.F., Al-Soud, W.A., Brejnrod, A. and Sørensen, S.J. 2017. Community structure of the metabolically active rumen bacterial and archaeal communities of dairy cows over the transition period. *PLoS ONE.* 12: e0187858. https://doi.org/10.1371/journal. pone.0187858.

Received on 11-01-2019 and accepted on 18-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 11-16
doi: 10.5958/2231-6744.2019.00002.1

Indian Journal of Animal Nutrition

Increasing Dairy Cows Productivity through New Balanced Concentrate Feed: A Study in Bihar, India

Dhiraj K. Singh*, Shree Prasad Sahu[1] and Nils Teufel[2]
Tata-Cornell Institute for Agriculture and Nutrition (TCI), E5, Qutab Hotel Campus, Shaheed Jeet Singh Marg, New Delhi-110016, India

ABSTRACT

The present study examined the effect of a new balanced feed on livestock productivity in Bihar in comparison to existing feeding practices. These included supplementing crop residues with either individual concentrate components or with commercial feed. The new balanced feed consisted of crushed grains (37%), cereal brans (30%), pulse husks (10%), oil cakes (20%) and minerals (3%). This resulted in higher ($P<0.05$) levels of metabolizable energy and digestibility compared to other commercial feeds. This new feed was introduced through a combination of participatory trainings on feeding and demonstrations on feed preparation and farm-based feeding trials on 1290 crossbred dairy cows. On an average, farmers were feeding 3.9 kg commercial and/or home-made feed per dairy animal/day adjusted to individual milk yields. After replacing the existing supplements with a reduced amount (3.3 kg) of new feed, average milk yield as well as fat and SNF contents increased ($P<0.05$) by 14, 14 and 4%, respectively. The dairy farmers reduced their cost of milk production and increased their revenue from increased milk sales simultaneously. The new balanced feed also showed better palatability and positive effects on health and reproductive performance in terms of animal appearance and early conception.

Key words: Balanced feed, Experimental trials, Livestock productivity, Milk composition

INTRODUCTION

Dairying is an integral part of small-holder farming systems as well as an important source of subsidiary income for most households in Bihar, India. Since crop production is mostly seasonal, dairy farming is one available option of finding regular employment and income throughout the year for many household members. In Bihar, the share of dairy in the gross value of output from agriculture and allied activities (GVOA) has increased from 13% in 2000-01 to 24% in 2014-15 while at the all India level, the share of dairy in GVOA increased only 2% in the same period. There has been robust growth of the livestock sector in Bihar and its share in GVOA has increased from 23% in 2000-01 to 33% in 2014-15. In comparison, the all India share of livestock in GVOA increased only 3% during the period and stood by 27% in 2013-14 (GOI, 2008, 2017).

Bihar's share of milch cows and buffaloes is about 6.8% and it contributes about 5.3% of the national milk production (GOI, 2017). Bihar figures ninth in milk production in the country. The milk production in the state witnessed tremendous increase in last decade from 2.5 million tonnes in 2000-01 to 8.7 million tonnes in 2016-17, the compounded growth rate of 8.8% being almost double the national average of 4.7% (GOI, 2006, 2017). Nevertheless, the per-capita milk availability in Bihar is still low (228 g/d) compared to India's national average (355 g/d) in 2016-17) which is mainly due to low dairy animal productivity. Low quality feeds and poor feeding practices have been identified as major causes of low productivity driven by the fact that feeding is the major cost of milk production and is, therefore, seen as a prime target for cost reductions (Wolf, 2010). Dependence on residue feeding, non-scientific livestock management, limited farm resources, weak support services and poor knowledge on nutrient requirements of dairy animals are main hurdles in improving livestock productivity in Bihar. The present study was undertaken to see the effect of a balanced concentrate feed on livestock productivity in Bihar in comparison to existing feeding practices.

*Corresponding author: E-mail: dks97@cornell.edu; dhiraj.in@gmail.com; [1]Assistant Professor, Bihar Animal Sciences University, Patna, Bihar, India; [2]Scientist International Livestock Research Institute, Nairobi, Kenya

MATERIALS AND METHODS

The data for this study were collected from 1290 dairy farmers feeding the new concentrate feed to their lactating cows. The experimental trials were conducted on lactating cows of the households who were the members of Self-Help Groups (SHGs) and Kisan Clubs. One lactating cow from each household was selected. The experimental trials were conducted in six districts of Bihar (Begusarai, Bhojpur, Muzaffarpur, Patna, Samastipur and Vaishali) during 2014-2015. A structured questionnaire was used to collect the data from all farmers which consisted of 3 days control (farmers' feeding practices) and 6 days experimental data on concentrate feed, milk yield, fat content and SNF content following the standard management practices of ILRI-CSISA project.

The current standard feeding practice of dairy farmers consisted of feeding crop residues with commercial cattle feed supplemented or with individual concentrate components such as crushed maize and/or oil cakes. The dairy farmers reported that without supplementation with individual concentrate components, milk yield reduced due to poor quality of commercial concentrates. Keeping this in view, a balanced concentrate feed was formulated with locally available feed ingredients according to the nutritional requirements for lactating cows given by ICAR (2013). To better understand the changes resulting from introducing new balanced feed, this feed and 14 locally available commercial dairy concentrate feeds were analysed for dry matter content, ash, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin, metabolisable energy and *in vitro* digestibility using Near-Infrared Spectroscopy (NIRS). The data were analysed statistically (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

It was observed that dairy farmers were feeding on an average 3.9 kg commercial and/or home-made supplements (crushed maize or in combination with mustard oil cake) per lactating animal/d (Table 1). After replacing the existing concentrate feed with a reduced amount (3.3 kg) of new balanced concentrate feed, average milk yield, fat content and SNF content increased ($P<0.05$) by 14, 14 and 4%, respectively. The results showed that dairy farmers could reduce cost of milk production while increasing their revenue from increased milk sales in terms of higher quantity (yield) and quality (fat and SNF contents). Similar results were reported by FAO (2012) which showed that milk yield and milk fat content increased *(*$P<0.05$) by 0.68 kg/d and 0.55% units in cows and 0.19 kg/d and 0.34% units in buffaloes, respectively in the northern region of India. In southern and central regions of India, milk yield increased ($P<0.05$) by 0.42 kg/d and 0.46% units, respectively in cows after ration balancing. The increase in milk production efficiency after ration balancing resulted in more (<0.05) milk from the same amount of feed. Thus, a decreased cost of the inputs increased profit.

The new balanced concentrate feed also showed

Table 1. Input and output of control and experiment animals

District	Concentrate feed (kg)		Milk yield(L)		Fat (%)		SNF (%)	
	C	E	C	E	C	E	C	E
Begusarai	$3.5^{c}\pm0.10$	$3.1^{b}\pm0.08$	$9.3^{a}\pm0.38$	$10.3^{a}\pm0.40$	$3.7^{b}\pm0.05$	$3.8^{d}\pm0.04$	$8.4^{a}\pm0.02$	$8.5^{a}\pm0.03$
Bhojpur	$2.5^{d}\pm0.13$	$2.5^{d}\pm0.10$	$7.7^{d}\pm0.41$	$8.5^{c}\pm0.43$	$5.3^{a}\pm0.18$	$5.4^{a}\pm0.23$	$7.9^{c}\pm0.11$	$8.2^{c}\pm0.09$
Muzaffarpur	$3.9^{b}\pm0.07$	$3.3^{b}\pm0.06$	$8.2^{d}\pm0.19$	$9.4^{b}\pm0.20$	$3.3^{c}\pm0.01$	$4.0^{c}\pm0.01$	$7.7^{d}\pm0.00$	$8.1^{d}\pm0.01$
Patna	$2.9^{d}\pm0.13$	$2.9^{c}\pm0.09$	$8.6^{c}\pm0.48$	$9.6^{b}\pm0.49$	$4.4^{d}\pm0.15$	$4.8^{b}\pm0.17$	$8.2^{b}\pm0.06$	$8.4^{b}\pm0.05$
Samastipur	$4.1^{a}\pm0.04$	$3.4^{a}\pm0.03$	$8.9^{c}\pm0.11$	$10.2^{a}\pm0.12$	$3.5^{b}\pm0.01$	$4.0^{c}\pm0.01$	$7.8^{d}\pm0.01$	$8.1^{d}\pm0.01$
Vaishali	$4.1^{a}\pm0.19$	$3.3^{b}\pm0.21$	$8.7^{c}\pm0.62$	$9.8^{b}\pm0.67$	$3.7^{b}\pm0.07$	$3.9^{d\pm}0.07$	$8.4^{a}\pm0.02$	$8.6^{a}\pm0.02$
Overall	3.9±0.03	3.3±0.03	8.7±0.09	9.9±0.10	3.5±0.01	4.0±0.01	7.9±0.01	8.2±0.01

^{a,b,c,d}Values bearing different superscripts in a column differ significantly ($P<0.05$); C: control/farmer's feeding and E: experimental feeding trial.

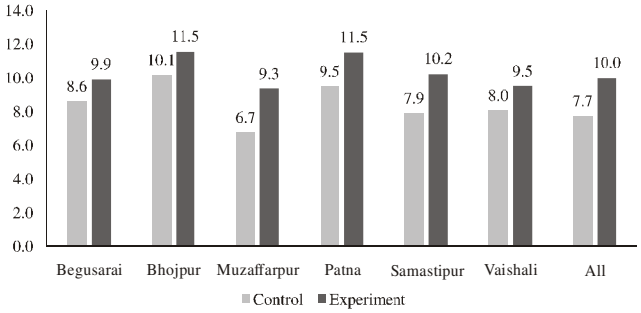

Fig. 1. Fat correct milk (FCM) yield per experiment animal across districts

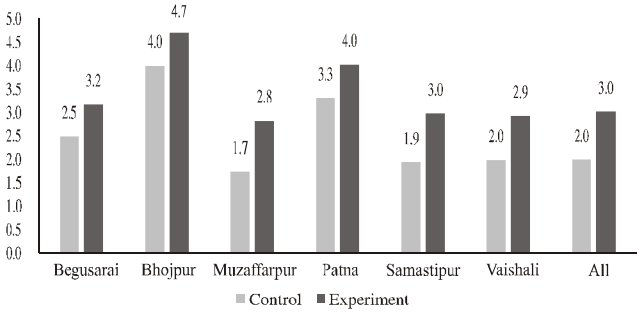

Fig. 2. Fat correct milk (FCM) yield per kg of ILRI-CSISA balanced concentrate feed

better palatability and positive effects on health and reproductive performance in terms of animal appearance, early conception and a lower incidence of ectoparasites. In addition, the new feed did not require cooking which is a common traditional practice in Bihar thus cutting down the cost incurred on fuel and labour.

Several studies have reported the effect of dietary energy on milk quality and animal health (Grieve *et al.,* 1986; Duffield *et al.,* 1997; Heur *et al.,* 2000; Weller *et al.,* 2002). Kennedy *et al.* (2001) revealed that increase in milk yield in response to supplementary concentrate feeding in recent decades stems partly from genetic improvement of cows. High genetic merit cows partition supplementary nutrients towards milk production instead of body reserves at the beginning of lactation. Similarly, Stefanon *et al.* (2002) reported that the milk yield response to concentrate supplementation depended on the overall energy content of the diet.

The farmers reduced their concentrate feed input by 0.6 kg/d/animal while milk yield increased (P<0.05) by 1.2 L/d/animal, fat content by 0.5 points and SNF content by 0.3 points (Table 2). On an average, FCM yield increased by 2.3 L/d/animal (Fig. 1). The lowest FCM yield increase was reported by Begusarai farmers (1.3 L/d/animal) and the highest by Muzaffarpur farmers (2.4 L/d/animal).

On an average, milk yield increased from 2.0 to 3.0 L during the experiment. Whelan *et al.* (2014) observed that milk yield improved with supplementary concentrates rich in protein. Highest output per kg of concentrate was observed in Muzaffarpur and Samastipur districts where milk yield increased by 1.1 L/kg balanced concentrate feed. Andersen *et al.* (2003) studied the effects of high concentrate proportion in diets on milk production and DMI during the first 16 weeks of lactation and DMI was not affected by percentage of concentrate although there was a strong tendency favouring the increase in milk yield with the high concentrate group.

The cost of the new balanced concentrate feed/L milk produced was ₹ 2.1 less than the commercial feed currently used by farmers during the control period

Table 2. Districtwise changes in input and output of experiment animals

District	Concentrate feed (kg)	Milk yield (L)	Fat (%)	SNF (%)
Begusarai	-0.3^{b}±0.05	1.0^{c}±0.04	0.2^{d}±0.02	0.1^{d}±0.02
Bhojpur	-0.1^{a}±0.07	0.8^{d}±0.07	0.2^{d}±0.09	0.3^{b}±0.08
Muzaffarpur	-0.6^{c}±0.06	1.1^{b}±0.04	0.7^{a}±0.01	0.4^{a}±0.01
Patna	-0.1^{a}±0.10	1.0^{c}±0.07	0.4^{c}±0.05	0.2^{c}±0.03
Samastipur	-0.7^{c}±0.04	1.3^{a}±0.02	0.5^{b}±0.01	0.3^{b}±0.01
Vaishali	-0.8^{d}±0.12	1.0^{c}±0.15	0.2^{d}±0.01	0.2^{c}±0.01
Overall	-0.6±0.03	1.2±0.02	0.5±0.01	0.3±0.01

^{a,b,c,d}Values bearing different superscripts in a column differ significantly (P<0.05)

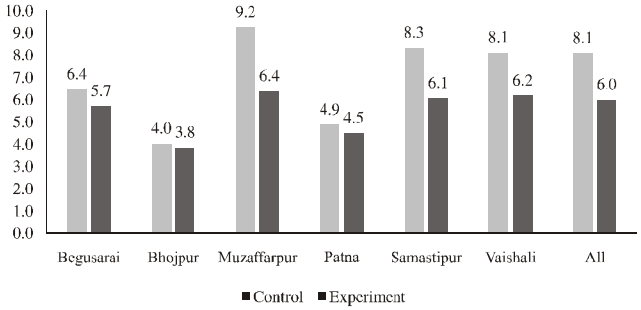

Fig. 3. Cost of balanced concentrate feed per litre of fat corrected milk (FCM) yield

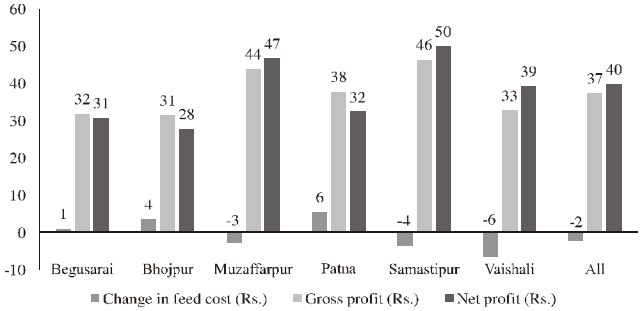

Fig. 4. Economics of ILRI-CSISA balanced concentrate feed (₹ per day/animal)

(Fig. 3). Muzaffarpur district showed the highest price gap (₹ 2.8) in cost of concentrate feed/L of milk between control and experiment while Bhojpur district showed the lowest difference (₹ 0.2). On an average, farmers gained from the new feeding practice *i.e.* they achieved positive net profits estimated at ₹ 40/ d/animal (Fig. 4). The observed variation in levels of benefits between districts was likely due to the variation in the levels of concentrate feed that farmers used during the feeding trials.

In some districts, farmers fed more balanced concentrate feed to obtain more milk while in other districts farmers used less concentrate during the experiment than control periods to reduce costs. The lowest benefit was reported in Bhojpur district where per animal/per day benefit was about ₹ 28 while the highest benefit was in Samastipur district (₹ 50) which was marginally higher than Muzaffarpur district and significantly higher than the other 4 districts. Buza *et al.* (2014) studied the effects of ration composition on profitability in dairy production on existing farms and reported that the lowered share of roughage in the feed ration had a positive effect on financial outcome. They also suggested that utilization of by-products has potential to enhance the dairy farm profitability. The amount of savings in dairy feed cost could have a large positive impact on reducing animal maintenance cost and thus, the profitability of dairy cooperation. Through

Table 3. Laboratory analysis of concentrate feeds (% on DM basis) available for dairy animals

Feed	DM[1]	Ash[2]	CP[3]	NDF[4]	ADF[5]	ADF[6]	ME[7]	IVOMD[8]
ILRI-CSISA Feed	91.0^{d}±0.10	7.4^{d}±0.13	22.5^{a}±0.20	35.9^{b}±0.39	14.2^{d}±0.36	3.3^{d}±0.11	10.3^{a}±0.09	72.7^{a}±0.45
Commercial Feed-1	91.1^{d}±0.16	13.5^{c}±0.34	16.8^{c}±2.31	35.7^{b}±0.84	18.6^{c}±0.36	4.7^{c}±0.23	9.0^{b}±0.11	65.9^{b}±0.61
Commercial Feed-2	94.2^{a}±0.54	18.5^{a}±0.34	21.8^{a}±0.82	23.6^{d}±1.83	22.9^{a}±0.77	5.2^{a}±0.17	7.2^{d}±0.11	57.8^{d}±0.20
Commercial Feed-3	93.0^{a}±0.43	16.0^{b}±0.33	22.6^{a}±0.35	25.8^{d}±1.33	19.7^{c}±0.64	5.0^{b}±0.27	8.1^{c}±0.10	62.3^{b}±0.57
Commercial Feed-4	92.2^{c}±0.47	14.7^{c}±0.94	19.9^{b}±0.80	34.0^{b}±3.60	23.1^{a}±1.38	5.3^{a}±0.27	8.2^{c}±0.38	61.1^{c}±1.81
Commercial Feed-5	92.0^{c}±0.25	16.9^{b}±0.77	19.5^{b}±0.70	32.1^{b}±1.31	22.4^{b}±0.55	5.2^{a}±0.07	8.2^{c}±0.13	61.6^{c}±0.56
Commercial Feed-6	91.8^{a}±0.24	15.4^{b}±0.29	21.6^{a}±0.56	31.5^{c}±0.81	20.1^{b}±0.58	4.7^{c}±0.22	8.4^{c}±0.07	62.7^{b}±0.55
Commercial Feed-7	91.0^{d}±0.17	15.1^{b}±0.43	15.0^{d}±2.25	35.6^{b}±0.94	20.7^{b}±0.55	4.8^{c}±0.24	8.5^{c}±0.11	63.0^{b}±0.65
Commercial Feed-8	90.9^{d}±0.08	12.9^{c}±0.35	19.0^{b}±0.38	40.9^{a}±0.71	22.7^{b}±0.90	5.3^{a}±0.14	9.0^{b}±0.15	64.8^{b}±0.85
Commercial Feed-9	91.6^{c}±0.11	14.0^{c}±0.41	22.7^{a}±0.49	31.7^{c}±0.97	19.3^{c}±0.63	5.1^{b}±0.16	8.7^{b}±0.15	64.5^{b}±0.79
Commercial Feed-10	93.0^{b}±0.51	16.2^{b}±0.68	20.0^{b}±0.57	30.9^{c}±1.51	24.4^{a}±0.95	5.5^{a}±0.18	7.5^{d}±0.24	58.3^{d}±1.19
Commercial Feed-11	92.2^{c}±0.24	17.3^{b}±0.39	21.1^{a}±1.18	28.0^{c}±0.74	21.9^{b}±0.72	5.1^{b}±0.15	7.7^{d}±0.15	59.4^{c}±0.82
Commercial Feed-12	93.67^{b}±0.65	19.7^{a}±0.32	22.5^{a}±0.00	23.1^{d}±0.75	21.4^{b}±0.30	4.4^{c}±0.05	7.3^{d}±0.00	59.1^{c}±0.08
Commercial Feed-13	92.1^{c}±0.37	16.0^{b}±0.81	22.4^{a}±0.92	30.6^{c}±2.07	22.2^{b}±1.14	5.3^{a}±0.21	7.9^{c}±0.35	60.5^{c}±1.71
Commercial Feed-14	91.1^{d}±0.14	12.8^{c}±0.46	20.4^{a}±1.20	38.4^{a}±0.96	21.9^{b}±0.58	5.2^{a}±0.15	8.9^{b}±0.13	65.1^{b}±0.69

^{a,b,c,d}Values bearing different superscripts in a column differ significantly (P<0.05);[1]dry matter, [2]ash, [3]crude protein, [4]neutral detergent fibre, [5]acid detergent fibre, [6]acid detergent lignin, [7]MJ metabolizable energy and [8]*in vitro* organic matter digestibility

the adoption of an optimum ration plan, it was possible to reduce the feed cost while maintaining a balanced diet for the lactating cows through the use of a linear programming model for formulatinga least cost diet (Munford, 1996; Torez, 2000; Djumaera *et al.,* 2009; Griffith, 2010).

The new balanced concentrate feed promoted by ILRI with the CSISA programme was of higher quality in many aspects (*e.g.* metabolizable energy and digestibility) than in any of the other commercial balanced concentrate feeds while only 3 commercial concentrates showed a higher protein value (Table 3). The lignin value of the new feed was lower than all other concentrate feeds which indicated that digestibility and energy content seemed to be more important for improving the feed quality than protein content.

This study was conducted only in 7 districts of Bihar. Therefore, the results applied mainly to this area. However, the type of crossbred dairy cows considered in this study, the feeding practices and the currently available concentrate feeds are similar in large areas of Eastern India. While slight adaptations to the locally appropriate balanced concentrate feed might be required to adjust to variations in dry fodder (*e.g.* wheat straw, paddy straw, maize stalks *etc.*).

CONCLUSIONS

The feeding of a new balanced concentrate feed for dairy animals increased the milk yield of dairy animals by 1.2 L/d/animal. The fat content also increased by 0.5 points while the SNF content increased by 0.3 points. On an average, farmers benefitted from increased net profits of ₹ 40.0/d/animal. New balanced concentrate feed was better than other commercial feeds in terms of protein, metabolizable energy and digestibility.

ACKNOWLEDGEMENTS

This research was conducted as part of the Cereal Systems Initiative for South Asia (CSISA) project funded by the Bill & Melinda Gates Foundation (BMGF) and the United States Agency for International Development (USAID).

REFERENCES

Andersen, J.B., Friggens, N.C., Sejrsen, K., Sørensen, M.T., Munksgaard, L. and Ingvartsen, K.L. 2003. The effects of low *vs.* high concentrate level in the diet on performance in cows milked two or three times daily in early lactation. *Livstc. Prod. Sci.* 81: 119-128.

Buza, M.H., Holden, L.A., White, R.A. and Ishler, V.A., 2014. Evaluating the effect of ration composition on income over feed cost and milk yield. *J. Dairy Sci.* 97: 3073-3080.

Djumaera, D., Djanibekov, N., Vlek, P.L.G., Martius, C. and Lamers, J P.A. 2009. Option of optimizing dairy feed ration with foliage of trees grown in the irrigated dry land of Central Asia. *Res. J. Agric. Biol. Sci*. 5: 698-708.

Duffield, T.F., Kelton, D F., Leslie, K.E., Lissemore, K.D. and Lumsden, J.H. 1997. Use of test day milk fat and milk protein to detect sub-clinical ketosis in dairy cattle in Ontario. *Can. Vet. J.* 38: 713-718.

FAO. 2012. *Balanced Feeding for Improving Livestock Productivity - Increase in Milk Production and Nutrient Use Efficiency and Decrease in Methane Emission* (M.R. Garg; author). FAO Animal Production and Health Paper No. 173. Rome, Italy.

GOI. 2006. *Basic Animal Husbandry Statistics*. Department of Animal Husbandry and Dairying, Ministry of Agriculture, Government of India, New Delhi.

GOI. 2008. *Statewise and Itemwise Estimates of Value of Output from Agriculture and Allied Activities (1999-2000 to 2005-06)*. Central Statistical Office, Ministry of Statistics and Programme Implementation, Government of India. New Delhi.

GOI. 2010. *Basic Animal Husbandry Statistics.* Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, Government of India, New Delhi. http://dahd.nic.in

GOI. 2017. *Basic Animal Husbandry and Fisheries Statistics*. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, New Delhi.

GOI. 2017. *Statewise and Itemwise Estimates of Value of Output from Agriculture and Allied Activities (2011-12 to 2014-15)*. Central Statistical Office, Ministry of Statistics and Programme Implementation, Government of India, New Delhi.

Goswami, S.N., Chaturvedi, A., Chatterji, S., Patil, N.G., Sen, T.K., Hajare, T.N. and Gawande, R. S. 2013. Least cost diet plan of cows for small dairy farmers of central India. *Afr. J. Agril. Res*. 8: 5989-5995.

Grieve, D.G., Kover, S., Rijpkema, Y.S. and Hof, G. 1986.

Relationship between milk composition and some nutritional parameters in early lactation. *Livstc. Prod. Sci.* 37: 239-254.

Griffith, M. 2010. Minimum cost feeding of dairy cows in Northern Victoria. Centre for Policy Studies, Monash University, Australia. General Paper No. G-195. http// .www.monash.edu.au/policy/.

Heur, C., Van Straalen, W.M., Schukken, Y.H., Dirkswager, A. and Noordhuizen, J.P.T.M. 2000. Prediction of energy balance in a high yielding dairy herd in early lactation: model development and precision. *Livstc. Prod. Sci.* 65: 91-105.

ICAR. 2013. *Nutrient Requirements of Cattle and Buffalo*. Indian Council of Agricultural Research, Krishi Bhawan, New Delhi, India.

Kennedy, J., Dillon, P., Faverdin, P., Delaby, L., Buckley, F. and Rath, M. 2001. Investigating the role and economic impact of concentrate supplementation at pasture. *Irish Grassland Assoc. J*. 35: 49-62.

Munford, A.G. 1996. The use of iterative linear programming in practical applications of animal diet formulation. *Math. Comput. Simul*. 42: 255-261.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* Oxford and IBH Publishing Company, Calcutta, India

Stefanon, B., Colitti, M., Gabai, G., Knight, C.H. and Wilde, C.J. 2002. Mammary apoptosis and lactation persistency in dairy animals. *J. Dairy Res*. 69: 37-52.

Torez, P.Z. 2000. Least cost ration formulation for Holstein dairy heifers by using linear and stochastic programming. *J. Dairy Sci*. 83: 443-451.

Weller, R.F., Jones, E.L. and Bowling, P.J. 2002. Comparison of the physical and financial performance of organic dairy farming systems 1998-2002. *Report for DEFRA on Project OF0146*.

Whelan, S., Mulligan, F.J., Gath, V., Flynn, B. and Pierce, K.M. 2014. Effect of dietary manipulation of crude protein content and non-fibrous-to-fibrous-carbohydrate ratio on energy balance in early-lactation dairy cows. *J. Dairy Sci.* 97: 7220-7224.

Wolf, C.A. 2010. Understanding the milk-to-feed price ratio as a proxy for dairy farm profitability. *J. Dairy Sci.* 93: 4942-4948.

Received on 06-08-2018 and accepted on 27-12-2018

Indian J. Anim. Nutr. 2019. 36 (1): 17-24
doi: 10.5958/2231-6744.2019.00003.3

Indian Journal of Animal Nutrition

Effect of UMMB Supplementation on Metabolic and Oxidative Parameters in Rambouillet Cross Sheep during Peripartum Period

A.P. Singh, R. Singh, A.M. Bhat, A. Tikoo and A.K. Pathak[1]
Department of Veterinary Medicine, Faculty of Veterinary Science and Animal Husbandry,
SKUAST-J, Jammu, J & K, India

ABSTRACT

Twelve crossbred Rambouillet sheep in advanced pregnancy were divided into 2 groups of 6 animals each. Group A was kept as control while group B was supplemented with UMMB (@ 50 g/d/animal. Blood samples were collected at 4 weeks and 1 week before lambing followed by 1, 4 and 8 weeks after lambing. The body weight gain and feed intake was better in UMMB supplemented group. In treatment group, there was increase ($P<0.05$) in Hb level at +4 and +8 weeks post lambing. No significant change was observed in PCV values. Significant ($P<0.05$) improvement was observed in plasma calcium, phosphorus, magnesium, iron and zinc levels, however, plasma copper level was similar in two groups. The oxidative stress parameters in both treatment and control group showed significant changes in both groups. Hence, UMMB supplementation improved metabolic and oxidative status of sheep during peripartum period.

Key words: Minerals, Oxidative stress, Peripartum period, Sheep, UMMB

INTRODUCTION

The peripartum period, also called transition period, is a critical phase in the life of ruminants. In small ruminants, due to an increased demand of nutrients by the foetus the animal is subjected to sudden and intense changes in metabolic, endocrine and immune status (Stefanon *et al.,* 2005). In dairy cows and goats, the transition period is marked by variations in oxidative parameters like glutathione peroxidase, glutathione, superoxide dismutase and ceruloplasmin (Stefanon *et al.,* 2005; Celi *et al.,* 2010). The maternal nutrition during peri partum period also plays an important role in the survival of lambs (McDowell *et al.,* 1996). The sheep in the Kandi areas of subtropical and intermediate agro-climatic zones of Shivalik hills of Jammu region in India are dependent mainly on grazing poor quality feed resources which are characteristically low in fermentable nitrogen, mineral and readily available carbohydrate. Developing alternate feeding strategies for ruminant production based on agro-industrial wastes is, therefore, of prime importance.

The use of urea molasses mineral blocks (UMMB) for supplementing crop residues based diet of livestock has been well documented in ruminants (Sansoucy, 1995; Singh *et al.,* 2010a) and has the potential to increase the viability of livestock production (Leng *et al.,* 1991). The UMMB provide nitrogen over a longer period of time than any other urea source. Further, ruminants have the unique ability to convert NPN compounds in their diet to a microbial protein of high biological value. (Thu and Uden, 2000, 2001). A UMMB prepared from locally available agro-industrial by-products has been an adoptable feed supplement which improved nutritional status of animals (Singh *et al.,* 2010b). The aim of this study was to determine the effect of urea molasses multinutrient blocks in ameliorating metabolic and oxidative stress during peripartum period in sheep.

MATERIALS AND METHODS

The study was conducted on Rambouillet sheep reared at Government Sheep Breeding Farm, Panthal, Udhampur, Jammu. History including pregnancy status, previous disease condition, feeding and managemental practices was obtained from the farm records. All the sheep were dewormed before the start of trial using fenbendazole @ 7.5 mg/kg body weight. Twelve crossbred Rambouillet sheep in advanced pregnancy were divided into two groups of 6 animals each. Group A was kept as control while group B was supplemented with UMMB (@ 50 g/d/animal. The trial lasted for 67

*Corresponding author: rajivrajiv101@gmail.com; [1]Department of Animal Nutrition

days and was conducted during January-March. The effect of UMMB supplementation was measured in terms of body weight, feed intake, hemato-biochemical parameters, oxidative and mineral status of animals. Blood samples were collected at 1 week before lambing followed by 1, 4 and 8 weeks after lambing. The UMMB were prepared using cold method by mixing molasses (35%), urea (10%), deoiled rice bran (10%), oiled rice bran (10%), groundnut meal (10%), cement (10%), area specific mineral mixture 14% (Singh, 2009) comprising of DCP-70%, $MgSO_4$-29%, $CuSO_4$-0.5%, $MnSO_4$-0.5%, Potassium iodate-0.09%, and common salt (1%).

For estimation of biochemical constituents and minerals, blood samples (~15 mL) were collected by jugular venipuncture into mineral free heparinised glass vials. The plasma was separated after centrifuging blood samples at 3000 rpm for 30 min. and stored at -10°C in deep freeze for subsequent analysis. For determining haemoglobin (Hb) and packed cell volume (PCV), 2 mL of blood sample was collected from each animal in sterile plastic tubes containing dipotassium salt of EDTA (Hi Media Mumbai, @ 2 mg/mL of blood) and analyzed (Jain *et al.,* 1986). Glucose was estimated immediately after collection of blood using gluco-chek strip. Standard kits (Span Diagnostics, Surat, India) were used for determination of total plasma protein, albumin, BUN, ALT and AST. Estimation of Ca, Inorganic fraction of phosphorus (Pi), Na and K was done using kits. Plasma samples (3 mL) were analysed for micromineral analysis by digesting in distilled concentrated nitric acid (15 mL). Digested samples were diluted to 10 mL with double glass distilled water. The concentrations of micro-elements *viz.*, Cu, Fe and Zn were measured by Polarized Zeeman Atomic Absorption Spectro-photometer (Z-2300, Hitachi).

Malondialdehyde (MDA; Shafiq-ur-Rehman, 1984), glutathione peroxidase (GPx; Hafeman *et al.,*1974), glutathione-s-transferase (GST; Habig *et al.,* 1974), superoxide dismutase (SOD; Marklund and Marklund, 1974) and catalase (CAT; Aebi; 1983) were estimated. The proximate analysis of feed stuff was carried out (AOAC, 1990). The statistical analysis of data was carried out using Turkey Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The CP, EE and CF contents of Dhaman leaves were 18.78, 2.80 and 3.65%, respectively. These values were in normal range (Singh *et al.,* 2010b). The minor variations found may be due to difference in cultivars, stage of agro-climatic conditions, season, stage of maturity, genetic makeup, soil fertility, harvesting methodology, post harvest storages and processing conditions like grinding and drying before analysis.

There was an average increase of 17.33% in BW of treated group compared with average decrease of -0.026% in control group (Table. 2). The findings are in agreement with Forsberg *et al.* (2002) and Singh *et al.* (2010a). UMMB has been suggested as a versatile supplement for sheep and goat (Golluscio *et al.,* 1998; Currier *et al.,* 2004; Singh *et al.,* 2010b). The overall DMI (kg/d) was higher ($P<0.05$) in treatment group as compared to control. These results clearly indicated that UMMB supplementation in peri-parturient sheep had a positive impact on weight gain and health status. UMMB supplementation enhances rumen microbial growth, feed intake of animals and increases feed digestion (FAO, 2007) allowing the animals to maintain and often improve productive performance (Bensalem and Nafzaoul, 2003). Sena *et al.* (2006) also reported that

Table 1. Proximate composition (% DM basis) of feedstuffs

Feed	Dry matter	Crude protein	Crude fibre	Ether extract	Total ash
Dhaman (*Grevia optiva*)	37.81	18.73	30.65	2.80	10.57
Oat grains	90.93	8.23	7.30	1.78	1.96
Pelleted feed	87.74	15.05	19.90	2.90	11.69
UMMB	86.35	21.75	1.10	6.60	28.21

Table 2. Effect of UMMB supplementation on body weight, haematology and biochemical parameters

Parameter	Week	Group	
		A	B
Body weight (kg)	-1 week	44.33±2.1	41.83±3.7
	+1 week	41.92±2.8	40.50±3.7
	+4 week	41.50±2.8	42.75±3.7
	+8 week	43.17±3.2	49.08±3.8
Overall DMI (kg/d)		1.87^{a}±2.03	1.98^{b}±2.11
Hb (g/dL)	-1 week	8.83^{a}±0.31	9.3^{a}±0.52
	+1 week	6.43^{b}±0.56	9.65^{a}±0.33
	+4 week	8.71^{a}±0.32	12.32^{b}±0.38
	+8 week	11.59^{b}±0.30	12.00^{b}±0.25
PCV (%)	-1 week	26.70^{a}±1.34	31.68^{a*}±2.25
	+1 week	23.58^{b}±1.67	32.79^{a*}±0.69
	+4 week	26.13±0.93	36.5^{a}±1.12
	+8 week	34.41^{c}±0.99	35.9^{a*}±0.76
Blood glucose (mg/dL)	-1 week	70.89^{a}±4.19	50.46^{a*}±5.20
	+1 week	82.56^{b}±5.64	60.07^{b}±3.31
	+4 week	61.33^{a}±3.87	65.77^{b*}±5.99
	+8 week	63.69^{a}±8.80	67.13^{b}±2.12
Total plasma protein (g/dL)	-1 week	7.75^{ab}±0.40	7.06^{a}±0.45
	+1 week	6.89^{c}±0.70	6.93^{a}±0.46
	+4 week	8.29^{a}±0.37	8.73^{a}±0.73
	+8 week	8.46^{b}±0.31	8.16^{a}±0.18
Albumin (g/dL)	-1 week	3.19^{a}±0.35	2.92^{ab}±0.14
	+1 week	2.06^{b}±0.13	2.44^{ab*}±0.13
	+4 week	2.41^{a}±0.13	2.55^{ab}±0.11
	+8 week	2.44^{a}±0.10	2.24^{c}±0.17
Globulin (g/dL)	-1 week	4.77^{a}±0.50	4.14^{a}±0.41
	+1 week	4.83^{a}±0.64	4.97^{ab}±0.18
	+4 week	5.87^{b}±0.37	6.18^{b}±0.78
	+8 week	6.02^{c}±0.32	5.93^{b}±0.09
BUN (mg/dL)	-1 week	21.87^{ab}±1.52	33.08^{a*}±2.03
	+1 week	19.22^{a}±1.98	30.58^{a*}±0.81
	+4 week	24.02^{b}±3.10	28.70^{b*}±1.15
	+8 week	20.97^{ab}±0.79	17.72^{c}±3.12
ALT activity (U/L)	-1 week	16.66^{ab}±1.72	18.51^{a*}±1.19
	+1 week	23.12^{c}±3.12	23.12^{a}±1.89
	+4 week	15.44^{a}±1.56	20.66^{a}±2.24
	+8 week	14.16^{a}±1.16	15.01±5.48^{a}
AST activity (U/L)	-1 week	80.93^{ab}±1.18	93.48^{a*}±2.32
	+1 week	101.38^{c}±1.67	96.47^{a}±2.78
	+4 week	95.42^{a}±2.13	94.92^{a}±2.63
	+8 week	92.72^{a}±1.62	93.21^{a}±2.14

^{a,b,c}Figures with different superscripts for a parameter in a column differ significantly (P<0.05); *Figures in a row differ significantly (P<0.05)

UMMB supplementation increased microbial activity by supplying nitrogen, minerals and energy in a balanced proportion.

The average values of Hb in groups A and B animals varied from 8.83 to 11.59 g/dL and 9.3 to 12.32 g/dL, respectively. In treatment group, there was increase (P<0.05) in Hb level at +4 and +8 weeks post lambing. Control group showed decrease (P<0.05) at +1 week and an increase (P<0.05) at +8 week compared with the level of Hb at 1 week before lambing (Table 2). The average values of PCV in groups A and B varied from 26.70 to 34.41% and from 31.68 to 36.5%, respectively. In control group, a decrease (P<0.05) in PCV level was observed at +1 week and an increase (P<0.05) was observed at +8 weeks as compared with the values observed at week 1. The elevated levels of hematological indices compared with control group could be attributed to higher DMI in the supplemented group. Singh *et al.* (2010a) also reported non-significant increase in Hb and PCV levels in animals supplemented with UMMB while Singh *et al.* (2017) reported rise (P<0.05) in PCV in beetal goats supplemented with UMMB on 30th day of trial.

The plasma glucose concentration in control and test group animals varied from 70.89 to 82.56 mg/dL and 50.46 to 67.13 mg/dL, respectively. An increase (P<0.05) in glucose level was observed at +1, +4 and +8 week in treatment group. In control group, significant increase (P<0.05) in glucose level was observed only at +1 week as compared to -1 week (Table. 3). The higher glucose level observed in supplemented group animals might be due to the fact that minerals in UMMB acted as cofactor and activator of many enzymatic systems associated with the

Table 3. Effect of UMMB supplementation on plasma minerals in sheep during peripartum period

Parameter	Week	Group	
		A	B
Ca (mg/dL)	-1 week	18.45^{b}±1.90	13.10^{ab*}±1.19
	+1 week	11.50^{a}±0.47	11.30^{a}±0.44
	+4 week	14.50^{b}±1.05	18.86^{c}±2.68
	+8 week	10.42^{a}±0.88	15.36^{b*}±0.97
Pi (mg/dL)	-1 week	8.02^{a}±0.83	5.70^{ab}±0.52
	+1 week	5.00^{b}±0.33	5.15^{a}±0.45
	+4 week	6.83^{c}±0.72	8.20^{c}±1.16
	+8 week	4.53^{b}±0.38	6.65^{d*}±0.43
Fe (µmol/L)	-1 week	385.53^{b}±94.67	234.38^{a}±78.60
	+1 week	106.66^{a}±23.11	131.48^{a}±21.22
	+4 week	58.87^{a}±19.70	122.25^{a*}±11.98
	+8 week	366.03^{b}±71.03	252.60^{a}±108.49
Zn (µmol/L)	-1 week	17.42^{b}±2.85	23.07^{ab}±3.24
	+1 week	21.52^{ab}±2.49	28.76^{ab*}±3.61
	+4 week	29.95^{a}±3.53	34.39^{c}±2.93
	+8 week	15.14^{b}±2.70	19.43^{b*}±1.45
Cu (µmol/L)	-1 week	20.42^{a}±4.66	12.36^{a}±3.12
	+1 week	23.39^{a}±6.91	19.99^{a}±4.77
	+4 week	11.19^{a}±2.59	23.48^{a}±7.56
	+8 week	19.06^{a}±2.52	8.05^{a}±1.43

^{a,b,c,d}Figures with different superscripts for a parameter in a column differ significantly (P<0.05); *Figures in a row differ significantly (P<0.05)

metabolism of nutrients (Mohapatra *et al.,* 2012).

The average values of total plasma protein (TPP) in control and test group animals varied from 6.89 to 8.46 g/dL and 6.93 to 8.73 g/dL respectively. In test group, there was no difference in TPP level at +1 week, +4 and +8 week whereas animals in control group showed decrease (P<0.05) at +1week as compared to -1 week. The average values of albumin among control and test group animals varied from 2.06 to 3.19 g/dL and 2.24 to 2.92 g/dL, respectively. In test group, an increase (P<0.05) in albumin level was observed at +1 week compared to control group. There was significant (P<0.05) decrease at +8 weeks as compared to -1 week. The average values of globulin in control and test group animals varied from 4.77 to 6.02 g/dL and from 4.14 to 6.18 g/dL, respectively. Significant (P<0.05) increase in globulin level was observed in treatment group at +4 and +8 weeks. Similar trend was observed in control group animals. Findings of the present study corroborated with those of Singh *et al.* (2010a) who reported non-significant effect of UMMB supplementation on total plasma protein and albumin level in anoestrus buffaloes after 4 weeks (Table 2). The role of mineral for improvement of serum protein level is mostly limited to the better availability of phosphorus in supplemented than un-supplemented group as phosphorus plays a significant role in amino acid and protein synthesis (Underwood and Suttle, 1999).

In treatment group, there was increase (P<0.05) in BUN level at +4 and +8 weeks as compared to -1 week. In control group, no significant (P<0.05) change in BUN level was observed. Qreshi *et al.* (2002)

Table 4. Effect of UMMB supplementation on oxidative stress indices of sheep during peripartum period

Parameter	Week	Group	
		A	B
MDA (µmol/mL)	-1 week	10.10^{b}±4.01	18.02^{b}±4.05
	+1 week	13.80^{b}±3.90	21.8^{ab}±3.9
	+4 week	20.34^{a}±4.29	28.34^{a}±4.29
	+8 week	24.57^{c}±1.21	32.57^{a}±1.21
SOD activity (U/mg of Hb)	-1 week	295.13^{a}±16.60	242.49^{bc}±14.81
	+1 week	252.94^{b}±8.85	219.06^{c*}±5.64
	+4 week	272.64^{ab}±9.02	289.14^{a}±14.32
	+8 week	282.45^{a}±15.82	289.14^{a}±14.32
Catalase activity (µmolH_2O_2 utilized/min/mg of Hb)	-1 week	112.75^{a}±4.44	105.74^{a*}±1.12
	+1 week	105.91^{a}±5.21	110.45^{a}±4.65
	+4 week	111.52^{a}±5.99	131.54^{b}±6.47
	+8 week	131.54^{b}±6.47	125.43^{a}±7.58
Glutathione S transferase activity (µmol of conjugate ofGSH-CDNB/min/ mg of Hb)	-1 week	0.03^{b}±0.00	0.02^{a}±0.00
	+1 week	0.01^{a}±0.00	0.02^{a}±0.00
	+4 week	0.02^{a}±0.00	0.03^{ab}±0.01
	+8 week	0.03^{b}±0.00	0.05^{b*}±0.00
GPx activity (U/mg of Hb)	-1 week	1.11^{a}±0.31	1.14^{a}±0.41
	+1 week	1.01^{a}±0.29	1.12^{a}±0.36
	+4 week	0.96^{a}±0.19	0.49^{b*}±0.14
	+8 week	1.34^{a}±0.20	1.15^{a}±0.20

[a,b,c]Figures with different superscripts for a parameter in a column differ significantly (P<0.05); [*]Figures in a row differ significantly (P<0.05)

reported that excessive levels of crude protein in the diet elevated BUN levels. Hosamani *et al.* (1998) also reported increase (P<0.01) in blood serum urea following UMMB supplementation in Murrah buffaloes. No significant change was observed in ALT and AST levels in treatment groups, however, in control group an increase (P<0.05) in ALT and AST level was observed at +1 week as compared to -1 week (Table 2). During the periparturient period, liver is over burdened by the increased gluconeogenesis and lipid infiltration causing injury to liver cells and consequently increased liver specific enzymes (Pechova *et al.,* 1997; Lubojacka *et al.,* 2005). However, in the present study animals supplemented with UMMB had better protein and energy metabolism. Feeding of UMMB had no direct effect on liver function (Cenesiz *et al.,* 2006).

The average values of plasma Ca in groups A and B varied from 10.42 to 18.45 mg/dL and 11.30 to 18.86 mg/dL, respectively. In treatment group, there was increase (P<0.05) in Ca level at +4 weeks as compared to -1 week whereas control group animals showed decrease (P<0.05) at +1 and +8 weeks (Table. 3). The average values of Pi in groups A and B varied from 4.53 to 8.02 mg/dL and from 5.15 to 8.20 mg/dL, respectively. In treatment group, there was an increase (P<0.05) in Pi level at +4 and +8 weeks as compared to -1 week. However, in control group there was decrease (P<0.05) in Pi level at +1, +4 and +8 weeks as compared to -1 week values. The average values of Ca (9.0-11.6 mg/dL) and Mg (2.10-2.90 mg/dL) were within the normal range. Singh *et al.* (2017) reported rise (P<0.05) in the mean plasma inorganic P and Mg concentration on d 60th compared to 0 d values in beetal goats supplemented with UMMB.

No significant change in plasma Fe level was observed in control group. The animals supplemented with UMMB showed increase (P<0.05) in plasma iron level at +4 weeks compared with control group. Singh *et al.* (2017) reported decrease (P<0.05) in concentration of Fe levels of both UMMB supplemented and non-supplemented goats from day 30th of the trial. A significant (P<0.05) variation in plasma Zn level was observed among test and control groups. In animals supplemented with UMMB blocks, significant (P<0.05) increase in Zn levels was found at +4 and +8 weeks. However, in control group there was decrease (P<0.05) in plasma Zn level upto +4 weeks compared with -1 week level. No significant change in plasma Cu level was observed in treatment group (Table. 3). Singh *et al.* (2017) reported significant (P<0.05) increase in Zn concentration in UMMB supplemented beetal goats after 30 days of supplementation.

There was significant (P<0.05) increase in the MDA levels and SOD activity in control groups at +4 and +8 weeks in both control and treatment groups (Table 4). No significant change was observed in CAT level. However, in control group there was increase (P<0.05) in CAT level at +8 weeks as compared with -1 week and decrease (P<0.05) in CAT activity was observed at -1 week in test group compared with control group. In treatment group, there was increase (P<0.05) in glutathione S transferse activity at +8 weeks whereas there was decrease (P<0.05) in control group at +1 week as compared with -1 week. There was no change in GPx activity in control group whereas there was increase (P<0.05) in GPx activity at 4 weeks post treatment. There was not much change in the oxidative stress due to UMMB supplementation. The reason could be the low dose of UMMB supplement. Aantioxidant status of blood is reflection of their corresponding concentration in rest of body (Bouwstra *et al.,* 2008). The increased SOD and MDA activity in both supplemented and control animals might have been due to high ROS production. SOD catalyzes the dismutation of $\bullet O_2^-$ into oxygen and hydrogen peroxide (H_2O_2) which is removed by the coordinated activity of catalase and GPx. Therefore, the increased activity of catalase and GPx in UMMB supplemented groups could be atributed to high production of H_2O_2.

CONCLUSIONS

Supplementary feeding of urea molasses multi-nutrient blocks during peripartum period in sheep increased body weight along with increase in Hb post lambing. Plasma calcium, phosphorus, magnesium, iron and zinc levels improved significantly, however, copper

level was similar in 2 groups. The oxidative stress parameters showed significant changes in both treatment and control group. Therefore, UMMB being good source of protein, energy and minerals improved metabolic status of sheep during peripartum period.

REFERENCES

Aebi, H.E. 1983. Catalase. In: *Methods of Enzymatic Analysis*. (H.U. Bergmeyer; ed.). New York Academic Press, New York, USA.

AOAC. 1990. *Official Methods of Analysis*. 15thedn. Association of Official Analytical Chemists, Washington DC., USA.

Bensalem, H., Nefzaoui, A. and Makkar, H.P.S. 2007. *Feed Supplementation Blocks for Increased Utilization of Tanniniferous Foliages by Ruminants*. pp 185-205.

Besalem, H. and Nefzaoui, A. 2003. Feed blocks as alternative supplements for sheep and goats. *Small Rumin. Res.* 49: 275-288.

Bouwstra, R.J., Goselink, R.M.A., Dobbelaar, P., Nielen, M., Newbold, J.R., Werven, T. 2008. The relationship between oxidative damage and vitamin E concentration in blood, milk and liver tissue from vitamin E supplemented and non supplemented periparturient heifers. *J. Dairy. Sci.* 91: 977-987.

Celi, P., Trana, A.D. and Claps, S. 2010. Effects of plane of nutrition on oxidative stress in goats during the peripartum period. *Vet. J.* 184: 95-99.

Cenesiz, S., Atakisi, O., Ozcan, A., Yuceyurt, R and Unal, Y. 2006. Effect of feed supplemented with urea molasses mineral blocks on activity of serum AST, ALT and levels of total protein, glucose, triglyceride, total lipid, total cholesterol in lambs, *Kafkas. Univ. Vet. Fak. Derg*. 12: 137-140.

Currier, T.A., Bohnert, D.W., Falck, S.J. and Bartle, S.J. 2004. Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: I. Effects on cow performance and the efficiency of nitrogen use in wethers. *J. Anim. Sci*. 82: 1508-1517.

Duncan, D.B. 1955. Multiple range and multiple F-test. *Biometrics.* 11: 1-42.

FAO. 2007. *Experiences with Urea-Molasses Multinutrient Blocks in Buffalo Production and Reproduction in Smallholder Dairy Farming, Punjab, India*. Food and Agriculture Organization of the United Nations, Rome. pp 59-70.

Forsberg, N.E., AI-Maqbaly, R., AI-Halhali, A., Ritchie, A. and Srikandakumar, A. 2002. Assessment of molasses urea blocks for goat and sheep production in the sultanate of Oman: Intake and growth studies. *Trop. Anim. Health. Prod.* 34: 231-239.

Golluscio, R.A., Paruelo, J.M., Mercau, J.L. and Deregibus, V.A. 1998. Urea supplementation effects on the utilization of low-quality forage and lamb production in Patagonian rangelands. *Grass. Forage. Sci.* 53: 47-56.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. 1974. Glutathione-S transferases: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 249: 7130-7139.

Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G. 1974. Effect of dietary selenium on erythrocyte and glutathione peroxidase in rats. *J. Nutr.* 104: 580-587.

Hosamani, S.V, Mehra, U.R and Dass, R S. 1998. Effect of different planes of nutrition on urea molasses mineral block intake, nutrient utilization, rumen fermentation pattern and blood profile in Murrah buffaloes (*Bubalus bubalis*). *Anim. Feed. Sci. Technol*. 76: 117-128.

Jain, N.C. 1986. *Schalms Veterinary Haematology*. Lei and Febiger, Philadelphia.

Leng, R.A., Preston, T. R., Sansoucy, R. and George, K.P.J. 1991. Multi-nutrient block as a strategic supplement for ruminants. *World Anim. Rev.* 67: 11-19.

Lubojacka, V., Pechova, A., Dvorak, R., Drastich, P., Kummer, V. and Poul, J. 2005. Liver steatosis following supplementation with fat in dairy cows diets. *Acta. Veterinaria. Brno*. 74: 217-224.

Marklund, S. and Marklund, M. 1974. Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay of superoxide dismutase. *Eur. J. Biochem*. 47: 469-474.

McDowell, L.R. 1996. Feeding minerals to cattle in pasture. *Anim. Feed Sci. Technol*. 60:241-246.

Mohapatra, P., Swain, R.K., Mishra, S.K., Sahoo, G., and Rout, K.K. 2012. Effect of supplementation area specific mineral mixture on reproductive performance of cows. *Indian. J. Anim. Sci.* 82: 1558-1563.

Pechova, A., Llek, J. and Halouzka, R. 1997. Diagnosis and control of the development of hepatic steatosis in dairy cows in the periparturient period. *Acta. Vet. Brno*. 66: 235-243.

Qreshi, M.S., Habib, G., Samad, H.A., Siddiqui, M.M., Ahmad, N. and Syed, M. 2002. Reproduction-nutrition relationship in dairy buffaloes, 1. Effect of intake of protein, energy and blood metabolites levels. *Asian-Australas. J. Anim. Sci.* 15: 330-339.

Sansoucy, R. 1995. New developments in the manufacture and utilization of multinutrient blocks. *World Anim. Rev.* 82: 78-83.

Sena, C., Ayla, A.O. and Yuceyurt, O.R. 2006. Effect of feed supplemented with urea molasses mineral blocks on activity of serum AST, ALT and levels of total protein, glucose, triglyceride, total lipid, total cholesterol in lambs. *Kafkas Univ. Vet. Fak. Derg.* 12: 137-140.

Shafiq-ur-rehman. 1984. Lead induced regional lipid peroxidation in brain. *Toxicol. Lett.* 21: 333-337.

Singh, R., Kumar, S. and Bhardwaj, R. 2010a. On-farm evaluation of urea molasses multinutrient blocks enriched with minerals in goats. *Indian J. Anim. Sci.* 80 : 358-361.

Singh, R., Kumar, S. and Brar, P.S. 2010b. Evaluation of urea molasses multi-nutrient blocks enriched with area specific mineral mixture in Buffaloes (*Bubalus bubalis*). *Indian J. Anim. Sci.* 80: 561-564.

Singh, V., Singh, R. and Hussain, K. 2017. Evaluation of urea molasses multinutrient block supplementation in beetal cross goats. *Indian J. Small Rumin.* 80: 358-361.

Stefanon, B., Sgorlon, S. and Gabai, G., 2005. Usefulness of nutraceutics in controlling oxidative stress in dairy cows around parturition. *Vet. Res. Commun*. 29: 387-390.

Thu, N.V. and Uden, P. 2000. Effect of urea molasses cake supplementation on live weight and milk yield of Murrah buffalo cows. *Asian-Australas. J. Anim. Sci.* 13:1329-1336.

Thu, N.V. and Uden, P. 2001. Effect of work and urea molasses cake supplementation of swamp buffaloes fed rice straw or grasses on rumen environment, feed degradation and intake. *Asian-Australas. J. Anim. Sci.* 14: 631-639.

Underwood, E.J. and Suttle, N. F. 1999. *The Mineral Nutrition of Livestock.* CAB International, Wallingford, Oxon OX10 8DE, UK. pp 397-420.

Received on 02-11-2018 and accepted on 18-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 25-29
doi: 10.5958/2231-6744.2019.00004.5

Indian Journal of Animal Nutrition

Evaluation of *kharif* Forage Crops for Biomass Production and Nutritional Parameters in Indo-Gangetic Plains of India

Phool Singh Hindoriya*, Rajesh Kumar Meena, Magan Singh, Rakesh Kumar, Hardev Ram, Vijendra Kumar Meena and Manish Kushwaha

Agronomy Section, ICAR- National Dairy Research Institute, Karnal, Haryana-132001, India

ABSTRACT

A field experiment was conducted during *Kharif*, 2017 at Research Farm of Agronomy Section, ICAR- National Dairy Research Institute, Karnal. The experiment was laid out in randomized complete block design with eight treatments and three replications. Among eight treatments, two perennial forage crops *viz.,* NBH and guinea grass cultivated sole as well as intercropped with cowpea and four seasonal fodder crops (cowpea, sorghum, baby corn and maize) were tested. Among different fodder crops, the highest DM content was recorded in guinea grass intercropped with cowpea in the 2:3 row ratio (25.1%) followed by sole guinea grass (24.9%) and cowpea (16.9%). The yields of protein (14.46 q/ha), ether extract (3.28 q/ha) and total ash (13.07 q/ha) were higher ($P<0.05$) in NBH intercropped with cowpea. The higher ADF content was observed in the sole sorghum (41.6%) followed by sole guinea (40.1%) and sole maize (35.3%). The higher value of NDF was recorded in the sole guinea grass (71.16%) as compared to other treatments. The ADL was found to be the highest in the sole cowpea (8.68%) crop. The NBH intercropped with cowpea in 2: 3 row ratio produced higher DM yield. The crude protein, ether extract and ash yield were also higher in the same combination of crop.

Key words: Chemical Composition, Fibre fractions, Forage and nutrient yield, Kharif crops

INTRODUCTION

Livestock plays an important role in Indian economy and about 20.5 million people depend upon livestock rearing for their livelihood. Livestock contributed 16% to the income of small farm households as against an average of 14% for all rural households (DAHD & F, 2012). India has vast livestock resources and contributes 4.1% of GDP and 25.6% of total agriculture GDP (GOI, 2017). At present, India is having 5.4% of the cultivated area under fodder crops which has resulted in a severe deficit of green fodder (36%), dry crop residues (11%) and concentrate feed ingredients (44%) as per IGFRI (2016) report. Due to shortage of feed and fodders, animals are not getting sufficient amount of quality feed and fodder resulting in adverse effect on animal's productivity. The two main attempts to fill this gap between fodder requirement and availability are either to increase the area under fodder production or to boost the productivity per unit area per unit time. Increase in area under fodder crops does not appear to be feasible. Therefore, second approach *i.e.* to boost productivity per unit area per unit time, remains the possible alternative.

Napier hybrid bajra and guinea grass are important forage grasses of the tropics known for good biomass production, palatability, persistence and fodder quality. The intercropping of legumes (cowpea) with guinea/napier grass will also ensure the availability of quality fodder in the existing cropping pattern. Halli *et al.* (2018) revealed that grasses grown with legume (cowpea) sustained the soil health and improved the feed quality which resulted in increase in milk production of cattle. The low CP content of these grasses can be enhanced through intercropping with forage legumes to maintain the desirable quality. Maize (*Zea mays* L.) is one of the most important and ideal forages. Sorghum (*Sorghum bicolor* L. Moench.) is most widely grown fodder under rainfed conditions owing to its low water requirement. In India, area under fodder sorghum is around 2.6 m ha (ICAR, 2012) which meets over 2/3rd of the fodder demand during *kharif* in western Haryana, U.P., Punjab, Delhi and Rajasthan. Thus, evaluation of different *kharif* forage crops alongwith perennial forage grasses and their fodder quality of all these crops at single site has not been done. Hence, a field experiment was conducted to evaluate the quality

*Corresponding author: E-mail: pshindoria2012@gmail.com

aspect of different *kharif* forage crops.

MATERIALS AND METHODS

The field experiment was conducted at the Agronomy Research Farm of ICAR- National Dairy Research Institute, Karnal, Haryana during *kharif*-2017. Karnal is situated in sub-tropical zone with annual rainfall of 1066 mm with bimodal distribution, 70% of which occurs during the main rainy season (June to September).

Soil of the experimental field was clay loam in texture, low in available nitrogen (196 kg/ha), medium in available phosphorus (20.80 kg/ha), high in available potassium (289.30 kg/ha) and neutral to alkaline in reaction (pH 7.18). The experiment was laid out in randomized complete block design (RCBD) with two perennial fodder crops NBH (variety NBH-37), guinea grass (Bundel guinea-1) intercropping combination with cowpea (C-152) and four annual crops cowpea, sorghum, baby corn and maize varieties (C-152, Sudan chari, HM-4 and J-1006, respectively) in the eight treatments with three replications using a total of 24 numbers of plots.

The crop was supplied with well decomposed farm yard manure (FYM) @ 10 t/ha three weeks prior to sowing of the crop. Half dose of nitrogen and full dose of phosphorus and potassium were applied in the form of urea, DAP and MOP, respectively as basal application and remaining half dose of nitrogen was supplied in split dose through broadcasting. In NBH and guinea grass, 25 kg/ha N was top dressed after each cutting. All standard agronomic and required plant protection measures were followed. The 1st cut was taken at 60 (DAP and DAS, respectively) in NBH, guinea grass and cowpea, sorghum and maize while baby corn was harvested at 65 DAS, 2nd cut at 30 days after 1st cutting was taken in the NBH, guinea grass and multicut sorghum. Samples taken from each plot were dried in shade followed by overnight drying in hot air oven at 70^{o}C for DM estimation. The DM, CP, EE and total ash (AOAC, 2005) and cell wall constituent's *viz.,* NDF, ADF, hemicellulose and ADL (Van Soest *et al.,* 1991) were determined. The data were analysed following ANOVA technique (Gomez and Gomez, 1984) in randomizes block design using SAS 9.3 software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Among different forage crops, the highest DM content was recorded in the intercropped guinea grass (25.1%) with cowpea in the 2:3 row ratio followed by sole guinea grass (24.9%) which was statistically at par with each other (Table 1). The higher DM content was observed in guinea grass because presence of more fibre content than sorghum (21.9%) and maize (21.51%). While, the lowest DM content was recorded in cowpea (16.93%). It might be due to more succulent shoots of cowpea fodder which led to reduction in DM content of

Table 1. DM content, dry fodder yield (DFY), CP content and crude protein yield (CPY) of different fodder crops

Treatment	DM (%)	DFY (t/ha)	CP (%)	CPY (q/ha)
NBH sole	19.27^{d}	11.68^{a}	9.78^{b}	11.43^{b}
Guinea sole	24.9^{a}	10.95^{a}	8.30^{d}	9.08^{c}
NBH + cowpea*	19.57^{d}	11.95^{a}	9.97^{b}	14.46^{a}
Guinea grass + cowpea*	25.1^{a}	10.59^{a}	8.50^{d}	11.67^{b}
Cowpea sole	16.93^{e}	5.68^{c}	17.10^{a}	7.93^{d}
Sorghum sole	21.96^{b}	9.91^{b}	8.01^{e}	9.72^{c}
Baby corn sole	19.57^{d}	6.50^{c}	8.94^{d}	5.84^{e}
Maize sole	21.51^{c}	9.99^{b}	9.45^{e}	9.43^{c}
SEm±	0.14	0.55	0.14	0.57
LSD (P= 0.05)	0.43	1.61	0.42	1.69

^{a,b,c,d,e} Values bearing different superscripts in a column differ significantly (P<0.05)

fodder cowpea plant. The present results are similar to those reported by Babasup *et al.* (2011).

Among different fodder crops, the highest dry fodder yield was recorded in the intercropping (2:3) row ratio of NBH + cowpea (11.95 t/ha) followed by sole NBH (11.68 t/ha). Sole NBH value was statistically at par with that of NBH + cowpea intercropping (Table 1). Mean relative yields of all intercropping treatments were greater (P<0.05) than sole fodder crops which indicated the advantages of intercropping over sole production. Higher dry fodder yield might be because these fodders showed the vigour and inherent growth performance. Nitrogen fixation ability of cowpea provided nitrogen carried over to NBH which enhanced the growth performance and production potentials by producing higher number of tillers and leaves/clumps and fast accumulation of sink component of photosynthesis. The lower dry fodder yield was noticed in the sole fodder cowpea due to higher moister content and lower fibre content. Anita *et al.* (2015) revealed that NBH and guinea grass intercropped with cowpea enhanced the total green fodder yield. The results of present investigation are in conformity with the findings of Aulakh *et al.* (2012).

The higher CP content was recorded in cowpea crop. The comparable values of CP content were also observed in cowpea (17.19 and 17.12%) intercropped with guinea and NBH grass, respectively in (2: 3) row ratio (Table 1). Among different non-leguminous fodder crops, higher CP content was recorded in NBH (9.97%) intercropped with cowpea followed by sole NBH (9.78%) while the lowest level was recorded in sole sorghum (8.01%). The highest total CP yield (14.46 q/ha) was recorded in NBH + cowpea intercropping followed by guinea + cowpea (11.67 q/ha) intercropping. The lowest value of total CP yield was recorded in sole baby corn (5.84 q/ha). This might be due to its lower DM yield as well as CP content while the highest CP yield in NBH + cowpea intercropping could be due to higher dry matter yield. These findings are in conformity with those of Kushwaha *et al.* (2018). Cowpea is usually intercropped with cereal fodders and grasses to improve the nutritive value of the herbage and similar results have been reported by Strydhorst *et al.* (2008). Sengul (2003) noticed that intercropping system provided better micro-environment that favoured higher protein content than those obtained from sole legume or grass stands.

The EE content was the highest in fodder cowpea (3.0%) intercropped with NBH followed by cowpea (2.95%) intercropped with guinea grass. It was comparatively higher than the rest of fodder crops. Among others crops, the lowest value of EE was observed in sole baby corn (1.54%). Ayub *et al.* (2002) also reported that EE content was positively correlated with nitrogen application. Total ether EE yield was the highest in intercropping of NBH + cowpea fodder (3.28 q/ha) followed by sole crop of NBH (3.03 q/ha) (Table

Table 2. Ether extract, ether yield, ash content, ash yield and NFE content of different fodder crops

Treatment	EE (%)	EE yield (q/ha)	Ash (%)	Ash yield (q/ha)	NFE (%)
NBH sole	2.60^{b}	3.03^{a}	10.81^{b}	12.63^{a}	47.11^{b}
Guinea sole	1.64^{d}	1.80^{c}	11.72^{a}	12.83^{a}	45.27^{c}
NBH + Cowpea*	2.65^{a}	3.28^{a}	10.85^{b}	13.07^{a}	47.66^{b}
Guinea grass + Cowpea*	1.67^{c}	2.21^{b}	11.88^{a}	12.31^{a}	45.39^{c}
Cowpea sole	2.85^{a}	1.62^{c}	10.88^{b}	6.19^{c}	42.9^{d}
Sorghum sole	1.88^{c}	1.85^{c}	9.60^{c}	9.51^{b}	50.43^{a}
Baby corn sole	1.54^{d}	1.00^{d}	8.53^{e}	5.55^{c}	50.77^{a}
Maize sole	1.63^{d}	1.63^{c}	9.15^{d}	9.14^{b}	50.92^{a}
SEm±	0.07	0.11	0.1	0.56	0.39
LSD (P= 0.05)	0.22	0.35	0.31	1.65	1.17

^{a,b,c,d,e} Values bearing different superscripts in a column differ significantly (P<0.05)

2). The total ash content was the highest in guinea grass (11.88%) intercropped with cowpea followed by guinea sole (11.72%). It was higher than other treatments. The lowest ash content was recorded in baby corn (8.53%) compared to other treatments which might be due to the higher DM content in guinea grass compared to other forage crops. The total ash yield (q/ha) was the highest in combination of NBH intercropping (13.07 q/ha) with cowpea in 2: 3 row ratio followed by sole guinea grass (12.83 q/ha) while the lowest total ash yield was obtained in the baby corn (5.55 q/ha). Similar findings were also reported by Rathore *et al.* (2015).

Among different forage crops, NFE content (Table 2.) was the highest in sole maize (50.92%), baby corn (50.77%) and sorghum (50.43%). Cereal forage crops showed higher NFE content compared to those of leguminous crops. The lowest NFE content was recorded in cowpea intercropped with grasses. The N application rate was inversely proportional to NFE content (Glamoclija *et al.,* 2011).

Among different cereals forage crops, the highest NDF content (Table 3) was obtained in sole guinea grass (71.16%) followed by sole sorghum (70.62%) and guinea grass intercropped with cowpea (70.57%). These were statistically at par with sole guinea grass. The lowest NDF content was noticed in the sole maize (62.67%). However, in cowpea, NDF content was lower (P<0.05) than all cereal forage crops which might be due to the fact that more rapidly synthesized carbohydrates are converted into proteins and protoplasm and only smaller portion is available for cell wall material. The results are in close conformity with the findings of Ayub *et al.* (2002) in sorghum.

The ADF content (Table 3) in sole sorghum (41.61%), sole guinea (40.07%), guinea intercropped (39.37%), sole NHB (38.84%), intercropped NBH (38.33%) were statistically at par with sole sorghum forage. The lowest ADF content was noticed in sole maize (35.3%) and it was lower than sole sorghum fodder crop. However, in cowpea, the ADF content (30.81%) was lower compared to all cereal forage crops. It is observed that legume fodder crops are more digestible than cereal fodder crops. Similar values of proximate principles and fibre fractions have been reported by Chander Datt *et al.* (2009).

The hemicellulose content was the highest in the guinea grass (31.2%) intercropped with cowpea in 2: 3 row ratio and sole guinea grass (31.09%) with cowpea. Cereal forage crops had higher hemicellulose content compared to legume fodder crops. The lowest hemicellulose content was observed in sole cowpea (16%). Hemicellulose content in plants possessed inverse relation with nitrogen uptake and CP content. Iqbal *et al.* (2012) reported that to get better CF yield, cereal fodder should be intercropped with legumes fodder preferably cowpea. The highest acid detergent lignin (ADL) content was recorded in the sole cowpea fodder crop (8.68%) followed by intercropping of

Table 3. Fibre fractions (% DM basis) in different treatments

Treatment	NDF (%)	ADF (%)	Hemicellulose (%)	ADL (%)
NBH sole	68.04^{b}	38.84^{a}	29.20^{a}	3.49^{c}
Guinea sole	71.16^{a}	40.07^{a}	31.09^{a}	3.25^{c}
NBH + cowpea*	67.63^{b}	38.33^{a}	29.3^{a}	3.28^{c}
Guinea grass + cowpea*	70.57^{a}	39.37^{a}	31.2^{a}	3.27^{c}
cowpea Sole	46.81^{d}	30.81^{c}	16.00^{b}	8.68^{a}
Sorghum sole	70.62^{a}	41.61^{a}	29.01^{a}	4.82^{b}
Baby corn sole	63.06^{c}	35.94^{b}	27.12^{a}	4.85^{b}
Maize sole	62.67^{c}	35.30^{b}	27.37^{a}	4.99^{b}
SEm±	0.55	1.66	1.75	0.18
LSD (P= 0.05)	1.64	4.88	5.15	0.53

^{a,b,c,d} Values bearing different superscripts in a column differ significantly (P<0.05)

cowpea (8.15%) with guinea grass. While the lowest ADL content was obtained in the sole guinea grass (3.25%) as also reported by Das *et al.* (2015).

CONCLUSION

The intercropping of NBH with cowpea in 2: 3 row ratio produced higher dry matter yield. The crude protein, ether extract and ash yield were also recorded higher in the same combination of crops. These all parameters are indicative of its good quality fodder production.

ACKNOWLEDGEMENT

The authors are obliged to Director, ICAR-NDRI, Karnal for providing necessary facilities and financial assistance for carrying out this study.

REFERENCES

Anita, M.R., Lakshmi, S. and Rani, T.S. 2015. Effects of row ratios of grass fodder cowpea mixtures on the yield and quality of forages. *Int. J. Forestry Crop Improv.* 6: 85-89.

AOAC. 2005. *Official Methods of Analysis.* 18th rev. edn. Association of Official Analytical Chemists, Arlington, Virginia. USA.

Aulakh, C.S., Gill, M.S., Mahey, R.K., Walia, S.S. and Singh, G. 2012. Effect of nutrient sources on productivity of fodder cropping systems in Punjab. *Indian J. Agron.* 57: 200-205.

Ayub, M., Nadeem, M.A., Tanveer, A. and Husnain, A. 2002. Effect of different levels of nitrogen and harvesting times on the growth, yield and quality of sorghum fodder. *Asian J. Plant Sci. Res*.1: 304-307.

Babasup, M., Halimsup, R.A., Alimonsup, A.R. and Abubakarsup, I. 2011. Grasslegume mixtures for enhanced forage production: Analysis of dry matter yield and competition indices. *Afr. J. Agric. Res*. 6: 5242-5250.

Chander Datt, Singh, N.P., Aruna Chhabra, Bujarbaruah, K.M. and Dhiman, K.R. 2009. Nutritional evaluation of cultivated fodders grown under agro-climate of Tripura. *Indian J. Anim. Sci*. 79: 1143-1148.

DAHD & F. 2012. *Livestock Census Report-2012.* Ministry of Agriculture and Farmers' Welfare, Government of India, New Delhi.

Das, L.K., Kundu, S.S., Kumar, D. and Chander Datt. 2015. Fractionation of carbohydrate and protein content of some forage feeds of ruminants for nutritive evaluation. *Vet. World*. 8: 197.

Glamoclija, D., Jankovic, S., Rakic, S., Maletic, R., Ikanovic, J. and Lakic, Z. 2011. Effects of nitrogen and harvesting time on chemical composition of biomass of Sudan grass, fodder sorghum and their hybrid. *J. Agric. Res. Dev.* 35: 127-138.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedure for Agricultural Research.* Willey Int. Sci. Pub. New York, USA.

GOI. 2017. *Annual Report- 2017.* Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Govt. of India, New Delhi. pp 1-162.

Halli, H.M., Rathore, S.S., Manjunatha, N. and Wasnik, V.K. 2018. Advances in agronomic management for ensuring fodder security in semi-arid zones of India. *Int. J. Curr. Microbiol. Appl. Sci.* 7: 1912-1921.

ICAR. 2012. *Handbook of Agriculture*. Indian Council of Agricultural Research, New Delhi, India.

IGFRI. 2016. A*nnual Report 2016-17*. ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India. pp 1-137.

Iqbal, A., Akbar, N., Khan, H.Z., Abbas, R.N. and Ahmad, J. 2012. Productivity of summer legume forages intercropped with maize as affected by mixed cropping in different sowing techniques. *J. Anim. Plant Sci.* 22: 758-763.

Kushwaha, M., Singh, M., Kumar, R., Tyagi, N., Soni, P.G., Choudhary, S., and Makarana, G. 2018. Yield and quality of multicut fodder sorghum as affected by nutrient levels and biofertilizer application. *Indian J. Anim. Nutr*. 35: 82-89.

Rathore, D.K., Kumar, R., Singh, M., Kumar, P., Tyagi, N., Chander Datt., Meena, B.S., Soni, P.G., Yadav, T. and Makarana, G. 2015. Effect of phosphorus and zinc application on nutritional characteristics of fodder cowpea (*Vigna unguiculata*). *Indian J. Anim. Nutr.* 32: 388-392.

Sengul, S. 2003. Performance of some forage grasses or legumes and their mixtures under dry land conditions. *Eur. J. Agron*. 19: 401-409.

Strydhorst, S.M., King, J.R., Lopetinsky, K.J. and Harker, K.N. 2008. Forage potential of intercropping barley with faba bean, lupin or field pea. *Agro. J.* 100: 182-190.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

Received on 25-09-2018 and accepted on 31-01-2019

Indian J. Anim. Nutr. 2019. 36 (1): 30-34
doi: 10.5958/2231-6744.2019.00005.7

Indian Journal of Animal Nutrition

Bioavailability of Major Minerals from Commonly Used Ruminants Feeds Using Dialyzability Technique

Abhilasha Singh*, Veena Mani, Bhawana Shukla, Anjila Kujur-, Suman Kapila[1]
Animal Nutrition Division, ICAR- National Dairy Research Institute, Karnal-132001, Haryana, India

ABSTRACT

Four feeds namely maize (*Zea mays*) grain, deoiled rice (*Oryza sativa*) bran, groundnut (*Arachis hypogaea*) cake and mustard (*Brassica campestris*) cake were used to assess the bioavailability of calcium, phosphorous, magnesium, sodium and potassium by dialyzability technique under *in vitro* conditions. For this, three Karan Fries calves kept on roughage based diet were used for collection of rumen liquor through stomach tube. *In vitro* dry matter digestibility (IVDMD) of different samples was estimated at three stages *i.e.* simulating ruminal, abomasal and intestinal conditions followed by determination of bioavailability of major minerals in the soluble fraction of intestinal stage sample through a 12 kD dialyzable membrane. Maize grain (69.91±1.11) possessed the highest (P<0.05) IVDMD value followed by mustard cake (68.51±1.087), groundnut cake (63.59±2.07) and deoiled rice bran (51.22±1.05) at the intestinal stage of digestion. Similarly, the highest (P<0.05) bioavailability of Ca, P and Mg (71.76±1.66, 87.57±0.37 and 85.05±0.12%, respectively) was found for maize grain followed by mustard cake, groundnut cake and deoiled rice bran, however, Na bioavailability was higher (98.10±0.10%) in case of groundnut cake and numerically similar values were obtained for K. Results suggested that *in vitro* method could be used for prediction of bioavailable minerals fraction from feeds, however, validation by actual *in vivo* experiment is required before accepting the bioavailability values while considering these for dietary mineral requirements.

Key words: Bioavailability, Dialyzability, In *vitro* digestion, IVDMD, Major minerals

INTRODUCTION

There is scanty information on bioavailability of minerals from different feed ingredients. Reliance on simplistic attributes such as solubility of the plant mineral in water or unphysiological extractants (*e.g.* citric acid) produces results having poor correlation with *in vivo* assessments of mineral bioavailability. Now a days, to assess the bioavailability of minerals, *in vitro* methods have gained importance because of accuracy of results, speed of analysis and relatively low costs. Solubility after the sequential simulation of gastric and intestinal digestion can give convincing values for ruminants. The accessibility of minerals to rumen microbes is equally important. Use of such *in vitro* techniques for prediction of availability can be made but account must be taken of possible interactions between feeds and results will always have to be validated *in vivo* (Suttle, 2010). Besides this, minerals in feeds and forages are associated with other compounds or trapped in the indigestible nutrient fractions resulting in slow release or making these unavailable for use. Therefore, mineral content can be determined chemically while bioavailability is difficult to be estimate (Evitayani *et al.,* 2006). Hence, this study was designed to investigate the bioavailability of major minerals (Ca, P, Mg, Na and K) present in mustard cake, groundnut cake, maize grain and deoiled rice bran under *in vitro* conditions.

MATERIALS AND METHODS

The samples of test feed *i.e.* maize grain, groundnut cake, mustard cake and deoiled rice bran were collected from feed mill of ICAR-National Dairy Research Institute, Karnal and dried in hot air oven at 80°C for 48 h and ground to pass through 1 mm sieve using Willey mill and stored in 200 mL capacity plastic bottles. These were analyzed for their proximate principles (AOAC, 2005) and fiber fractions (Van Soest *et al.,* 1991). Residues left after NDF and ADF determination were pooled separately for further analysis of NDF and ADF bound minerals.

Three stage *in vitro* experiment was conducted to determine DM digestibility of different feed ingredients. Ground samples were subjected to a

*Corresponding author: E-mail: abhilasha1495@gmail.com [1]Animal Biochemistry Division

simulated ruminal, abomasal and intestinal phase digestion by following the method of Tilley and Terry (1963) with certain modification as described by Calsamiglia and Stern (1995). Rumen liquor was collected from three male Karan Fries calves (Av. age= 12 month; BW=120 kg) using stomach tube. After collection of rumen liquor in pre warmed thermos flask, brought to laboratory and used as a source of rumen microbes. About 0.5 g of ground sample was taken for ruminal digestion to which 10 mL of fresh strained rumen liquor and 40 mL of McDougall buffer (McDougall, 1948) were added, CO_2 was passed through the contents for about 10 sec. and the stoppard flasks were incubated for 24 h at 38ºC with periodic shaking. Ruminal digestion was followed by addition of 2 mL of 6N HCl and 0.1 g pepsin enzyme (from porcine mucosa) and pH was then adjusted to 2.0 with additional HCl. After 24 h of incubation at 38ºC in abomasal followed by intestinal with addition of 0.5 mL of 1N NaOH and 13.5 mL of a pH 7.8 phosphate buffer containing 37.5 mg of pancreatin (pancreatic enzymes from porcine pancreas, P1750, Sigma-Aldrich). The contents were incubated at 38°C for 6 h. After incubation, the flasks were removed and centrifuged at 3000 rpm for 10 min. and supernatant was collected for further analysis.

In dialysis procedure, pre treatment of dialyzable membrane (flat width 25 mm, internal diameter 16 mm, molecular weight 12 kD, Sigma-Aldrich) was done by boiling it for 5 min. in Millipore water. The ready to use membranes were kept in 20% ethanol solution in refrigerator at 4ºC and rinsed several times with Millipore water. A known quantity of sample (10 mL) was taken from the supernatant collected in third stage of *in vitro* digestion and was filled in dialyzable membrane and tied with thread. Samples were kept in 1000 mL beaker containing 500 mL Millipore water for 24 h. The contents were continuously shaken using magnetic stirrer (Kaushik, 2013). The bioavailabilty of minerals was the amount of dialyzable mineral expressed as a percentage of total mineral in the feed sample (Hansen, 2008). Bioavailability of minerals was determined by estimating concentration of the mineral in dialysate.

The modified method of closed system of acid digestion of samples (EPA, 2001) was followed for digestion of samples for mineral estimation. The contents of Ca, Mg, Na, and K were analysed using atomic absorption spectrophotometer (Hitachi Model Z – 5000 with Zeeman correction). The P content was estimated by spectrophotometer (AOAC, 2005). The data were statistically analyzed using SPSS (2010).

Table 1. Chemical composition (% DM basis) and mineral profile of the feeds

Parameter	Mustard cake	Groundnut cake	Maize grain	Deoiled rice bran
Dry matter	91.52	91.62	91.76	90.97
Crude protein	37.70	45.45	9.81	16.60
Ether extract	5.90	6.12	4.22	1.10
Crude fibre	8.37	7.61	2.42	13.09
Nitrogen free extract	39.91	34.52	83.56	54.32
Ash	8.12	6.32	1.53	14.90
Neutral detergent fibre	29.50	18.12	11.70	41.10
Acid detergent fibre	20.62	14.32	2.80	25.50
Calcium	0.48	0.32	0.15	0.80
Phosphorous	1.58	0.74	0.41	1.90
Magnesium	0.63	0.51	0.34	1.14
Sodium	0.082	0.05	0.025	0.033
Potassium	1.84	1.25	0.78	1.25

Table 2. Mineral (% of total) found in NDF and ADF fraction of feeds

Parameter	Fibre fraction	Mustard cake	Groundnut cake	Maize grain	Deoiled rice bran
Calcium	NDF	3.63	3.79	3.23	6.52
	ADF	2.69	2.36	1.91	2.79
Phosphorous	NDF	2.135	2.417	2.432	5.52
	ADF	0.76	0.78	0.457	1.751
Magnesium	NDF	2.57	2.72	1.75	3.37
	ADF	1.13	1.19	0.83	1.12
Sodium	NDF	0.718	0.752	0.372	0.775
	ADF	0.274	0.282	0.153	0.313
Potassium	NDF	0.693	0.714	0.412	1.331
	ADF	0.355	0.321	0.166	0.51

(n=6, replicates per feed)

RESULTS AND DISCUSSION

The chemical composition of the four ingredients has been presented in Table 1. Deoiled rice bran had higher NDF (41.10%) followed by mustard cake (29.50%). Lowest values for NDF (11.70%) and ADF (2.30%) were observed in maize grain. The values are in agreement with those reported by ICAR (2013). The highest values for Ca (0.80%), P (1.90%) and Mg (1.14%) were found in DORB. The lowest levels of Ca (0.15%,), P (0.41%), Mg (0.34%), Na (0.025%) and K (0.78%) were found in maize grain. Kumar and Kaur (2007) reported that Ca and P contents in different concentrate ingredients varied from 0.11 to 0.67% and from 0.68 to 1.61%, respectively. The results of present study are comparable with the values given by ICAR (2013).

The maximum association of Ca with NDF and ADF fraction was found in DORB (6.52 and 2.79%). The association of all the minerals with fiber fractions were lowest in case of maize grain among all four feedstuffs (Table 2). Among all minerals, solubility of Ca was quite low. No information is available on minerals associated with NDF and ADF fractions in concentrate ingredients. However, reports are available indicating variation in association of minerals with cell wall constituents in forages. Serra *et al.* (1996) reported 0.7 Ca, 14.3 P, 1.9 Mg and 3.7% K associated with NDF and 0.2 Ca, 4.4 P, 0.7 Mg and 2.8% K with ADF fraction in same forages. Evitayani *et al.* (2006) showed that 27.5 Ca, 8.3 P and 18.1% Mg was associated with NDF and 7.8 Ca, 1.8 P and 2.2% Mg was associated with ADF in grasses.

There was increasing trend in digestibility (Table 3) when incubation proceeded from ruminal to intestinal stage in all feeds and a similar trends was also reported by Gupta *et al.* (2016) and Parihar *et al.* (2017). Significantly ($P<0.05$) higher IVDMD values were found in maize grain (66.52-72.10%) and lowest in deoiled rice bran (42.90-57.27%). The highest digestibility was 69.91% in maize grain. Digestibility of

Table 3. *In vitro* DM digestibility (%) estimated by three stage digestion technique

Feed	Ruminal stage	Up to abomasal stage	Up to intestinal stage
Mustard cake	$65.59^{Ca}\pm0.7$	$68.74^{Cab}\pm0.6$	$71.19^{Bb}\pm1.04$
Groundnut cake	$57.44^{Ba}\pm1.8$	$65.32^{Bb}\pm0.51$	$68.04^{Bb}\pm2.1$
Maize grain	$66.52^{Ca}\pm0.9$	$71.12^{Db}\pm0.7$	$72.10^{Cb}\pm0.03$
Deoiled rice bran	$42.90^{Aa}\pm0.64$	$53.49^{Ab}\pm0.24$	$57.27^{Ab}\pm1.7$

Figures with different superscripts in a row^{a,b} and in a column$^{A, B,C, D}$ differ significantly ($P<0.05$): (n= 9 replicate per feed)

Table 4. Bioavailability (%) of various major minerals from different feeds using dialyzability technique

Parameter	Mustard cake	Groundnut cake	Maize grain	Deoiled rice bran
Calcium	65.28^{ab}±0.69	61.88^{a}±1.23	71.76^{c}±1.66	66.70^{b}±1.03
Phosphorous	81.20^{a}±0.13	82.73^{b}±0.27	87.57^{c}±0.37	81.22^{a}±0.25
Magnesium	82.42^{a}±0.06	84.34^{b}±0.09	85.05^{c}±0.12	82.49^{a}±0.15
Sodium	97.99^{b}±0.35	98.10^{b}±0.10	95.55^{a}±0.50	96.04^{a}±0.36
Potassium	95.89±0.61	94.33±0.75	96.06±0.11	95.88±0.25

^{a,b,c}Values with different superscripts in a row differ significantly (P<0.05); (n= 9 per feed)

mustard cake at ruminal, abomasal and intestinal stage of incubation was found to be 65.59±0.7, 68.74±0.6, 71.19±1.04% and the corresponding values were 57.44±1.8, 65.32±0.51, 68.04±2.1% for groundnut cake. Mondal *et al.* (2010) reported comparable values of DM digestibility in mustard cake (69.63%) and groundnut cake (60.88%) but it was lower for maize grain (50.3%).

The Ca bioavailability (%) was 61.88±1.23 for groundnut cake and 65.28±0.69 for mustard cake. Kumar *et al.* (2015) reported slightly lower values than the present values. Maize grain had higher (P<0.05) bioavailability values for Ca (71.76±1.66), P (87.57±0.37) and Mg (85.05±0.12%), respectively than those from mustard cake, groundnut cake and de oiled rice bran which might be due to the lower fibre content, lower association of these minerals with NDF, ADF and higher degradability of maize grain. Values for Na and K were quite high (94.33 to 98.10%) though significantly higher values were found for Na release for both the cakes but considering the extent of release these values seemed be of no practical relevance. Brown *et al.* (2004) reported similar pattern of ruminal release of K from corn and cotton seed meal and reported values of more than 80%. Ceresnakova *et al.* (2007) conducted *in sacco* study on some grasses such as hybrid rila, hybrid niva, grass silage and reported the disappearance of K up to 98 to 99%. Trinacty *et al.* (2000) observed total tract release of K to be 98.4% from lucerne hay using nylon capsule method. Bamikole (2009) studied the macro-mineral bioavailability in goats fed forages of nitrogen fertilized guinea grass and guinea grass verano stylo mixture and observed that K all forages was well utilized with no significant variations.

CONCLUSIONS

Though, *in vitro* method could be used for prediction of bioavailable minerals fraction from feeds, however, validation by actual feeding trial is required before accepting the bioavailability values while formulating rations as the present bioavailability values were much higher than those reported by NRC (2001) under practical situations.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-NDRI, Karnal, Haryana for providing financial support to carry out this study.

REFERENCES

AOAC. 2005. *Official Methods of Analysis*. Association of Official Analytical Chemists. 18th edn. Virginia, USA.

Bamikole, M.A. and Babayemi, O.J. 2009. Macro mineral utilization in West African dwarf goats fed combinations of fertilized and unfertilized Guinea grass, Verano stylo and a concentrate mix. *Livestc. Res. Rural Dev.* 21 (9).

Brown, M.S., Drager, C.D., Cochran E.M. and Lauterbach, E.A. 2004. Ruminal mineral release from corn and cottonseed meal. *Beef Cattle Research in Texas*. pp 109-111.

Ceresnakova, Z., Flak, P., Polacikova, M. and Chrenkova, M. 2007. *In sacco* macromineral release from selected forages. *Czech. J. Anim. Sci*. 52: 175-182.

Calsamiglia, S. and Stern, M.D. 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminant. *J. Anim. Sci.* 73: 1459-1465.

E.P.A. 2001. Method-1631. *Development of Digestion Procedures to Determine Mercury In Solid and Semisolid Samples.* Frontier Geosciences, available from EPA sample control centre DYN Crop I & ET, Alexandria, VA22304 (703-461-2100., SCC@ dyncorp.com.).

Evitayani, W.L., Fariani, A., Ichinohe, T., Hayashida, M., Razak, S.A.A. and Fujihara, T. 2006. Macro mineral distribution of forages in South Sumatra during rainy and dry seasons. *J. Food, Agric. Environ*. 4: 155-160.

Gupta, S., Tomar, S.K., Mani, V., Kumar, R. D., Parihar, D.S. and Kujur S.T.A. 2016. Bioaccessibility of copper, molybdenum and zinc from selected oilseed cakes using three stage *in vitro* technique. *Indian J. Anim. Nutr*. 33: 149-153.

Hansen, S.L. 2008. *Nutritional Interrelationships between Iron, Copper and Manganese in Domestic Livestock*. Ph.D. Thesis. North Carolina State University, North Carolina, USA.

ICAR. 2013. *Indian Feeds and Fodder*. Krishi Bhawan, New Delhi, India.

Kaushik, R. 2013. *Development and Evaluation of Vitamin and Calcium Fortified Milk*. Ph.D. thesis, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India.

Kumar, R.D., Kewalramani, N., Mani, V., Gupta, S. and Parihar, D.S. 2015. Bioaccessibility of calcium, phosphorous and magnesium from different oil seed cakes. *Indian J. Anim. Nutr*. 32: 457-459.

Kumar, R.S. and Kaur, H. 2007. Macro mineral status and dietary cation-anion difference value of some Indian feeds and fodders. *Anim. Nutr. Feed Technol*. 7: 111-117.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan, C.A. 2009. *Animal Nutrition.* 6th edn. Dorling Kindersley (India) Pvt. Ltd. pp 635-638.

McDougall, E.I. 1948. Studies on ruminant saliva. The compostion and output of sheep saliva. *Biochem.* 43: 99.

Mondal, G., Chopra, R.C. and Barman, K. 2010. Nitrogen fractions, digestion kinetics and in vitro digestibility of some ruminant feeds. *Indian J. Anim. Nutr*. 27: 25-30.

NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. edn. National Academy of Sciences. Washington, D.C., USA

Parihar, D.S., Mani, V., Kewalramani, N., Gupta, S., Kumar, D. and Kujur, S.T.A. 2017. Bioaccessibility of trace minerals from different oilseed cakes under *in vitro* conditions. *Indian J. Anim. Nutr*. 34: 70-73.

Serra, A.B., Serra, S.D., Ichinohe, T. and Fujihara, T. 1996. Amount and distribution of dietary minerals in selected philipins forages. *Am. J. Anim. Sci*. 9: 139-147.

SPSS. 2010. *Statistical Packages for Social Sciences*. Version 16, SPSS Inc., Illinois, USA.

Suttle, N.F. 2010. *Mineral Nutrition of Livestock*. 4th edn. CAB International, Oxfordshire, UK. p 27.

Tilley, J.M.A. and Terry, R.A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grassland Soc.* 18: 104-111.

Trinacty, J., Simek, M., Loucka, R. and Takahashi, J. 2000. Total tract digestibility of alfalfa hay minerals evaluated by nylon capsule method. *Czech. J. Anim. Sci*. 45: 65-69.

Van Soest, P.J., Robertson, J.B. and Lewis, B. 1991. Methods for dietary fibre, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

Received on 18-10-2018 and accepted on 21-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 35-39
doi: 10.5958/2231-6744.2019.00006.9

Indian Journal of Animal Nutrition

Effect of Poly-Herbal Mixture Supplementation during Post partum Period on Feed Intake and Reproductive Performance of Sahiwal Cows

Piyali Kuri, Parveen Kumar*, Nishant Kumar, Anjali Aggarwal and Mahendra Singh
Animal Physiology Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India

ABSTRACT

To investigate effect of poly-herbal mixture supplementation on feed intake and reproductive parameters, 12 Sahiwal cows at peri-parturient period were selected and divided into two groups of 6 animals each on the basis of body weight, parity and expected producing ability. Control group was fed basal diet alone while in supplemented group alongwith basal diet, poly-herbal mixture was given @ 450 g/animal/d from day of calving up to 7 days continuously and then on alternate days up to day 21 and then at weekly interval up to day 60. Body weight, feed intake and milk production were better (P<0.05) in supplemented group. There were no cases of RFM and metritis in supplemented group and rapid rate of cervix and uterine involution was observed in supplemented group compared to control. There was significant (P<0.05) reduction in interval from calving to 1st observed estrus, number of services per conception and service period while increase (P<0.05) in first service conception rate in cows of poly-herbal mixture supplemented group. Hence, poly-herbal mixture supplementation in diet of Sahiwal cows @ 450 g/animal/d improved post partum reproductive performance.

Key words: Feed intake, Milk production, Poly-herbal mixture, Reproduction, Sahiwal cows

INTRODUCTION

Sahiwal is one of the most important milch breeds of cattle in Indian subcontinent. Improvement of post partum reproductive efficiency of Sahiwal cattle is of major concern. Multifarious strategies are emerging worldwide to improve reproductive performance of dairy animals which include use of hormones, antibiotics, nutritional supplements (Narvariya *et al.,* 2018; Raheja *et al.,* 2018), semi-synthetic products *etc.* but many of them are associated with suppressing symptoms, less accessibility, cost and side effects (Malviya *et al.,* 2011). Thereby, people are again oriented back to herbal and ayurvedic medicines having the holistic approach to improve performance of dairy animals. Herbal remedies are gaining more attention as an alternative to supplementation for female fertility. As recognized by the World Health Organization, local ethno-veterinary medicines could play an important role in ensuring general well-being and welfare of livestock in the developing world (WHO, 2008). Previous studies have documented that herbs exert multiple effects such as growth promoting, anti-inflammatory and anti-oxidant activity (Ao *et al.,* 2011; Huang *et al.,* 2010) and can regulate metabolism in livestock (Grela and Semeniuk, 2006). Ginger is one of the famous traditional medicinal herbs because of its anti-oxidant, anti-inflammatory and immuno-modulatory effects (Ali *et al.,* 2008). Turmeric (*Curcuma longa*) has been shown to possess anti-inflammatory (Santoskar *et al.,* 1986) and antioxidant properties (Sreejayan and Rao, 1994). Krishnamoorthy and Madalageri (1999) reported that seeds of Ajwain act as anthelmintic, carminative, laxative and stomachic. It also cures abdominal tumors, abdominal pain and piles. *Shatavari* has been documented in dairy animals as reproductive system tonic, immune-modulator, anti-oxidant and anti-stress compound (Kumar *et al.,* 2008). Fenugreek is one of the world's oldest medicinal herbs whose immunomodulatory effect, anti-inflammatory and anti-neoplastic effect, antimicrobial effect and antioxidant effect have been reported (Priyanjali *et al.,* 2005). Synergistic interactions of bioactive compounds in herbal plants are mainly responsible for their potent health beneficial effect (Liu, 2004).

Supplementation of poly-herbal improved performance of Murrah buffaloes (Chandra, 2015). However, very scanty literature is present regarding its efficacy in improvement of reproductive parameters in indigenous breeds of cattle. Hence. the present work

*Corresponding author: E-mail: drpk1959@gmail.com

was conducted to assess the effect of supplementation of poly-herbal mixture on feed intake and reproductive performance of Sahiwal cows.

MATERIALS AND METHODS

Twelve pregnant Sahiwal cows were selected during transition period from Livestock Research Center, NDRI, Karnal. The animals were free from any anatomical, physiological and infectious disorders. Present study was conducted at Livestock Research Center of ICAR-National Dairy Research Institute, Karnal, Haryana, India during September 2017 to March, 2018. Karnal is situated at an altitude of 250 m above mean sea level, latitude and longitude position being 29°42" N and 79°54" E, respectively. Experiment was approved and conducted under the established standards of the Institutional Animal Ethics Committee (IAEC).

The cows of treatment group were fed individually at the time of poly-herbal mixture supplementation daily during the experiment. Experimental animals were maintained as per the standard conditions of feeding (ICAR, 2003) and management followed at NDRI.

Control group was fed basal diet alone and poly-herbal mixture was supplemented in treatment group @ 450 g/animal/d during first 7 days after calving, then at alternate day up to day 21 (9, 11, 13, 15, 17, 19 and 21) and then at weekly intervals up to day 60 (28, 35, 42, 49, 56 and 60). The composition and quantity of poly-herbal mixture has been presented in Table 1. Individual herb was purchased from local market after assessing their quality in consultation with ayuverdic practitioners. Each herb was ground separately. The herbal mixture was prepared after mixing the herbs in correct quantity. Body weight and feed intake of all animals was recorded at weekly intervals. Daily milk yield of individual cows was recorded.

Involution status of cervix and uterus was assessed by ultrasound examination. Ultrasonography of cervix and uterus was performed at weekly interval by a real time B-Mode diagnostic ultrasound scanner equipped with a 7.5 MH linear array transducer. Diameter of cervix and uterus was measured after freezing the ultrasound image and recorded.

The reproductive parameters (incidence of retention of fetal membranes (RFM), metritis, involution status of cervix, involution status of uterus, interval from calving to 1st observed estrus, calving to day of 1st service, 1st service conception rate, number of services per conception and service period) were recorded. For calculating days to first observed estrus, both groups of cows were observed twice daily in the

Table 1. The composition and quantity of poly-herbal mixture

Name of Herb (in Hindi)	Common name	Botanical name	Part of herb used	Quantity
Ajwain	Carom	*Trachyspermum ammi*	Seed	25 g
Jeera	Cumin	*Cuminum cyminumlinn*	Seed	25 g
Methi	Fenugreek	*Trigonella foenum graecum*	Seed	25 g
Sundh	Ginger	*Zingiber officinale*	Rhizome	25 g
Saunf	Fennel	*Foeniculum vulgare*	Seed	25 g
Sowa	Indian dill	*Anethum graveolens*	Foliage	25 g
Haldi	Turmeric	*Curcuma longa*	Rhizome	25 g
Satavari	Shatavari	*Asparagus racemosus*	Root	50 mg
Other ingredients				
Kala Namak	Black salt			25 g
Gur	Jaggery			250 g
Total				450 g

Table 2. Body weight (kg) of Sahiwal cows in post partum period

Group	Week							
	1	2	3	4	5	6	7	8
Control	356.63	362.03	353.58^{A}	364.67	365.20^{A}	363.54^{A}	363.67^{A}	360.33^{A}
	±4.76	±3.18	±2.42	±9.45	±6.17	±8.34	±6.23	±5.65
Treatment	360.23^{A}	368.23	373.39^{B}	380.79	386.80^{B}	395.33^{B}	386.70^{B}	384.72^{B}
	±4.14	±2.67	±5.09	±7.6	±7.6	±8.72	±7.34	±3.24

^{A,B}Mean having different superscripts within column differs significantly (P≤0.05)

morning and evening to determine the signs of estrus by the experienced and skilled personnel and estrus chart was prepared for each cow so that they were brought for A.I. after voluntary waiting period (VWP) which was approximately set as 60 days postpartum period at LRC, NDRI. Karnal. Service period was calculated by counting the number of days from calving to the service that resulted in pregnancy (effected service) and this was confirmed by pregnancy diagnosis after 45-60 days of last service. Number of inseminations per conception was calculated as the number of insemination required for successful conception. 1st service conception rate are based on a rectal diagnosis of pregnancy conducted 6-8 weeks after insemination.

$$\text{First-service conception rate (\%)} = \frac{\text{No. pregnant first service}}{\text{No. bred first service}} \times 100$$

Statistical analysis was done using student's t-test which was performed for comparison of various parameters between the control and treatment group using the SPSS statistical software program (version 21.0).

RESULTS AND DISCUSSION

Results pertaining to body weight, DM intake and milk yield of Sahiwal cows of control and supplemented groups have been presented in Tables 2, 3 and 4, respectively. Body weight of Sahiwal cows was higher (P<0.05) in treatment group compared to control at 3rd and 5th week onwards. Increased body weight in treatment group might be attributed to higher DM intake in treatment group. Weekly milk yield was better (P<0.05) in treatment group compared to control group which might be attributed to galactopoietic activity of herbs like ajwain, fenugreek, shatavari *etc.*

It is clear from the results that supplementation of herbal mixture exerted positive influence on rate of cervix as well as uterine involution as demonstrated by lower diameter of cervix and uterine horn in supplemented group compared to control group throughout the experiment (Table 5). The overall mean values of diameter for supplemented and control group were 4.16±0.19 and 4.79±0.17 cm, respectively. Chandra (2015) in Murrah buffaloes and Barjibhe (2016) in Sahiwal cows reported similar results. The overall diameter of uterine horn in supplemented and control group was 4.33±0.14 and 5.09±0.16 cm, respectively. This lowering of uterine diameters occurred at rapid rate in supplemented group of animals compared to control from 1st to 6th week of post partum period. Chandra (2015) found similar positive influence of herbal mixture supplementation on involution of uterus of Murrah buffalo. Beneficial effects of herbal extracts may arise from activation of feed intake, immune-stimulation, anti-inflammatory and anti-oxidant

Table 3. DM intake in Sahiwal cows

Parameter	Group	
	Control	Treatment
DM intake (kg/d/animal)	9.00^{A}±0.46	9.96^{B}±0.31
DM intake (kg//100 kg BW)	2.57	2.62

Table 4. Milk yield (kg/animal/d) of Sahiwal cows in post partum period

Group	Week							
	1	2	3	4	5	6	7	8
Control	6.89^{A}	6.93^{A}	7.53^{A}	6.77^{A}	7.06^{A}	6.43^{A}	5.94^{A}	6.08^{A}
	±0.32	±0.21	±0.23	±0.35	±0.14	±0.21	±0.27	±0.36
Treatment	7.73^{B}	7.73^{B}	7.48^{B}	7.69^{B}	7.73^{B}	7.63^{B}	7.55^{B}	7.17^{B}
	±0.12	±0.15	±0.15	±0.20	±0.15	±0.37	±0.26	±0.32

^{A,B}Means having different superscript within column differ significantly (P≤0.05)

properties (Yan *et al.,* 2011).

In the present study, 2 cows of control group suffered from RFM and metritis while cows of herbal mixture supplemented group had no incidence of metritis which might be attributed to anti-oxidant and immune-modulatory action of herbal mixture which prevent infections (Ao *et al.,* 2011; Huang *et al.,* 2010). The interval from calving to day of first observed estrus was shorter (P<0.05) in cows of herbal mixture supplemented group. In our study, herbal mixture supplemented cows showed shorter interval between calving and 1st observed estrus which indicated beneficial role of herbal mixture in improving uterine health as indicated by rapid involution rate of cervix and uterus, and strengthening of estrus expression intensity. Consequently, 1st observed estrus after calving occurs earlier in cows of herbal mixture supplemented group.

Number of services per conception was lower (P<0.05) in cows of herbal mixture supplemented group. Our results regarding the effect of herbal mixture in reducing the number of services required per conception is correlated with no incidence of RFM and metritis and better uterine involution in herbal mixture supplemented cows suggesting the favorable action of herbal mixture in improving conception rate with minimum services.

Service period was shorter (P<0.05) in cows of herbal mixture supplemented group. In cows of control group, incidence of RFM and metritis was higher as compared to cows in herbal mixture supplemented group where there was no case of uterine infection supporting our results of positive effect of herbal mixture in reducing service period (Chandra, 2015).

CONCLUSIONS

Supplementation of poly-herbal mixture in the diet of Sahiwal cattle improved in post partum reproductive performance in terms of involution of uterus and cervix, incidence of RFM and metritis, interval from calving to 1st observed estrus, interval from calving to 1st observed estrus, service period, number of insemination per conception and 1st service conception rate.

ACKNOWLEDGEMENT

The financial grant for the study was provided by National Initiative on Climate Resilient Agriculture, Indian Council of Agricultural Research (NICRA-

Table 5. Cervix and uterus diameter (cm) during post partum period in Sahiwal cows

Group	Week					
	1	2	3	4	5	6
Cervix diameter (cm)						
Control	6.08^{a}±0.27	5.49^{a}±0.23	4.95$^{a\pm}$0.18	4.38$^{a\pm}$0.13	4.04$^{a\pm}$0.05	3.84$^{a\pm}$0.07
Treatment	5.4$^{b\pm}$0.21	4.78$^{a\pm}$0.16	4.31$^{b\pm}$0.13	3.87$^{b\pm}$0.19	3.39$^{b\pm}$0.16	3.21$^{b\pm}$0.16
Uterus diameter (cm)						
Control	6.208^{a}±0.15	5.72^{a}±0.15	5.27$^{a\pm}$0.13	4.83$^{a\pm}$0.13	4.4^{a}±0.13	4.09^{a}±0.07
Treatment	5.44$^{b\pm}$0.16	4.99^{a}±0.13	4.44^{b}±0.16	4.00$^{b\pm}$0.16	3.7^{b}±0.21	3.42^{b}±0.16

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

Table 6. Reproductive performance in control and treatment groups of Sahiwal cows

Reproductive parameter	Group	
	Control	Treatment
Incidence of RFM and metritis	2	0
Interval from calving to first observed estrus	$75^{b\pm}2.9$	$46.67^{a\pm}3.81$
Service period (days)	$121.33^{b}\pm6.94$	$93.34^{a}\pm4.41$
Number of insemination per conception	$2.6^{b}\pm0.33$	$1.6^{a}\pm0.33$
1st service conception rate (%)	16.66	50.0

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

ICAR), New Delhi. The authors would like to thank the Director, ICAR-NDRI for providing necessary facilities to conduct this research work.

REFERENCES

Ali, B.H., Blunden, G., Tanira, M.O. and Nemmar, A. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): A review of recent research. *Food Chem. Toxicol.* 46: 409-420.

Ao, X., Yan, L., Zhou, T.X., Wang. J.P., Kim, H.J., Cho, J.H. and Kim, I.H. 2011. Effects of *Saururus chinensis* extract supplementation on growth performance, meat quality and slurry noxious gas emission in finishing pigs. *Livestc. Sci*. 138: 187-192.

Barjibhe. 2016. *Effect of Polyherbal Mixture, Butyrate and Mustard Oil Supplementation During Transition Period on Productive and Reproductive Performance of Sahiwal Cows.* M.V.Sc. Dissertation. National Dairy Research Institute (Deemed University), Karnal-132001, Haryana, India.

Chandra, S. 2015. *Effect of Poly Herbal Mixture and Butyrate Feeding on Postpartum Reproductive Performancei in Murrah Buffaloes*. M.V.Sc. Dissertation. National Dairy Research Institute (Deemed University), Karnal-132001, Haryana, India.

Huang,Y.,Yoo, J.S., Kim, H.J., Wang, Y., Chen, Y.J., Cho, J.H. and Kim, I.H. 2010. Effects of dietary supplementation with blended essential oils on growth performance, nutrient digestibility, blood profiles and fecal characteristics in weanling pigs. *Asian- Australas. J. Anim. Sci.* 23: 607-613.

ICAR. 2013. *Nutrient Requirement of Cattle and Buffalo.* Indian Council of Agricultural Research, New Delhi, India.

Krishnamoorthy, V. and Madalageri, M.B. 1999. Bishop weed (*Trachyspermum ammi*): An essential crop for north Karnatka. *J. Med. Aromatic Plant Sci.* 21: 996-998.

Kumar, S., Mehla, R.K. and Dang, A.K. 2008. Use of shatavari (*Asparagus racemosus*) as a galactopoietic and therapeutic herb: A review. *Agric. Rev.*29: 132-138.

Liu, R.H. 2004. Potential Synergy of photochemicals in cancer prevention : Mechanism of action. *J. Nutr.* 134: 3479-3485.

Malviya, N., Jain, S., Gupta, V.B. and Vyas, S. 2011. Recent studies on aphrodisiac herbs for the management of male sexual dysfunction : A review. *Acta Pol Pharm.* 68: 3-8.

Narvariya, D.S., Chandra, R., Kumar, N., Roy, A.K., Chander Datt and Mehla, R.K. 2018. Supplementary feeding: Effect on growth and blood metabolites in Sahiwal heifers *Indian J. Anim. Nutr.* 35: 161-165.

Priyanjali., Saroj, D., Mohan, G.H. and Devasagayam, T.P.A. 2005. Anti-oxidant properties of germinated fenugreek seeds. *Phytotherapy Res*.19: 977-983.

Raheja, N., Kumar, N., Patel, B. and Lathwal, S.S. 2018. Effect of dietary betaine on reproductive performance of Karan Fries cows during hot humid season *Int. J. Curr. Microbiol. Appl. Sci* 7: 1451-1460.

Santoskar, R.R., Shah, S.J. and Shenoy, S.G. 1986. Evaluation of anti-inflammatory property of curcumin (diferuloy1 methane) in patients with postoperative inflammation. *Int. J. Clin. Pharmacol. Ther. Toxicol*. 24: 651-654.

Sreejayan, N. and Rao, M.N. 1994. Curcuminoids as potent inhibitors of lipid peroxidation. *J. Pharm. Pharmacol*. 46: 1013-1016.

WHO. 2008. http://www.who.int/dg/speeches/2008/20081107/en/ index.html

Yan, L. and Kim, I.H. 2011. Effect of dietary grape pomace fermented by *Saccharomyces boulandi* on the growth performance, nutrient digestibility and meat quality in finishing pig. *J. Anim. Sci.* 12: 1763-1770.

Received on 01-01-2019 and accepted on 13-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 40-46
doi: 10.5958/2231-6744.2019.00007.0

Indian Journal of Animal Nutrition

Effect of Supplementation of Synthetic Lysine and Methionine on Serum Biochemical Profile, Carcass Characteristics and Meat Composition in Broiler Chicken

Bornalee Handique, G. Saikia, Runjun Dowarah*, B.N. Saikia and S. Tamuly
Department of Animal Nutrition, College of Veterinary Science,
Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India

ABSTRACT

A total of 144 day old broiler chicks of Vencobb strain 400 randomly distributed into four groups (3 replicates of 12 chicks) using randomized block design *viz.* T_0 - basal diet (control), T_1, T_2 and T_3 were offered the basal diet supplemented with synthetic L-lysine at 20, 30 and 40% and DL-methionine at 30, 40 and 50% in excess of the total BIS (2007) recommendations upto 42 days of age. Non-significant effect of supplementation of synthetic lysine and methionine were observed on feed intake. However, total gain in body weight and FCR were significantly ($P<0.01$) influenced by supplementation. Significant increase ($P<0.001$) in serum total protein was observed in the birds of T_2 group whereas serum cholesterol and triglyceride level decreased ($P<0.01$) with increased level of synthetic amino acids. Statistically non-significant effect of supplementation was observed on dressing percentage, yield of prime cuts, percent weight of visceral organs and chemical composition of meat except that the abdominal fat pad was lower in groups T_2 and T_3. The study revealed that supplementation of 30% synthetic L-lysine and 40% DL-methionine higher than BIS (2007) recommendation in broiler rations reduced serum cholesterol and triglyceride levels.

Key words: Blood biochemical parameters, Broiler chicken, Carcass characterstics, DL-methionine, L-lysine

INTRODUCTION

India is the 5th largest producer of poultry meat in the world producing about 2.337 million tonnes of chicken meat annually (Prabakaran, 2012). Broiler farming is fast growing and profitable meat industry in the country. It can supply readily available source of animal protein to fight against protein malnutrition in Indian population. The major objective of commercial broiler farming is to produce protein rich and fat free meat for the consumers. The change in market scenario and consumer preference have forced the producers to produce high quality carcass with low price by using different feed additives.

Lysine is essential for mobilization of fatty acid into the mitochondria and also helps in enhancing growth performance, breast meat yield, carcass protein retention and reduces fat deposition. Lysine has also been shown to exhibit specific effects on carcass composition and breast meat yield (Schutte and Pack, 1995). Increasing lysine over and above of NRC (1994) recommendations improved weight gain, feed efficiency and breast meat yield (Si *et al.,* 2004) and reduced deposition of extra fat in the carcass (Moran and Bilgili, 1990). The blood biochemical profile may monitor the quality of nutrition and health of birds (Etim *et al.,* 2014). Blood glucose, cholesterol, triglyceride and total protein level indicate changes in carbohydrate, fat and protein metabolism in the body affecting growth, meat production and quality of meat. Lysine and methionine in excess of NRC (1994) recommendations improved plasma triglyceride and cholesterol and also enhanced economical performance (Bouyeh, 2013). Therefore, the present study was conducted to evaluate the effect of synthetic L-lysine and DL-methionine on blood biochemical profile and carcass characterstics of the commercial broiler chicken.

MATERIALS AND METHODS

The experiment was conducted in the experimental poultry shed, Department of Animal Nutrition, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. A total of 144 day old broiler chicks (Vencobb strain 400) randomly distributed into 4 groups (3 replicates of 12 chicks)

*Corresponding Author: Email: runjundowarah03@gmail.com

using randomization block design *viz.* T_0-basal diet (control), T_1, T_2 and T_3 were offered the basal diet supplemented with synthetic L- lysine at 20, 30 and 40% and DL- methionine at 30, 40 and 50% in excess of the total BIS (2007) recommendation. The basal diet was prepared as per BIS (2007) without supplementation of L-lysine and DL-methionine (Table 1). DL-methionine (MetAMINO®) and L- lysine (Biolys®) were procured from Evonik India, Mumbai.

All the chicks were reared in deep litter system under similar environmental conditions (temperature 20-27°C and humidity 78-88%) and had access to *ad lib.* feed and water during entire experimental period. The chicks were offered *ad lib.* pre starter ration containing 23% crude protein and 3000 kcal ME per kg (BIS, 2007) for the first 7 days of age. The experimental diets were offered *ad lib.* to the starter and finisher chicken. The chicks were vaccinated against Ranikhet disease with Lasota strain (F1) and Infectious Bursal Disease (IBD) vaccine on 7th and 14th of age, respectively. Daily feed offered and residue left was weighed and recorded to monitor daily feed intake. The body weight of the individual birds was recorded weekly in the morning prior to feeding during the entire experimental period of 42 days to calculate average body weight change, gain in weight and feed conversion ratio (FCR).

At the end of the experiment, six birds from each group were selected for collection of blood sample from the wing vein. The blood samples were brought to the laboratory without disturbing the clots and centrifuged at 3000 rpm for 15 min. to collect serum and stored at -20°C till further analysis. After thawing, serum samples were analyzed for concentration of glucose, total protein, cholesterol, triglyceride and alanine amino transferase (ALT) by spectrometric method (dual beam UV-Spectrometer) using commercial kits (Coral Clinical System, Uttrakhand, India).

Table 1. Ingredient (%) and nutrient composition (% DM basis) of the basal diet

Attribute	Starter	Finisher
Ingredient composition		
Maize	51	54.3
De-oiled rice polish	5.5	7.2
De-oiled ground nut cake	15	10
Soybean meal	23.5	22.5
Vegetable oil	3	4
Mineral mixture	1.5	1.5
Common salt	0.5	0.5
Nutrient composition on DM basis		
Dry matter	92.23	92.09
Crude protein	22.08	20.01
Crude fibre	5.78	5.10
Ether extract	4.20	4.30
NFE	54.79	57.59
Total ash	13.15	13.00
*ME (Kcal/kg)	3130	3202
*Lysine	1.20	1.00
*Methionine	0.50	0.45

*Calculated values (Vitamin premix (Vitablend vit A, B_2, D_3, K) was added @ 20 g per quintal of diet in both starter and finisher diet. Mineral mixture contained calcium 25%, Phosphorus 5%, Sodium chloride 23%, Iodine 10 ppm, Copper 100 ppm, Manganese 2000 ppm and Cobalt 10 ppm.

Birds were slaughtered for the carcass parameters and composition of meat at the end of the experimental trial. The birds were fasted overnight and pre-slaughter weights were recorded. The dressed weight of each group was obtained separately after complete bleeding and removal of feathers, viscera, head and legs by keeping the skin intact with the carcass and calculated as percent of pre-slaughter weight. The neck, wing, back, drumstick, thigh and breast meat were weighed seperately and divided by pre-slaughter weight to determine relative weight and expressed as percentage. Fat around the abdominal wall was removed and weighed and calculated as percentage pre-slaughter weight.

The edible visceral organs (heart, liver and gizzard) and lymphoid organs were weighed individually after separating from viscera. The total weight of small intestine along with caecal content was taken and calculated as percent of pre-slaughter weight. The representative meat samples (100 g) were collected from breast and thigh portion after slaughter of the birds. Samples were mixed together and thoroughly chopped with the help of chopper and kept at -20°C for further analysis. The proximate constituents of diets and meat were analysed (AOAC, 2005). Data were analyzed by using IBM-SPSS version 20. One way ANOVA was used for comparison of means according to Duncans multiple range test at 5% level of significance (Duncan, 1955).

RESULTS AND DISCUSSION

Gain in weight (g/bird) showed significant ($P<0.001$) effect due to supplementation of synthetic L- lysine and DL-methionine in the diet (Table 2). The present findings are in accordance with the report of Kalbande *et al.* (2009) in broilers fed on different levels of synthetic methionine supplemented diets. It might be due to the effect of balance of both lysine and methionine and their combined effect in improving feed and nutrient utilization and more availability of lysine and methionine for protein synthesis. However, the feed intake was similar in different groups. Contrary to the present findings, Pillai *et al.* (2006) reported higher ($P<0.05$) feed intake with addition of high level of methionine in the broiler ration. Significant difference ($P<0.001$) was observed in FCR due to supplementation, which might be due to better effect of lysine and methionine in nutrient utilization resulting in higher body weight gain. Similar pattern was reported by Osti and Pandey (2004).

Blood glucose level of broilers in different groups varied from 180.9 to 182.2 mg/dL (Table 3) and the values were similar in all groups. The blood glucose values were comparable to the findings of Sonowal (2008) in broiler chicken. The serum protein values in different groups varied from 4.30 to 6.11 g/dL. Significantly ($P<0.001$) higher serum total protein values were obtained in the higher supplementation groups as compared to control (T_0) and T_1 groups. However, the highest value was found in group T_2 where 30 and 40% synthetic L-lysine and DL-methionine were supplemented. Similar trend of serum protein values of broiler chicken were reported by Hosseintabar *et al.*

Table 2. Effect of dietary supplementation of synthetic L-lysine and DL-methionine on growth performance of commercial broiler chicken

Parameter	Group				SEM	P value
	T_0	T_1	T_2	T_3		
Total gain in weight (g/bird)	1713.15^{a}±23.90	1799.96^{b}±26.80	1923.35^{c}±27.00	1882.60^{c}±23.50	14.20	<0.001
Total feed intake (g/bird)	3291±21.00	3214±25.10	3353±37.80	3408±14.10	73.17	0.881
Overall FCR	1.92^{b}±0.05	1.79^{b}±0.03	1.75^{a}±0.01	1.81^{b}±0.11	0.13	0.042

^{abc}Mean values with different superscripts within row differ significantly

Table 3. Effect of dietary supplementation of synthetic L-lysine and DL-methionine on blood biochemical profile of commercial broiler chicken

Parameter	Group				SEM	P value
	T_0	T_1	T_2	T_3		
Glucose (mg/dL)	181.10±10.60	182.20±15.10	181.90±16.00	180.90±14.60	6.37	1.000
Protein (g/dL)	4.30^{a}±0.45	5.02^{ab}±0.76	6.11^{c}±0.88	5.52^{bc}±0.12	0.20	0.001
Cholesterol (mg/dL)	166.50^{c}±2.83	150.50^{b}±4.78	112.30^{a}±2.26	107.50^{a}±1.43	6.59	<0.001
Triglycerides (mg/dL)	102.00^{c}±3.05	83.00^{b}±2.37	55.86^{a}±2.28	52.74^{a}±2.69	5.37	<0.001
ALT (U/mL)	26.79±0.69	25.86±0.49	25.17±0.36	25.15±0.34	0.28	0.105

[a,b,c]Mean values with different superscripts within a row differ significantly (P<0.001)

(2015) when broilers were supplemented with lysine and methionine in excess of NRC (1994) recommendation. The higher values of serum protein in T_2 and T_3 group might be attributed to higher metabolizability of crude protein with higher nitrogen retention due to proper balance of amino acids in diet leading to increased absorption of amino acid into the blood.

The values of serum cholesterol decreased (P<0.001) with increased level of supplementation. The values were lower in groups T_2 and T_3 compared to groups T_0 and T_1 which might be attributed to the hypocholesterolemic activity of methionine. The findings of current experiment corroborated with those of Kalbande *et al.* (2009). Serum triglycerides levels were influenced (P<0.001) by supplementation of synthetic amino acids. Lower values were observed in groups given high level of supplementation (T_2 and T_3 group) as compared to the unsupplemented group (T_0) and low supplemented group (T_1). The lower level of serum triglyceride in supplemented group might be attributed to the lipolytic action of both lysine and metionine since higher concentration of lysine and methionine stimulate pancrease for secretion of insulin into blood. Insulin in poultry can exert glucagon effect on release of fatty acids and amino acids from body store and leading to protein synthesis lowering triglyceride levels (Sturkie, 1986). Similar finding were reported by Bouyeh and Gevorgytan (2011) when basal diet was supplemented with Lys and Met (as TSAA) in 0, 10, 20, 30 or 40% more than NRC (1994) recommendations. The serum ALT activity was found to be similar in all the groups. These findings were in

Table 4. Effect of dietary supplementation of synthetic L-lysine and DL-methionine on dressing percentage and prime cuts of commercial broiler chicken

Parameter	Group				SEM	P value
	T_0	T_1	T_2	T_3		
Slaughter wt. (g)	1774^{a}±57.8	1874^{ab}±61.2	2079^{b}±43.6	1944^{ab}±87.4	40.6	0.036
Dressed wt. (g)	1244^{a}±40.1	1348^{a}±69.4	1558^{b}±38.9	1420^{ab}±43.1	31.9	0.028
Dressing percentage	70.12±0.88	71.95±1.40	74.95±0.50	73.19±1.31	0.568	0.271
Prime cuts						
Neck	6.17±0.25	6.12±0.47	6.19±0.27	6.27±0.08	0.14	0.989
Wing	10.56±0.52	10.73±0.96	10.04±0.38	10.62±0.25	0.27	0.846
Back	17.43±0.49	17.57±1.09	17.64±1.09	15.91±1.67	0.55	0.690
Breast	32.74±1.34	32.61±1.12	35.20±0.67	35.38±0.43	0.55	0.110
Thigh	13.97±1.19	13.97±0.82	14.71±0.46	15.77±0.37	0.40	0.354
Drumstick	12.63±0.61	13.18±0.91	13.53±0.38	12.83±0.27	0.28	0.723

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

accordance with those of Kalbande *et al.* (2009) and Halder and Roy (2007).

Dressing percentage was similar in all the groups (Table 4). Bouyeh (2013) observed dressing percentage of 71-78 in broilers fed with the rations supplemented with lysine and methionine @ 1.1, 1.2, 1.3 and 1.4 times in excess of NRC (1994) recommendations. Similar dressing percentage were reported in broiler chicken due to feeding of excess lysine and methionine (Tang *et al.,*2007; Ahmed and Abbas, 2015). Highest dressing percentage in group T_2 might be due to proper balance of amino acids in diet leading to more protein synthesis and deposition in body causing muscle development especially in breast muscle. Si *et al.* (2004) showed that increasing lysine over and above NRC (1994) recommendation improved weight gain, feed efficiency and breast muscle yield in broilers.

Percent yield of different prime cuts was similar in all groups (Table 4). While Hesabi *et al.* (2006) and Bouyeh (2013) reported that breast muscle yield, thigh and leg percentage was significantly higher with increased level of methionine and lysine in excess of NRC (1994) recommendations. The relative percent weight of visceral organs except that of abdominal fat did not differ significantly among the groups (Table 5). Onu *et al.* (2010) also reported similar results on supplementation of different levels of synthetic lysine and methionine in excess of NRC (1994) recommendations in the ration of broiler chicken. The mean relative percent weight of abdominal fat was lower ($P<0.01$) in groups T_2 and T_3 compared to groups T_1 and T_0. Similarly, Melaku *et al.* (2014) also obtained lower abdominal fat in broilers by feeding of rations supplemented with different levels of lysine. Lower percentage of abdominal fat in groups T_2 and T_3 indicated that high level of lysine and methionine reduced fat accumulation which might be due to the effect of lysine and methionine as precursor of L-carnitine and augment its supply for use in metabolism thereby facilitating fatty acid oxidation and

Table 5. Effect of dietary supplementation of synthetic L-lysine and DL-methionine on visceral and lymphoid organs of commercial broiler chicken

Attribute	Group				SEM	P value
	T_0	T_1	T_2	T_3		
Visceral organ weight						
Liver	3.63±0.02	3.61±0.03	3.59±0.03	3.64±0.02	0.01	0.410
Heart	0.59±0.03	0.53±0.03	0.57±0.02	0.55±0.03	0.01	0.400
Gizzard	3.29±0.15	3.28±0.12	3.29±0.10	3.27±0.12	0.06	0.999
Intestine	4.79±0.16	4.80±0.49	4.62±0.29	4.90±0.38	0.16	0.950
Kidney	0.26±0.05	0.24±0.04	0.28±0.06	0.26±0.04	0.02	0.970
Abdominal fat	1.32^{c}±0.05	1.14^{b}±0.06	0.71^{a}±0.02	0.66^{a}±0.02	0.08	<0.01
Lymphoid organ weight						
Spleen	0.14±0.02	0.13±0.01	0.15±0.02	0.13±0.03	0.01	0.898
Thymus	0.39±0.03	0.37±0.03	0.38±0.04	0.36±0.06	0.02	0.938
Bursa	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.00	0.01	0.174
Percent chemical composition of meat (DM basis)						
Moisture	74.33±0.20	74.23±0.93	74.14±1.99	74.30±0.90	0.45	0.999
Crude protein	18.16±0.95	18.19±0.60	18.90±1.38	18.26±1.06	0.39	0.940
Ether extract	6.52±0.09	6.46±0.48	6.03±0.07	6.32±0.92	0.21	0.908
Total ash	0.74±0.06	0.70±0.12	0.72±0.07	0.74±0.02	0.03	0.977

[a,b,c]Mean values with different superscripts within a row differ significantly ($P<0.01$)

reducing the amount of long chain fatty acids available for storage of fat as abdominal fat pad. Relative percent yield of lymphoid organs (spleen, bursa and thymus) was similar in different groups. The relative percent yields of spleen, thymus and bursa of fabricius observed in the present experiment were similar to the finding of Cengiz *et al.* (2008) in broiler chicken fed rations in excess of lysine and methionine of NRC requirements. Other workers (Konashi *et al.,* 2000; Kidd and Fancher, 2001) reported non-significant effect of dietary amino acid concentration on immune organs.

The moisture, crude protein, ether extract and total ash contents in breast and thigh muscle were similar in different groups (Table 5). However, higher protein (18.90±1.38%) and lower fat (6.03±0.07%) content was observed in group T_2 where ration was supplemented with 30% lysine and 40% methionine in excess of BIS (2007) recommendations which might be due to proper ratio of essential and non-essential amino acids in the diet. Bedford and Summers (1985) also observed that the increase in the ratio of essential and non-essential amino acids in broiler diet increased the carcass protein with decrease in carcass fat. Reduction in the amount of fat in the carcass might be due to higher level of lysine in diet which increased energy expenditure for protein deposition and maintained greater muscle mass providing a lower amount of energy for fat deposition (Lesson, 1995).

CONCLUSION

Dietary supplementation of synthetic L-lysine and DL-methionine @ 30 and 40% higher than BIS (2007) reduced the accumulation of abdominal fat pad and decreased serum cholesterol and triglycerides level in the commercial broilers, however, further studies are required to ascertain the findings of the present study.

REFERENCES

Ahmed, M.E. and Abbas, T.E. 2015. The effect of feeding herbal methionine versus DL-methionine supplemented diets on broiler performance and carcass characteristics. In: *"Proc. Int. Conf. Agricult. Ecol. Med. Sci*." held at Penang, Malaysia from Feb. 10-11, 2015.

AOAC. 2005. *Official Methods of Analysis.* 18th edn. Association of Official Analytical Chemists, Washington, DC, USA.

Bedford, M.R. and Summers, J.D. 1985. Influence of the ratio of essential to non-essential amino acids on performance and carcass composition of the broiler chick. *Br. Poult. Sci.* 26: 483-491.

BIS. 2007. *Bureau of Indian Standards: Poultry Feed Specification.* 4th Review. Manak Bhawan, 9 Bahadur Shah Zafar Marg, New Delhi, India.

Bouyeh, Mehrdad. 2013. Effect of excess dietary lysine and methionine on performance and economical efficiency of broiler chicks. *Annals Biol. Res.* 4: 241-246.

Bouyeh, M. and Gevorgyan, O.X. 2011. Influence of excess lysine and methionine on cholesterol, fat and performance of broiler chicks. *J. Anim. Vet. Adv.* 10: 1546-1550.

Cengiz, O., Onol, A.G., Sevim, O., Ozturk, M., Sari, M. and Daskiran, M. 2008. Influence of excessive lysine and/or methionine supplementation on growth performance and carcass traits in broiler chicks. *Revue Med. Vet*. 159: 230-236.

De-Jonge, W.J., Kwikkers, K.L., Te. Velde, A.A., Van Deventer, S.I.H., Nolte, M.A., Mebius, R.E., Ruuter, P.M., Lamers, M.C. and Lambers, W.H. 2002. Arginine deficiency affects early B cell maturation and lymphoid organ development in transgenic mice. *J. Clin. Innest.* 110: 1539-1548.

Duncan, D.B. 1955. Multiple range and multiple F-test. *Biometrics.* 11: 1-42.

Etim, N., Akpabio, U., Okpongete, R.O. and Offiong, E.E.A. 2014. Do diets affect haematological parameters of poultry. *Br. J. Appl. Sci. Technol.* 4: 1953-1965.

Halder, G. and Roy, B. 2007. Effect of herbal or synthetic methionine on performance, cost benefit ratio, meat and feather quality of broiler chicken. *Int. J. Agril. Res.* 2: 987-996.

Hesabi, A., Nasiri, H. and Birjandi, M. 2006. Effect of supplemental methionine and lysine on performance and carcass yield characteristics in broiler chicks. In: "*Proc 12th European Poultry Conference"* held at Verona, Italy from Sept. 10 to 14, 2016. p 112.

Hosseintabar, B., Dadashbeiki, M., Bouyeh, M., Seidavi, A., Hoven, R.V.D. and Gamboa, S. 2015. Effect of different levels of L-carnitine and lysine-methionine on broiler blood parameters. *Rev.MVZ Cordoba* 20: 4698-4708.

Kalbande, V.H., Ravikanth, K., Maini, S. and Rekhe, D.S. 2009. Methionine supplementation options in poultry. *Int. J. Poult. Sci.* 8: 588-591.

Kidd, M.T. and Fancher, B.I. 2001. Lysine needs of starting

chicks and subsequent effects during the growing period. *J. Appl. Poult. Res.* 10: 385-393.

Konashi, S., Takahashi, K. and Akiba, Y. 2000. Effect of dietary essential amino acid deficiencies on immunological variables in broiler chickens. *Br. J. Nutr.* 83: 449-456.

Kwaka, H., Austic, R.E. and Diettert, R.R. 1999. Influence of dietary arginine concentration on organ growth in chicken. *Poult. Sci*. 78: 1536-1541.

Lesson, S., Diaz, G. and Summers, J.D. 1995. *Poultry Metabolic Disorder and Mycotoxin.* University Books, Guelph, Ontario, Canada.

Melaku, M., Urge, M., Animut, G. and Getu, A. 2014. Effect of supplementation with synthetic lysine on the performance of finisher broiler chicks. *Iranian J. Appl. Anim. Sci.* 5: 179-187.

Moran, E.T., Jr. and Bilgili, S.F. 1990. Processing losses carcass quality and meat yield of broiler chicks receiving diets marginally deficient to adequate in lysine prior to marketing. *Poult. Sci.* 69: 702-710.

NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. edn. National Academy Press, Washington, DC. USA.

Onu. P.N., Ayo-Enwerm, M.C. and Ahaotu, E.O. 2010. Evaluation of carcass characteristics and cost effectiveness of broiler chicks fed synthetic lysine and methionine supplemented soyabean based diet. *Int. J. Nutr.* 1: 22-26.

Osti, N.P. and Pandey, S.B. 2004. Methionine and lysine supplementation in low quality feed ingredient based diets on the performance of broiler chicken. In: "*Proc. Fourth National Conference on Science and Technology*" organized by Royal Nepal Academy of Science and Technology (RONAST), Kathmandu, Nepal from March 23 to 26, 2004.

Pillai, P.B., Fanatico, A.C., Beers, K.W., Blair, M.E. and Emmert, J.L. 2006. Homocysteine remethylation in young broilers fed varying levels of methionine, choline and betaine. *Poult. Sci.* 85: 90-95.

Prabakaran, R. 2012. Overview of poultry production in India *vis-à-vis* global scenario. In: *"Proc XXIX IPSACON"* held from December 5 to 7, 1212 at Hyderabad, India. pp 3-20.

Schutte, J.B. and Pack, M. 1995. Sulphur amino acid requirement of broiler chicks from fourteen to thirty eight days of age. 1. Performance and carcass yield. *Pout. Sci*. 74: 480-487.

Si, J., Fritts, C.A., Waldroup, P.W. and Burnham, D.J. 2004. Effect of excess methionine from meeting needs for total sulfuramino acids on utilization of diets low in crude protein by broiler chicks. *J. Appl. Poult. Res*. 13: 579-587.

Sibbald, I.R. and Wolynetz, M.S. 1986. Effects of dietary lysine and feed intake on energy utilization and tissue synthesis by broiler chicken. *Poult. Sci*. 65: 98-105.

Sonowal, M. 2008. *Growth Performance and Nutrient Utilization of Broiler Chicken at Different Dietary Levels of Treated and Untreated Mustard Oil Cake*. Ph.D. thesis Assam Agricultural University, Khanapara, Guwahati, India.

Sturkie, P.D. 1986. *Avian Physiology*. 4th edn. Springer Verlag. New York, USA.

Tang, M.Y., Ma, Q.G., Chen, X.D. and Ji, C. 2007. Effects of meat dietary metabolizable energy and lysine on carcass characteristics and quality in arbor acres broilers. *Asian-Australas. J. Anim. Sci.* 20: 1865-1873.

Received on 11-12-2018 and accepted on 09-01-2019

Indian J. Anim. Nutr. 2019. 36 (1): 47-50
doi: 10.5958/2231-6744.2019.00008.2

Indian Journal of Animal Nutrition

Influence of Incorporation of Azolla Meal on Performance of Laying Japanese Quails

R. Kasi Sowjanya Lakshmi*, Ch. Venkata Seshiah, P. Ravikanth Reddy, K. Nagaraja and I. Kumar

NTR College of Veterinary Sciences, Sri Venkateswara Veterinary University, Gannavaram-521102, Andhra Pradesh, India

ABSTRACT

This study was conducted to evaluate the performance of laying Japanese quails fed on different levels of azolla meal (AZM). Ten weeks old 150 quails (*Coturnix coturnix japonica*) were randomly divided into 3 groups with 5 replicates of 10 birds each. Control group was fed with a diet containing 0% AZM while the other two groups received diets with 3 and 6% AZM, respectively for a period of 6 weeks. Feed consumption and egg production were recorded and feed efficiency (g feed consumed/12 eggs) was calculated during the entire trial period. Ten eggs from each treatment were examined for quality. At the end of the trial, 5 birds from each treatment group were slaughtered to study the carcass characteristics. There were no significant differences in both performance and egg quality parameters by incorporation of AZM. Carcass characteristics were also found to be similar in 3 groups except for giblet, back and wings percentage. It was concluded that AZM could be incorporated in laying quail's diet up to 6% without affecting the performance and egg quality parameters.

Key words: Azolla meal, Carcass traits, Egg quality, Japanese quails, Performance

INTRODUCTION

In poultry production, feed cost accounts for nearly 60% of the total cost of production (Shaikh and Zala, 2011). The shrinking feed resources of the world and their escalating cost has triggered search for cheap unconventional feeds for poultry production. There is a conscientious effort to switch on to non-conventional feed items to slash feed cost in poultry production.

Azolla has been established as a potential feed ingredient for livestock and poultry by many researchers (Pillai *et al.,* 2005). Azolla (*Azolla pinnata*), an aquatic fern, abundantly available in stagnant water in tropical and subtropical regions of the world, has been recommended for feeding broiler and layer chicken (Basak *et al.,* 2002). It is very rich in proteins, essential amino acids, vitamins, growth promoter intermediaries and minerals (Pillai *et al.,* 2005; Henry *et al.,* 2017). Inclusion of azolla in the poultry diet economises production (Dhumal *et al.,* 2009) but very limited studies have been conducted on evaluating its effects on the laying performance and carcass traits of Japanese quails. Further, in the recent past, small and marginal poultry farmers of India are more interested in rearing Japanese quails rather than other species due to increasing consumer demand especially in urban areas. Hence, an attempt was made to investigate the effect of feeding azolla (*Azolla pinnata*) meal (AZM) on laying performance, egg quality and carcass traits of Japanese quails (*Coturnix coturnix japonica*).

MATERIALS AND METHODS

Azolla was collected from the ponds maintained at the farm, dried under shade, ground and stored in plastic bags. One hundred and fifty quails of 10 weeks belonging to single hatch were weighed individually and allotted randomly to 3 groups with 5 replicates of 10 quails each. Three experimental diets were prepared by incorporating 0, 3 and 6% of azolla meal (AZM) in quail layer ration of groups T_1, T_2 and T_3, respectively. The diets were isocaloric and isonitrogenous and prepared to meet the requirements (NRC, 1994) of laying quails (ME-2800 Kcal/kg and CP-20%). Birds were kept in quail layer cages and feed and water were provided *ad lib.*

Ingredient composition and calculated nutrient content of three diets have been presented in Table 1. Daily egg production, mortality of birds and weekly feed

*Correspnding author: E-mail: soujanyavetty@gmail.com, Assistant Professor (Animal Nutrition) Livestock Farm Complex, NTRCVSc, Gannavaram, Andhra Pradesh

Table 1. Ingredient composition and calculated nutrient content

	Level of azolla meal (%)		
	0	**3**	**6**
Feed ingredient			
Maize	40	38.8	37.6
Soyabean meal	24	23.3	22.5
Gingelly cake	12	11.6	11.3
Azolla meal (AZM)	0	3	6
Deoiled rice bran	15	14.5	14.1
Shell grit	7	6.85	6.6
DCP	1.5	1.45	1.4
*Premix	0.5	0.5	0.5
Total	100	100	100
Chemical composition (calculated)			
CP (%)	19.84	19.92	19.98
ME (Kcal/kg)	2790	2756	2783
Ca (%)	3.06	3.04	2.99
P (%)	0.6	0.59	0.59

*Premix: : 0.1%, heptocare 0.05%, coccidiostat 0.05%, 0.065% choline chloride, B-complex- 0.09% (mg/kg) (thiamin-1; pyridoxine-2; cyanocobalamine-0.01; niacin,15; pantathonic acid-10; ±-tocopherol-10; biotin-0.08); menadione-0.002%; retinol acetate-0.03%; Cholecalciferol -0.03%

consumption were recorded. FCR was calculated based on feed intake and egg production. Ten eggs per treatment were collected for studying the egg quality parameters. Egg weight, yolk color, yolk index, albumen index and Haugh unit were calculated (Card and Nesheim, 1972). At the end of feeding trial, 5 birds from each treatment (total 15 birds) were slaughtered to study the carcass characteristics like dressed weight, weight of legs, wings, breast, back, neck thigh, giblet and dressing percentage.

Azolla meal was analysed for proximate principles, Ca and P contents (AOAC, 2005). Data were statistically analyzed by one way ANOVA using SPSS windows (SPSS, Inc., 2002). Significant differences ($P<0.05$) between means were determined by Duncans multiple comparison test (Duncan, 1955).

RESULTS AND DISCUSSION

The proximate composition of azolla meal has been presented in Table 2. The CP content (22.56%) of AZM was comparable to the values repoted by Alalade and Iyayi (2006) and Shaukat *et al.*(2015). The Ca content (1.93%) was in close conformity with the values reported by Parthasarathy *et al.* (2001). The P content (0.64%) found in present study was in close agreement with value (0.64%) reported by Shaukat *et al.* (2015).

Incorporation of AZM in diets of quails up to 6% level did not affect egg production, feed intake and FCR (feed consumed/dozen of eggs produced) for the experimental period of 6 weeks (Table 3). However, Shamna *et al.* (2013) reported that performance of broiler quails depressed beyond 5% by substitution of AZM in the diet. Inconsistent results on AZM inclusion have been reported which could be due to differences

Table 2. Chemical composition (% DM basis) of azollla meal

Constituent	Percentage
Dry matter	89.65
Organic matter	83.35
Total ash	16.65
Crude protein	22.56
Ether extract	1.96
Crude fiber	16.80
Nitrogen free extract	42.03
Calcium	1.93
Phosphorus	0.61

Table 3. Effect of different levels of AZM in quail ration on production performance

Group	Feed intake (g per bird/d)	Egg Production (%)	FCR (g/Feed consumed 12 eggs produced)
T_1 (0% AZM)	24.03	69.38	0.407
T_2 (3% AZM)	22.81	65.18	0.435
T_3 (6% AZM)	24.34	63.38	0.459
SEM	0.459	1.979	0.023
P value	0.378	0.479	0.699

in species, physiological status, percent levels of AZM and type of concentrate replaced. Several workers (Basak *et al.,* 2002; Bholka, 2011; Naghshi *et al.,* 2014) reported that feeding of AZM upto 5% level in diets of commercial broiler chicken had positive effect on production performance. Including AZM up to 7.5% of body weight increased body weight gain by 2.6% with higher Ranikhet virus titers in commercial broilers (Prabina and Kumar, 2010). However, Alalada *et al.* (2007) observed non-significant variations in growth performance of Nera brown pullets when AZM was fed upto 10% level. Shaukat *et al.* (2015) noticed a linear reduction in feed consumption of broiler chicken with increased AZM levels in the diets. Similar findings on production performance of ducks were reported elsewhere (Lawas *et al.,* 1998; Sujatha *et al.,* 2013). Recently, Henry *et al.* (2017) found that fresh azolla supplementation @ 30 g/bird/d reduced feed consumption without affecting the growth performance in turkeys at 7 weeks age which might be due to high protein and mineral content of azolla.

There were no significant variations in egg quality parameters in different groups except for yolk color which was found more pronounced (3.5 to 4.0) with increased levels of AZM in the diet. Similarly, Sujatha *et al.* (2013) also reported an increase in yolk color from 6.0 to 7.4 on feeding fresh azolla to ducks. Incorporating AZM in layer diet resulted in better yolk color which might be attributed to the carotenes present in AZM (Bholka, 2011). However, Alalade *et al.* (2007) reported no impact of feeding AZM during grower phase of pullets on egg quality characteristics including yolk color in laying phase. Lawas *et al.* (1998) observed a non-significant difference in average egg weight values on feeding fresh azolla to laying ducks. On the contrary, Bholka (2011) reported higher egg weights in layers fed with 7.5% AZM.

There was no significant influence of level of AZM on carcass traits except for giblet, back and wings percentage which were higher ($P<0.05$) in 3% AZM fed group (Table 5). Shaukat *et al.* (2015) also reported no effect on carcass traits on feeding azolla up to 20% level. Basak *et al.* (2002) reported higher dressing percentage of broiler chicken in treatment group fed 5% AZM due to the higher body weight gains. Naghshi *et al.* (2014) also reported better carcass efficiency with 5% azolla feeding in commercial broilers except for abdominal fat, liver, gizzard and breast relative percentage.

CONCLUSION

It could be concluded that azolla meal might be fed to laying quails up to 6% as a replacement without

Table 4. Effect of different levels of AZM in quail ration on egg quality traits

Group	Egg weight	Shape index	Albumin index	Haugh unit score	Yolk index	Yolk color
T_1 (0% AZM)	10.36	75.327	0.086	81.325	0.512	3.5
T_2 (3% AZM)	10.26	78.639	0.083	81.499	0.487	3.7
T_3 (6% AZM)	10.28	79.748	0.077	81.810	0.499	4.0
SEM	0.171	2.856	0.003	0.695	0.016	-
P value	0.994	0.816	0.477	0.962	0.824	-

Table 5. Effect of different levels of AZM in quail ration on carcass traits

Group	Live wt	Head, leg, wing (g)	Giblet (%)	Back (g)	Breast (g)	Wings (g)	Thigh (g)	Dressed weight (g)	Dressing (%)
T_1 (0% AZM)	169.20	16.38	08.84[b]	32.58[ab]	38.54	09.08[b]	23.86	109.22	64.706
T_2 (3% AZM)	176.92	16.36	13.18[a]	34.68[a]	44.66	11.28[a]	21.94	111.48	63.462
T_3 (6% AZM)	174.14	16.96	10.16[b]	27.48[b]	38.68	08.22[b]	22.81	104.86	60.402
SEM	4.222	0.415	0.675	1.218	1.358	0.425	1.576	1.982	1.240
P value	0.779	0.822	0.013	0.031	0.103	0.002	0.898	0.412	0.372

[ab]Means bearing different superscripts in a column differ significantly (P<0.05)

affecting feed consumption, egg production, FCR, egg quality parameters and carcass traits.

REFERENCES

Alalade, O.A. and Iyayi, E.A. 2006. Chemical omposition and the feeding value of azolla (*Azolla pinnata*) meal for egg-type chicks. *Int. J. Poult. Sci.* 5: 137-141.

Alalade, O.A., Eustace, A., Iyayi, E.A. and Alalade. T.O. 2007. The nutritive value of azolla (*Azolla pinnata*) meal in diets for growing pullets and subsequent effect on laying performance. *J. Poult. Sci.* 44: 273-277.

AOAC. 2005. *Official Methods of Analysis*. 18th edn. Association of Official Analytical Chemists, Arlington, VA, USA.

Basak, B., Pramanik, M.A.H., Rahman, M.H., Tarafdar, S.U., Roy, B.C. 2002. Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. *Int. J. Poult.* Sci.,1: 29-34.

Bholka, P.C. 2011. *Nutritional Evaluation of Azolla (Azolla pinnata) in Broilers and Layers*. Ph.D. thesis, KVAFSU, Bidar, Karnataka, India.

Card, L.E. and Nesheim, M.C. 1972. *Poultry Production*. 11th edn. Lea and Febiger, Philadelphia.

Dhumal, M.V., Siddiqui M.F., Siddiqui M.B.A. and Avari, P.E. 2009. Performance of broilers fed on different levels of azolla meal. *Indian J. Poult. Sci*. 44: 65-68.

Duncan, D.B. 1955. Multiple range F- tests. *Biometrics.* 11: 1-42.

Henry, A.C.E., Reetha T.L., Paramasivam, A. and Mehala, C. 2017. Effect of azolla supplementation on production performance of nandanam–ii turkey growers. *Indian Vet. J*. 94: 28-30.

Lawas, M.V.P., Roxas, D.B. and Lambio, A.L., 1998. Laying performance of Philippine Mallard ducks fed diets substituted with fresh azolla. In: *"Proc. Philippine Society of Animal Science".* 35th Annual Convention, College, Laguna, Philippines:. pp 220-225.

Naghshi, H., Khojasteh, S., and Jafari, M. 2014. Investigation of the effect of different levels of azolla (azolla pinnata) on performance and carcass characteristics of cobb broiler chicks. *Int. J. Farm. Alli Sci.* 3: 45-49.

NRC. 1994. *Nutrient Requirements of Poultry.* 9th edn. Natl. Acad. Press, Washington, DC, USA.

Parthasarathy, R., Kadrivel, R. and Kathaperumal, V. 2001. Chemical evaluation of azolla as a poultry feed ingredient. *Cheiron.* 30: 35-37.

Pillai, P.K., Premalatha, S. and Rajamony, S. 2005. Azolla: a sustainable feed for livestock. *LEISA, Leusden*. 21: 26-27.

Prabina, B.J. and Kumar, K. 2010. Dried azolla as a nutritionally rich cost effective and immuno-modulatory feed supplement for broilers. *Asian J. Anim. Sci*. 5: 20-22.

Shaikh, A.S. and Zala, Y.C. 2011. Production performance and economic appraisal of broiler farms in Anand district of Gujarat. *Agric. Econ. Res. Rev.* 24: 317-323.

Shamna, T.P., Peethambaran, P.A,. Jalaludeen, A., Joseph, L.and Aslam, M.K.M. 2013. Broiler characteristics of Japanese quails (*coturnix coturnix japonica)* at different levels of diet substitution with *Azolla pinnata*. *Anim. Sci. Rep*. 7: 75-80.

Shaukat, A., Adil, S., Banday, M.T. and Manzoor A. Khan. 2015. Feeding potential of aquatic fern-azolla in broiler chicken ration. *J. Poult. Sci. Technol.* 3: 15-19.

SPSS Inc. 2002. *Stastical Packages for Socail Sciences.* SPSS for windows: release 11.5, standard version, 1982-2002. SPSS Inc, Chicago, II.

Sujatha, T., Kundu, A., Jeyakumar, S. and Kundu M.S. 2013. Azolla supplementation: feed cost benefit in duck ration in andaman islands. *Tamilnadu J. Vet Anim. Sci*. 9: 130-136.

Received on 26-08-2018 and accepted on 28-01-2019

Indian J. Anim. Nutr. 2019. 36 (1): 51-57
doi: 10.5958/2231-6744.2019.00009.4

Indian Journal of
Animal Nutrition

Growth Performance and Nutrient Utilization of Male Broiler Chicken as Affected by Feed Restriction with or without Garlic Supplementation

Vishavdeep Singh, Udeybir Singh*, A.P.S. Sethi and J.S. Lamba
Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University,
Ludhiana-141004, Punjab, India

ABSTRACT

One hundred and twenty, day old male broiler chicks were randomly distributed into 5 treatments each having 4 replicates with 6 birds in each replicate. Diets were formulated as per ICAR (2013) specifications and were fed in 3 phases. Treatment T_1 served as control. The other treatments comprised: $T_2 = T_1$ with feed restriction (10-12 h) at 8-17 days of age (DOA) with garlic supplementation, $T_3 = T_1$ with feed restriction at 8-17 DOA (10-12 h) without garlic supplementation, $T_4 = T_1$ with feed restriction at 18-27 DOA (10-12 h) with garlic supplementation, $T_5 = T_1$ with feed restriction at 18-27 DOA (10-12 h) without garlic supplementation. Early feed restriction (8-17 DOA) with or without garlic supplementation reduced ($P<0.05$) the average body weight gain and feed intake at 2nd week of age. Garlic supplementation and late feed restriction from 18-27 DOA reduced ($P<0.05$) the average body weight gain and feed intake at 4th week of age. There was no significant effect on feed conversion ratio (FCR) of early and late feed restriction with or without garlic supplementation expect at 5th week of age where FCR was observed to be better ($P<0.05$) in T_2 and T_4 as compared to control. There was no significant difference in protein efficiency ratio and calorie efficiency ratio among the groups. Early and late feed restrictions with garlic supplementation (T_2 and T_4) had higher ($P<0.05$) digestibility of crude protein and organic matter. It was concluded that garlic supplementation irrespective of restriction period (early or late) improved the FCR at the 5th week of age, crude protein digestibility and organic matter digestibility.

Key words: Feed restriction, Garlic supplementation, Male broiler chicken, Nutrient digestibility

INTRODUCTION

Poultry industry is supporting the nutrient supply of the huge population of the world. Broilers make up a large part of this industry with chicken meat accounting for 86% of the world poultry meat. Indian poultry sector has been growing @ 8-10% annually with broiler meat volumes growing @ of 10% due to increased domestic consumption (Panigrahy *et al.,* 2016). If feed is offered *ad lib.*, broilers consume feed 2-3 times above maintenance requirements. This enhanced growth due to *ad lib*. feeding is unfortunately accompanied by certain ill effects like high metabolic rate, high mortality, increased body fat, metabolic and skeletal defects (Zubair and Leeson, 1996). Feed restriction is a method of feeding in which the time or duration or amount of feed is limited. Plavnik and Hurwitz (1988) observed that the timing, severity and duration of restriction had significant effect on the subsequent ability of broilers to recover from a growth defect. Early feed restriction programs used to reduce abdominal and carcass fat in broiler chickens rely on the event called compensatory growth. Male broilers have a greater ability to exhibit compensatory growth following a period of under nutrition than females (McMurty *et al.,* 1988; Plavnik and Hurwitz, 1990 and 1991). Feed restriction strategy in broilers can improve feed efficiency, reduce feed cost and mortality along with the production of quality meat at cheaper rates. So, feed restriction strategies have been introduced at 8-17 days of age (DOA) and 18-27 DOA to reduce these metabolic problems and hence improve economy of broiler production.

Antibiotics as feed supplement are under serious criticism due to their ill effects like development of microbial resistance to the pathogens and their potential harmful effects on human health (Rahmatnejad *et al.,* 2009). At present, there is increasing pressure to reduce or eliminate the use of antibiotics in poultry feed due to the negative human health issue of antibiotic resistance. In garlic (*Allium sativum*), major active ingredients like allicin, ajoene, dialkyl polysulphides,

*Corresponding author: E-mail: udeybirchahal@gmail.com

s-allylcysteine *etc.* may be responsible for the various properties of garlic (Canogullari *et al.,* 2010). Allicin rapidly decomposes to several volatile organosulphur compounds which possibly reduce low density lipoprotein, triglyceride and cholesterol in serum (Alder *et al.,* 2003). Garlic as natural growth promoter can improve growth rate, feed conversion ratio and carcass characteristics (Tollba and Hassan, 2003; Makwana *et al.,* 2015). Supplementation of garlic powder @ 1.5% gave best response in terms of growth performance (Singh *et al.,* 2015). This study was, therefore, conducted to assess the effect of feed restriction along with garlic supplementation @ 1.5% on growth performance and nutrient digestibility in male broiler chicken.

MATERIALS AND METHODS

The study was conducted at the poultry farm and poultry nutrition laboratory of the Department of Animal Nutrition, GADVASU, Ludhiana, Punjab. One hundred and twenty male, day old meat type chicks (IBL-80) were procured from GADVASU hatchery and distributed randomly into 5 groups having 24 birds per treatment with 4 replicates having 6 chicks in each replicate representing different treatments *viz.,* T_1 - Control male group fed *ad lib.* as per ICAR (2013) specification *i.e.* starter diet (0-14 DOA) *i.e.* 22% CP and 3000 Kcal/kg ME, grower diet (14-21 DOA) *i.e.* 21.5% CP and 3050 Kcal/kg ME and finisher diet (21-35 DOA) *i.e.* 19.5% CP and 3100 Kcal/kg ME. The other treatments comprised: $T_2 = T_1$ with feed restriction (10-12 h) at 8-17 DOA (10-12 h) with garlic supplementation, $T_3 = T_1$ with feed restriction at 8-17 DOA (10-12 h) without garlic supplementation, $T_4 = T_1$ with feed restriction at 18-27 DOA (10-12 h) with garlic supplementation, $T_5 = T_1$ with feed restriction at 18-27 DOA (10-12 h) without garlic supplementation.

Six broilers diets were formulated for the study for weekly observations. The percent ingredient composition of all the phases was kept as per ICAR (2013) specifications (Table 1). Each diet was fed to quadruplicate group of chicks having 6 birds in each replicate during all the phases of growth. The feeders were removed from 10-12 h during 8 p.m. to 8 a.m. (next day) to apply feed restriction.

A metabolic trial was conducted at the end of experiment. Four birds with comparable body weight were selected from each treatment and were housed in battery brooders. There were 2 replicates of each treatment having 2 birds in each replicate. Birds were fed the same treatment ration for five days as in growth study to provide them adaptation time in the metabolic cages. After adaptation period of five days, the measured quantity of feed for next three consecutive days was offered to each replicate both in the morning and evening. The residual feed left was removed on 4th day and weighed to record the actual consumption of feed for each replicate. Three days total collection method was used for faeces. The sample of faeces were ground and analyzed for various proximate parameters (AOAC, 2005).

The data were subjected to statistical analysis using one way ANOVA in Software Package for Social Sciences (SPSS, version 22.0) to test the difference between various treatments. The treatment means were compared by Duncan's Multiple Ranged Test (Duncan, 1995) at 5% level of significance.

RESULTS AND DISCUSSION

There was no significant difference in average body weight gain at 1st week of age. But early feed restriction with or without garlic supplementation (T_2 and T_3) reduced ($P<0.05$) the average body weight gain at 2nd week of age (Table 2). Garlic supplementations in early (T_2) and late (T_4) feed restricted groups had no significant effect on average body weight gain as compared to their non-garlic supplemented T_3 and T_5 treatments. However, numerically higher value was observed due to garlic supplementation in corresponding feed restricted treatments at different weeks of age. There was no significant effect of garlic supplementation and feed restriction at 3rd and 5th week of age. But, garlic supplementation and late feed restriction (T_4 and T_5) from 18-27 days of age reduced ($P<0.05$) the average body weight gain at 4th week of age. Similarly, significantly ($P<0.05$) reduced average

Table 1. Ingredient composition (%) of experimental diets

Phase	Ingredient (kg/100 kg)	Treatment				
		T_1	T_2	T_3	T_4	T_5
Starter	Maize	54.85	54.35	54.85	54.35	54.85
	Soybean meal	33.5	33.5	33.5	33.5	33.5
	Groundnut extraction	4.5	4.0	4.5	4.0	4.5
	De-oiled rice bran	1.0	0.5	1.0	0.5	1.0
	Garlic	-	1.5	-	1.5	-
	Oil	2.5	2.5	2.5	2.5	2.5
	Di-calcium phosphate	1.5	1.5	1.5	1.5	1.5
	Limestone powder	1.5	1.5	1.5	1.5	1.5
	Methionine (g)	150	150	150	150	150
	Salt (g)	300	300	300	300	300
	Additives*(g)	200	200	200	200	200
	Total (kg)	100	100	100	100	100
Grower	Maize	57.0	57.0	57.0	57.0	57.0
	Soybean Meal	27.0	27.0	27.0	27.0	27.0
	Groundnut extraction	4.0	4.0	4.0	4.0	4.0
	De-oiled rice bran	5.0	3.5	5.0	3.5	5.0
	Garlic	-	1.5	-	1.5	-
	Oil	3.5	3.5	3.5	3.5	3.5
	Di-calcium phosphate	1.9	1.9	1.9	1.9	1.9
	Limestone powder	1.0	1.0	1.0	1.0	1.0
	Methionine (g)	100	100	100	100	100
	Salt (g)	300	300	300	300	300
	Additives*(g)	200	200	200	200	200
	Total (Kg)	100	100	100	100	100
Finisher	Maize	57.0	57.0	57.0	57.0	57.0
	Soybean meal	27.0	27.0	27.0	27.0	27.0
	Groundnut extraction	4.0	4.0	4.0	4.0	4.0
	De-oiled rice bran	5.0	3.5	5.0	3.5	5.0
	Garlic	-	1.5	-	1.5	-
	Oil	3.5	3.5	3.5	3.5	3.5
	Di-calcium phosphate	1.9	1.9	1.9	1.9	1.9
	Limestone powder	1.0	1.0	1.0	1.0	1.0
	Methionine (g)	100	100	100	100	100
	Salt (g)	300	300	300	300	300
	Additives*(g)	200	200	200	200	200
	Total (kg)	100	100	100	100	100

*Additives include Vit A 8,25,000 IU, Vit D_3 1,20,000 IU/, Vit K 100 mg, Riboflavin 500 mg, Thiamine 80 mg, Pyridoxine 160 mg, Vit E 800 mg, Cyanocobalamine 100 mcg, Niacin 1200 mg, Calcium pantothenate 80 mg, Manganese sulphate 25 g, Ferrous sulphate 10 g, Copper sulphate 500mg, Zinc oxide 8g Potassium Iodide 100 mg, Coccidiostat 60g

body weight gain due to early feed restriction was reported by other workers (Omosebi *et al.,* 2014; Malpotra *et al.,* 2017; Sidhu *et al.,* 2017). But, various other studies reported that there was no significant difference in the final body weight due to feed restrictions (Saber *et al.,* 2011; Jahanpour *et al.,* 2015). Moreover, Khetani *et al*. (2009) did not find any compensatory growth in the feed restricted groups. In contrast, the other researchers (Konjufca *et al.,* 1997; Ademola *et al.,* 2004; Onibi *et al.,* 2009), observed no effect of garlic supplementation on body weight gain. Whereas, higher (P<0.05) body weight gain in the garlic supplemented group than the control group has been reported (Noman *et al.,* 2015; Varmaghany *et al.,* 2015; Khan *et al.,* 2017).

Significantly (P<0.05) reduced feed intake was observed in early feed restriction with garlic (T_2) and without garlic (T_3) at 2nd week of age and late feed

Table 2. Effect of feed restriction and garlic supplementation on growth performance in male broilers

Item	Treatment					SEM
	T_1	T_2	T_3	T_4	T_5	
Average body weight gain (g)						
1st wk	92.80	89.20	86.37	91.66	88.13	1.136
2nd wk	163.37[a]	143.43[b]	140.04[b]	163.16[a]	156.43[a]	3.168
3rd wk	238.10	234.04	231.50	238.27	231.39	1.876
4th wk	278.02[a]	282.39[a]	275.91[a]	270.47[ab]	265.33[b]	1.439
5th wk	353.29	355.22	348.22	362.41	347.29	2.607
Average feed intake						
1st wk	155.60	147.39	144.25	150.8	145.63	1.351
2nd wk	280.40[a]	248.45[b]	246.90[b]	278.48[a]	274.78[a]	3.383
3rd wk	447.33[a]	439.22[ab]	436.11[b]	446.61[a]	446.85[a]	2.091
4th wk	556.02[a]	557.90[a]	553.60[a]	541.20[b]	533.32[b]	1.654
5th wk	808.62	805.33	802.20	810.12	806.16	2.822
Feed conversion ratio (kg feed consumed/kg gain)						
1st wk	1.67	1.65	1.67	1.64	1.65	0.023
2nd wk	1.71	1.73	1.76	1.70	1.75	0.025
3rd wk	1.87	1.87	1.88	1.87	1.93	0.014
4th wk	1.99	1.97	2.00	2.00	2.01	0.013
5th wk	2.28[ab]	2.26[b]	2.30[a]	2.23[b]	2.32[a]	0.032
Protein efficiency ratio						
1st wk	2.70	2.73	2.71	2.75	2.73	0.070
2nd wk	2.63	2.61	2.56	2.65	2.57	0.072
3rd wk	2.48	2.48	2.47	2.48	2.41	0.061
4th wk	2.57	2.60	2.56	2.57	2.56	0.060
5th wk	2.25	2.27	2.23	2.30	2.22	0.079
Calorie efficiency ratio						
1st wk	0.199	0.202	0.200	0.203	0.202	0.010
2nd wk	0.194	0.192	0.189	0.195	0.190	0.002
3rd wk	0.174	0.173	0.172	0.175	0.169	0.034
4th wk	0.161	0.163	0.161	0.161	0.160	0.001
5th wk	0.141	0.142	0.140	0.144	0.139	0.002

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

Table 3. Effect of feed restriction and garlic supplementation on nutrient digestibility (%) in male broilers

Digestibility	Treatment					SEM
	T_1	T_2	T_3	T_4	T_5	
Crude protein	68.8[b]	71.69[a]	69.09[b]	71.18[a]	67.65[b]	0.918
Ether extract	88.57	87.96	87.93	89.05	87.11	0.797
Fiber	37.15	35.97	37.34	36.39	37.09	0.847
Organic matter	71.17[b]	75.33[a]	71.13[b]	75.31[a]	71.21[b]	0.786
Calcium	50.82	50.87	50.60	50.89	50.65	0.248
Phosphorus	46.58	46.65	46.41	46.63	46.36	0.316

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

restriction with garlic (T_4) and without garlic (T_5) at 4th week of age as compared to control (Table 2). It indicated that feed restrictions with or without garlic supplementation reduced (P<0.05) the feed intake. Results of various previous studies (Zhan *et al.,* 2007; Mehmood *et al.,* 2013; Omosebi *et al.,*2014) showed that duration of restriction and level of restriction reduced (P<0.05) feed intake in poultry. However, other workers (Yalcin *et al.,* 2006; Onibi *et al.,* 2009; Saber *et al.,* 2011; Afsharmanesh *et al.,* 2016) observed non-significant feed intake due to feed restriction and garlic supplementation. Whereas, Al massad *et al.*(2018) and Islam *et al.* (2018), reported highery (P<0.05) feed consumption due to garlic supplementation. There was no significant effect on FCR in different treatments as compared to control upto 4th week of age. Saber *et al.* (2011) and Sidhu *et al.* (2017) did not found any significant effect of feed restrictions on FCR. Also, Onibi *et al.*(2009) did not found any significant difference in FCR in garlic supplemented broilers (8-56 days). But, garlic supplemented groups *i.e.* T_2 and T_4 had better FCR than the non-garlic supplemented group *i.e.* T_3 and T_5 at 5th week of age. Rincon and Leeson (2002), Omosebi *et al.* (2014) and Malpotra *et al.* (2017) reported better FCR with feed restriction. A better (P<0.05) FCR in garlic powder supplemented groups.has been reported (Fadlalla *et al.* 2010; Raessi *et al.,* 2010; Patel *et al.,* 2017; Ratika *et al.,* 2018).

There was no significant difference in PER and CER due to early and late feed restriction with or without garlic supplementation in different weeks of age from 1st to 5th week of age as well as in different phases and overall period. In this context, Butzen *et al.* (2015) and Sidhu *et al.* (2017) reported that PER and CER were not affected by feed restrictions during different phases. While Saleh *et al.* (2005) and Ratika *et al.* (2018) reported that PER and CER were better in garlic supplemented and feed restricted groups as compared to control group. Furthermore, Malpotra *et al.* (2017) reported that PER and CER were higher for 2nd week feed restricted group during 2nd week and 4th week feed restricted group during 4th week. Overall improved protein feed efficiency due to feed restrictions followed by compensatory growth was also reported (Mollison and Guenter, 1984); Ramlah *et al.* 1996; Al-Taleb, 2003).

Early and late feed restrictions with garlic supplementation (T_2 and T_4) had higher (P<0.05) digestibility of crude protein and organic matter. The digestibility of of ether extract, fibre, calcium and phosphorus due to early and late feed restriction with or without garlic supplementation was similar in all the groups. Feed restriction increased nutrient digestibility by 5% with 25% feed restriction from 28-39 days of age (Teeter and Smith 1985). However, significantly lower value for phosphorus retention was observed in third week restrictions as compared to second week restrictions as reported by Malpotra *et al.* (2017). Singh *et al.* (2017) reported higher crude fibre digestibility in garlic supplemented diets as compared to control and also calcium retention was found to be higher in garlic supplemented diets but dry matter metabolizability, ether

extract digestibility, percent nitrogen and phosphorus retention remained unaffected.

CONCLUSION

It was concluded that garlic supplementation irrespective of restriction period (early or late) improved the FCR at the 5th week of age, crude protein digestibility and organic matter digestibility.

REFERENCES

Ademola, S.G., Farinu, G.O., Ajayi-Obe, A.O. and Babatunde, G.M. 2004. Growth, haematological and biochemical studies on garlic and ginger fed broiler chicken. *Moor J. Agric. Res.* 5: 122-128.

Afsharmanesh, M., Lotfi, M. and Mehdipour, Z. 2016. Effects of wet feeding and early feed restriction on blood parameters and growth performance of broiler chickens. *Anim. Nutr.* 2: 168-172.

Al-Massad, M., Al-Ramamneh, D., Al-Sharafat, A., Abdelqader, A. and Hussain, N., 2018. Effect of using garlic on the economical and physiological characteristics of broiler chickens. *Russian Agric. Sci.* 44: 276-281.

Al-Taleb, S.S. 2003. Effect of an early feed restriction of broiler's on productive performance and carcass quality. *Online J. Biol. Sci.* 3: 607-611.

Alder, R., Lookinland, S., Berry, J.A. and Williams, M. 2003. A systematic review of the effectiveness of garlic as an antihyperlipidemic agent. *J. Am. Assoc. Nurs. Pract.* 15: 120-129.

Ankri, S. and Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. *Microb. Infect.* 1: 125-129.

AOAC. 2005. *Officials Methods of Analysis*. 18th rev. edn. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Butzen, F.M., Vieira, M.M., Kessler, A.M., Aristimunha, P.C., Marx, F.R., Bockor, L. and Ribeiro, A.M.L. 2015. Early feed restriction in broilers. II: Body composition and nutrient gain. *J. Appl. Poult. Res.* 24: 198-205.

Canogullari, S., Baylan, M., Erdogan, Z., Duzguner, V. and Kucukgul, A. 2010. The effects of dietary garlic powder on performance, egg yolk and serum cholesterol concentrations in laying quails. *Czech J. Anim. Sci.* 55: 286-293.

Duncan, D.B. 1955. Multiple range and multiple F test. *Biometrics* 11: 1-42.

Fadlalla, I.M.T., Mohammed, B.H. and Bakhiet, A.O. 2010. Effect of feeding garlic on the performance and immunity of broilers. *Asian J. Poult. Sci.* 4: 182-189.

ICAR. 2013. *Nutrient Requirements of Poultry.* Indian Council of Agricultural Research, Krishi Bhawan, New Delhi, India.

Islam, M.A., Haque, M.E., Shikhauu, M.S.A., Uddin, M.J., Uddin, M.N., Islam, M.T. and Islam, M.S. 2018. Effect of red chili and garlic nutrition as feed additives on growth performance of broiler chicken. *Int. J. Nat. Soc. Sci.* 5: 16-24.

Jahanpour, H., Seidavi, A., Qotbi, A.A.A., Van Den Hoven, R., Rocha de Silva, S., Laudadio, V. and Tufarelli, V. 2015. Effects of the level and duration of feeding restriction on carcass components of broilers. *Arch. Anim. Breed.* 58: 99-105.

Konjufca, V.H., Pesti, G.M. and Bakalli, R.I. 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper. *Poult. Sci.* 76: 1264-1271.

Khan, M.S.I., Prodhan, M.S., Islam, M.S., Hasan, M.N. and Islam, M.S. 2017. Effect of garlic extract on growth performances and hematological parameters of broilers. *Asian J. Med. Biol. Res.* 3: 317-322.

Khetani, T.L., Nkukwana, T.T., Chimonyo, M. and Muchenje, V. 2009. Effect of quantitative feed restriction on broiler performance. *Trop. Anim. Health Prod.* 41: 379.

Makwana, R.B., Raval, A.P., Chauhan, H.D., Kulkarni, R.C., Srivastava, A.K., Bhagwat, S.R. and Rajgor, B.B. 2015. Effects of garlic (*Allium sativum*) supplementation on growth performance, carcass characteristics and economics of broilers. *J. Anim. Res.* 5: 843-848.

Malpotra, K., Singh, U. and Sethi, A.P.S. 2017. Effects of feed restriction and additional fat supplementation on chemical composition and sensory evaluation of broiler's meat. *Indian J. Poult. Sci.* 52: 327-331.

McMurtry, J.P., Rosebrough, R.W., Plavnik, I. and Cartwright, A.I. 1988. Influence of early plane of nutrition on enzyme systems and subsequent tissue deposition. In: *Biomechanisms Regulating Growth and Development* (G.L. Steffens and T.S. Rumsey; eds). pp 329-341.

Mehmood, S., Sahota, A.W., Akram, M., Javed, K., Hussain, J., Sharif, H. and Jatoi, A.S. 2013. Influence of feed restriction regimes on growth performance of broilers with different initial weight categories. *J. Anim. Plant Sci.* 26: 1522-1526.

Mollison, B. and Guenter, W. 1984. Abdominal fat deposition and sudden death syndrome in broilers: the effects of restricted intake, early life caloric (fat) restriction and calorie: protein ratio. *Poult. Sci.* 63: 1190-1200.

Noman, Z.A., Hasan, M.M., Talukder, S., Sarker, Y.A., Paul, T.K. and Sikder, M.H. 2015. Effects of garlic extract on growth, carcass characteristics and haematological

parameters in broilers. *The Bangladesh Vet.* 32: 1-6.

Onibi, G.E., Adebisi, O.E. and Fajemisin, A.N. 2009. Response of broiler chickens in terms of performance and meat quality to garlic (*Allium sativum*) supplementation. *Afr. J. Agric. Res.* 4: 511-517.

Omosebi, D.J., Adeyemi, O.A., Sogunle, O.M., Idowu, O.M.O. and Njoku, C.P. 2014. Effects of duration and level of feed restriction on performance and meat quality of broiler chickens. *Arch. de Zoote*. 63: 611-621.

Panigrahy, K.K., Behera, K., Panda, S. and Kumar, S. 2016. Study of growth performance and carcass characteristics in male and female Vanaraja chickens. *Int. J. Sci. Env. Technol.* 5: 4334-4338.

Patel, R.M., Garg, D., Patel, V.R., Vahora, S., Raval, A. and Choubey, M. 2017. Effect of Dietary Supplementation of Garlic (*Allium sativum*) and Fenugreek (*Trigonella foenum-graecum*) seed powder on growth performance, carcass characteristics and economics of feeding in broilers. *J. Anim. Res*. 7: 313-318.

Plavnik, I. and Hurwitz, S. 1988. Early feed restriction in chicks: effect of age, duration, and sex. *Poult. Sci.* 67: 384-390.

Plavnik, I. and Hurwitz, S. 1990. Performance of broiler chickens and turkey poults subjected to feed restriction or to feeding of low sodium diets at an early age. *Poult. Sci.* 69: 945-952.

Plavnik, I. and Hurwitz, S. 1991. Response of broiler chickens and turkey poults to food restriction of varied severity during early life. *Br. Poult. Sci.* 32: 343-352.

Raeesi, M., Hoeini-Aliabad, S.A., Roofchaee, A., Zare Shahneh, A. and Pirali, S. 2010. Effect of periodically use of garlic (*Allium sativum*) powder on performance and carcass characteristics in broiler chickens. *World Acad. Sci. Eng. Technol.* 68: 1213-1219.

Rahmatnejad, E., Roshanfekr, H., Ashayerizadeh, O., Mamooee, M. and Ashayerizadeh, A. 2009. Evaluation of the effect of several non-antibiotic additives on growth performance of broiler chickens. *J. Anim. Vet. Adv.* 8: 1757-1760.

Ramlah, A.H., Halim, A.S, and Siti-Sara, A.R. 1996. Effect of early feed restriction on performance of broilers. *Asian-Australas. J. Anim. Sci.* 9: 63-67.

Ratika, K., Singh, R.J. and Singh, R.K. 2018. Effect of garlic (*Allium sativum*) and turmeric (*Cucurma longa*) powder supplementation on blood parameters of starter and finisher growth phase of broilers. *Int. J. Pure Appl. Biosci.* 6: 562-567.

Rincon, M.U. and Leeson, S. 2002. Quantitative and qualitative feed restriction on growth characteristics of male broiler chickens. *Poult. Sci.* 81: 679-688.

Saber, S.N., Maheri-Sis, N., Shaddel-Telli, A., Hatefinezhad, K., Gorbani, A. and Yousefi, J. 2011. Effect of feed restriction on growth performance of broiler chickens. *Annal. Bio. Res.* 2: 247-252.

Saleh, E.A., Watkins, S.E., Waldroup, A.L. and Waldroup, P.W. 2005. Effects of early quantitative feed restriction on live performance and carcass composition of male broilers grown for further processing. *J. Appl. Poult. Res.* 14: 87-93.

Sidhu, N.S., Singh, U., Sethi, A.P.S., Hundal, J.S., Lamba, J.S. and Sharma, A. 2017. Effect of black pepper and jaggery supplementation with or without feed restriction on broilers performance. *J. Anim. Res.* 7: 903-912.

Singh, J., Sethi, A.P.S., Sikka, S.S., Chatli, M.K. and Kumar, P. 2015. Effect of sun dried whole bulb garlic powder on growth, carcass characteristics and meat quality of commercial broilers. *Indian J. Anim. Sci.* 85: 67-71.

Singh, J., Sharma, M., Singh, N.D., Kaur, P., Sethi, A.P.S. and Sikka, S.S. 2017. Effect of sun dried whole bulb garlic powder on nutrient utilization, blood parameters, duodenum morphology and faecal microbial load in broiler chickens. *Indian J. Anim. Sci.* 87: 195-198.

Teeter, R.G. and Smith, M.O. 1985. Feed intake effects upon gain, carcass yield and ration digestibility in broilers force fed five feed intakes. *Poult. Sci.* 64: 2155-2160.

Tollba, A.A.H. and Hassan, M.S.H. 2003. Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions: Black cumin (*Niglla sativa*) or garlic (*Allium sativum*). *Poult. Sci.* 23: 327-340.

Varmaghany, S., Amir, M., Torshizi., K. Rahimi., S., Lotfollahian, H. and Hassanzadeh, M. 2015. The effects of increasing levels of dietary garlic bulb on growth performance, systolic blood pressure, hematology and ascites syndrome in broiler chickens. *Poult. Sci.* 94: 1812-1820.

Yalcin, S., Onbaþýlar, E.E., Reisli, Z. and Yalcýn, S. 2006. Effect of garlic powder on the performance, egg traits and blood parameters of laying hens. *J. Sci. Food Agric.* 86: 1336-1339.

Zhan, X.A., Wang, M., Ren, H., Zhao, R.Q., Li, J.X. and Tan, Z.L. 2007. Effect of early feed restriction on metabolic programming and compensatory growth in broiler chickens. *Poult. Sci.* 86: 654-660.

Zubair, A.K. and Leeson, S. 1996. Compensatory growth in the broiler chicken: A review. *World's Poult. Sci.* 52: 189-201.

Received on 27-07-2018 and accepted on 07-01-2019

Indian J. Anim. Nutr. 2019. 36 (1): 58-64
doi: 10.5958/2231-6744.2019.00010.0

Indian Journal of Animal Nutrition

Performance of Broiler Chicken Fed Diets Supplemented with a Phytogenic Mixture

V.V. Singh, V.K. Singh*, D. Tewari, S. Gautam, V.B. Singh and P. Singh
Department of Animal Nutrition, College of Veterinary Science & Animal Husbandry,
Narendra Dev University of Agriculture & Technology, Kumarganj-224229, Ayodhya, Uttar Pradesh, India

ABSTRACT

Two hundred fifty (Vencobb-400Y strain), day old broiler chicks were randomly divided into 5 groups of 50 birds each. Each group was further divided in to 5 replicates of 10 birds each. These were kept either on basal diet without any supplement (NCON), basal diet with antibiotic (PCON), basal diet with 1% phytogenic mixture (LPH), basal diet with 2% phytogenic mixture (MPH) and basal diet with 3% phytogenic mixture (HPH) for 35 days in order to evaluate the effect of a phytogenic mixture on the performance of broiler chicken. The phytogenic mixture supplemented group's birds had higher body weight than the control group birds and the mean body weight of LPH, MPH and HPH was higher (P<0.05) than NCON group. After 35 days of experimental period, it was found that the total feed intake of phytogenic supplemented birds was similar to NCON group but higher than that of PCON birds. Among the phytogenic mixture supplemented birds, lowest feed intake was observed in MPH group. Among the herbal supplemented groups, lowest FCR was found in MPH group and it was similar to PCON group. The energy efficiency ratio (EER) of herbs supplemented groups was higher (P<0.05) than NCON group but lower than that of PCON group. The protein efficiency ratio (PER) of phytogenic mixture supplemented birds was better (P<0.05) than NCON group and lower than that of PCON group. Among herbal groups, the highest PER was found in MPH group. The performance index of phytogenic mixture supplemented birds was higher (P<0.05) in comparison to NCON birds. Rearing of broilers supplemented with phytogenic mixture was more economical than NCON group but less economical than the birds reared on conventional system of rearing *i.e.* on AGP. Therefore, 2% phytogenic mixture was economical and has potential to use as an alternative to AGP in broiler rearing.

Key words: Broiler, Chicken, Economics, Growth Performance, Phytogenic mixture

INTRODUCTION

Broiler chicken grow very fast and it is ready for marketing within 35-42 days. The rapid growth rate in broilers imposes severe stress on birds which leads to poor performance, immuno-suppression and high mortality (Xie and Song, 2005). To reduce stress in birds, antibiotics are added in feed. But the European Union in January, 2006 banned the majority of commercial antibiotic growth promoters used in poultry feed in order to protect consumer health. This has led to the interest in finding alternatives to antibiotics for poultry production (Hassan *et al.,* 2011).

Herbs can serve as safer alternatives as growth promoters due to their suitability and preferences, reduced mortality, reduced risk of diseases, minimum health hazards and environment friendliness. Our country abounds in herbal plants having pharmacological properties which needs exploitation by the modern methods. Tulsi (O*cimum sanctum*) is an analgesic and antistress agent and has antimicrobial, anti-inflammatory, immuno-stimulant and anti-oxidative property (Singh *et al.,* 2014). Neem (*Azadirachta indica*) leaves have different medicinal properties like immunostimulant, antiviral, antibacterial, antifungal, hepatoprotective and antiprotozoal, and has no side effects (Ong *et al.,* 2014). Sahijan (*Moringa oleifera*) leaves are good sources of fats, proteins, minerals and have antimicrobial effects (Olugbemi *et al.,* 2010). Eucalyptus is a well known medicinal plant because of its biological and pharmacological properties. It has antibacterial activities against *Salmonella, Klebsiella, Streptococcus, Proteus, Staphylococcus and Escherichia* coli (Hassan *et al.,* 2011). Giloy *(Tinospora cardiofolia)* also known as guduchi has immune-potentiating, antibacterial, antidiabetic, analgesic and antioxidant properties. It has hepatoprotective effect

*Corresponding author : E-mail: vksinghnduat@gmail.com

(Ganguly and Prasad, 2011). Most of polyherbal mixtures could be used as an alternative to antibiotic growth promoters (AGP). Considering the above facts, a phytogenic mixture was formulated by using tulsi, neem, sahijan, eucalyptus leaves and its effect on performance of birds was evaluated.

MATERIALS AND METHODS

Day-old commercial Vencobb-400Y broiler chicks (n=250) were distributed in a completely randomized design into five groups of 50 chicks each and they were further subdivided into five replicates of ten birds each. The chicks were placed on one of the five dietary treatments *i.e.* either a basal diet (NCON) or that supplemented with antibiotic noseheptide (PCON), phytogenic mixture at level of 1% (LPH), 2.0% (MPH) and 3% (HPH). The indigenous economical phytogenic mixture containing blend of neem, tulsi, sahijan and eucalyptus leaves and giloy stem powder. The experimental diets were formulated for pre-starter (1-7 d), starter (8-21 d) and finisher (22-35 d) phases separately as per BIS (2007) and ingredients composition of diets has been presented in Table 1.

The chicks were kept hygienically on floor litter system in separate pens. All the birds were reared adopting uniform management conditions. Replicatewise body weight of chicks was recorded at day 0 and then at weekly intervals upto 35 d. Replicatewise total feed consumed during the experimental period was recorded weekly. These data were used to calculate feed conversion ratio (FCR).The energy efficiency ratio (EER; g of weight gain ×100/total ME intake) and protein efficiency ratio (PER; grams of weight gain/g of protein intake) were calculated. Performance index (PI) was calculated (North and Bell, 1990). To find out the commercial viability of phytogenic mixture as an alternative of antibiotic growth promoters, economic efficiency was also calculated. The diets were analysed for proximate principles (AOAC, 2000).

Table 1. Ingredients and chemical composition of basal diet

Ingredient	Pre-starter (1-7 d)	Starter (8-21 d)	Finisher (22-35 d)
Ingredient composition (% as fed basis)			
Maize	50.42	55.52	59.82
Soybean meal	42.00	36.40	27.40
Rice polish	-	-	5.00
Vegetable fat	4.48	5.18	5.43
Dicalcium phosphate	1.15	0.97	0.80
Limestone powder	0.90	1.04	0.75
Common salt	0.40	0.38	0.36
DL-Methionine	0.25	0.18	0.16
Choline chloride	0.15	0.08	0.03
Vitamin premix[1]	0.05	0.05	0.05
Mineral premix[2]	0.10	0.10	0.10
Nutrient composition (% DM basis)			
Dry matter	87.15	87.77	89.22
Crude protein	23.14	22.09	20.30
Crude fiber	3.05	3.25	3.45
Ether extract	4.98	5.80	7.78
Metabolizable energy[*] (kcal/kg)	3003	3107	3203

[1]Supplies (per kg diet): vitamin A 16500 IU; vitamin D_3 3200 ICU; vitamin E 12 mg; vitamin K 2 mg; vitamin B_2 10 mg; vitamin B_6 2.4 mg; vitamin B_{12} 12 mg; niacin 18 mg and pantothenic acid 12 mg; [2]Supplies (per kg diet): manganese 90 mg; zinc, 72 mg; iron, 60 mg copper, 10 mg and iodine 1.2 mg; [*]Calculated value

Data were subjected to statistical analysis using completely randomized design employing one way analysis of variance (Snedecor and Cochran, 1989). The means of different treatments were compared with Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The chemical composition of diets has been presented in Table 1. The information on effect of phytogenic mixture supplementation on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) has been presented in Table 2. The overall body weight gain of birds from d 1 to 35 in phytogenic mixture as well as in antibiotic supplemented chicks was similar and higher (P<0.05) than the NCON group. Body weight gain was not affected by treatments during prestarter period. However, in starter period BWG in phytogenic mixture supplemented groups was higher (P<0.05) than PCON and NCON groups. the BWG in LPH and MPH groups in finisher phase was higher (P<0.05) than NCON group but similar to that of PCON group. The present findings of higher body weight gain of broilers due to indigenous polyherbal mixture supplementation are in concurrence with the reports of Bhattacharya and Bhattacharya (2013) and Alam *et al.* (2015). Similarly, Khatun *et al.* (2013) supplemented tulsi and neem leaves extract @ 1, 2 and 3 ml/L drinking water of broiler chicken and found that the chicks supplemented with herbal extracts had higher live weight. Rahman *et al.* (2014) also reported that supplementation of neem, turmeric, cinnamon extracts in the feed of broiler chicken improved (P<0.05) the mean live weights of birds in comparison to control group birds. Tazi (2012) showed a positive effect of moringa on body weight gain of broiler chicken. On the contrary, Namagirilakshmi (2005) and Varaprasad *et al.* (2007) found no effect on body weight gain of chicks due to supplementation of herbal mixture. The differences reported in other studies could be attributed to difference in inclusion level of herbs, sources of herbs, basal diet composition or microbial environment in which the birds were reared.

In phytogenic mixture supplemented groups total feed intake were comparable to NCON group but was higher (P<0.05) than PCON group. In different phases of growth, it was found that in initial phase *i.e*. prestarter period, feed intake of phytogenic mixture supplemented groups was similar to PCON but lower (P<0.05) than NCON group. In starter and finisher phase, feed intake of HPH group was higher than PCON group and

Table 2. Effect of experimental diets on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broiler chicken

Period	Attribute	Group*					SEM
		NCON	PCON	LPH	MPH	HPH	
1-7 d (Pre-starter)	BWG (g/bird)	170.60	169.20	163.40	165.30	164.90	1.699
	FI (g/bird)	149.60^{a}	126.00^{b}	127.40^{b}	127.20^{b}	128.40^{b}	2.19
	FCR	0.88^{a}	0.77^{b}	0.78^{b}	0.75^{b}	0.78^{b}	0.010
8-21 d (Starter)	BWG (g/bird)	725.60^{b}	758.00^{b}	808.40^{a}	808.60^{a}	849.20^{a}	10.99
	FI (g/bird)	979.00^{ab}	964.40^{b}	949.40^{bc}	937.00^{c}	999.60^{a}	10.51
	FCR	1.35^{a}	1.14^{b}	1.18^{b}	1.16^{b}	1.18^{b}	0.017
22-35 d (Finisher)	BWG (g/bird)	963.60^{c}	1092.00^{a}	1063.20^{ab}	1060.60^{ab}	992.60^{bc}	15.90
	FI (g/bird)	1976.00^{ab}	1921.00^{b}	2003.00^{a}	1972.00^{ab}	2037.00^{a}	12.97
	FCR	2.05^{a}	1.77^{b}	1.89^{b}	1.87^{b}	2.05^{a}	0.030
1-35 d (Overall)	BWG (g/bird)	1859.80^{b}	2019.20^{a}	2035.00^{a}	2034.50^{a}	2006.70^{a}	20.32
	FI (g/bird)	3104.60^{ab}	2911.40^{c}	3079.80^{ab}	3036.20^{b}	3165.00^{a}	23.18
	FCR	1.67^{a}	1.45^{d}	1.51^{c}	1.50^{cd}	1.58^{b}	0.017

^{a,b,c,d}Means with different superscripts in a row differ between groups significantly (P<0.05); *NCON- basal diet; PCON- basal diet+ antibiotic; LPH- basal diet + 1% phytogenic mixture; MPH- basal diet + 2% phytogenic mixture; HPH- basal diet + 3% phytogenic mixture

similar to that of NCON group. The higher feed intake in HPH group and NCON group birds than PCON might be due to the less efficient utilization of nutrient for energy need of birds, because birds feed intake is directly influenced by its energy requirements. Singh *et al.* (2014) observed that incorporation of tulsi leaf powder in broiler diet did not affect feed intake. Rahman *et al.* (2015) also revealed no significant differences in feed consumption of broiler chicken due to supplementation of neem leaf and ginger. Contrary to these findings, Nath *et al.* (2012) reported that supplementation of herbal formulation containing tulsi @ 1 ml/L in drinking water exhibited significant positive effect on the feed intake. Qamar (2015) reported that supplementation of a commercial herbal products showed a significant positive effect on the feed consumption of broilers. However, Iheukwumere *et al.* (2008) reported decline in feed intake by supplementing neem leaf meal in broiler feed. Ogbe and Affiku (2012) found decline in feed intake in broilers by supplementing a poly-herbal extract that have high concentration of *M. oleifera* leaves extract.

The overall FCR of phytogenic mixture supplemented groups was better (P<0.05) than NCON group and in MPH group FCR was comparable to PCON group. In prestarter and starter period, FCR of phytogenic mixture and antibiotic supplemented birds was similar and lower (P<0.05) than NCON group. However, this FCR trend was only observed in LPH and MPH group in finisher period. The positive results in indigenous polyherbal supplemented groups are compatible with the earlier findings in which broilers fed rations containing tulsi had better feed conversion ratio (Singh *et al.,* 2014; Nath *et al.,* 2012) compared to its absence in ration. Similarly, it was also reported that supplementation of neem and ginger in diet of broilers reduced FCR (Rahman *et al.,* 2015). Chicks fed on moringa based diets performed better (P<0.05) in term of FCR (Ebenebe *et al.,* 2012). Hasan *et al.* (2011) found that FCR was significantly improved by using both eucalyptus and antibiotic and their mixtures when compared with control group in laying quail. However, non-significant change in FCR of broiler chickens was reported on supplementation of neem leaf powder (Sabeeha *et al.,* 2015).

In the prestarter and starter phase EER and PER of phytogenic mixture supplemented birds were similar to PCON group and higher (P<0.05) than NCON group

Table 3. Effect of experimental diets on energy efficiency ratio (EER), protein efficiency ratio (PER) and performance index (PI) of broiler chicken

Period	Attribute	Group					SEM
		NCON	PCON	LPH	MPH	HPH	
1-7 d (Pre-starter)	EER	38.07^{b}	44.74^{a}	42.72^{a}	43.25^{a}	42.74^{a}	0.560
	PER	4.94^{b}	5.81^{a}	5.54^{a}	5.61^{a}	5.55^{a}	0.073
	PI (%)	23.75^{b}	27.00^{a}	25.76^{ab}	26.92^{a}	26.00^{ab}	0.400
8-21 d (Starter)	EER	23.91^{b}	28.27^{a}	27.45^{a}	27.84^{a}	27.40^{a}	0.358
	PER	3.36^{b}	3.97^{a}	3.85^{a}	3.91^{a}	3.85^{a}	0.050
	PI (%)	69.22^{c}	84.73^{b}	86.00^{b}	87.25^{b}	89.34^{a}	1.810
22-35 d (Finisher)	EER	15.23^{b}	17.73^{a}	16.60^{a}	16.77^{a}	15.22^{b}	0.262
	PER	2.40^{b}	2.80^{a}	2.62^{a}	2.65^{a}	2.40^{b}	0.042
	PI (%)	92.52^{c}	117.06^{a}	110.15^{ab}	111.65^{ab}	99.64^{bc}	2.550
1-35 d (Overall)	EER	18.94^{d}	21.89^{a}	20.89^{b}	21.16^{b}	20.03^{c}	0.231
	PER	2.88^{d}	3.34^{a}	3.19^{b}	3.23^{b}	3.05^{c}	0.036
	PI (%)	113.74^{c}	142.62^{a}	137.09^{ab}	138.85^{ab}	129.59^{b}	2.650

^{a,b,c,d}Means with different superscripts in a row differ between groups significantly (P<0.05)

(Table 3). In finisher phase, EER, PER and PI of LPH and MPH groups was similar to that of PCON group and higher (P<0.05) in comparison to NCON group. The overall EER and PER values of phytogenic mixture supplemented groups were lower (P<0.05) than PCON group and higher than NCON group. The overall PI was similar in LPH and MPH groups compared to traditional antibiotic supplement *i.e*. PCON and higher (P<0.05) than NCON group. Among the phytogenic mixture supplemented groups, the highest PI value was found in MPH group. The improvement in performance indices *i.e*. BWG, FCR, EER, PER and PI of broilers supplemented with a phytogenic mixture might be due to the synergistic action of the active principles of the herbs blend to prepare the herbal product. It could be partly explained by the increase in the apparent digestibility of dietary protein and the pre-caecal digestive capacity in general which increased the intestinal availability of nutrients for absorption and consequently birds grew faster (Windisch *et al.,* 2008). Another mode of action of growth-promoting herbs arises from stabilizing the ecosystem of the gastrointestinal microbiota (Windisch *et al.,* 2008) by decreasing microbial activity and controlling potential pathogenic microorganisms in the gastrointestinal tract of animals (Castillo *et al.,* 2006). In fact, animals receiving feed supplemented with polyherbal preparation had more stabilized intestinal health and less exposed to microbial toxins and other undesired microbial metabolites such as ammonia and biogenic amines (Jamroz *et al.,* 2003; Windisch *et al.,* 2008). Moreover, decreasing microbial activity led to reduced production of VFA which contributed to the stabilization of the intestinal pH and ensured an optimum activity of digestive enzymes (Jamroz *et al.,* 2003). In addition, Jamroz *et al.* (2006) demonstrated that essential oils present in some herbs improved the absorptive capacity of the intestinal mucosa by increasing intestinal villi size and crypt depth.

Total expenditure to rear an individual bird in different groups ranged from ₹ 107.82 to ₹ 118.69 and exhibited that rearing of birds on phytogenic mixture

Table 4. Economics of broiler production kept under different treatments from one day old to 5 weeks age

Attribute	Group					SEM
	NCON	PCON	LPH	MPH	HPH	
Chick cost (₹)	20.00	20.00	20.00	20.00	20.00	0.000
Feed cost (₹)						
a. Prestarter	4.61^{a}	3.88^{b}	3.92^{b}	3.92^{b}	3.95^{b}	0.068
b. Starter	27.41^{ab}	24.20^{d}	26.58^{bc}	26.24^{c}	27.99^{a}	0.294
c. Finisher	56.51^{ab}	54.94^{b}	57.28^{a}	56.40^{ab}	58.25^{a}	0.371
d. Total	88.53^{ab}	83.03^{c}	87.79^{ab}	86.55^{b}	90.20^{a}	0.660
Herbal/antibiotic cost (₹)	0.00^{e}	0.29^{d}	1.29^{c}	2.55^{b}	3.99^{a}	0.303
Miscellaneous (₹)	4.50	4.50	4.50	4.50	4.50	0.000
Total expenditure (₹)	113.03^{b}	107.82^{c}	113.59^{b}	113.60^{b}	118.69^{a}	0.833
Total expenditure/ kg live wt. (₹)	59.58^{a}	52.51^{c}	54.81^{b}	54.94^{b}	58.09^{a}	0.580
Total gain (₹)	119.52^{b}	129.58^{a}	130.55^{a}	130.51^{a}	128.77^{a}	1.285
Net profit (₹)	6.42^{b}	10.49^{a}	8.19^{b}	8.06^{b}	4.91^{c}	0.580
Economic efficiency	0.08^{d}	0.26^{a}	0.19^{b}	0.19^{b}	0.11^{c}	0.015
Relative economic efficiency	0.00^{d}	2.73^{a}	1.77^{b}	1.78^{b}	0.59^{c}	0.224

^{a,b,c,d,e}Means with different superscripts in a row differ significantly (P<0.05)

was costlier than the birds reared on antibiotics (Table 4). It was also observed that cost of rearing on this phytogenic mixture in LPH and MPH groups was not higher than the birds reared only on basal diet (NCON). However, production cost/kg live weight in LPH (₹ 54.81) and MPH (₹ 54.94) groups was lower (P<0.05) in comparison to NCON group (₹ 59.58). The net profit per bird ranged from ₹ 4.91 to 10.49 and it showed no variation among LPH, MPH and NCON groups. However, net profit was lower (P<0.05) in phytogenic mixture supplemented groups than PCON group. The economic efficiency and relative economic efficiency data revealed that rearing of broilers on phytogenic mixture was more economical than NCON group but less economical than the birds reared on conventional system of rearing *i.e*. AGP. The results of the present study are in line with the findings of Mahmood *et al.* (2014) who reported that supplementation of various commercial herbal growth promoters in the ration exhibited an increase in the profit margin of broilers as compared to those using ration without supplementation. Similarly, Ahmad (2005) reported that dietary inclusion of polyherbal mixture in the feed was more economical in broiler production. Singh *et al.* (2014) found lower cost of production per broiler in 1% tulsi leaf powder supplemented group in comparison to control group. Reddy *et al.* (2012) also reported that the feed cost per kg live weight gain was lower (P<0.01) in 0.25% tulsi and 0.25% herbals combination supplemented birds. Sabeeha *et al.* (2015) reported that neem leaf powder supplementation @ 100 g/50 kg diet was more economical than other groups.

CONCLUSION

The dietary supplementation of a phytogenic mixture @ 2% showed potential as an alternative of antibiotic growth promoters and improved the overall performance of broiler chickens.

REFERENCES

Ahmad, S. 2005. *Comparative Efficiency of Turmeric, Garlic, Cinnamon and Kalongi as Growth Promoter in Broiler.* M.Sc. (Hons.). Thesis submitted to Department of Poultry Sciences, Univ. Agric., Faisalabad, Pakistan.

Alam, M.M., Rakib, A.T.M.F.K., Abdullah-Al-Hasan, M., Hasan, M.S. and Ali, M.A. 2015. Effects of neem leave powder as a growth promoter in broilers. *Int. J. Nat. Soc. Sci.* 2: 22-26.

AOAC. 2000. *Official Methods of Analysis*. 17th edn. Association of Official Analytical Chemists, Verginia, Washington, D.C., USA.

Bhattacharya, C. and Bhattacharya, G. 2013. Therapeutic potential of Giloe, *Tinospora cordifolia*. *Int. J. Pharm. Bio. Arch.* 4: 558-584.

BIS. 2007. *Poultry Feeds Specifications. 5th* Review. Bureau of Indian Standards, New Delhi, India.

Castillo, M., Martin-Orue, S.M., Roca, M., Manzanilla, E.G., Badiola, I., Perez, J.F. and Gasa, J. 2006. The response of gastrointestinal microbiota to avilamycin, butyrate and plant extracts in early-weaned pigs. *J. Anim. Sci.* 84: 2725-2734.

Duncan, D.B. 1955. Multiple Range and Multiple F Tests. *Biometrics* 11:1-42.

Ebenebe, C.L., Umegechi Aniebo, C.O. and Nweze, B.O. 2012. Comparison of haematological parameters and weight changes of broiler chicks fed different levels of *Moringa oleifera* diet. *Int. J. Agric. Biol. Sci.* 1: 23-25.

Ganguly, S. and Prasad, A. 2011. Role of plant extract and cow urine distillate as immunomodulators: Review. *J. Med. Plant Res.*5: 649-651.

Hassan, M.S.H., El Sanhour, M.H., Ali, W.A.H. and Ahmed, A.M.H. 2011. Effect of using eucalyptus leaves as natural additives on productive, physiological, immunological and histological performance of laying Japanese quail. *Egypt. Poult. Sci.* 31: 305-329.

Iheukwumere, F.C., Ndubuisi, E.C., Mazi, E.A. and Onyekwere, M.U. 2008. Performance, nutrient utilization and organ characteristics of broilers fed cassava leaf meal (*Manihot esculenta Crantz*), *Pak. J. Nutr.* 7: 13-16.

Jamroz, D., Orda, J., Kamel, C., Wiliczkiewicz, A., Wertelecki, T. and Skorupinska, J. 2003. The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics and gut microbial status in broiler chickens. *J. Anim. Feed Sci.*12: 583-596.

Jamroz, D., Wertelecki, T., Houszka, M. and Kamel, C. 2006. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *J. Anim. Physiol. Anim. Nutr.* 90: 255-268.

Khatun, S., Mostofa, M., Alom, F., Uddin, J., Alam, M. N. and Moitry N.F. 2013. Efficacy of tulsi and neem leaves

extract in broiler production. *Bangladesh J. Vet. Med.* 11: 1-5.

Mahmood, S., FurqanSaleem, M., Ahmad, F., Abbas, G., Mahmood, A., Qamar, S.H. and Zia ur Rehman, M. 2014. Comparative effect of different commercial herbal growth promotors on performance, minor body parts weight and immune response in broilers. *Adv. Zool. Bot.* 2: 69-74.

Namagirilakshmi, S. 2005. *Turmeric (Curcuma longa) as Nutraceutical to Improve Broiler Performance*. M.V.Sc. thesis submitted to Tamilnadu Veterinary and Animal Sciences University, Chennai, Taminadu, India.

Nath, D.D., Rahman, M.M., Akter, F. and Mostofa, M. 2012. Effects of tulsi, black pepper and cloves extract as a growth promoter in broiler. *Bangladesh J. Vet. Med.* 10: 33-39.

North, M.O. and Bell, D.D. 1990. *Commercial Chicken Production Manual.* AVI Publishing Inc, New York, USA.

Ogbe, A.O. and Affiku, J.P. 2012. Effect of polyherbal aqueous extracts (*Moringa oleifera*, Gum arabic and wild *Ganoderma lucidum*) in comparison with antibiotic on growth performance and haematological parameters of broiler chickens. *Res. J. Recent Sci.* 1: 10-18.

Olugbemi, T.S., Mutayoba, S.K. and Lekule, F.P. 2010. Effect of *Moringa oleifera* inclusion in cassava based diets fed to broiler chickens. *Int. J. Poult. Sci.* 9: 363-367.

Ong, G.H., Syamsiah, A.S., Hasrul, A.H.M., Zunaida, B., Maizatul, Z.M., Jihan, R.M., Redzwan, S., Leow, L., Faizul, F.M.Y., Chandrawathani, P. and Ramlan, M. 2014. Antiviral effect of aqueous neem extract from branches of neem tree on Newcastle disease virus. *Malays. J. Vet. Res.* 5: 5-9.

Qamar, S.H., Ul-Haq, A., Asghar, N., Ur Rehman, S., Akhtar, P. and Abbas, G. 2015. Effect of herbal medicine supplementations (Arsilvon Super, Bedgen40 and Hepa-cure Herbal Medicines) on growth performance, immunity and haematological profile in broilers. *Adv. Zool. Bot.* 3: 17-23.

Rahman, M.A., Ali, M.A., Saha, B.K. Abdullah-Al-Hasan, M., Rahman, M.A. and Mostofa, M. 2015. Use of neem leaf and ginger extracts for cost effective broiler production. *Int. J. Nat. Soc. Sci.* 2: 11-16.

Rahman, S., Mostafa, M., Fatema, M.N., Latif, M.A. and Afrin, S. 2014. Effects of neem leaves, turmeric and cinnamon extract as a growth promoter in broilers. *Bangladesh Res. Publ. J.* 10: 7-13.

Reddy, T.E., Sudhakara Reddy, Satyanarayana, P., Reddy, P.V.V. and Shakila, S. 2012. Effect of herbal preparations (*Phyllanthus emblica, Curcuma longa and Ocimum sanctum*) on the performance of broilers. *Tamilnadu J. Vet. Anim. Sci.* 8: 209-214.

Sabeeha Abed Ali, Kumar, P. and Neeraj. 2015. Growth performance of broilers Fed with neem leaf powder (*Azadirachta indica*) and amproliumas coccidiostat feed additive. *Eur. Acad. Res*. 2: 1275-1271.

Singh, A., Neeraj., Sushma and Doley, P. 2014. Effect of dietary supplementation of tulsi (O*cimum sanctum)* leaf powder on growth performance of broilers. *Bioved.* 25: 169-171.

Snedecor, G.W. and Cochran, W.G. 1989. *Statistical Methods*. 8th edn. Iowa State University Press, Iowa, USA.

Tazi, S.M.A.E. 2012. Effect of feeding different levels of *Moringa oleifera* leaf meal on the performance and carcass quality of broiler chicks. *Int. J. Sci. Res. (IJSR).* 3: 147-151.

Varaprasad Reddy, L.S.S., Thangavel, A., Leela, V. and Narayana Raju, K.V.S. 2007. Effect of dietary supplementation of tulasi (*Ocimum sanctum*) and selenium on lipid peroxides levels and growth rate in broiler chickens. *Tamilnadu J. Vet. Anim. Sci.* 3: 144-149.

Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. 2008. Use of phytogenic productsas feed additives for swine and poultry. *J. Anim. Sci.* 86: 140.

Xie, Y.X. and Song, Z.Y. 2005. Causes and control measures of ND immunization failure. *Livestc. Poult. Industry* 178: 21.

Received on 14-08-2018 and accepted on 01-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 65-72
doi: 10.5958/2231-6744.2019.00011.2

Indian Journal of Animal Nutrition

Seasonal Variations in Proximate Composition of Nine Freshwater Fish

B.N. Paul*, S. Bhowmick, P. Singh, S. Chanda, N. Sridhar[1] and S.S. Giri[2]
Regional Research Centre of CIFA. P.O. Rahara, Kolkata-700 118, West Bengal, India

ABSTRACT

The variation in proximate composition was determined in relation to season and body weight of nine freshwater fish *i.e. Mystus vittatus, Ompok bimaculatus, Channa striata, Wallago attu, Pangasianodon hypophthalmus, Labeo bata*, *Labeo calbasu*, *Cirrhinus reba* and *Puntius javanicus*. The samples were collected in different seasons from different places and divided into small and big body weight groups. Nutritional composition of fish species was determined during the three seasons *i.e.* S_1 (January-April), S_2 (May-August) and S_3 (September-December) of the year. Protein content ranged from 12.52-19.19% among the species on wet weight basis. Protein content was higher (P<0.01) in *C. reba* (>100 g) during January-April. Protein, fat and ash content of *P. javanicus* was higher (P<0.01) in fish of size (>100 g) during S_3 (September-December). Protein content was higher (P<0.05) in *O. bimaculatus* and *M. vitattus* during S_1 (January-April) irrespective of body weight. Protein content was higher (P<0.01) during the month of May-August in *C. striata* of (>500 g) size group. Fat content of the *L. bata* was higher (P<0.01) during S_1 (January to April) irrespective of body size. Fat content in *L. calbasu* was similar in different seasons. Fat content of *C. striata* was higher (P<0.01) in >100g size group during S_1 (January-April). Protein content was higher in *W. attu* of >1000 g size group during S_2 (May-August). *P. hypopthalamus* of (>1000 g) size group revealed that fat content was higher (P<0.01) during S_3 (September-December). Thus, Indian freshwater fish are rich in protein, fat and ash contents and qualify as health food though there were some seasonal variations in proximate composition within the fish species.

Key words: Body weight, Freshwater fish, Proximate composition, Seasonal variations

INTRODUCTION

Food is an important component of public health as the quality and quantity of food components greatly influence the health status of the consumers (Bamji, 2011). Fish is one of the cheapest sources of quality animal protein and availability and affordability of protein is better for fish in comparison to other animal protein sources (Louka *et al.,* 2004; Mohanty *et al.,* 2019). The nutrient compositions of a particular species often appear to vary from season to season (Deka *et al.,* 2012).

Protein in the form of enzymes and hormones are concentrated with a wide range of vital metabolic process in the body. On a fresh-weight basis, fish contain about 12-22% protein and all 10 essential amino acids with easily digestible protein of high biological value (Mohanty *et al.,* 2014). The fat content of fish ranges from 1.0 to 15% and it varies depending on the species as well as season. Lipids are one of the most important components of fish muscle providing energy reserves and components of cell bio-membranes. Though some information is available on nutrient composition of Indian major carp (Paul *et al.,* 2015a; 2016), catfish (Paul *et al.,* 2015), climbing perch (Paul *et al.,* 2017) and minor carps (Paul *et al.,* 2018). The chemical composition of fish varies according to individual and species depending on age, sex, environment, season and geographical location (Boran and Karacam, 2011). The knowledge of fish composition is essential for its maximum utilization (Silva and Chamul, 2000). Keeping in view of importance of eating fish, the seasonal variation in proximate composition of nine freshwater fish *(Labeo. bata, Labeo. calbasu, Cirrhinus. reba, Puntius. javanicus*, *Ompok. bimaculatus, Mystus. vittatus*, *Channa. striata*, *Wallago. attu* and *Pangasius. hypophthalmus*) was studied which could

*Corresponding author: E-mail: bnpaulcifa@gmail.com:[1]Regional Research Centre of CIFA, Hessaraghatta Lake, P.O.: Hessaraghatta, Bengaluru-560 089, Karnataka, India; [2]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751002, Odisha, India

be helpful to dieticians, nutritionists, researchers, fish farmers and related stakeholders to promote fish as health food in human nutrition.

MATERIALS AND METHODS

The samples were collected from various places of West Bengal and Odisha throughout the year. The samples were further categorized into two groups according to their weight ranges based on the harvesting size available in market *i.e.,* for *L. bata* small (<100 g) and big (>100 g); *L. calbasu* small (<300 g) and big (>300 g); *C. reba* small (<100 g) and big (>100 g); *P. javanicus* small (<100g) and big (>100 g); *O. bimaculatus* small (<100 g) and big (>100 g); *M. vittatus* small (<15 g) and big (>15 g); *C. striata* small (<500 g) and big (>500 g); *W. attu* small (<100 g) and big (>100 g) and *P. hypophthalamus* small (<100 g) and big (>100 g). Nutritional composition of fish species were analyzed during the three seasons *i.e.* S_1 (January-April), S_2 (May-August) and S_3 (September-December) of the year. The number of fish samples such as *L. bata*, *L. calbasu, C. reba, P. javanicus, M. vittatus*, *O. bimaculatus, C. striata, W. attu and P. hypophthalmus* collected were 52, 54, 51, 53, 59, 52, 48 and 56, respectively.

Fish scales and fins were removed and then washed with running water followed by filleting of edible muscle, which were oven dried at 60°C until achieving constant weight powdered in mixer grinder and stored until chemical analysis. The sampling procedure and the sample preparation for analysis were followed as per Sankar *et al.* (2010).

The moisture content of the wet muscle was estimated by heating it in an oven to a constant weight at 60°C under atmospheric pressure. Dried samples were used for determination of proximate composition of fish muscle (AOAC, 1995). The data were statistically analysed as per Snedecor and Cochran (1994) by one way ANOVA and the least significant difference (LSD) was used for comparison of the mean values.

RESULTS AND DISCUSSION

The moisture content of *Labeo bata* (Table 1) was higher (P<0.01) in fish of (>100 g) during second season (S_2) *i.e.* May-August of the year in comparison to fish of <100 g during first season (January-April) of the year. It did not vary among other groups. Within the same season of the year, moisture content did not vary between small and large fish. The protein content of *Labeo bata* was higher (P<0.01) in <100 g group during S_1 (January to April). The fat content of the species (Table 1) was (P<0.01) higher during January to April irrespective of their body weights in comparison to the fish in other seasons of the year. However, the ash content was similar among the groups according to the size and different seasons of the year.

The moisture content of *L.calbasu* was higher (P<0.01) in size groups <300 g during S_2 (May-August). The protein content of *L. calbasu* was higher (P<0.01) in size group <300 g during Januar-April in comparison to the fish of same size group during May-

Table 1. Proximate composition (on % wet weight basis) of *Labeo bata* in different seasons

Particular	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<100g)	Big (>100g)	Small (<100g)	Big (>100g)	Small (<100g)	Big (>100g)	
Moisture	70.95^{a}±1.29	71.73^{ab}±0.29	74.01^{ab}±0.60	75.05^{b}±0.38	74.09^{ab}±0.42	73.19^{ab}±0.86	0.0058
Protein	18.27^{b}±1.01	16.28^{ab}±0.89	14.84^{a}±0.52	16.76^{ab}±0.33	14.32^{a}±0.23	16.27^{ab}±0.56	0.0002
Fat	4.94^{b}±0.13	4.86^{b}±0.21	3.29^{a}±0.27	2.93^{a}±0.14	3.35^{a}±0.27	3.29^{a}±0.34	0.0001
Ash	2.67±0.34	2.67±0.25	2.56±0.08	2.61±0.15	2.26±0.08	2.68±0.20	0.6054

[a,b]Means bearing different superscripts in a row differ significantly

Table 2. Proximate composition (on % wet weight basis) of *L. calbasu* in different seasons

Particular	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<300g)	Big (>300g)	Small (<300g)	Big (>300g)	Small (<300g)	Big (>300g)	
Moisture	74.51^{ab}±0.36	74.64^{ab}±0.29	76.36^{c}±0.55	73.70^{a}±0.55	76.08^{bc}±0.23	74.54^{ab}±0.25	0.0003
Protein	15.33^{b}±0.29	14.19^{ab}±0.14	13.34^{a}±0.50	15.22^{b}±0.45	14.02^{ab}±0.39	14.04^{ab}±0.12	0.0023
Fat	2.69±0.21	2.62±0.17	2.32±0.28	3.41±0.26	3.38±0.28	3.02±0.12	0.1500
Ash	2.30^{b}±0.05	2.19^{b}±0.04	1.80^{a}±0.12	2.12^{ab}±0.10	2.29^{b}±0.07	2.40^{b}±0.05	0.0005

[a,b,c]Means bearing different superscripts in a row differ significantly

August (Table 2). Moreover, during this period, the bigger fish (>300 g) showed higher (P<0.01) body protein content than their smaller counterparts (<300 g). Fat content in *L. calbasu* did not differ significantly according to the size of the fish and season of the year. However, the ash content of *L. calbasu* irrespective of body weight was higher (P<0.01) during S_1 (January-April) and S_3 (September-December) in comparison to the <300 g fish during S_2 (May-August).

The moisture content of *C. reba* was higher (P<0.01) during September-December months irrespective of their body weight in comparison to the fish of all size group during January-April (Table 3). Moreover, larger fish (>100 g) exhibited lower moisture content than their smaller counterparts (<100 g). However, protein content in *C. reba* of both size groups during January-April was higher (P<0.01) than the fish of other seasons of the year. Moreover, the larger fish (>100 g) sampled during May-August showed higher (P<0.01) protein content than the larger fish sampled during September-December. Fat content was more (P<0.01) in *C. reba* of >100 g size group during September to December. Ash content was similar in the groups according to the body weight and seasons.

The moisture content of *P. javanicus* was higher (P<0.01) in fish of <100 g size group during May-August whereas the lowest (P<0.01) moisture content was recorded in fish of >100 g size group during September-December (Table 4). Protein, fat and ash contents were higher (P<0.01) in fish of >100 g size group during September-December. Moisture content in *O. bimaculatus* fish (<100 g size group) was more (P<0.01) during May-August and September-December. Protein content was the highest (P<0.01) in both groups of *Ompok* during S_1 (January-April). The highest (P<0.01) fat content was recorded in fish of >100 g size group during January-April month. Total ash was also higher (P<0.01) in both groups during January-April and September-December.

The moisture content *M. vitattus*. was higher (P<0.01) in fish of <15 g size group during S_2 (May-August). Protein content was the highest (P<0.05) in

Table 3. Proximate composition (on % wet weight basis) of *Cirrhinus reba* in different seasons

Particular	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<100g)	Big (>100g)	Small (<100g)	Big (>100g)	Small (<100g)	Big (>100g)	
Moisture	71.99^{b}±0.53	69.52^{a}±0.29	73.29^{bc}±0.40	73.95^{bc}±0.17	74.43^{c}±0.40	74.72^{c}±0.18	0.0002
Protein	17.07^{cd}±0.55	18.61^{d}±0.31	15.05^{ab}±0.39	15.44^{bc}±0.68	$14.0^{ab}8$±0.25	13.31^{a}±0.19	0.0005
Fat	3.07^{a}±0.15	3.14^{a}±0.44	3.95^{a}±0.34	4.42^{a}±0.83	5.15^{ab}±0.48	6.79^{b}±0.34	0.0001
Ash	2.56^{b}±0.07	3.19^{c}±0.07	2.24^{ab}±0.05	2.46^{ab}±0.10	2.24^{ab}±0.06	2.22^{a}±0.06	0.0003

[a,b,c]Means bearing different superscripts in a row differ significantly

Table 4. Proximate composition (on % wet weight basis) of *Puntius javanicus* in different seasons

Particular	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<100g)	Big (>100g)	Small (<100g)	Big (>100g)	Small (<100g)	Big (>100g)	
Moisture	73.80^{b}±1.06	72.78^{b}±0.65	77.28^{c}±0.29	73.73^{b}±0.53	75.27^{bc}±0.46	65.75^{a}±1.10	0.0001
Protein	14.72^{ab}±0.60	15.33^{bc}±0.34	13.09^{a}±0.25	15.21^{bc}±0.33	13.90^{ab}±0.25	17.14^{c}±0.87	0.0001
Fat	3.03^{a}±0.22	4.46^{ab}±1.10	3.28^{a}±0.18	4.32^{ab}±0.16	5.54^{b}±0.56	9.40^{c}±0.76	0.0002
Ash	2.14^{a}±0.07	2.06^{a}±0.09	2.41^{a}±0.05	2.43^{a}±0.06	2.50^{a}±0.07	3.05^{b}±0.27	0.0003

^{a,b,c}Means bearing different superscripts in a row differ significantly

Mystus of both size group during the first season of the year *i.e.,* January-April (Table 6). The fat content was more (P<0.01) in fish of >15 g size group during S_2 (May-August). Total ash content was higher (P<0.01) in both groups during S_1 (January-April) and in >15 g size group during S_2 (May-August).

Moisture content *C. striata* was higher in fish of >500 g size group during S_3 (September-December) in comparison to fish of >500 g during both January-April and May-August. However, fish of >500 g size group during both January-April showed lower moisture content than the fish of <500 g during both April-August and September-December (Table 7). Protein content was more (P<0.01) during May-August in *Channa* of >500 g size group. Fat content was higher (P<0.01) in fish of >500 g size group during January-April. Ash content was also higher in *Channa* of >500 g size group during January-April. However, <500 g size group of channa also showed higher (P<0.01) ash content in S_1 (January-April) and S_2 (May-August).

Moisture contents of fish of <1000 g size group of *W. attu* was the highest (P<0.01) during January-April. Protein content was higher in fish of >1000 g during second season *i.e.,* May-August. Fat and ash content was similar in both groups during the year. The proximate composition of *P. hypophthalamus* has been given in Table 9. The moisture content was higher (P<0.01) in big sized (>1000 g) fish during May-August and small sized (<1000 g) fish during September-December. Protein content was higher (P<0.01) in small sized (<1000 g) fish during the first and second season of the year *i.e.* January-April and May-August. Big sized (>1000 g) *Pangas* exhibited higher (P<0.01) fat content during S_3 (September-December). Ash content was higher (P<0.01) in species of both the groups during January-April.

Moisture content of the fish in the present findings ranged from 64.53 to 77.34 which was within the reported values in IMC (Joseph *et al.,* 1990; Shakir *et al.,* 2013 and Paul *et al.,* 2016). However, Sankar and Ramachandran (2001) reported higher moisture content (77-81%) in IMC than our findings. The

Table 5. Proximate composition (on % wet weight basis) of *O. bimaculatus* in different seasons

Particular	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<100g)	Big (>100g)	Small (<100g)	Big (>100g)	Small (<100g)	Big (>100g)	
Moisture	74.86^{bc}±0.99	70.28^{a}±1.70	76.25^{c}±0.61	75.73^{bc}±0.40	77.34^{c}±0.16	72.39^{ab}±0.70	0.0002
Protein	15.97^{c}±0.79	15.87^{c}±0.75	13.79^{ab}±0.49	13.45^{ab}±0.24	12.71^{a}±0.05	14.34^{b}±0.45	0.0001
Fat	3.02^{a}±0.36	6.64^{c}±0.38	4.44^{b}±0.26	3.63^{ab}±0.21	3.02^{a}±0.18	4.20^{ab}±0.23	0.0004
Ash	1.82^{a}±0.07	2.45^{b}±0.12	1.78^{a}±0.07	2.09^{ab}±0.08	2.50^{b}±0.20	2.32^{ab}±0.08	0.0002

^{a,b,c}Means bearing different superscripts in a row differ significantly

Table 6. Proximate composition (on % wet weight basis) of *Mystus vittatus* in different seasons

Particular	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<15g)	Big (>15g)	Small (<15g)	Big (>15g)	Small (<15g)	Big (>15g)	
Moisture	$70.00^{a}\pm0.76$	$70.03^{a}\pm0.32$	$74.18^{b}\pm0.93$	$71.60^{ab}\pm0.53$	$73.70^{ab}\pm0.45$	$72.71^{ab}\pm0.92$	0.0003
Protein	$19.19^{b}\pm0.45$	$18.36^{b}\pm0.27$	$13.89^{a}\pm0.45$	$14.50^{a}\pm0.28$	$14.29^{a}\pm0.22$	$14.95^{a}\pm0.35$	0.0004
Fat	$5.63^{a}\pm0.36$	$5.76^{a}\pm0.25$	$7.24^{ab}\pm0.52$	$8.47^{b}\pm0.38$	$6.33^{a}\pm0.25$	$7.07^{ab}\pm0.20$	0.0004
Ash	$2.99^{b}\pm0.09$	$2.87^{b}\pm0.09$	$2.57^{ab}\pm0.13$	$2.94^{b}\pm0.19$	$2.50^{ab}\pm0.08$	$2.08^{a}\pm0.09$	0.0015

[a,b]Means bearing different superscripts in a row differ significantly

moisture content of *C. striata*, *O. bimaculatus* and *W. attu* in the present study was similar to Magur and Singhi as reported by Paul *et al.* (2015) and Pal and Ghosh (2013). Moisture content of *Anabas testudineus* (68.00%) reported by Paul *et al.* (2017) was similar to our findings.

In the present investigations, CP content of fish was in the range of 12.52 to 19.19% which was in agreement with protein levels for carp (FAO, 2008). Hossain *et al.* (2015) reported that protein content in small indigenous fishes ranged from 14.29 to 17.95%. In agreement with our findings, Chakraborty *et al.* (2015) reported that the protein content of *Amblypharyngodon mola* was 18.31%. Protein content of *C. reba* (19.74%) as reported by Mridha *et al.* (2005) was higher than our findings. The protein content of these fish species in the present study were in agreement with the protein content of *A. testudineus* (16.91%) as reported by Paul *et al.* (2017).

The fat content of nine freshwater fish was higher than the fat content of Indian major carps (Paul *et al.,* 2016). The fat content of freshwater fish ranged from 2.09 to 7.17%. There is an inverse relationship between moisture and lipid content of fish tissue (Jankowska *et al.,* 2007; Dempson *et al.,* 2008) as observed in fat and moisture content of *M. vittatus* and *P. hypophthalmus*. Our finding on fat contents are similar to those reported in magur and singhi (Paul *et al.,* 2015), small indigenous fish species of Bangladesh (Mazumder *et al.,* 2008), freshwater eel (Pal and Ghosh, 2013) and *Anabas testudineus* (Paul *et al.,* 2017). Shakir *et al.* (2013) also observed that crude fat content in Indian major carps varied from 1.75 to 2.71%. Begum and Minar (2012) found that crude fat content in small and large carp fishes of Bangladesh was 3-4%. The similar concentration of lipid in small indigenous fish in Bangladesh was found (Ahmed *et al.,* 2012).

The ash content of freshwater fish ranged from 1.37 to 9.40%. Our results are in agreement with earlier reports (Sankar, 2001; Mazumder *et al.,* 2008; Paul *et al.,* 2016 and 2015; Bogard *et al.,* 2015; Chrisolite *et al.,* 2015). In the present study, the protein

Table 7. Proximate composition (on % wet weight basis) of *C. striata* in different seasons

Particular	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<500g)	Big (>500g)	Small (<500g)	Big (>500g)	Small (<500g)	Big (>500g)	
Moisture	$75.68^{abc}\pm0.58$	$74.66^{a}\pm0.34$	$76.76^{bc}\pm0.39$	$75.06^{ab}\pm0.38$	$76.67^{bc}\pm0.22$	$77.19^{c}\pm0.35$	0.0009
Protein	$15.61^{cd}\pm0.58$	$14.95^{bc}\pm0.27$	$14.11^{ab}\pm0.50$	$16.95^{d}\pm0.72$	$13.43^{ab}\pm0.19$	$12.85^{a}\pm0.21$	0.0004
Fat	$1.77^{b}\pm0.31$	$2.68^{d}\pm0.18$	$1.37^{a}\pm0.12$	$2.31^{c}\pm0.20$	$1.89^{b}\pm0.13$	$2.50^{cd}\pm0.32$	0.0002
Ash	$2.39^{b}\pm0.10$	$2.48^{b}\pm0.05$	$2.55^{b}\pm0.09$	$2.37^{ab}\pm0.11$	$2.08^{ab}\pm0.07$	$1.98^{a}\pm0.07$	0.0002

[a,b,c]Means bearing different superscripts in a row differ significantly

Table 8. Proximate composition (on % wet weight basis) of *Wallago attu* in different seasons

Particular	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<1000g)	Big (>1000g)	Small (<1000g)	Big (>1000g)	Small (<1000g)	Big (>1000g)	
Moisture	76.78^{b}±0.57	74.50^{ab}±0.82	76.27^{ab}±0.61	71.86^{a}±1.22	73.19^{ab}±1.44	73.16^{ab}±0.58	0.0016
Protein	12.52^{a}±0.21	14.40^{bc}±0.59	12.96^{ab}±0.43	15.23^{c}±0.30	14.57^{bc}±0.67	14.72^{bc}±0.28	0.0008
Fat	3.49±0.09	4.13±0.41	3.58±0.23	3.61±0.35	4.11±0.78	2.91±0.09	0.1889
Ash	2.13±0.06	1.81±0.09	1.94±0.04	1.91±0.12	1.82±0.21	2.09±0.14	0.3792

[a,b]Means bearing different superscripts in a row differ significantly

and ash contents of *P. hypophthalmus* were similar to that of Thai Pangas whereas fat content was lower and moisture content was higher than Thai Pangas (Bogard *et al.,* 2015). The fat and ash content of *C. striata* in the present study was higher than shoal whereas protein content was lower than shoal (18.70%) (Bogard *et al.,* 2015). However, the protein content of *C. striata* in our study was similar to earlier study in the same species (Chrisolite *et al.,* 2015). The protein and ash content of *M. vittatus* was similar to tangra whereas fat and moisture content was lower than tangra (Bogard *et al.,* 2015). Hossain *et al.* (2015) reported that *Puntius puntio, Anabas testudineus* and *Channa punctata* contained more than 3% of ash which was also similar as observed in this study. In corroboration with our findings, Chakraborty *et al.* (2015) reported that the ash content of *Channa gachua* and *Channa punctatus* was 1.88 and 2.01%, respectively. The proximate composition of 9 fishes in our study was also similar to that reported by Mazumdar *et al.* (2008) and Sharma *et al.* (2009).

The study provided a base line data on the proximate composition of 9 indigenous freshwater fishes round the year suggesting greater variation in protein and fat content of muscle tissue. Highest fat content for *Bata* and *Channa* was recorded in January-April. However, *reba, Puntius* and *Pangas* showed higher fat content in second season of the year *i.e.,* May-August. The results were in agreement with the findings of Chrisolite *et al.* (2015) in sardine fish. Protein content of *Bata, Calbasu, reba, Puntius, Ompok* and *Mystus* was higher in January-April. However, moisture content was found to be lower during that period. It was observed that *Channa* and *Wallago* have higher protein content during May-August. However, moisture content was higher in September-December and January-April for both of the species, respectively.

CONCLUSION

Thus, it might be concluded that Indian freshwater fish are rich in protein, fat and ash and thus qualify as health food though there were some seasonal variations in proximate composition within the fish

Table 9. Proximate composition (on % wet weight basis) of *P. hypophthalmus* in different seasons

Particulars	S_1 (January-April)		S_2 (May-August)		S_3 (September-December)		P value
	Small (<1000g)	Big (>1000g)	Small (<1000g)	Big (>1000g)	Small (<1000g)	Big (>1000g)	
Moisture	64.53^{a}±0.68	72.59^{bc}±0.66	67.47^{ab}±1.35	74.07^{c}±0.44	74.88^{c}±0.66	68.77^{ab}±0.94	0.0001
Protein	17.42^{c}±0.22	14.39^{ab}±0.42	17.83^{c}±0.77	14.25^{ab}±0.19	13.03^{a}±0.38	16.16^{bc}±0.59	0.0001
Fat	7.32^{a}±1.13	5.12^{a}±0.36	8.44^{a}±1.07	6.27^{a}±0.62	6.37^{a}±0.68	13.15^{b}±0.50	0.0001
Ash	2.70^{b}±0.19	2.43^{b}±0.11	1.52^{a}±0.08	1.73^{a}±0.12	1.55^{a}±0.14	1.33^{a}±0.05	0.0002

[a,b,c]Means bearing different superscripts in a row differ significantly

species.

ACKNOWLEDGEMENTS

This work was supported by Ministry of Agriculture and Farmers Welfare, Government of India under ICAR Outreach Activity on Nutrient Profiling and Evaluation of fish as a Dietary component. The authors greatly acknowledge the help of DDG (Fisheries, ICAR) and Director, CIFA for providing necessary support and facility to conduct the work.

REFERENCES

Ahmed, S., Rahman, A.F.M.A., Mustafa, M.G., Hossain, M.B. and Nahar, N. 2012. Nutrient composition of indigenous and exotic fishes of rainfed waterlogged paddy fields in Lakshmipur, Bangladesh. *World J. Zool.* 7: 135-140.

AOAC. 1995. *Official methods of Analysis.* 16th edn. Association of Official Analytical Chemists, Washington, DC., USA.

Bamji, M.S. 2011. Can a malnourished nation become a sporting nation? *Curr. Sci*. 101: 602-604.

Begum, M. and Minar, M.H. 2012. Comparative study about body composition of different sis, shell fish and ilish: commonly available in Bangladesh. *Trends in Fish Res*. 1: 38-42.

Bogard, J.R., Thilsted, S.H., Marks, G.C., Wahab, M.A., Hossain, M.A.R., Jakobsen, J. and Stangoulis, J. 2015. Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. *J. Food Comp. Analysis* 42: 120-133.

Boran, G. and Karacam, H. 2011. Seasonal changes in proximate composition of some fish species from black sea. *Turk J. Fish Aquatic Soc.* 11: 1-5.

Chakraborty, S., Brahma, B.K. and Goyal, A.K. 2015. Proximate composition of three Small indigenous fish species encountered in the local fish market of Kokrajhar, BTAD, Assam. *Indian J. Appl. Res.* 5: 712-714.

Chrisolite, B., Shanmugam, S.A. and Arumugam, S.S. 2015. Proximate and mineral composition of fifteen freshwater fishes of Toothukudi, Tamilnadu. *J. Aqua Tropics* 30: 33-43.

Dempson, I.B., Schwarz, C.J., Shears, M. and Furey, G. 2004. Comparative proximate body composition of Atlantic salmon with emphasis on part from fluvial and lacustrine habitats. *J. Fish Biol*. 64: 1257-1271.

Deka, K.B., Mahanta, R. and Goswani, U. 2012. Seasonal variation of protein and essential amino acid contents in *Labeo gonius* from Lotic and Lentic water bodies. *World J. Sci. Med. Res*. 2: 71.

FAO. 2008. *FAO Fisheries and Aquaculture- Chemical Elements of Fish.* http:// www.fao.org/fishery/topics/14820/en.

Hossain, Md. N., Afroz, H., Haque, Md. Z. and Begum, M. 2015. Evaluation of nutritional properties of small indigenous fish species in Bangladesh. *Int. J. Biosci.* 6: 102-109.

Jankowska, B., Zakes, Z., Zmijewski, T., Szczepkowski, M. and Kowalska, A. 2007. Slaughter yield, proximate composition and flesh colour of cultivated and wild perch *(Perca fluviatilis* L.). *Food Chem*. 118: 764-768.

Joseph, J., Perigreen, P.A., Surendran, P.K. and Gopakumar, K. 1990. I.I.F-I.I.R, Commission 2: 117, Aberdeen, U.K.

Louka, N., Juhel, V., Fazilleau, V. and Loonis, P. 2004. A novel colorimetry analysis used to compare different drying fish processes. *Food Control* 15: 327-334.

Mazumder, M.S.A., Rahman, M.M., Ahmed, A.T.A., Begum, M. and Hossain, M.A. 2008. Proximate composition of some small indigenous fish species (SIS) in Bangladesh. *Int. J. Sustainable Crop Prod*. 3: 18-23.

Mohanty, B.P., Ganguly, S., Mahanty, A., Mitra, T., Patra, S., Karunakaran, D., Mathew, S., Chakraborty, K., Paul, B.N., Sarma, D., Dayal, S., Singh, S. and Ayyappan, S. 2019. Fish in human health and nutrition. In: *Advances in Fish Research*. (B.P. Mohanty; ed.). Narendra Publishing House, Delhi, India. Vol: VII. pp 189-218.

Mohanty, B.P., Mahanty, A., Ganguly, S., Sankar, T.V., Chakraborty, K., Anandan, R., Paul, B.N., Sarma, D., Mathew, S., Asha, K.K., Behera, B.K., Afftabuddin, M., Debnath, D., Vijaygopal, P., Sridhar, N., Akhtar, M.S., Sahi, N., Mitra, T., Banerjee, S., Paria, P., Das, D., Das, P., Vijayan, K.K., Lamanan, P.T., Sharma, A.P. 2014. Amino acid composition of 27 Food fishes and their importance in clinical nutrition. *J. Amino Acid*. 1: 1-7. Doi.org/10.1155/2014/269797

Mridha, M.A., Lipi, S.Y., Narejo, N.T., Uddin, M.S., Kabir, M.S. and Karim, M. 2005. Determination of biochemical composition of *Cirrhinus reba* (Hamilton 1822) from Jessore, Bangladesh. *J. Sci. Technol. Univ. Peshawar*. 29: 1-5.

Pal, M. and Ghosh, M. 2013. Assay of biochemical composition of two Indian freshwater eel with special emphasis on accumulation of toxic heavy metals. *J. Aqua Food Product Technol.* 22: 27-35.

Paul, B.N., Bhowmick, S., Chanda, S., Sridhar, N., Saha, G.S. and Giri, S.S. 2018. Nutritional values of minor carps. *SAARC J. Agric*. 16: 215-231.

Paul, B.N., Chanda, S., Bhowmick, S., Sridhar, N., Saha, G.S. and Giri, S.S. 2017. Nutrient Profile of Indian Climbing Perch, *Anabas testudineus*. *SAARC J Agricult*. 15: 99-109.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S. and Giri, S.S. 2016. Proximate, mineral and vitamin contents of Indian major carp. *Indian J. Anim Nutr*. 33: 102-107.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S. and Giri, S.S. 2015. Proximate and mineral composition of Magur *(Clarias batrachus*) and Singhi *(Heteropneustes fossilis*). *Indian J. Anim Nutr*. 32: 453-456.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S. and Giri, S.S. 2015a. Fatty acid profile of Indian major carp. *Indian J. Anim Nutr*. 32: 221-226.

Sankar, T.V. and Ramachandran, A. 2001. Changes in biochemical composition in Indian major carps in relation to size. *Fish Technol.* 38: 22-27.

Sankar, T.V., Susheela, M., Anandan, R., Asha, K.K., Mohanty, B.P. 2010. *Nutrient Profiling of Fish.* ICAR-Central Institute of Fisheries Technology, Handbook, Cochin, Kerala, India. pp 1-61.

Shakir, H.A., Qazi, J.I., Chaudhary, A.S., Hussain, A. and Ali, S. 2013. Nutritional comparison of three fish species co-cultured in an earthen pond. *Biologia.* 59: 353-356.

Sharma, P., Kumar, V., Sinha, A.K., Ranjan, J., Kithsiri, H.M.P. and Venkateshwarlu, G. 2009. Comparative fatty acid profiles of wild and farmed tropical freshwater fish rohu *(Labeo rohita*). *Fish Physiol. Biochem.* 36: 365-379.

Silva, J.J. and Chamul, R.S. 2000. Composition of marine and fresh water finfish and shellfish species and their products. In: *Marine and Fresh Water Handbook* (R.E Martin, E. Paine Carter, E.J. Flic and L.M.DaviS; eds.). Technomic Publishing Company Inc, USA. pp 31-46.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* Oxford and IBH Publishing Company, Calcutta, India.

WHO. 2007. Protein and amino acid requirement in human nutrition: *Report of a Joint WHO/FAO/UNO Expert Consultation.* WHO Technical Report Series. World Health Organisation, Geneva, Switzerland.

Received on 10-12-2018 and accepted on 04-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 73-76
doi: 10.5958/2231-6744.2019.00012.4

Indian Journal of Animal Nutrition

Effect of Supplementation of L- Threonine on Growth Performance and Carcass Characteristics in Japanese Quails

Pramod Namdeo, S.P. Tiwari, Raina Doneria*, Meenu Dubey and Rajkumar Yadav
Department of Animal Nutrition, College of Veterinary Science & A.H., Anjora, Durg, Chhatisgarh, India

ABSTRACT

Day old Japanese quail chicks (n = 150) were randomly allocated to 5 groups with three replicates of 10 birds in each. Total five isocaloric diets (2900 kcal ME/kg feed) were prepared containing 24, 23, 22, 21 and 20% CP for the groups T_1 T_2, T_3, T_4 and T_5, respectively. L-threonine was supplemented in diet of each group at the rate of 0.0, 0.05, 0.1, 0.15 and 0.2% in the diet of groups T_1 T_2, T_3, T_4 and T_5, respectively. Body weight at 28 d was higher ($P<0.01$) in group T_3 as compared to other groups. The feed intake was similar in all the groups. The FCR was better in group T_3 where L-threonine was supplemented @ 0.1% in the diet. Supplementation of threonine resulted in increase ($P<0.05$) in the weight of thigh, however, other carcass traits did not differ among the groups. Hence Japanese quail could be raised on lower CP diet (22%) with 0.1% L-threonine supplementation to achieve better body weight gains and FCR without affecting carcass characteristics

Key words: Growth performance, Japanese quail, L-threonine, Supplementation

INTRODUCTION

Recently, Japanese quails began to attract the interest of quail producers with the perspective of reaching a different range of the consumer market and to enable the productive exploitation of birds to augment meat production. Quails are able to withstand environmental constraints due to their inherent ability to grow under natural conditions. Protein and amino acids play important role in least cost feed formulations for poultry. Progressive reduction in crude protein (CP) content can induce a situation in which other amino acids such as threonine become limiting to support better performance (Ramalho de Lima *et al.,* 2013). Now a days, crystalline amino acids are available commercially that can be added to the feed in specific quantities and quality of the feed protein can be enhanced and reduction of crude protein in diet is possible. As threonine is the third limiting amino acid in low CP based diets for poultry after methionine and lysine (Kidd and Kerr, 1996), therefore, by using L- threonine further reduction in dietary CP beyond requirement is possible (Abbasi *et al.,* 2014). A lot of research on amino acid requirements in chicken has been conducted but the quails have received less attention. Hence, the present study was conducted to see the effect of dietary supplementation of L- threonine on growth performance and carcass characteristics in Japanese quails.

MATERIALS AND METHODS

Day old Japanese quail chicks (n=150) were randomly divided into 5 groups, each group had 3 replicates of 10 chicks in each. The chicks were reared under deep litter system. Total five isocaloric diets (2900 kcal ME/kg feed) were prepared containing 24, 23, 22, 21 and 20% CP for the groups T_1 T_2, T_3, T_4 and T_5, respectively. L-threonine was supplemented in diet of each group at the rate of 0.0, 0.05, 0.1, 0.15 and 0.2% in the diet of groups T_1 T_2, T_3, T_4 and T_5, respectively. The total concentration of L-threonine in the diet was 1.001, 1.013, 1.017, 1.024 and 1.041% in the respective groups. Diets were prepared using conventional feed ingredients (Table 1).

Growth performance of the birds was recorded in terms of weight gain, feed consumption and feed conversion ratio from 0 to 28 days of age. Feed intake was recorded weekly. Chicks were weighed individually at weekly intervals and the average gain in body weight under each dietary treatment was calculated. Feed conversion ratio (FCR) was calculated as kg feed intake/kg gain. On day 28, 3 birds from each replicate were sacrificed for evaluation of carcass

*Corresponding author: E-mail: raina.doneria@gmail.com

Table 1. Ingredient composition (%) of Japanese quails (0-28 d) diet

Feed ingredient	Group				
	T_1	T_2	T_3	T_4	T_5
Yellow maize	50.00	49.5	51.4	52.25	53
Soya DOC	43.50	40	37	33.5	30.4
De oiled rice bran	2.00	5.8	7	9.5	12
Soya Oil	1.55	1.75	1.65	1.8	1.65
DCP	1.00	1.00	1.00	1.00	1.00
LSP	1.00	1.00	1.00	1.00	1.00
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.05	0.05	0.05	0.05	0.05
Feed premix*	0.80	0.80	0.80	0.80	0.80
L-threonine supplemented (%)	0	0.05	0.1	0.15	0.2
L-threonine present in feed	1.001	0.969	0.926	0.874	0.844
Overall L-threonine (%)	1.001	1.013	1.017	1.024	1.041

Trace mineral premix mg/kg diet: Mg 300, Mn 55, Fe 56, Zn 30, Cu 4; vitamin premix (per kg diet) : vit. A 8250IU, vit. K 1mg, vit.E 26.84 mg, vit. B1 2 mg,vit. B2 4 mg, vit. B12 100µg, Niacin 60 mg, pantothenic acid 10 mg; choline 500 mg and 30 ppm salinomycin (Coxistac 12%), 55 ppm bacitracin methylene di salicyclate (BMD110)

characteristics. The diets were analysed for proximate principles, Ca and P contents (AOAC, 2005). Data were subjected to one way ANOVA (Snedecor and Cochran, 1994) using SPSS package (SPSS ver. 10.0).

RESULTS AND DISCUSSION

The chemical composition of different diets has been given in Table 2. Overall, the body weight at 4 week was higher ($P<0.05$) in quails fed diet containing 22% CP with threonine @ 0.10% (Table 3). Further increase in the level of threonine with reduced protein did not have any significant effect on body weight. The findings are in accordance with Shi *et al.* (2010) who reported that increase in dietary threonine resulted in an increase and then a decrease in body weight in Yangzhou geese. This pattern indicated that excess threonine might cause an imbalance in the amino acid profile of the diet which altered the body metabolism. Improved body weight gain in broiler with increase in L-threonine level has also been reported by Rezaeipour *et al.* (2012) and Estalkhzir *et al.* (2013). In contrast, Ayasan *et al.* (2011) reported that threonine supplementation had no significant difference on body weight gain but a quadratic response was observed in final body weight of broiler.

Dietary supplementation of threonine did not influence feed intake in quails (Table 3) as also reported

Table 2. Nutrient composition (% DM basis) of Japanese quail diet

Particular	Group				
	T_1	T_2	T_3	T_4	T_5
Moisture	9.74	9.43	9.32	9.35	9.20
CP	24.02	23.01	22.03	21.01	20.02
EE	3.08	3.79	2.35	3.05	3.97
CF	4.51	5.04	4.60	4.98	5.06
Ca	0.89	0.82	0.85	0.86	0.81
P	0.34	0.46	0.43	0.39	0.30
ME (kcal/kg)	2904	2902	2909	2902	2907

Table 3. The effect of L-threonine supplementation on feed intake, body weight and feed conversion ratio in Japanese quails

Group	Cumulative feed intake (g/bird)				Cumulative body wt (g)				Feed conversion ratio			
	7 d	14 d	21 d	28 d	7 d	14 d**	21 d	28 d*	7 d	14 d*	21 d	28 d
T_1(0%	46.71	160.0	315.99	488.95	28.18	64.84[b]	106.9	139.30[b]	3.96	2.47[a]	2.96	3.51
L-threonine)	±1.27	±2.14	±5.79	±3.98	±1.62	±1.61	±1.81	±2.65	±0.18	±0.03	±0.04	±0.04
T_2(0.05%	48.11	166.9	329.57	505.88	28.08	64.03[b]	104.0	138.0[b]	3.95	2.61[a]	3.19	3.67
L- threonine)	±1.06	±4.78	±8.67	±5.75	±1.20	±1.19	±4.81	±1.88	±0.03	±0.07	±0.22	±0.13
T_3(0.10%	48.11	160.06	316.37	502.59	32.39	74.44[a]	118.2	151.6[a]	3.45	2.15[b]	2.68	3.31
L- threonine)	±0.56	±5.46	±8.45	±7.94	±1.01	±1.05[a]	±2.04	±2.63	±0.11	±0.06	±0.03	±0.03
T_4(0.15%	47.50	158.2	310.1	487.3	28.94	64.14[b]	104.1	137.1[b]	3.87	2.48[a]	2.99	3.56
L- threonine)	±1.66	±4.72	±7.49	±7.41	±2.23	±2.26	±3.86	±3.47	±0.35	±0.16	±0.19	±0.19
T_5(0.2%	45.48	161.09	314.02	505.25	26.13	64.62[b]	106.0	130.3[b]	4.09	2.50[a]	2.97	3.90
L- threonine)	±0.66	±3.22	±8.82	±21.16	±0.51	±2.11	±5.06	±470	±0.08	±0.04	±0.11	±0.31

[a,b] Means bearing different superscripts in a column differ significantly (*P< 0.05; **P< 0.01)

by previous researchers (Dozier *et al.* 2000, 2001; Ayasan *et al.* 2011; Mazraeh *et al.,* 2013). Feed conversion ratio was similar in different groups on first, third and fourth week, however, it was better (P<0.05) in group T_3 at 2nd week which might be due to increased nutrient utilization in this group though the CP level was below the recommended level in the diet. The findings corroborated with those of Rezaeipour *et al.* (2012) and Dozier *et al.* (2000). Similarly, significant effect on feed/gain ratio has been reported in geese (Shi *et al.,* 2010) in quails (Abdel-wareth *et al.,* 2014) when threonine was supplemented in the diet.

The dressing percent weight of internal organs and various cuts of Japanese quails were similar in different groups. Weight of thigh in terms of percent live weight was higher (P<0.01) in group T_5 as compared to groups T_3 and T_4, however, weights of heart, liver, gizzard, back and neck, breast and wing were similar in different grousp (Table 4). The observations were in accordance with the findings of Shayan *et al.* (2013) who reported that dressing percentage, weights of internal organs were not affected by reducing CP in quail diets. Similarly, Baylan *et al.* (2006) reported that dietary threonine in the feed did not influence chilled

Table 4. Effect of L-threonine supplementation on various carcass cuts of Japanese quails as percentage of live weight at day 28

Group	Weight of carcass cuts as percentage of live weight									
	Live wt.	Dressed wt.	Liver	Heart	Gizzard	Breast	Thigh	Wing	Back and neck	Giblet
T_1(0%	141.38	70.00	2.11	0.94	2.10	10.31	17.03[a]	22.80	14.80	5.15
L-threonine)	±8.03	±0.73	±0.19	±0.06	±0.19	±0.40	±0.34	±0.72	±0.94	±0.38
T_2(0.05%	136.00	70.67	2.00	0.93	2.07	11.99	17.08[ab]	23.21	12.88	4.99
L- threonine)	±5.08	±1.21	±0.14	±0.08	±0.02	±0.65	±0.40	±0.52	±0.95	±0.21
T_3(0.10%	149.88	69.83	2.40	1.17	2.12	11.92	16.43[b]	23.21	13.67	5.69
L- threonine)	±3.03	±0.34	±1.31	±0.12	±0.10	±0.10	±0.19	±0.87	±0.26	±0.39
T_4(0.15%	140.00	69.82	1.86	1.00	1.92	11.57	16.84[b]	23.30	12.91	4.81
L- threonine)	±7.69	±0.85	±0.19	±0.04	±0.16	±1.08	±0.23	±0.58	±0.28	±0.13
T_5(0.2%	142.00	69.75	2.10	1.01	2.12	10.87	16.94[b]	23.38	13.30	5.23
L- threonine)	±5.40	±0.49	±0.13	±0.04	±0.16	±0.55	±0.27	±0.61	±1.09	±0.15

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

carcass weight nor its relative yield in quail. Similar findings were also reported in broilers (Rezaeipour *et al.,* 2012; Dozier *et al.,* 2000). Shi *et al.,* (2010) observed no significant linear or quadratic responses for carcass traits in geese. Kerr *et al.* (1999) also reported that carcass yield was unaffected by dietary threonine supplementation. Increased thigh weight due to supplementation of threonine as reported in the experiment corroborated with the findings of Kerr *et al.* (1999) and Dozier *et al.* (2000).

CONCLUSION

It is concluded that Japanese quails could be raised by feeding lower CP diet (22%) with 0.1% L-threonine supplementation to achieve better performance in terms of body weight and feed conversion ratio without affecting carcass characteristics.

REFERENCES

Abbasi, M.A., Mahdavi, A.H., Samie, A.H. and Jahanian, R.I. 2014. Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. *Br. J. Poult. Sci.* 16: 35-44.

Abdel-wareth, A.A.A. and Esmail, Z.S.H. 2014. Some productive, egg quality and serum metabolic profile responses due to L-threonine supplementation to laying hen diets. *Asian J. Poult. Sci.* 8: 75-81.

AOAC. 2005. *Officials Methods of Analysis*. 18th rev. edn. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Ayasan, T. and Okan, F. 2011. Threonine requirement of female broilers from 22 to 42 days of age. *Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi*. 6: 15-21.

Baylan, M., Canogullari, S., Ayasan, T. and Sahin, A. 2006. Dietary threonine supplementation for improving growth performance and edible carcass parts in Japanese quails, *Coturnix coturnix Japonica*. *Int. J. Poult. Sci.* 5: 635-638.

Dozier, W.A., Moran, E.T. and Kidd, M.T. 2000. Threonine requirement of broiler males from 42 to 56 days in a summer environment. *J. Appl. Poult. Res.* 9: 496-500.

Dozier, W.A., Moran, E.T. and Kidd, M.T. 2001. Male and female broiler responses to low and adequate threonine on nitrogen and energy balance. *Poult. Sci.* 80: 926-930.

Estalkhzir, M.F., Khojasteh, S. and Jafari, M. 2013. The effect of different levels of threonine on performance and carcass characteristics of broiler chickens. *J. Appl. Sci.* 2: 382-386.

Kerr, B.J., Kidd, M.T., McWard, G.W. and Quarles, C.L. 1999. Interactive effects of lysine and threonine on live performance and breast yield in male broilers. *J. Appl. Poult. Res.* 8: 391-399.

Kidd, M.T. and Kerr, B J. 1996. L-threonine for poultry: A review. *J. Appl. Poult. Res.* 5: 358-367.

Mazraeh, A.A., Shahryar, H.A., Nobar, R.S. and Gorbani, A. 2013. The effects of different levels of L-threonine on growth performance and carcass traits of broiler chickens during phase growth. *Bull. Env. Pharm. Life Sci.* 2:122-124.

Ramalho de Lima, M., Guilherme Perazzo Costa, M., Romao Guerra, R.R, Vilar da Silva, J., Boa-Viagem Rabello, J., Miglino, M.A., Vieira Lobato, G. B., Sena Netto, S B. and Dantas, L.D. 2013. Threonine: lysine ratio for Japanese quail hen diets. *J. Appl. Poult. Res.* 22: 260-268.

Rezaeipour, V., Fononi, H. and Irani, M. 2012. Effects of dietary L-threonine and *Saccharomyces cerevisiae* on performance, intestinal morphology and immune response of broiler chickens. *South Afr. J. Anim. Sci.* 42: 266-273.

Shayan, B.S., Eila, N. and Norozian, H. 2013. The effect of decreased crude protein diets on performance, immune response and carcass traits of Japanese quail chickens. *Annals Biol. Res.* 4: 313-317.

Shi, S. R., Wang, Z. Y., Zou, J.M., Yang, H. M. and Jiang, N. 2010. Effects of dietary threonine on growth performance and carcass traits of Yangzhou geese. *Czech J. Anim. Sci.* 55 (9): 382-387.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. 8th edn. Iowa State University Press, Ames, Iowa, USA.

Received on 11-10-2018 and accepted on 02-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 77-82
doi: 10.5958/2231-6744.2019.00013.6

Indian Journal of Animal Nutrition

Development of Calcium Fortified Biscuits Incorporated with Chicken Slaughter House Byproducts and Evaluation of Their Palatability in Dogs

Khushpreet Singh Virk, Om Prakash Malav*, Manish Kumar Chatli, Nitin Mehta, Pavan Kumar and Rajesh V. Wagh

Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India

ABSTRACT

This study was designed to optimize the level of poultry by-products *viz.,* liver and gizzard along with calcium fortification for the development of dog biscuits. Poultry liver and gizzard were minced, air dried at 60°C for 15 -16 h and ground. Three different levels of poultry liver and gizzard *viz.*, 10, 20 and 30% were incorporated separately in standardized dog biscuits formulation after replacing refined wheat flour. The developed product was analyzed for physico-chemical properties (pH and cooking yield), proximate composition (moisture, protein, fat and ash), mineral content (Ca and P), instrumental color and texture profile analysis. Dicalcium phosphate was incorporated at 2% level in the 30% liver powder incorporated dog biscuits with acceptable physico-chemical and sensory qualities. Among the selected levels, incorporation of liver powder at 30% level was found to be better for the preparation of dog biscuits.

Key words: Dog biscuits, Gizzard, Liver, Sensory evaluation, Texture profile

INTRODUCTION

Large number of poultry birds are slaughtered annually in India leading to production of 2.72 million tonnes of meat (BAHS, 2016-17). It leaves huge loads of slaughter house by-products *viz.*, offals, feathers, blood *etc.* In general, the total by-products range from 5 to 6% of the live weight of chicken. Efficient utilization of these by-products has direct impact on the economy and environmental pollution of the country. Meat by-products are produced by slaughter houses, meat processors, wholesalers and rendering plants. These can be used as feeds for the poultry, fish and pets like dogs and cats. In India, pet food production is mostly cereal based. Slaughterhouse wastes or animal by-products such as liver, lung, kidney, brain, spleen and tripe have high nutritive value and these can be efficiently utilized for the production of pet foods as the animal proteins are the integral part of their diet. Liver is richer source of protein, vitamins and minerals than fresh meat. It is excellent source of readily digestible haeme iron, vitamins particularly riboflavin, niacin, vitamin B_{12} and vitamin A, D, E and K. Gizzard is one of the principal edible byproducts of poultry processing which is being marketed as variety meats along with dressed chicken. Gizzard contains approximately 20% protein and could be of potential use in preparation of dog biscuits. Further, utilization of this byproduct would increase the profitability of broiler industry.

Pets play a significant role as companion animals. There are about 4.0 million pets in the Indian households with the population increasing by 26% every year. Pet food market has huge potential in India with the growth rate of 10-15% per annum. Dogs, cats and other pet food contribute 80, 15 and 5%, respectively to pet food market. Meat and meat products are deficient in calcium. Calcium requirement in pets during peak growth and lactation ranges from 1.0-1.8% of total diet on dry matter basis. Calcium deficiency is very common nutritional disorder among pets. It results primarily from animals fed diets high in meat and organ meats which are high in phosphorous and low in calcium that could lead to metabolic diseases. Therefore, the present study was designed to develop dog biscuits based on the slaughter house byproducts and fortified with calcium.

MATERIALS AND METHODS

Poultry byproducts *i.e.* liver and gizzard required for the experiments were collected from the

*Corresponding author: Email: drommalav@gmail.com

Table 1. Ingredient composition (%) of dog biscuits in control diet

Ingredient	Percentage
Refined wheat flour (maida)	55
Vegetable oil	20
Whole egg liquid	16
Spice mix	3
Sugar	3
Table salt	2
Baking powder	1

Instructional Poultry Processing Plant of Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. After slaughtering poultry birds, poultry liver and gizzard were collected, cleaned and packed in LDPE bags and stored in freezer. The refined soybean oil (Fortune, Adani Wilmar Ltd.),refined wheat flour (maida), common salt (Tata Chemicals Ltd., Mumbai, India), spice mix, chicken eggs, sugar, baking powder (Weikfield) and packaging material (LDPE and LDPE/ polyester/polyethylene laminated plastic bags) were procured from local market of Ludhiana, Punjab, India. Analytical grade chemicals, media and high purity standards required for analyzing the products were procured from different firms (SRL, Fisher Scientific, LobaChemie, Himedia). Formulation and processing protocols of the dog biscuit was standardized on the basis of available literature and various preliminary trials conducted in laboratory. The standardized formulation has been given in Table 1. Poultry liver and gizzard were minced in the meat mincer (Mado Eskimo Mew-714, Mado, Germany). Minced poultry liver and gizzard were air dried at 60°C for 15-16 h in industrial tray dryer and ground.

For the preparation of dog biscuits, first of all, sugar powder was added in paddle mixer followed by vegetable oil and whole egg liquid. All other ingredients left were mixed. Ingredients were used according to formulation and mixed in paddle mixture and dough was prepared by mixing the ingredients. Mixing of dough was done uniformally. Biscuits were made by filling dough in stainless steel mold of different shapes and cooking was done in hot air oven at temperature of 180°C for 10 min. Both liver and gizzard incorporated biscuits were prepared separately. The liver and gizzard powder was incorporated at 10, 20 and 30% levels after replacing the refined wheat flour in the formulation (Table 2).

The pH of finely grounded dog biscuits (10 g of sample was homogenized with 50 mL of distilled water for 1 min. using pestle and mortar) was determined (Trout *et al.,* 1992) with digital pH meter (FE-20-1-KIT, Mettler-Toledo India Pvt. Ltd., Mumbai). The weight of each product was recorded before and after cooking. The cooking yield was calculated and expressed as percentage by a formula:

$$\text{Cooking yield}\,(\%) = \frac{\text{Weight of baked product}}{\text{Weight of raw product}} \times 100$$

The acceptability of dog biscuits was tested (Griffin *et al.,* 2003). In this test, 12 dogs (BW= 4±2 kg) were selected and 100 g of dog biscuits were served to the dogs for 5 days after their regular meals. Food intake was recorded. The same procedure was repeated for other treatment dog biscuits. Under this test, 500 g dog biscuits from each treatment were served to all the dogs.

Hardness and fracturability of dog biscuits was

Table 2. Levels of liver powder and gizzard powder in different groups

Ingredient	Group						
	Control	T_1	T_2	T_3	T_4	T_5	T_6
Refined wheat flour	55	45	35	25	45	35	25
Liver powder	-	10	20	30	0	0	0
Gizzard powder	-	0	0	0	10	20	30

Where, $T_{1=}$ 10% liver powder; T_2=20% liver powder; T_3=30% liver powder; T_4=10% gizzard powder; T_5=20% gizzard powder; T_6=30% gizzard powder

analysed using texture analyzer (TMS-PRO, Food Technology Corporation, USA). Texture parameters hardness (N) and shear force value (kg/cm^2) were determined (Bourne, 1978) and interpreted as follows. Hardness (N) = maximum force required to compress the sample, shear force value (kg/cm^2) = the force required to cut the given product.

The moisture, protein, fat and ash contents of the product were estimated (AOAC, 1995). Color profile was measured using Lovibond Tintometer (Lovibond House, United Kingdom) set at 2^o of cool white light (d_{65}) and known as '*l*', *a*, and *b* values. '*l*' value denotes (brightness 100) or lightness (0), *a* (+ redness/ - greenness), *b* (+ yellowness/-blueness) values were recorded on/in a hundreds of dog biscuit kept in a plate. The experienced panel of seven members evaluated the samples for the sensory attributes *viz.*, color and general appearance, meat odour intensity and overall acceptability using 5-point descriptive scale where 5 represented excellent and 1 as extremely poor. The data were analyzed statistically using SPSS-16.0" (SPSS Inc., Chicago, II USA) software package as per standard methods (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The scores for all the sensory attributes showed increasing (P<0.05) trend with increasing level of chicken liver powder in the formulation whereas the scores for the gizzard powder incorporated dog biscuits showed decrease (P<0.05) with the increasing incorporation level of gizzard powder which might be associated with increase in the dark colour of developed dog biscuits. Appearance scores for group T_3 were higher (P<0.05) than other treatment products which might be due to increase in redness value as shown in instrumental colour analysis. Similarly, there was increasing (P<0.05) trend in colour scores with the increase in level of chicken liver powder but there was decreasing trend noticed on incorporation of gizzard powder. Colour score for group T_3 was higher (P<0.05) than that found in groupsT_1 and T_4 but the colour values for both these treatment products were higher (P<0.05) than in groups T_1, T_5 and T_6. There was increasing (P<0.05) trend in meat flavour intensity scores with the increase in incorporation level of chicken liver powder and gizzard powder. Flavour scores for group T_3 were higher (P<0.05) than other products which might be due higher level of chicken liver powder which was more liked by the sensory panelists. Meat flavour intensity scores for gizzard powder incorporated meat biscuits and that in group T_2 were comparable to each other whereas group T_1 showed the lowest score among the groups.

The sensory scores for all the parameters in the dog biscuits containing 30% chicken liver powder (T_3) were highest among all the treatment products and highly acceptable. The overall acceptability score was higher (P<0.05) for dog biscuits containing 30% chicken liver powder (T_3) compared to the other treatment products which is reflective of scores of other sensory parameters (Table 4). The intake of dog biscuits in groups

Table 3. Effect of different levels of incorporation of liver powder and gizzard powder on the sensory parameters of dog biscuits

Sensory parameter	Liver and gizzard powder level					
	T_1 (10% liver)	T_2 (20% liver)	T_3 (30% liver)	T_4 (10% gizzard)	T_5 (20% gizzard)	T_6 (30% gizzard)
General appearance	2.95^{c}±0.14	3.90^{b}±0.12	4.33^{a}±0.12	3.57^{b}±0.12	3.08^{c}±0.11	2.83^{c}±0.10
Colour	2.82^{c}±0.13	3.66^{b}±0.14	4.19^{a}±0.14	3.43^{b}±0.14	2.99^{c}±0.12	2.73^{c}±0.12
Meat flavour intensity	2.68^{d}±0.12	3.57^{b}±0.16	4.39^{a}±0.13	2.91^{cd}±0.19	3.18^{bc}±0.17	3.21^{bc}±0.17
Overall acceptability	2.84^{c}±0.11	3.71^{b}±0.14	4.48^{a}±0.12	3.10^{c}±0.17	3.13^{c}±0.15	3.21^{c}±0.15

^{a,b,c,d}Means with different superscripts in a row differ significantly (P<0.05); N=21; T_1= 10% liver powder, T_2= 20% liver powder; T_3= 30% liver powder; T_4= 10% gizzard powder, T_5= 20% gizzard powder; T_6= 30% gizzard powder

T_2 and T_3 was the highest. A total of six kg of each treatment biscuit was served to all the dogs. The consumption of biscuits in groups T_2 and T_3 was higher than other groups. The consumption of biscuits in group T_3 was the highest which might be due to the more meat flavour intensity as shown in the sensory analysis of dog biscuits. Similarly Regenstein *et al.* (2003) and Karthikeyan *et al.* (2002) reported that incorporation of fish and animal waste in pet foods supplied high protein feed ingredients and palatability enhancing agents in animal foods. Mahender *et al.* (2013) also reported that incorporation of poultry slaughter waste in dog biscuits results in increased in palatability of dog biscuits by conducting an experiment on adult Labrador dogs.

The results for instrumental colour profile (redness (a^*), yellowness (b^*) and lightness (L) and texture profile (fracturability and shear force value) of all the liver and gizzard powder incorporated dog biscuits is presented in Table 5. Redness (a^* value) is an indicator of freshness of the meat and criteria for

Table 4. Effect of incorporation of liver and gizzard powder on acceptability of biscuits by dogs

Item	Dog No.	Liver and gizzard powder level					
		T_1 (10% liver)	T_2 (20% liver)	T_3 (30% liver)	T_4 (10% gizzard)	T_5 (20% gizzard)	T_6 (30% gizzard)
Amount served/dog (g)		500	500	500	500	500	500
Intake (g)/d/dog)	Dog 1	91.60^{b}	94.00^{ABa}	95.20^{ABCa}	90.80^{ABb}	90.40^{ABb}	88.40^{Ab}
		±2.29	±1.34	±0.80	±2.13	±2.11	±2.98
	Dog 2	$91.80\pm^{b}$	93.00^{BCb}	95.40^{ABCa}	90.80^{AB}	85.60^{BCc}	81.60^{ABd}
		±2.60	±0.70	±1.21	±2.48	±1.63	±1.96
	Dog 3	91.80^{b}	97.60^{Aa}	97.60^{Aa}	91.80^{ABb}	87.80^{ABbc}	83.80^{ABc}
		±2.60	±0.93	±0.93	±2.60	±2.52	±3.99
	Dog 4	91.60^{b}	97.20^{Aa}	97.20^{ABa}	90.00^{ABb}	90.00^{ABb}	82.80^{ABc}
		0±2.29	±1.07	±1.07	±1.60	±1.64	±4.35
	Dog 5	91.80^{b}	94.00^{ABa}	95.80^{ABCa}	91.80^{ABb}	87.80^{ABc}	83.80^{ABd}
		±2.60	±1.34	±1.53	±2.60	±2.52	±3.31
	Dog 6	91.80^{b}	94.60^{ABa}	94.60^{ABCa}	89.80^{AB}	89.80^{ABbc}	81.80^{ABc}
		±2.60	±0.51	±0.51	±1.59	±1.59	±2.78
	Dog 7	94.00^{a}	93.00^{BCa}	93.00^{Ca}	93.40^{Aa}	93.40^{a}	91.40^{Ab}
		±0.71	±0.71	±0.71	±1.08	±1.08	±2.56
	Dog 8	91.80^{b}	94.00^{ABa}	94.00^{BCa}	91.80^{ABb}	86.80^{ABCc}	85.40^{ABc}
		±2.60	±1.34	±1.34	±2.60	±3.35	±3.61
	Dog 9	91.60^{b}	90.00^{CDb}	95.20^{ABCa}	90.00^{ABb}	90.00^{ABb}	84.00^{ABc}
		±2.29	±2.30	±0.37	±1.64	±1.64	±4.76
	Dog 10	89.20^{b}	87.60^{Db}	93.20^{Ca}	85.80^{Bb}	80.80^{Cc}	75.40^{Bd}
		±1.24	±0.93	±1.28	±1.32	±1.93	±1.72
	Dog 11	90.80^{b}	93.00^{BCa}	94.00^{BCa}	90.80^{ABb}	89.80^{ABb}	87.80^{Ab}
		±1.71	±0.71	±0.71	±1.71	±1.36	±3.10
	Dog 12	91.60^{b}	94.00^{ABb}	97.20^{ABa}	91.60^{ABb}	90.00^{ABbc}	86.00^{ABc}
		±2.29	±1.34	±0.97	±2.29	±1.64	±4.32
Average intake (g/d/dog)		91.67^{b}	93.50^{a}	95.20^{a}	90.70^{b}	88.55^{bc}	84.35^{d}
		±1.39	±1.80	±1.27	±1.83	±1.19	±2.34

^{a,b,c,d}Means with different superscripts in a row differ significantly (P<0.05); ^{A,B,C,D}Means with different superscripts in a column differ significantly (P<0.05); N=12; T_1= 10% liver powder, T_2= 20% liver powder; T_3= 30% liver powder; T_4= 10% gizzard powder, T_5= 20% gizzard powder; T_6= 30% gizzard powder

Table 5. Effect of different levels of incorporation of liver and gizzard powder on colour and textural parameters of dog biscuits

Parameter	Liver and gizzard powder level					
	T_1 (10% liver)	T_2 (20% liver)	T_3 (30% liver)	T_4 (10% gizzard)	T_5 (20% gizzard)	T_6 (30% gizzard)
Redness (a^*)	10.73^{a}±0.09	10.69^{a}±0.06	9.04^{b}±0.13	6.77^{c}±0.05	6.68^{c}±0.15	7.03^{c}±0.20
Yellowness (b^*)	25.84^{a}±0.08	26.07^{a}±0.15	25.83^{b}±0.29	25.89^{a}±0.17	26.04^{a}±0.25	22.83^{b}±0.29
Lightness (L)	45.49^{b}±0.22	46.71^{ab}±0.23	45.62^{c}±0.73	47.54^{a}±0.27	47.65^{a}±0.22	47.47^{a}±0.67
Fracturability (N)	1.85^{c}±0.03	1.60^{d}±0.03	1.13^{f}±0.06	2.27^{a}±0.06	2.18^{b}±0.03	1.49^{e}±0.05
SFV (kg/cm^2)	3.71^{a}±0.08	3.36^{b}±0.06	2.87^{c}±0.05	3.56^{a}±0.07	3.30^{b}±0.07	2.89^{c}±0.08^{c}

^{a,b,c,d,e,f}Means with different superscripts in a row differ significantly (P<0.05); N=6; T_1= 10% liver powder, T_2= 20% liver powder; T_3= 30% liver powder; T_4= 10% gizzard powder, T_5= 20% gizzard powder; T_6= 30% gizzard powder

quality evaluation by the consumers. Redness (a^*) value showed the pattern in the order of $T_1>T_2>T_3>T_6>T_4>T_5$ and followed a decreasing trend in liver powder incorporated dog biscuits. Redness (a^*) value of dog biscuits incorporated with chicken gizzard powder was lower (P<0.05) than the liver incorporated dog biscuits. The yellowness (b^*) in groups T_3 and T_6 dog biscuits was also lower (P<0.05) than other groups. Lightness (L^* value) of chicken liver powder incorporated dog biscuits was higher (P<0.05) than the chicken gizzard powder incorporated dog biscuits. Meat oxidation caused a decrease in a^* value (Lavieri and Williams 2014; Kumar *et al.,* 2015). Realini *et al* (2015) also reported decrease in b^* values of beef patties containing Acerola fruit extract. Selgas *et al.* (2009) reported same trend for a^* and b^* values in hamburgers containing dried tomato powder.

The values for both fracturability and shear force showed declining trend with increase in the incorporation of liver and gizzard powder which might be due to the replacement of refined wheat flour with by-products powder having low starch content and lower gelatinization on cooking of the developed biscuits. Similarly, Malav *et al.* (2017) also reported higher (P<0.05) hardness and fracturability values in spent hen meat papad incorporated with black gram flour. The highest values for fracturability was observed for dog biscuits incorporated with 10% gizzard powder whereas the highest values for shear force was observed for the dog biscuits incorporated with 10% liver powder.

CONCLUSIONS

The poultry slaughter byproducts *viz.*, liver and gizzard could be effectively utilized for the development of biscuits for dogs. Dog biscuits with high nutritive value and sensory quality were developed by incorporation of chicken liver powder at 30% level.

REFERENCES

AOAC. 1995. *Official Methods of Analysis*. 16th edn. Association of Official Analytical Chemists, Washington, DC., USA.

BAHS. 2016. *Basic Animal Husbandry & Fisheries Statistics-2016* http://dahd.nic.in/Division/statistics/animal-husbandry-statistics-division. -Cover.

Bourne, M.C. 1978. *Food Texture and Viscosity: Concept and Measurement*. 2nd edn. Academic Press, London, U.K. pp 257-290.

Griffin, R.W., Scott, G.C. and Cante, C.J. 1984. Food preferences of dogs housed in testing-kennels and in consumers' homes: Some comparisons. *Neurosci. Biobehav. Rev*. 8: 253-259.

Karthikeyan, N., Singh, R.P., Johri, T.S. and Tanwar, V.K. 2002. Nutritional quality and palatability of pet food from poultry by-product meal. *Indian J. Anim. Sci.* 72: 410-413.

Kumar, V., Chatli, M.K., Wagh, R.V., Mehta, N. and Kumar, P. 2015. Effect of the combination of natural antioxidants and packaging methods on quality of pork patties during storage. *J. Food Sci. Technol.* 52: 6230-6241.

Lavieri, N. and Williams, S.K. 2014. Effects of packaging systems and fat concentrations on microbiology, sensory and physical properties of ground beef stored at 4±1°C for 25 days. *Meat Sci.* 97: 534-541.

Mahender, M., Sudhakar, K. and Harikrishna, C.H. 2013. Effect of feeding poultry slaughter waste based dog diet biscuits on intake, palatability and nutrient utilization in Labrador dogs. *Indian J. Anim. Sci*. 1: 79-82.

Malav, O.P., Chatli, M.K., Kumar, P. and Mehta, N. 2017. Optimization and quality evaluation of spent hen meat papad incorporated with corn and black gram flour. *Indian J. Poult. Sci*. 52: 312-317.

Realini, C.E, Guardia, M.D., Diaz, I., Garcia-Regueiro, J.A. and Arnau, J. 2015. Effects of Acerola fruit extract on sensory and shelf-life of salted beef patties from grinds differing in fatty acid composition. *Meat Sci.* 99: 18-24.

Regenstein, J., Goldhor, S. and Graves, D. 2003. Increasing the value of Alaska Pollock byproducts. In: *"Proc. Advances in Seafood Byproducts"*: (P.J. Bechtel; ed.) Alaska Sea Grant College Program, Univ. Alaska, Fairbanks. pp 459-482.

Selgas, M.D., Garcia, M.L. and Calvo, M.M. 2009. Effects of irradiation and storage on the physico-chemical and sensory properties of hamburgers enriched with lycopene. *Int. J. Food Sci. Technol.* 44: 1983-1989.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. 8th edn. Ames, Iowa State University Press, Iowa, USA.

SPSS. 2007 *Stastical Packages for Social Sciences*. SPSS Version 16.0, SPSS Inc., Chicago, II, USA.

Trout, E.S., Hunt, M.C., Johnson, D.E., Claus, J.R., Kastner, C.L. and Kropt, D.H. 1992. Characteristics of low fat ground beef containing texture modifying ingredients. *J. Food Sci.* 57: 19-24.

Received on 11-12-2018 and accepted on 13-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 83-87
doi: 10.5958/2231-6744.2019.00014.8

Indian Journal of Animal Nutrition

SHORT COMMUNICATION

Supplementary Effect of Different Levels of Nano Zinc Oxide on Zinc Bioavailability and Blood Metabolites in Lambs

K.K. Singh, S.B. Maity and A. Maity

Plant Animal Relationship Division, ICAR-Indian Grassland and Fodder Research Institute, Jhansi-284003, Uttar Pradesh, India

ABSTRACT

Twenty five *Jalauni* male lambs (average body weight = 4.5 kg, age 35 d) were randomly divided into 5 groups of 5 animals each. All the lambs were maintained on basal ration comprising grass hay and concentrate mixture (Maize grain, mustard cake and common salt in the ratio of 64: 35: 1). The group G_1 served as control supplemented with 40 ppm zinc from ZnO while the lambs in groups G_2, G_3, G_4 and G_5 were supplemented with 40, 30, 20, 10 ppm Zn from nano ZnO (nZnO 30 nm) in the concentrate mixture over and above the basal ration (24.12 ppm) for a period of 150 d. Results indicated no difference in the intake and digestibility of nutrients amongst groups. The Zn intake was higher ($P<0.05$) in group G_1 and G_2 as compared to groups G_3 to G_5. A similar trend was also observed for faecal and urinary Zn excretion which was higher ($P<0.05$) in group G_1 as compared to groups G_2, G_3, G_4 and G_5 that led to higher Zn retention (2.01 and 1.88 mg/d) in groups $G_2$and G_3 as compared to groups G_1, G_4 and G_5 (1.50, 1.40 and 1.33 mg/d) and absorption was higher ($P<0.05$) in nZnO supplemented lambs in comparison to ZnO supplemented lambs (G_1). The blood metabolites were within the normal physiological range except that plasma Zn concentration was higher ($P<0.05$) in nZnO supplemented lambs. Hence, nZnO improved ($P<0.05$) Zn availability in lambs. Thus, nZnO could be a better source of Zn supplementation at a supplementary level of 20 ppm.

Key words: Blood metabolites, Lamb growth, Nano zinc oxide, Zinc bioavailability

Zinc (Zn) is an important part of a number of enzymes which play a important role in the metabolism of nutrients in animals (Jia *et al.,* 2008). As Zn is not stored in the body, a continuous dietary supply is necessary for proper physiological functions (Zalewski *et al.,* 2005). Inspite of the poor solubility of zinc oxide, it is the main source of Zn used by the animal feed industry (Wedekind and Baker, 1990). Recently, the use of nano zinc oxide (nZnO) for supplementation in ruminant diets has begun but whether nano forms are more effective than normal zinc oxide (ZnO) remains unclear. The changeover from micro particles to nanoparticles (<100 nm in diameter) involves an increase of the surface area among other changes in properties. A larger surface area of the nanoparticles allows greater solubility which might lead to better utilization in animals. Limited knowledge of the effects of this substance highlights the need to ascertain their possible use as a nutritional supplement in ruminants. Hence, an experiment was conducted to find out the effect of different levels of nZnO on Zn availability in lambs.

For this study, twenty five *Jalauni* male lambs (BW= 4.5 kg; age 35 d) were randomly divided into 5 groups of 5 animals each. All the lambs were maintained on basal ration comprising grass hay and concentrate mixture (Maize grain, mustard cake and common salt in the ratio of 64: 35: 1). The group G_1 served as control supplemented with 40 ppm Zn from ZnO while the lambs in groups G_2, G_3, G_4 and G_5 were supplemented with 40, 30, 20, 10 ppm Zn from nano ZnO (nZnO 30 nm) in the concentrate mixture over and above the basal ration (24.12 ppm) for a period of 150 d (ICAR, 2013). Live weight of each animal was recorded weekly. In the middle of feeding trial, a metabolism trial of 5 days duration was conducted to evaluate the effect of nZnO on zinc availability as well as blood metabolites in lambs. The proximate composition (AOAC, 2000) and fibre fractions (Van Soest *et al.,* 1991) in biological samples were determined. For estimation of Zn, feed, faeces and urine samples were digested in tri-acid

*Corressponding author: E-mail: krisksingh@gmail.com

Table 1. Chemical composition (% DM basis) of feed ingredients

Feed ingredient	CP	CF	EE	Ash	NFE	NDF	ADF	Zinc
Grass hay	6.20	32.20	1.10	9.88	52.80	70.44	35.45	10.20
Mustard cake	30.02	6.60	7.90	6.90	48.58	25.25	18.62	40.02
Maize	10.00	2.20	4.30	2.10	81.30	12.45	4.32	22.01

mixture while plasma samples were diluted to 1/5 with deionized water and analysed using VARIAN AA240 Atomic Absorption Spectrophotometer. Blood samples were collected in heparinized test tubes before (0 d) and at the end of experimental feeding (90 d) to estimate creatinine (Wootton, 1964) and superoxide dismutase (Sun *et al.,* 1988). Data were analyzed statistically (Snedecor and Cochran, 1994).

The chemical composition of feed ingredients has been presented in Table 1. The DM intake ranged from 350.95 to 374.22 g/d with the corresponding values of 3.60 to 3.79 kg/100 kg body weight which was similar in all the groups (Table 2). Zaboli *et al.* (2013) also observed no effect of supplementary zinc oxide on DM

Table 2. Effect of different levels of nano zinc oxide on intake and utilization of nutrients in lambs

Parameter	Group				
	I	II	III	IV	V
Body wt. (kg)	9.75±0.29	9.83±0.32	9.82±0.22	9.82±0.18	9.88±0.16
DM intake (g/d)	350.95±8.20	355.85±7.62	360.48±10.45	355.50±8.42	374.22±14.65
DM intake (% BW)	3.60±0.08	3.62±0.07	3.67±0.09	3.62±0.09	3.79±0.10
CP intake (g/d)	54.34±0.25	54.84±0.40	55.31±0.15	54.80±0.22	55.79±0.20
TDN intake (g/d)	200.52±4.16	204.72±5.11	208.76±4.55	207.53±4.62	210.45±6.42
Digestibility (%)					
DM	64.22±0.52	66.42±0.81	67.36±0.88	65.22±0.74	66.88±0.78
OM	66.12±0.66	67.88±1.24	69.42±1.32	67.65±1.40	68.45±1.00
CP	66.22±0.52	65.96±0.27	66.40±0.30	65.98±0.25	65.48±0.29
CF	58.70±3.10	60.40±3.10	60.45±2.68	57.99±3.12	60.45±2.30
NFE	69.24±1.70	71.00±1.60	72.05±2.45	68.88±2.32	70.12±2.13
EE	78.22±2.41	80.00±2.65	80.55±2.44	78.65±2.66	76.55±3.46
NDF	60.14±1.47	60.24±2.64	58.44±2.14	61.65±1.88	60.21±2.15
ADF	54.60±1.56	52.80±1.66	55.45±1.99	54.68±2.15	52.45±2.10
Nitrogen balance (g/d)					
N intake	8.69±0.12	8.77±0.14	8.85±0.22	8.74±0.16	8.93±0.22
Faecal N	3.05±0.18	3.15±0.20	3.20±0.16	3.24±0.10	3.34±0.12
Urinary N	3.54±0.14	3.57±0.16	3.45±0.11	3.41±0.12	3.43±0.16
N balance	2.10±0.11	2.05±0.12	2.20±0.11	2.12±0.10	2.16±0.14
Zinc retention (mg/d)					
Zn intake	22.99^{a} ±0.32	23.09^{a} ±0.45	19.69^{b} ±0.25	16.09^{c} ±0.18	12.84^{d}±0.15
Faecal Zn	20.2^{a} 8±0.41	19.76^{a} ±0.62	16.92^{b} ±0.19	13.81^{c} ±0.12	10.81^{d} ±0.17
Urinary Zn	1.19^{a} ±0.10	1.33^{a} ±0.16	1.01^{b} ±0.14	0.87^{bc} ±0.09	0.70^{c}±0.11
Zn retained	1.50^{c}±0.11	2.01^{a} ±0.09	1.77^{b} ±0.10	1.40^{c} ±0.09	1.33^{c}±0.08
Absorption (%)	6.55±0.37	8.67±0.43	9.01±0.58	8.70±0.11	10.34±0.32

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

Table 3. Growth performance of lambs on different feeding regime

Parameter	Group				
	I	II	III	IV	V
Initial body wt. (kg)	3.64±0.04	3.55±0.08	3.60±0.09	3.50±0.07	3.72±0.22
Final body wt. (kg)	11.75±0.10	12.36±0.11	12.22±0.08	11.82±0.07	12.44±0.12
Weight gain (kg)	8.11±0.10	8.81±0.13	8.62±0.11	8.32±0.09	8.72±0.10
ADG (g)	54.06±3.12	58.73±2.26	57.47±1.88	55.46±2.11	58.13±3.22

intake in groups supplemented with different levels (*e.g.* 0, 20 and 40 ppm) of zinc oxide and nano-zinc oxide in Iranian Angora goat kids. Similarly, supplementation of Zn to a basal diet containing more than 25 mg Zn/kg DM had no effect on DMI in growing lambs (Garg *et al.,* 2008). Intake of CP and TDN followed the trend exhibited by DM intake. The apparent digestibility of DM, OM, CP, NDF and ADFwas similar in all the groups (Table 2). Jadhav *et al.* (2008) reported that supplementation of Zn at 0, 35 and 70 ppm level in buffalo calves did not affect nutrient digestibility. Similarly, there was no difference in digestibility of CP, EE, NDF, ADF and cellulose in crossbred cattle supplemented with 0, 35 and 70 ppm Zn in basal diet containing more than 25 ppm Zn (Mandal *et al.,* 2007). All the animals were in positive N balance.

The average daily gain (Table 3) was similar (54.06 to 58.73 g/d) in all the groups. Zaboli *et al.* (2013) also did not find any effect due to supplementation of 20 or 40 ppm nZnO on average daily gain in Iranian Angora goat kids fed control diet containing 22 ppm Zn. However, nZnO has been reported to enhance growth performance in poultry (Mishra *et al.,* 2014). As the Zn level (30 ppm) in the basal ration was adequate (Mc Dowell, 1985) for normal growth of *Jalauni* lambs which could be the reason for non-significant effect on growth performance.

Zn intake was higher in groups G_1 and G_2 as compared to groups G_3 to G_5 owing to higher supplementation levels. A similar trend was also observed with the faecal and urinary Zn excretion which was higher ($P<0.05$) in groups $G_1$and G_2as compared to groups G_3, G_4 and G_5 (Table 2) which might have led to higher Zn retention (2.01and 1.88 mg/d) in groups $G_2$and G_3, respectively as compared to groups G_1, G_4 and G_5 (1.50, 1.40 and 1.33 mg/d) and the absorption was higher ($P<0.05$) in nZnO supplemented lambs (G_2 to G_5) in comparison to ZnO supplemented lambs (G_1). A similar excretion pattern of Zn has been reported earlier by Singh *et al.* (2009) in kids. There has been variable effects of different Zn sources on its retention. Jadhav *et al.* (2008) observed higher Zn retention in buffalo calves supplemented with 0, 35 and 70 ppm Zn from zinc sulphate. Singh *et al.* (2018) supplemented 60 ppm nZnO in the diet of pre-ruminant *Jalauni* lambs over and above the level in basal ration (30 ppm Zn) and

Table 4. Effect of different levels of nano zinc oxide on plasma metabolites in lambs

Parameter	Group				
	I	II	III	IV	V
Zn initial (µg/dL)	0.98±0.16	0.86±0.18	0.82±0.22	0.92±0.16	0.85±0.14
Zn final (µg/dL)	1.22^{d} ±0.06	1.66^{a} ±0.10	1.55^{ab} ±0.09	1.42^{bc} ±0.11	1.16^{d} ±0.08
Creatinine (0 d mg/dL)	0.95±0.09	0.91±0.10	0.94±0.12	0.92±0.11	0.98±0.14
Creatinine (90 d mg//dL)	0.99±0.06	1.02±0.11	1.01±0.14	1.06±0.16	1.10±0.15
SOD (0 d, U/dL)	225.12±6.88	228.12±7.41	227.98±7.62	232.15±6.55	230.32±7.22
SOD (90 d, U/dL)	236.14±8.45	242.65±8.56	240.45±6.55	244.52±5.68	245.85±7.55

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

reported improved Zn availability and plasma Zn levels in pre-ruminant lambs.

Initial plasma level of Zn was similar in different groups (Table 3). However, after 90 d, there was an increase ($P<0.05$) in the plasma Zn levels in both groups. But the increase was more prominent in groups G_2, G_3 and G_4 (1.66, 1.55 and 1.42 µg/dL) as compared to groups G_1 and G_5 (1.22 and 1.16 µg/dL). The higher plasma Zn levels in nZnO supplemented lambs (G_2 to G_4) might be due to the greater retention of Zn than in group G_5. Lower plasma Zn levels in group G_1 might be due to the lower Zn retention due to low solubility of ZnO in GI tract (Wedekind and Baker, 1990; Singh *et al.,* 2018) and in group G_5 due to the comparatively lower Zn intake. It has been shown that nano particles are absorbed in duodenum by active transport and nano-elemental forms can cross the small intestine and further distribute into the blood (Hillyer and Albrecht, 2001). Najafzadeh *et al.* (2013) also observed increased serum Zn levels after oral administration of nano zinc oxide in lambs.

The initial and final plasma creatinine and SOD levels were similar and within the normal physiological range in lambs (Table 4). Najafzadeh *et al.* (2013) observed increased creatinine level in the serum of lambs supplemented orally with nano zinc particle at the rate of 20 mg/kg BW. High serum creatinine levels in lambs might be due to the exposure of high doses (360-400 mg/d) of nZnO. Further, the toxicity of nZnO is reported to be associated with dose and duration of exposure to nano particles (Swain *et al.,* 2016). Whereas, in the present study the nZnO intake was very low (13 mg/d) as compared to the former study (360-400 mg/d).

Therefore, nutrient utilization and growth performance was similar in groups supplemented with both sources of Zn supplementation, however, nZnO significantly improved Zn bioavailability. Thus, nZnO could be a better source of Zn supplementation at 20 ppm level of supplementation.

REFERENCES

AOAC. 2000. *Official Methods of Analysis*. Association of Official Analytical Chemists. 16th edn. USDA, Washington, DC., USA.

Garg, A.K., Vishal, M. and Dass, R.S. 2008. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. *Anim. Feed Sci. Technol*. 144: 82-96.

Hillyer, J.F. and Albrecht, R.M. 2001. Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. J. *Pharmacol. Sci.* 90: 1927-1936.

ICAR. 2013. *Nutrient Requirement of Cattle and Buffalo.* Indian Council of Agricultural Research, New Delhi, India.

Jadhav, S.E., Garg, A.K. and Dass, R.S. 2008. Effect of graded levels of zinc supplementation on growth and nutrient utilization in male buffalo calves. *Anim.Nutr. Feed Technol.* 8: 65-72.

Jia, W., Xiaoping, Zh., Wei, Zh., Jianbo, Ch., Cuihua, G. and Zhihai, J. 2008. Effects of source of supplemental zinc on performance, nutrients digestibility and plasma zinc status in Cashmere goats. *Small Rumin. Res*. 80: 68-72.

Mandal, G.P., Dass, R.S., Isore, D.P., Garg, A.K. and Ram, G.C. 2007. Effect of zinc supplementation from two sources on growth, nutrient utilization and immune response in male crossbred cattle. *Anim. Feed Sci. Technol.* 138: 1-12.

Mc Dowell, L.R. 1985. *Nutrient Requirement of Grazing Ruminant in Warm Climate*. Academic Press. Inc. California, USA.

Mishra, A., Swain, R.K., Mishra, S.K., Panda, N. and Sethy, K. 2014. Growth performance and serum biochemical parameters as affected by nano zinc supplementation in layer chickens. *Indian J. Anim. Nutr.* 31: 384-388.

Najafzadeh, H., Ghoreishi, S.M., Mohammadian, B., Rahimi, E., Afzalzadeh, M.R, Kazemivarnamkhasti, M. and Ganjealidarani, H. 2013. Serum biochemical and histopathological changes in liver and kidney in lambs after zinc oxide nano particles administration. *Vet. World* 6: 534-537.

Singh, K.K., Maity, S.B. and Maity, A. 2018. Effect of nano zinc oxide on zinc bioavailability and blood biochemical changes in pre-ruminant lambs. *Indian J. Anim. Sci.* 88: 805-807.

Singh, K.K., Misra, A.K. and Maity, S.B. 2009. Effect of excess iron in the diet on copper and zinc utilization in growing lambs. *Indian Vet. J.* 86: 66-68.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* I.B.H. Publishing Company, Calcutta, West Bengal, India.

Sun, Y., Oberley, L.W. and Li, Y. 1988. A simple method for clinical assay of superoxide dismutase. *Clinic. Chem*.34: 497-500.

Swain, P.S., Rao, S.B.N., Rajendran, D., Dominic, G. and Selvaraju, S. 2016. Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Anim. Nutr.* 2: 134-141.

Van Soest, P.J., Robertson, J. B., Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharide in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

Wedekind, K.J. and Baker, D.H. 1990. Zinc bioavailability in feed-grade sources of zinc. *J. Anim. Sci.* 68: 684-689.

Wootton, I.D.P. 1964. *Micro Analysis in Medical Biochemistry*. 4th edn. J. and A. Churchill Ltd. London. pp. 174-175.

Zaboli, K., Aliarabi, H., Bahari, A. A. and Abbasalipourkabir, R. 2013. Role of dietary nano-zinc oxide on growth performance and blood levels of mineral. *J. Pharmaceut. Health Sci.* 2: 19-26.

Zalewski, P.D., Ai, Q.T., Dion, G., Lata, J., Chiara, M. and Richard, E.R. 2005. Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets: A review: *Pharmacol. Therap.*105:127-149.

Received on 17-01-2019 and accepted on 18-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 88-90
doi: 10.5958/2231-6744.2019.00015.X

Indian Journal of Animal Nutrition

SHORT COMMUNICATION

Growth Performance and Nutrient Utilization in Different Goat Breeds in the Plain Agro-cllimatic Zone of Chhattisgarh

Raj Kumar Yadav, Meenu Dubey*, Raina Doneria and S.P. Tiwari
Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Anjora, Durg, 491001, Chhatisgarh, India

ABSTRACT

Growth performance and nutrient utilization was studied in three goat breeds in the plain agro climatic zone of Chhattisgarh. Five kids (4-5 months of age) each of Jamunapri, Barbari and Sirohi breed were regard in three groups under semi intensive system of goat production. Kids were allowed grazing for 4-5 h followed by concentrate mixture feeding @ 100, 150 and 200 g/d/ kid in the evening for 1st, 2nd and 3rd month of experiment, respectively. The average daily gain was similar in Jamunapari (47.50 g), Barbari (45.83 g) and Sirohi (39.30 g) kids. The digestibility of CP was higher (P<0.05) in Jamunapari (72.17%) and Barbari (70.52%) compared to Sirohi kids (65.17%). Similarly, the digestibility of CF, EE and NFE was better (P<0.05) in Jamunapari compared to Sirohi. Jamunapari and Barbari kids showed better N retention than Sirohi kids. The retention of P was also higher (P<0.05) in Jamunapari compared to Sirohi breed. It might be concluded that the growth performance and efficiency of nutrient utilization was better in Jamunapari and Barbari breed in comparison to Sirohi breed in plain region of Chhattisgarh.

Key words: Barbari, Goats, Growth performance, Jamunapari, Nutrient utilization, Sirohi

Goats occupy a unique place among domestic livestock because of their ability to survive and produce under extreme climatic and management conditions. Goats play an important role to small-scale resource poor livestock keepers, efficient utilization of feed resources, small size and early maturity, short generation interval and higher digestion efficiency of roughage make them suitable for small land holders for income generation, increased farm productivity and for improved family nutrition. Therefore, improvement of goat productivity is one way of reducing poverty among the poor's (Ahuya *et al.,* 2009). Chhattisgarh state has wide variations in climate and geology and the state has been divided in three agro-climatic zones *i.e.* Northern hill, plain and plateau. Variation in soil type affects the vegetation of respective area and nutritive value of grown crop which ultimately affect the population and performance of animal to a great extent. Various studies showed that a number of environmental factors affect the growth and lactation traits and thus directly obscure the recognition of the genetic potential of animals. Present experiment was conducted to assess the growth performance and nutrient utilization in Jamunapari, Barbari and Sirohi breeds of goat kids in plain agro climatic zone of Chhattisgarh.

For this study, five kids of each breed (Jamunapari, Barbari and Sirohi) of 4-5 months age were raised in three groups. A feeding cum growth trial was conducted for 90 days. The kids were housed in a well-ventilated goat shed with facilities for individual feeding under hygienic conditions and maintained under semi-intensive system of goat kid production. All kids were dewormed by Albendazole @ 7.5 mg/kg BW orally. The kids were allowed to graze on pasture grass available in the agro climatic zone for 4-5 h and in the evening concentrate mixture was offered @ 100, 150 and 200 g/d/kid during 1st, 2nd and 3rd month of feeding, respectively. Concentrate mixture contained (parts) maize 44, deoiled soybean cake 18, deoiled rice bran 26, wheat bran 10 along with premixes (2 parts) containing vitamins, minerals and feed additives. The concentrate mixture contained 16% DCP and 70% TDN.

The kids were weighed at fortnightly intervals and average daily gain was calculated. After 90 days of

*Corresponding author: E- mail: drmeenu.dubey@gmail.com

feeding, a metabolism trial of 5 days collection period was conducted to determine digestibility of nutrients, N, Ca and P balances. The kids were kept individually in the metabolic cage having provision for individual feeding and collection of faeces and urine separately. During metabolism trial, animals were not allowed to graze and weighed quantity of feed was offered daily in the morning at 8.00 A.M. and evening at 4.00 PM. The left over feed was collected daily and weighed. Samples of feed offered, residue left and faeces voided were analyzed for proximate principles (AOAC, 2000). The samples were also analyzed for calcium (Talapatra *et al.,* 1940) and inorganic phosphorus (Fiske and Subbarow, 1925). The data were subjected to analysis of variance following Completely Randomized Design as per Snedecor and Cochran (1994) and significance of differences among groups was analyzed by Duncan multiple range test using SPSS software.

The results showed that initially up to 45 day no significant difference in the average daily weight gain (ADG) among the breeds was observed, however, between 45-60 days, the highest ADG was recorded in Jamunapari (53.33 g) followed by Barbari (50.00 g) kids which was higher (P<0.01) than in Sirohi (35.33 g) kids (Table 1). Similarly, during the last fortnight weight gain was higher (P<0.05) in Barbari and Jamunapari as compared to Sirohi kids. Mean ADG was, however, similar in different groups. The weight gain during 90 d trial period in Jamunapari, Barbari and Sirohi was 4.28, 4.13 and 3.54 kg, respectively. The total weight gain was similar in 3 groups. In present study, the growth performance of Jamunapari and Barbari kids was similar. The ADG of Jamunapari kid (47.50 g) in the present study corroborated with findings of Das and Yadav (2015) and Khan and Naznin (2013) under semi intensive system of goat kid production. The ADG of Barbari kid (45.83 g) in this study was lower than 66.22 g/d as reported by Paramasivam *et al.* (2002) under semi intensive conditions. In contrast, Bharathidhasan *et al.* (2009) reported that there was no significant effect of non-genetic factors on early production parameters of the Barbari goats.

The DM intake was found to be similar in 3 groups. The digestibility of CP was higher (P<0.05) in Jamunapari and Barbari as compared to Sirohi kids. The CF digestibility was also higher (P<0.05) in Jamunapari as compared to Sirohi kids though the variation in CF digestibility between Barbari and Sirohi kids was not significant. The digestibility of EE and NFE was higher in Jamunapari as compared to Sirohi, though the value differed non- significantly between Barbari and Sirohi. The reason for increased digestibility of CP, EE, CF and NFE in Jamunapari kid might be due to better adaptability of this breed in the agro climatic condition of Chhattisgarh state. Chaturvedi *et al.* (2010) also reported that the digestibility of DM, OM, CP, NDF and ADF was higher in group where lambs were maintained on grazing and concentrate supplementation as compared to group where lambs were maintained on sole grazing. The N retention was higher (P<0.05) in Jamunapari and Barbari goats as compared to Sirohi (Table 2). The Ca retention was

Table 1. Average daily weight gain (g/d) in kids of Jamunapari, Barbari and Sirohi breeds in plain region of Chhattisgarh

Period (d)	Group			Level of significance
	Jamunapari	Barbari	Sirohi	
0-15	37.33±2.41	38.66±4.23	32.66±3.78	NS
15-30	46.00±4.23	42.66±3.78	42.66±4.28	NS
30-45	50.66±5.29	43.33±4.68	45.33±2.12	NS
45-60	53.33^{a}±2.12	50.00^{a}±5.67	35.33^{b}±5.12	**
60-75	46.66±6.46	48.66±3.39	40.00±4.17	NS
75-90	50.66^{a}±4.78	52.66^{a}±4.19	40.66^{b}±5.12	*

Table 2. DM intake and nutrient utilization in goat kids in plain agro climatic zone of Chhattisgarh

Particular	Group			Level of significance
	Jamunapari	Barbari	Sirohi	
DM intake				
DMI (g/d)	489.25±49.96	421.97±73.98	389.33±20.96	NS
DMI (kg/100 kg BW)	4.08	4.20	3.12	NS
Digestibility (%)				
DM	62.63±0.37	62.02±0.49	61.41±0.34	NS
CP	72.17^{a}±0.45	70.52^{a}±0.21	65.17^{b}±1.31	*
CF	68.74^{a}±1.29	65.84^{ab}±2.19	61.83^{b}±1.83	*
EE	66.32^{a}±2.59	64.05^{ab}±1.27	58.33^{b}±2.55	*
NFE	65.34^{a}±2.04	63.35^{ab}±0.74	58.84^{b}±2.04	*
Nutrient retention (%)				
N	72.18^{a}±0.45	70.55^{a}±0.21	65.19^{b}±1.30	*
Ca	64.30±2.45	62.00±2.89	58.82±5.55	NS
P	69.53^{a}±3.39	65.15^{ab}±2.11	58.97^{b}±2.38	*

^{a,b} Means with different superscript in a row differ significantly ($^{*}P<0.05$, NS- not significant)

similar in 3 groups. The retention of P was higher (P<0.05) in Jamunapari as compared to Sirohi though the difference between Barbari and Sirohi was not significant.

It could be concluded that the growth performance and nutrient utilization was better in Jamunapari and Barbari kids as compared to Sirohi kids in plain region of Chhattisgarh.

REFERENCES

Ahuya, C.O., Ojango, J.M.K., Mosi, R.O., Peacock, C.P. and Okeyo, A.M. 2009. Performance of Toggenburg dairy goats in small holder production systems of the eastern highlands of Kenya. *Small Rumin. Res.* 83: 7-13.

AOAC. 2000. *Official Methods of Analysis*. 17th edn. Association of Official Analytical Chemists, Verginia, Washington, D.C., USA.

Bharathidhasan, A., Narayanan, R., Gopu, P., Subramanian, A., Prabakaran, R. and Rajendran, R. 2009. Effect of non-genetic factors on birth weight, weaning weight and pre weaning gain of Barbari goat. *Tamilnadu J. Vet. Anim. Sci.* 5: 99-103.

Chaturvedi, O.H, Bhatta, R., Shakhyan, S.K. and Karim, S.A. 2010. Nutrient utilization and production performance of lambs grazing on community rangeland with and without concentrate supplementation. *Indian J. Anim. Sci.* 80: 693-696.

Das, S.K. and Yadav, B.P.S. 2015. Productive performance of Jamunapari goat under semi intensive system of management. *Indian J. Anim. Res.* 49 : 254-257.

Fiske, C.H. and Subbarow, Y. 1925. The colorimetric determination of phosphorus. *J. Biochem. Chem.* 66: 375-400.

Khan, M.K.I. and Naznin, M. 2013. Study the live weight gain of Black Bengal and Jamunapari goat breeds by fitting the linear regression under semi intensive condition. *Pak J. Biol Sci.* 16: 998-1003.

Paramasivam, A., Sasirekha, A., Sivakumar, T. and Ramesh, V.P 2002. Growth performance and carcass traits of Barbari goats under different systems of management. *Indian J. Anim. Sci.* 72: 1016-1018.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* 8th edn. Iowa State University Press, Ames, Iowa.

Talapatra, S.K., Ray, S.N. and Sen, K.C. 1940. Estimation of phosphorus, chlorine,calcium, magnesium, sodium and potassium in food stuffs. *Indian J. Vet. Sci. Anim. Husb.*10: 243-246.

Received on 02-11-2018 and accepted on 02-02-2019

Indian J. Anim. Nutr. 2019. 36 (1): 91-93
doi: 10.5958/2231-6744.2019.00016.1

Indian Journal of Animal Nutrition

SHORT COMMUNICATION

Evaluation of Berseem (*Trifolium alexandrinum* L.) Varieties for Fodder and Seed Production

J.J. Gupta* **and A. Dey**
ICAR Research Complex for Eastern Region, Patna-800014, Bihar, India

ABSTRACT

Three varieties of berseem (*Trifolium alexandrinum* L.) seeds *viz.*, JHB-146, Wardan and Hybrid were sown in first week of November with seed rate of 20 kg/ha in line with distance of 15 cm. Fodder was harvested three and/or four times at 55, 90, 110 and/or 130 days of crop duration. Irrigation with top dressing of urea @ 60 kg/ha was provided after each harvest. Finally crop was harvested at 160 days of crop duration to record the total biomass and seed yield. The maximum ($P<0.01$) cumulative fodder yield was recorded from Wardan variety irrespective of total numbers of cuttings. The highest berseem seed yield was recorded in Wardan variety after third cut of fodder in March first week than JHB-146 and Hybrid berseem variety. However, seed yield reduced at forth cut of last week of March. Hence, berseem fodder could be harvested only thrice and then left for maximum seed yield. However, reasonably good seed yield could be harvested from Wardan berseem variety even after forth cut of fodder in last week of March.

Key words: Berseem, Fodder production, Seed production, Variety

Being the source of major rabi forage for dairy animal feeding, berseem (*Trifolium alexandrinum* L.) occupies 54% of total cultivated fodder area in India (Kumar, 2013). It is well adapted to most places of north and eastern India under sub-tropical condition with assured irrigation facilities due to its rapid growth, multi-cut nature, good fodder recovery after cutting, long period of fodder supply, high tonnage with excellent palatability and high nutritive value (Saini and Chowdhury, 1993; Gupta *et. al*., 2016). In eastern India, sowing period of berseem starts from middle of October to November and first harvest is taken after 45-50 days of sowing. A total of 4-6 cuttings with interval of 25-30 days were taken for a period of 115-125 days from end of November to March with cumulative biomass production of 55-60 t/ha depending upon the agronomical package of practices (Gupta *et. al*., 2017). However, farmers depend on local market for availability of seed though it is not available in time resulting in late start of cultivation operations. Consequently, there is scarcity period which extends from October to middle of December. As such *Trifolium sp*. and *Medicago sp*. are poor seed producer or shy-seeder due to their narrow genetic base (Hazra and Sinha, 1996). Besides, not much attention has been paid in India towards its seed production because it is mainly cultivated for forage. It is estimated that the annual availability of seeds of all fodder crops in India is only 20% of the total requirement (Hazra, 1992). Hence, the present study was undertaken to evaluate the performance of different cultivars of berseem for production of forage biomass and seed so as to increase the availability of both berseem forage and seed to mitigate the future fodder requirements.

The study was conducted at ICAR Research Complex for Eastern Region, Patna, Bihar, India farm during 2016-17 having tropical agro-climatic conditions, clay-loam type soil with neutral pH (6.74), 0.67% organic carbon and N, P, K values of 248, 23, 267 kg/ha, respectively. Land was prepared with addition of FYM @ 5 t/ha and DAP @ 60 kg/ha. Three main plots of 300 m^2 each were prepared and further divided into 2 sub-plots of 150 m^2 each. Three varieties of berseem seeds *viz.,* JHB-146, Wardan and Hybrid were sown in first week of November with seed rate of 20 kg/ha in line with row to row distance of 15 cm. Irrigation was provided just after sowing. Fodder was harvested thrice from all sub-plots at 55, 90 and 110 days. Irrigation with

*Corresponding author e-mail: guptajj@rediffmail.com

Table 1. Performance of different berseem varieties in production of forage and seed

Particular	Variety			LSD±
	JHB-146	Wardan	Hybrid	
Cumulative fodder yield 3 cuts (t/ha)	28.98^{a}±0.42	35.63^{b}±0.37	32.95^{b}±0.15	2.74**
Cumulative fodder yield 4 cuts (t/ha)	39.67^{a}±0.47	47.25^{b}±0.75	42.72^{ab}±0.43	4.67**
Cumulative fodder yield 3 cuts (t DM/ha)	3.92^{a}±0.04	5.14^{b}±0.05	4.80^{b}0.02	0.34**
Cumulative fodder yield 4 cuts (t DM/ha)	5.36^{a}±0.07	6.82^{b}±0.11	6.24^{b}±0.06	0.68**
Straw yield after 3 cuts (t DM/ha)	1.47^{a}±0.01	1.75^{b}±0.03	1.87^{b}±0.07	0.18*
Straw yield after 4 cuts (t DM/ha)	0.70^{a}±0.02	0.91^{b}±0.03	1.12^{c}±0.03	0.19**
Seed yield after 3 cuts (q/ha)	2.53^{b}±0.06	3.24^{c}±0.04	1.07^{a}0.07	0.49**
Seed yield after 4 cuts (q/ha)	1.55^{b}±0.06	1.89^{b}±0.02	0.85^{a}±0.05	0.39**
Total biomass yield after 3 cuts (t DM/ha)	5.65^{a}±0.03	7.21^{c}±0.07	6.78^{b}±0.04	0.40**
Total biomass yield after 4 cuts (t DM/ha)	6.21^{a}±0.09	7.92^{b}±0.08	7.44^{b}±0.09	0.74**

^{a,b,c}Value having different superscripts within a row differ significantly (P<0.05*; P<0.01**)

top dressing of urea @ 60 kg/ha was provided after each harvest. Forth cut fodder was harvested at 130 days of crop duration from 50% area (75 m^2) of all sub-plots and the rest was left for seed production. However, the sub-plots from where 4th cut fodder was harvested were left for seed production. Finally, crops of all sub-plots were harvested at 160 days of crop duration to record total biomass and seed yield. Dry matter content in fodder, straw and seeds was estimated (AOAC, 2005). The data were analysed for test of significance as per Snedecor and Cochran (1994).

The results showed that the highest (P<0.01) cumulative fodder yield was recorded in Wardan variety irrespective of total cuttings than JHB-146, however, fodder yield of hybrid berseem was at par with Wardan variety. Cumulative maximum fodder yield from Wardan variety was found to be 35.63±0.37 and 47.25±0.75 t/ha in 3rd and 4th cuts, respectively which appear to be sufficient to meet fodder needs (25 kg/d/head) of 20 adult cattle unit for 70 or 100 days. Sardana and Narwal (2000) also reported maximum cumulative fodder yield from more number of cuttings. Similar type of trend was observed for DM yield. The cumulative fresh yield of Mescavi variety of berseem (3 cuts) ranged from 35.0 to 42.5 t/ha in Tripura state of north-east India having acidic soil conditions (Chander Datt *et al.,* 2009). The straw yield after third cut of fodder was recorded to be highest (P<0.05) in hybrid berseem than 4th cut. However, no significant difference was observed between hybrid and Wardan varieties with respect to straw yield. Fourth cut was obtained in last week of March when ambient temperature increases and humidity reduces which results into retarded plant growth and straw yield.

The maximum berseem seed yield was recorded in Wardan variety (3.24±0.04 q/ha) after third cut fodder in Ist week of March than JHB-146 (2.53±0.06 q/ha) and Hybrid (1.07±0.07 q/ha) varieties. However, seed yield reduced to the level of 38.73, 41.67 and 20.56% in JHB-146, Wardan and Hybrid berseem varieties, respectively after 4th cut of fodder in last week of March. Maximum flowering was observed after 3rd cut fodder in Ist week of March with favourable weather conditions that might be the probable reason for maximum seed yield (Yadav *et. al*., 2015). Total biomass yield was the highest in Wardan variety irrespective of cuttings of fodder. However, total biomass yield increased after 4th cut in each variety.

Hence, berseem fodder may be harvested only three times and then left for maximum seed yield, however, reasonably good seed yield could be taken from Wardan variety even after 4th cut of fodder in last week of March.

REFERENCES

AOAC. 2005. *Official Methods of Analysis*. 18th edn. Association of Official Analytical Chemists, Washington, DC, USA.

Chander Datt, Singh, N.P., Aruna Chhabra, Bujarbaruah, K.M. and Dhiman, K.R. 2009. Nutritional evaluation of cultivated fodders grown under agro-climate of Tripura. *Indian J. Anim. Sci.* 79: 1143-1148.

Gupta, J.J., Dey, A. and Bhatt, B.P. 2017. Fodder production and feeding technology for livestock in eastern India. Technical Bulletin No. R-61/Patna-35, ICAR Research Complex for Eastern Region, Patna, India. pp.27.

Gupta, J.J., Dey, A., Bhatt, B.P. and Barari, S.K. 2016. Potential yield and nutritive value of promising forages of eastern India. *Range Management and Agroforestry,* 37: 248-252.

Hazra, C.R. 1992. Requirements and strategies in forage seed production. In: "*Proc. National Symposium on Strategy for Forage Production and Improvement by 2000 A.D.*" (R.S. Paroda, S.K. Arora and A.S. Faroda; eds.) held at Trivandrum, Kerala, India. pp.211-219.

Hazra, C.R. and Sinha, N.C. 1996. *Forage Seed Production - A Technological Development.* South Asian Publishers, New Delhi, India. pp 91-115.

Kumar, V. 2013. Area under fodder production in India. http://agropedia.iitk.ac.in/content/area-under-fodder-production-india.

Saini, S.L. and Chowdhury, R.K. 1993. Effect of finalcutting for fodder on seed yield and seed quality in berseem. In: "*Proc. XVII International Grassland Congress*", Palmerston, Hamilton, Lincoln, New Zealand. Volume II, pp 1690-1691.

Sardana, V. and Narwal, S.V. 2000. Seed yield and quality of late-sown berseem (*Trifolium alexandrinum* L.) under different sowing dates and cutting management. *Indian J. Agron.* 45: 437-442.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Yadav, P.S., Vijay, D. and Malaviya, D.R. 2015. Effect of cutting management on seed yield and quality attributes of tetraploid berseem. *Range Manage. Agroforest.* 36: 47-51.

Received on 16-08-2018 and accepted on 10-01-2019

Indian J. Anim. Nutr. 2019. 36 (1): 94-98
doi: 10.5958/2231-6744.2019.00017.3

Indian Journal of Animal Nutrition

SHORT COMMUNICATION

Influence of Supplementing Giloe and Cinnamon on Production Performance in Commercial Broiler Chicken

Garima Tiwari, Anshu Rahal*, Ashoka Kumar and Anil Kumar
Department of Animal Nutrition, College of Veterinary & Animal Sciences,
GBPUAT, Pantnagar-263145, Uttarakhand, India

ABSTRACT

Feeding trial of 42 days was conducted to study the effect of dietary incorporation of giloe and cinnamon powder on growth performance in broiler chicken. A total of 120, day-old broiler chicks were divided randomly into 6 groups with 2 replicates of 10 chicks each *viz.*, T_1 (control) were fed basal diet whereas in treatment group T_2, T_3, T_4, T_5 and T_6, basal diet was incorporated with 1% giloe, 2% giloe, 1% cinnamon, 2% cinnamon and combination of 1% giloe and 1% cinnamon, respectively. During the whole experimental period (42 d), incorporation of 1% giloe improved broiler performance in terms of body weight, weight gain, feed conversion ratio and performance index. Hence, increased body weight gain, better feed conversion ratio and performance index was attained by inclusion of giloe @ 1% in diet of broiler chicken.

Key words: Broiler chicken, Cinnamon, Giloe, Growth performance

Cinnamon (*Cinnamomum zeylanicum)* belongs to the *Lauraceae* family. Cinnamon primarily contains essential oils and other derivatives such as cinnamaldehyde, cinnamic acid and cinnamate. Cinnamon has activities against neurological disorders such as Parkinson's and Alzheimer's diseases (Sangal, 2011). Cinnamon helps in reducing colon cancer (Long *et al*, 2015). *Giloe (Tinospora cordifolia),* a deciduous climbing shrub, protects the body against diseases. Giloe leaves, barks and roots contain-various bioactive compounds. It has anti-spasmodic, anti-inflammatory, anti-arthritic and anti-allergic properties (Chopra *et al.,* 1982). The aqueous extract of giloe plant possessed hepato-protective effect (Ganguly and Prasad, 2011). Herbal extracts in diet of broilers enhanced their performance (Koochaksaraie *et al.,* 2011; Petrolli *et al.,* 2012; Faghani *et al.,* 2014; Shirzadegan., 2014; Hussein *et al.,* 2016). Realizing their beneficial effects both giloe and cinnamon either singly or in combinations were used in diets of broiler chicken to study their growth performance.

For the experimental feeding trial, 120, day-old broiler chicks (Vencobb) weighing 35- 45 g were purchased from the adjoining local market, Bajpur. All the chicks were individually weighed and wing banded. Then chicks were randomly allotted to six different groups each with two replicates of 10 chicks in such a way that average body weight was similar for all the groups. Feeding trial was carried out in completely randomised design. Feed used for experimental broiler chicks were as per BIS specification (1997). Giloe stem and cinnamon bark were collected from Medicinal Plant Research Development Centre (MRDC) of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttrakhand. The stems and bark were dried, ground and stored in moisture free bags until used. The proximate composition of different diets was analysed (AOAC, 2005). Group T_1 was taken as control while in groups T_2 and T_3, giloe was added @ 1 and 2% to the basal diet, respectively. Cinnamon was supplemented @ 1 and 2%, respectively in groups T_4 and T_5 while in group T_6, a combination of giloe @ 1%+cinnamon @ 1% was added to the basal diet. The broiler chicks were housed in deep litter system and provided *ad lib.* feed and water throughout the trial period. Adequate light, temperature and ventilation were provided during the experiment. A layer of 4-5 cm thick rice husk was provided as litter material. All the housing and

*Corresponding author: E-mail: anshurahal@rediffmail.com

managemental conditions were similar in all the groups. The starter diet was provided during 0-3 weeks while, finisher diet was provided during 4-6 weeks of age in clean feeder. Fresh and clean water was made available to all the birds during the study. The body weight of individual birds and residual feed was recorded weekly replicatewise. The birds were vaccinated for IBD on 14^{th} d and RD vaccine (Lasota strain) booster on 28^{th} d. Growth performance was studied by recording and calculating feed intake and body weight gain. Feed conversion ratio (FCR) was calculated by dividing feed consumed by weight gain for a particular period. The performance index (PI) of broilers during different periods of growth was calculated by using the formula (Bird, 1995) *i.e.* body weight gain (g) divided by FCR. The data were analysed statistically (Snedecor and Cochran, 1994) by using software SPSS 16 with the one way Analysis of Variance (ANOVA) technique.

The chemical composition of broiler starter and finisher diets has been presented in Table 2. The average body weight gain was higher (P<0.05) in chicken fed diets incorporated with giloe and cinnamon powder. There was improvement in body weight gain due to incorporation of giloe and cinnamon powder, however, the body weight gains were similar in groups T_2, T_3, T_4 and T_6 were statistically similar. The average weight gain in group T_1 differed (P<0.05) from groups T_2, T_3, T_4 and T_6. The highest weight gain was observed in group T_2 (1% giloe). The cumulative feed intake was also similar in different groups. The FCR varied from 1.76 in group T_2 (1% giloe) to 1.95 in group T_1 (control). The better FCR was observed in group T_2 followed by groups T_6 and T_3. The performance index during 0-42 days of feeding trial was higher (P<0.01) in group T_2 followed by groups T_6 and T_3. The overall cumulative performance in terms of weight gain, FCR and performance index improved due to incorporation of giloe and cinnamon powder in the broiler chicks compared to control. Significant improvement in body weight on supplementation of giloe alone has also been reported by Gujral *et al.* (2002) and Kumar *et al.* (2006). The use of giloe alone as well as in combination with bael showed significant effect on body weight gain. The results of the present study are corroborated by findings of other workers (Kulkarni *et al.,* 2011; Bhardwaj *et al.,* 2011; Rajeshwari *et al.,* 2012;

Table 1. Ingredient composition (%) of basal diets

Ingredient	Starter diet (0-3 wk)	Finisher diet (4-6 wk)
Maize	55.00	60.00
Deoiled soyabean meal	36.00	32.00
Rice polish	4.60	3.10
Soyabean oil	0.50	1.00
Marble stone	1.00	1.00
Dicalcium phosphate	2.00	2.00
DL- methionine	0.10	0.10
Coccidiostat (Meduramycin)	0.05	0.05
Copper sulphate	0.01	0.01
Common salt	0.30	0.30
Merivite -100 (Vitamin B_{12})	0.02	0.02
Phosphoric acid	0.10	0.10
Lipocare (choline chloride)	0.05	0.05
Hepatocare	0.10	0.10
Vitamin mixture	0.03	0.03
Trace minerals	0.14	0.14

Table 2. Chemical composition (% DM basis) of the experimental diets

Parameter	Group					
	T_1	T_2	T_3	T_4	T_5	T_6
Starter diets (0-3 wk)						
Dry matter	93.97	94.22	94.18	94.06	94.48	94.1
Crude protein	22.89	22.92	22.96	22.90	22.92	22.90
Ether extract	3.85	3.75	3.77	3.80	3.81	3.79
Crude fibre	4.11	4.78	4.81	4.25	4.29	4.60
Ash	10.24	6.92	6.97	6.87	6.90	6.90
Nitrogen-free extract	58.91	61.63	61.49	62.18	62.08	61.81
Finisher diets (4-6 wk)						
Dry matter	94.06	94.62	94.92	94.31	94.27	95.06
Crude protein	19.23	20.70	20.75	20.52	20.58	20.64
Ether extract	3.60	3.42	3.47	3.54	3.58	3.51
Crude fibre	4.17	4.97	5.02	4.72	4.78	4.92
Ash	9.46	7.81	7.86	16.97	6.99	7.10
Nitrogen-free extract	63.54	63.1	62.9	64.25	64.07	63.83

Bhardwaj *et al.,* 2012). Sarag *et al.* (2001) and Gujral *et al.* (2002) also recorded increase in feed intake due to inclusion of giloe in the diet of broiler chicks. The inclusion of giloe improved FCR (Sarag *et al.,* 2001). Improvement in body weight gain and feed conversion ratio in supplemented groups might be due to active ingredients in giloe and cinnamon causing greater feed utilization efficiency.

The growth performance of broiler chicks fed diets containing cinnamon powder increased when compared to that of control chicks which could be related to the fact that aromatic herbs have some growth

Table 3. Growth performance of broiler chicken from 0-42 d fed diets incorporated with giloe and cinnamon powder

Parameter	Group						SEM	P value
	T_1	T_2	T_3	T_4	T_5	T_6		
Initial body weight (g)	44.40 ±1.20	44.00 ±1.20	41.95 ±0.55	44.10 ±1.10	44.60 ±0.60	43.20 ±0.20	0.38	0.40
Body weight at d 42* (g)	1453^{b} ±65.8	1624^{a} ±3.85	1582^{a} ±1.46	1561^{a} ±17.95	1519^{ab} ^{b}±20.06	1594^{a} ±8.75	19.04	0.05
Weight gain (g)	1408^{b} ±67.05	1580^{a} ±2.65	1540^{a} ±0.91	1517^{a} ±16.85	1474^{ab} ±19.46	1551^{a} ±8.55	19.19	0.05
Cumularive feed intake* (g)	2747 ±143.93	2774 ±57.47	2830 ±30.74	2832 ±27.20	2784 ±44.83	2817 ±36.37	22.68	0.93
Feed conversion ratio**	1.95^{a} ±0.01	1.76^{d} ±0.04	1.84^{bc} ^{c}±0.00	1.87^{bc} ±0.00	1.89^{b} ±0.01	1.82^{cd} ±0.01	0.02	0.01
Performance index**	722.23^{d} ±30.92	900.52^{a} ±21.67	838.10^{bc} ±0.12	812.87^{bc} ±10.25	780.73^{c} ±8.04	853.95^{ab} ±1.61		

^{a,b,c,d}Values bearing different superscripts in a row differ significantly ($^{*}P<0.05$; $^{**}P<0.01$)

promoting, appetite, digestion and antimicrobial effects (Kamel, 2001) and anti- heat stress properties for growing birds and may have stimulating effects on the animal digestive system (Langhout, 2000). Sang-Oh *et al.* (2013) also observed an increase in body weight, weight gain and better FCR in cinnamon fed broiler chicks which might be due to improved health and immune status of birds associated with increased serum levels of immunoglobulins. Dietary cinnamon powder has also been reported to have antibacterial (Valero and Salmeron, 2003; Hernandez *et al.,* 2004) and antioxidant properties (Park and Park, 2000).

Hence, increased body weight gain, better feed conversion ratio and performance index was attained by inclusion of giloe @ 1% in diet of broilers.

REFERENCES

AOAC. 2005. *Official Method of Analysis*. 18th edn. Association of Official Analytical Chemists. Washington, DC, USA.

Bhardwaj, U., Tiwary, B.K., Prasad, A. and Ganguly, S. 2011. Use of *Tinospora cordifolia* as poultry feed supplement. *Double Helix in Res.* 1: 18-22.

Bhardwaj, U., Tiwary, B.K., Prasad, A. and Ganguly, S. 2012. Effect of *Tinospora cordifolia* extract on immune response and serum biochemical profile in broilers. *Indian J. Anim. Sci*. 82: 379-381.

Bird, J. M. 1995. 'Performance index' of growing chicken. *Poult. Sci.* 34: 1163-64.

BIS. 1997. *Bureau of Indian Standards. Poultry Feeds-Specification*s IS- 1374. Manak Bhawan, New Delhi, India.

Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapur, L.D. 1982. *Indigenous Drugs of India*. Academic Publishers, New Delhi, India.

Faghani, M., Rahimian, Y., Rafiee, A. and Namjoo, A.R. 2014. Effect of garlic and cinnamon in comparison to virginiamycin on performance and some haematological parameters in broiler chicks. *Res. Opinions Anim. Vet. Sci.* 4: 504-507.

Ganguly, S. and Prasad, A. 2011. Role of plant extracts and cow urine distillate as immunomodulators: A review. *J. Med. Plants* Res. 5: 649-651.

Gujral, D., Jogi, S., Kumar, A. and Bais, R.K.S. 2002. Effect of herbal liver stimulants on efficacy of feed utilization in commercial broiler chicken. *Indian J Anim. Res.* 36: 43-45.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Megias, M.D. 2004. Influence of two plant extracts on broilers performance, digestibility and digestive organ size. *Poult. Sci.* 83: 169-174.

Hussein, T.K., Hwaidi, E.H. and Mohammad, A.H. 2016. The effects of cinnamon powder feeding on some blood aspects and performance of broiler chicken. *Kufa J. Vet. Med.l Sci.* 6: 118-122.

Kamel, !. 2001. Tracing methods of action and roles of plant extract in non-ruminant. In: *Recent Advances in Animal Nutrition* (Authors: P.C. Garnsworthy and J. Wiseman). *Nottingham University Press*. pp 135-150.

Koochaksaraie, R.R., Irani, M. and Gharavysi, S. 2011. The effects of cinnamon powder feeding on some blood metabolites in broiler chicks. *Revista Brasileira de Ciencia Avicola.* 13: 197-202.

Kulkarni, R.C., Mandal, A.B., Munj, C.P., Dan, A., Saxena, A. and Tyagi, P. K. 2011. Response of coloured broilers to dietary addition of geloi (*Tinospora cordifolia*) during extreme summer. *Int. J. Poult. Sci.* 46: 70-74.

Kumar, P., Tiwary, B.K., Singh, S.K., Shekhar, P. and Shee, N.C. 2006. Effect of an adaptogenic herbal preparation on humoral immune response and on some economic traits in broiler chicks. *J. Res.-Birsa Agricultural University* 18: 295.

Langhout, P. 2000. New additives for broiler chickens. *World Poult.* 16: 22-27.

Long, M., Tao, S., De La Vega, M.R., Jiang, T., Wen, Q., Park, S.L., Zhang, D.D. and Wondrak, G.T., 2015. Nrf2-dependent suppression of azoxymethane/dextran sulfate sodium-induced colon carcinogenesis by the cinnamon-derived dietary factor cinnamaldehyde. *Cancer Prev. Res. 8:* 444-454.

Park, R.J. and Park, Y.G. 2000. Studies on the effects of the parts of *Cinamomum cassia* PRESL on the antixidation. *Korea J. Herbol.* 15: 45-55.

Petrolli, T.G., Albino, L.F.T., Rostagno, H.S., Gomes, P.C., Tavernari, F.D.C. and Balbino, E.M. 2012. Herbal extracts in diets for broilers. *Revista Brasileira de Zootecnia*. 41: 1683-1690.

Prince, M., Stanely, P., Padmanabhan, M. and Menon, V.P. 2004. Restoration of antioxidant defence by ethanolic *Tinospora cordifolia* root extract in alloxan induced diabetic liver and kidney. *Phytother. Res.* 18: 785-787.

Rajeswari, P.R., Velmurugan, S., Babu, M.M., Dhas, S.A., Kesavan, K. and Citarasu, T. 2012. A study on the influence of selected Indian herbal active principles on enhancing the immune system in *Fenneropenaeus indicus* against *Vibrio harveyi* infection. *Aquac. Int*. 20: 1009-

1020.

Sangal, A. 2011. Role of cinnamon as beneficial antidiabetic food adjunct: A review. *Adv. Appl. Sci. Res.* 2: 440-450.

Sang-Oh, P., Chae-Min, R., Byung-Sung, P. and Jong, H. 2013. The meat quality and growth performance in broiler chickens fed diet with cinnamon powder. *J. Environ. Biol.* 34: 127.

Sarag, A.N., Khobragade, R.S., Rekhate, D.H. and Dhok, A.P. 2001. Supplementation of *T. cordifolia* (0.5%) and *L. reticulate* (0.5%) has positive effects on liveweight gain and feed efficiency of broilers. *Punjabrao Krishi Vidyapeeth Res. J.* 25: 114-115.

Shirzadegan, K. 2014. Reactions of modern broiler chickens to administration of cinnamon powder in the diet. *Iranian J. Appl. Anim. Sci.* 4: 367-371.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* 8th edn. The lowa State University Press, Ames, Iowa, USA.

Valero, M. and Salmeron, M.C. 2003. Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. *Int. J. Food Microbiol.* 85: 73-81.

Received on 16-10-2018 and accepted on 28-01-2019

Indian J. Anim. Nutr. 2019. 36 (1): 99-102
doi: 10.5958/2231-6744.2019.00018.5

Indian Journal of Animal Nutrition

SHORT COMMUNICATION

Effect of Rumen Modifier on Methanogenesis and Feed Digestibility under *in Vitro* Conditions

Neeti Lakhani*, Devki Nandan Kamra, Preeti Lakhani[1] and Anju Kala
Animal Nutrtion Division, ICAR- Indian Veterinary Research Institute,
Izatnagar-243122, Bareilly, Uttar Pradesh, India

ABSTRACT

Neem seed cake (*Azadirachta indica*), mahua seed cake (*Madhuca longifolia),* fennel seed (*Foeniculum vulgare*), harad (*Terminalia chebula)*, fruit pulp of bahera (*Terminalia bellirica*), fruit pulp of fruit of amla (*Phyllanthus emblica*) and ajwain seed (*Trachyspermum ammi*) were dried, powdered and mixed in a ratio of 2: 2: 2: 1: 1: 1: 1 to prepare a mixture of rumen modifier-7 (RM-7) and was tested at 0 (control) 5, 10, 15 and 20% of the substrate along with 0.06% of sodium sulphate (except control) to study their effects on rumen fermentation *in vitro.* Inclusion of RM-7 at 5, 10, 15 and 20% of substrate along with 0.06% of sodium sulphate did not affect gas production but there was significant (P<0.01) reduction (18.5 and 34.1%) in methane production as well as feed digestibility (60.88 to 52.71%)). Total volatile fatty acids (VFA), individual VFA (acetate, propionate and butyrate) and ammonia N levels were not affected by any of the treatments. Hence, inclusion of rumen modifier (RM-7 at 5, 10, 15 and 20% + 0.06% sodium sulphate of substrate) did not affect total gas production but there was significant reduction in methane production upto 10% level.

Key words: *In vitro* true digestibility, Methane, Rumen modifier, Sodium sulphate

The feed consumed by ruminants is fermented by synergistic activity of bacteria, protozoa, fungi, archaea and bacteriophages as a result of which the polysaccharides of feed are converted into volatile fatty acids and microbial protein, the two main sources of energy and protein for the host animals. But during feed fermentation in the rumen, CO_2 and H_2 gases are produced as by products and rumen methanogenic archaea reduce CO_2 into methane by utilizing H_2 which is eructated out through mouth. In rumen, methanogenesis is an essential metabolic process to maintain a low H_2 pressure but wasteful as 2-12% dietary energy (Johnson and Johnson, 1995) is wasted in the form of methane. Also, methane being a potent green house gas having 23 times more global warming potential than CO_2. Enteric methane production contributes significantly to global warming. Hence, the inhibition of methanogenesis has been of great concern for nutritionists since long back and now a days due to global warming, in the perspective of green house gas emission.

A number of feed additives like antibiotics, ionophores, defaunating agents and antimethanogenic compounds have been experimented to manipulate rumen fermentation to improve feed utilization efficiency with a reduction in methanogenesis in the rumen. But due to increasing awareness of the adverse effects associated with these chemical feed additives their use is being discouraged owing to several reasons. Several plant secondary metabolites (PSM) such as essential oils (EOs), saponins, tannins and flavonoids have been documented for their ability to reduce methane emission and improve feed utilization efficiency by modulating rumen microbial fermentation process (Inamdar *et al.,* 2015). PSMs are quite effective in methane mitigation at higher doses but fibre digestibility is also reduced (Pawar *et al.,* 2014). Hence, an *in vitro* experiment was designed to find out a combination of PSMs and sulphate to achieve maximum methane inhibition with no adverse effect on other fermentation parameters.

For this study, neem seed cake (*Azadirachta*

*Corresponding author-E-mail: lakhani10aishi@gmail.com; [1]Division of Animal physiology, ICAR- National Dairy Research Institute, Karnal-132001, Haryana, India

indica), mahua seed cake (*Madhuca longifolia),* fennel seed (*Foeniculum vulgare*), harad (*Terminalia chebula)*, fruit pulp of bahera (*Terminalia bellirica*), fruit pulp of fruit of amla (*Phyllanthus emblica*) and ajwain seed (*Trachyspermum ammi*) were dried, powdered and mixed in a ratio of 2: 2: 2: 1: 1: 1: 1 to prepare a mixture of rumen modifier-7 (RM-7). This RM-7 was used at the rate of 0, 5, 10, 15 and 20% along with sodium sulphate at 0.06% of the substrate. The plant parts selected in this study were those which showed promising results in the series of *in vitro* experiments conducted in the author's laboratory using several plant parts rich in PSMs (Patra *et al.* 2006a). The estimations were done in triplicates. *In vitro* gas production tests were conducted as per the procedure of (Menke and Steingass, 1988). The substrate (200 mg per syringe) used was wheat straw and concentrate mixture (maize grain 32, de-oiled soyabean meal 26, wheat bran 39, mineral mixture 2 and salt 1%) in 1 :1 ratio and rumen liquor was collected from two fistulated buffaloes fed on the same diet. The rumen liquor was then pooled together and used as inoculum. Incubation medium (30mL) was dispensed anaerobically in each syringe. Each set of syringe comprised of one treatment in triplicate and two blank syringes (without substrate). Three such sets were run for each percentage of RM-7. An incubation period of 24 h at 39°C for each set of syringes was allowed to study rumen fermentation parameters.

After 24 h of incubation, total gas production was estimated by piston displacement. For methane estimation, 100 µL of gas sampled from headspace of caliberated syringe was injected into Nucon-5765 gas chromatograph equipped with PorapakQ column and flame ionization detector (Agarwal *et al.,* 2008). A mixture of 50% carbon dioxide and 50% methane (Spancan; Spantech Products Ltd, Godstone, UK) was used as standard. For VFA estimation, 0.5 mL fermented medium was mixed with 0.1 mL of 25% metaphosphoric acid and allowed to stand at room temperature for 1 h. The mixture was centrifuged at 10,000 rpm for 10 minute and 1 µL of the supernatant was injected on Nucon-5765 gas chromatograph equipped with chromosorb 101 column and FID as per the procedure (Cottyn and Boucque, 1968) with some modifications (Agarwal *et al.,* 2008). Fermented medium was analyzed for NH_3-N (Weatherburn, 1967). The proximate principles (AOAC, 1995) and cell wall constituents (Van Soest *et al.,* 1991) in wheat straw and concentrate mixture and RM-7 were estimated. The means were compared using Tukey's test if the main effect was significant (*i.e.,* $P<0.05$) using SPSS Version 16.0 (Inc, Chicago, USA).

The chemical composition of concentrate mixture, wheat straw and RM-7 has been presented in Table 1. The total gas production in 24 h ranged from 138.4 to 141.6 mL/g DM. No significant increase in gas production was observed by inclusion of increasing level of RM-7 as compared to the control (Table 2). Arif *et al.* (2015) tested various plant parts and few oil cakes rich in different secondary metabolites like tannins, saponins, essential oils, flavonoids *etc.* There was no effect on *in vitro* gas production by inclusion of any of the plant parts at the rate of 10% of the substrate. Phuong (2012) reported significant ($P<0.05$) reduction in gas production by inclusion of sulphur (as sodium

Table 1. Chemical composition (% DM basis) of concentrate mixture, wheat straw and RM-7

Attribute	Concentrate mixture	Wheat straw	RM-7
Dry matter	93.78	94.18	92.13
Organic matter	89.12	90.92	88.90
Crude Protein	17.95	3.01	9.44
Ether extract	3.59	2.13	2.69
Neutral detergent fibre	28.38	86.50	43.31
Acid detergent fibre	12.20	53.38	37.63

RM-7, blend of neem, fennel, mahua, harad, bahera, ajwain and amla in 2: 2: 2: 1: 1: 1:1 proportion

sulphate) @ 0.4 and 0.8%.

Unlike gas production, methane production decreased (P<0.01) linearly by including graded levels of the rumen modifier. The reduction in methane production (mL/g DM) was 8.41, 22.97, 31.34 and 25.84% at 5, 10, 15 and 20% of substrate, respectively when compared to control. Similar results were obtained by Kumar *et al.* (2016) using fenugreek seeds @ 1, 2 and 3% level under *in vitro* conditions. Chaturvedi *et al.* (2016) reported that methane production (mg/g of substrate DM) reduced (P<0.05) by the inclusion of combinations of amla and neem. The significant reduction in level of methane production was due to the various components present in the rumen modifier. The RM 7 contained mixture of plant parts rich in tannins, saponins and essential oils which have already been tested for their antimethanogenic activity in the author's laboratory (Kumar *et al.* 2011; Patra and Yu, 2014). *In vitro* inclusion or feeding of sulphur resulted in reduction in methane production confirming its role as alternate electron acceptor (Van Zijderfeld *et al.,* 2010). The rumen modifier (RM-7 + sodium sulphate) also exhibited antimethanogenic activity in a dose dependent manner.

A significant linear decrease (P<0.01) in the feed IVTD was observed with increasing level of rumen modifier with maximum reduction at 20% level when compared to control. The values of IVTD ranged from 52.7 to 60.8%. In the present study, RM-7 was used which contained tannins (amla, harad and bahera), saponin (mahua), essential oils (ajwain and fennel), azadiractin and oils (Agarwal *et al.,* 2008). The ingredients were selected in a manner to achieve maximum methane inhibition with either increase in feed IVTD or no effect. Lila *et al.* (2003) reported decreased IVDMD with sarsaponin (methanol extract) at the level of 1.2-3.2 g/L of incubation medium. Gupta *et al.* (2017) reported increase in feed IVTD by including sodium sulphate either alone or in combination with blend of plant parts in the incubation mixture.

No significant differences in the level of TVFA, its fractions and ammonia were observed due to increasing level off RM-7. Gupta *et al.* (2017) found changes in TVFA and its fractions by inclusion of a blend of plant parts but were not affected by inclusion of sodium sulphate. The difference in the two experiments might be due to difference in the two blends and also the levels of the blend of plant parts. Ammonia nitrogen concentration in the fermented medium was not affected either by inclusion of the blend of plant parts or sodium sulphate alone or in combination (Gupta *et al.,* 2017) indicating no change in nitrogen metabolism due to addition of feed additive.

Therefore, rumen modifier had no significant effect on total gas production but was potent inhibitor of methane up to 10% level under *in vitro* conditions,

Table 2. Effect of rumen modifier on *in vitro* rumen fermentation parameters

Attribute	RM-7 (% of substrate)					SEM
	0 (control)	5.0	10.0	15.0	20.0	
Gas production(mL/g DM)	138±1.30	129±3.15	133±2.91	128±4.75	141±4.49	5.01
Methane(mL/g DM)	28.69^{b}±0.83	23.37^{b}±0.87	21.48^{a}±1.02	18.93^{a}±1.02	21.65^{a}±0.84	1.30
IVTD (%)	60.88^{c}±0.87	57.95^{b}±0.93	58.91^{b}±1.39	54.94^{ab}±1.47	52.71^{a}±0.81	1.60
NH_3-N (mg/dL)	9.52±0.47	10.54±0.15	9.81±0.32	10.87±0.84	9.84±0.59	0.85
TVFA (mM/L)	47.99±0.07	49.43±0.08	46.81±0.12	46.71±0.18	49.05±0.10	0.17
Acetate (%)	76.51±0.53	74.06±1.34	77.89±1.46	75.88±1.03	75.18±0.76	1.53
Propionate(%)	15.68±0.76	17.86±1.63	13.77±1.07	15.10±1.23	16.60±0.81	1.62
Butyrate (%)	7.62±0.30	8.39±0.73	7.76±0.73	8.07±0.79	8.81±0.64	0.94
A: P Ratio	4.96±0.29	4.32±0.48	5.38±0.28	5.25±0.47	4.75±0.18	0.57

[a,b,c]Mean values bearing different superscripts in a row differ significantly (P<0.001)

however, *in vivo* trials need to conducted for valid conclusions.

ACKNOWLEDGEMENT

The financial assistance in the form of a fellowship provided to the first author by the Director, ICAR-Indian Veterinary Research Institute, Izatnagar, India is gratefully acknowledged.

REFERENCES

Agarwal, N., Kamra, D.N., Chatterjee, P.N., Kumar, R. and Chaudhary, L.C. 2008. *In vitro* methanogenesis, microbial profile and fermentation of green forages with buffalo rumen liquor as influenced by 2-bromoethanesulphonic acid. *Asian-Australas. J. Anim. Sci.* 21:818-823.

AOAC. 1995. *Official Methods of Analysis*, 16th edn. Association of Official Analytical Chemists, Arlington, VA, USA.

Arif, M., Sarwar,M., Mehr-un-Nisa, Hayat, Z. and Younas, M. 2016. Effect of supplementary sodium nitrate and sulphur on methane production and growth rates in sheep and goats fed forage based diet low in true protein. *J. Anim. Plant Sci.* 26: 69-78.

Chaturvedi, I., Dutta, T.K., Singh, P.K. and Sharma, A. 2016. Effect of combined herbal feed additives on methane, total gas production and rumen fermentation. *Bioinfor.* 11: 261-266.

Cottyn, B.G. and Boucque, C.V. 1968. Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. *J. Agric. Food Chem.* 16:105-107.

Gupta, V.P., Kamra, D.N., Agarwal, N. and Chaudhary, L.C. 2017. Effect of sulphate and blend of plant containing secondary metabolites on *in vitro* methanogenesis, digestibility and feed fermentation with buffalo rumen liquor. *Indian J. Anim. Sci.* 87: 199-202.

Inamdar, A.I., Chaudhary, L.C., Agarwal, N. and Kamra, D.N. 2015. Effect of *Madhuca longifolia* and *Terminalia chebula* on methane production and nutrient utilization in buffaloes. *Anim. Feed Sci. Technol.* 201: 38-45.

Johnson, K.A. and Johnson, D. E.1995. Methane emissions from cattle. *J. Anim. Sci.* 73: 2483-2492.

Kumar, R., Kamra, D.N., Agarwal, N., Chaudhary, L.C. and Zadbuke, S.S. 2011. Effect of tree leaves containing plant secondary metabolites on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. *Anim. Nutr. Feed Technol*. 11: 103-114.

Lila, Z.A., Mohammed, N., Kanda, S., Kamada, T and Itabashi, H. 2003. Effect of sarsaponin on ruminal fermentation with particular reference to methane production *in vitro*. *J. Dairy Sci.* 86: 3330-3336.

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.* 28: 7-55.

Patra, A.K. and Yu, Z. 2014. Combinations of nitrate, saponin, and sulfate additively reduce methane production by rumen cultures *in vitro* while not adversely affecting feed digestion, fermentation or microbial communities. *Bioresource Technol.* 155: 129-135.

Patra, A.K., Kamra, D.N. and Agarwal, N. 2006. Effect of plants containing secondary metabolites on *in vitro* methanogenesis, enzyme profile and fermentation of feed with rumen liquor of buffalo. *Anim. Nutr. Feed Technol.* 6: 203-213.

Pawar, M., Kamra, D.N., Agarwal, N. and Chaudhary, L.C. 2014. Effects of essential oils on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. *Agric Res.* 3: 67-74.

Phuong, L.T.B. 2012. *Mitigation of Methane Production from Ruminants: Effect of Nitrate and Urea on Methane Production in an in Vitro System and on Growth Performance And Methane Emissions In Growing Cattle*. Thesis. M.Sc. Can Tho University. Vietnam.

SPSS. 2010. *Statistical Packages for Social Sciences*. Version 16, SPSS Inc., Illinois, USA.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Method of dietary fiber, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

Van Zijderveld, S.M., Gerrits, W.J.J., Dijkstra, J., Newbold, J.R., Hulshof, R.B.A. and Perdok, H.B. 2011. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. *J. Dairy Sci.* 94: 4028-4038.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chem.* 39: 971-974.

Received on 20-07-2018 and accepted on 24-12-2018

Indian J. Anim. Nutr. 2019. 36 (1): 103-106
doi: 10.5958/2231-6744.2019.00019.7

Indian Journal of Animal Nutrition

SHORT COMMUNICATION

Supplementation of Brown Seaweed (*Turbinaria conoids*) Powder and its Effect on Blood Metabolites and Mineral Profile in Adult Goats

Rojita Yengkhom*, Putan Singh, Nirmala Muwel, Kanti Raje, Bornalee Handique and K. Venkateswaran

Animal Nutrtion Division, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, India

ABSTRACT

Fifteen adult goats (14.59±1.41kg kg BW; 11-12 months of age) were assigned to 3 groups (CON, T_1 and T_2) using completely randomised design. Goats in CON group were fed a standard ration of concentrate mixture, straw and green with no seaweed supplement whereas, goats in T_1 and T_2 groups were given concentrate mixture having 4 and 8% of brown seaweed (*Turbinaria conoids*), respectively. The values of PCV, hemoglobin, albumin, globulin and A: G ratio were similar in all the groups, however, serum glucose and total protein levels were higher (P<0.01) in group T_2 as compared to groups T_1 and CON. Serum mineral concentation of Ca, P, Fe, and Mn did not differ among the groups whereas Cu level was found to be higher in T_1 as compared to CON and T_2 and serum Zn concentration was higher (P<0.01) in group T_1 followed by groupsT_2 and CON. Hence, incorporation of brown seaweed at 4% level in the concentrate mixture of adult goats increased the concentrations of glucose, total protein, Cu and Zn without any adverse impact on other blood metabolites.

Key words: Blood parameters, Brown seaweed, Goats, Serum minerals

Seaweeds are rich in complex carbohydrates, amino acids, vitamins like B_{12}, C, E and carotenoids (Burtin, 2003), minerals (Ito and Hori, 1989), natural bioactive compounds (Chojnacka *et al.,* 2012), growth promoters and fucoidan *etc.* (Urbano and Goni, 2002) and thus the supplementation of suitable amount of seaweeds to animal feed may promote the growth and thus improve animal health as well as quality of animal product. Brown algae (*Turbinaria conoids*) is largest seaweed and has been more exploited than other algae types for their use in animal feeding. Brown seaweed possesses large amount of iodine (I) and calcium (Ca) compared with other foods (Kaufmann *et al.,* 1998). Using seaweed as an animal feed supplement has great potential but little attention has been paid in India to exploit these as animal feed. Considering the functional properties of seaweeds, it would be most appropriate to use them as feed additive (Makkar *et al.,* 2016). Research conducted so far was mainly focused on therapeutic uses of red seaweed meals mostly in laboratory animals. Considering the functional properties of brown seaweed, it is hypothesized that supplementation of brown seaweed meal would satisfy the minerals requirement of goats without affecting vital organ functions.

The experiment was carried out at Animal Nutrition Research Shed, ICAR-Indian Veterinary Research Institute, Izatnagar. Fifteen adult goats (mean BW= 14.59±1.41 kg) were randomly divided into 3 groups with 5 goats each in completely randomized block design. The goats were randomly allocated into three dietary treatments; control (CON) and 2 treatment groups (T_1 and T_2). In the control group, goats were offered a concentrate mixture without brown seaweed, however, goats in groups T_1 and T_2 were given concentrate mixture having 4 and 8% of brown seaweed, respectively. The composition of respective concentrate mixtures has been given in Table 1. All the experimental animals were provided weighed amount of respective concentrate mixture daily (NRC, 2001). Wheat straw was offered *ad lib.* after ensuring complete consumption of the concentrate mixture. A small amount of green oats fodder (500 g) was given to all the experimental goats. All the goats were housed in

*Corresponding author: E-mail: rojitayeng11@gmail.com

a well ventilated shed having provision for individual feeding. The animals were provided with fresh and clean tap water free choice twice daily. Proper ethical care and management procedures were adopted throughout the study period according to the guidelines for Institutional Animal Ethics Committee for experimentation.

About 6 mL of blood samples were collected from all experimental goats early in the morning before feeding at 0, 30 and 60 d of experimental period from jugular vein. Out of 6 mL, 2 mL was added with EDTA for subsequent estimation of haematological parameters and the remaining was taken in a well cleaned sterilized centrifuge tubes to separate sera. Serum was separated and preserved at -20°C for further analysis. Haemoglobin (Hb) and packed cell volume (PCV) were estimated in whole blood immediately after blood collection by cyanomethaemoglobin method and Wintrobe's tube, respectively. All the serum biochemical parameters and concentration of Ca and P were estimated by using diagnostic kits (Cogent) manufactured by Span Diagnostics Pvt. Ltd., Surat, India. Iron, zinc, copper, manganese concentration in serum samples were estimated by Atomic Absorption Spectrophotometer (Electronics Corporation of India Ltd., Hyderabad, India, Model No. 4141). The data were analysed statistically (Snedecor and Cochran, 1994).

The data on haematological and biochemical have been presented in Table 2. The values of PCV (%), Hb (gdL^{-1}), albumin (gdL^{-1}), globulin (gdL^{-1}) and A: G ratio were similar in 3 groups and within the normal range reported for the species (Kaneko *et al.,* 1997). El-Banna *et al.* (2005) also reported similar results when rabbits fed with green seaweed (*Ulva lactuca*) and did not show any negative effects on haematological parameters. However, Nogueira *et al.* (2017) reported that feeding of brown algae (*Padina sanctae-crucis*) to mice at 2000 ppm raised blood metabolites significantly. The present results indicated that the dietary addition of brown seaweed to adult goats increased ($P<0.01$) blood glucose levels (mg dL^{-1}) in group T_2 followed by T_1 and CON groups although values remained within normal ranges (Braun *et al.,* 2007). Similarly, higher blood glucose levels have been reported when brown seaweed was added to the diets of goats (Kannan *et al.,* 2007) or lambs (Archer *et al.,* 2007). Total protein (g dL^{-1}) level was also higher ($P<0.01$) in group T_2 as compared to groups T_1 and CON. On the other hand, EL-Banna *et al.* (2005) and Okab *et al.* (2003) found comparable levels of glucose and protein among the groups when rabbits were fed with green seaweed (*Ulva lactuca*).

The concentrations of serum Ca, P, Fe and Mn were found to be similar in all the groups (Table 2). Venkateswaran, (2018) also observed comparable levels of serum Ca, P, Fe and Mn when Ca enriched brown seaweed was supplemented to crossbreed calves. Similarly, Singh *et al.* (2017) found no significant difference in serum Fe and Mn levels when lactating cows were supplemented with brown seaweed

Table 1. Ingredient composition (%) of concentrate mixtures

Ingredient	Group		
	CON	T_1	T_2
Maize	40	40	40
Wheat bran	40	40	40
Soyabean meal	17	17	17
Mineral mixture	2	2	2
Brown seaweed	0	4	8
Common salt	1	1	1
DCP (%)	13.85	13.72	13.75

CON: Conc. mix. without seaweed; T_1: Conc. mix. with 4% seaweed; T_2: Conc. mix. with 8% seaweed

Table 2. Effect of brown seaweed supplementation on blood metabolites and serum minerals in adult goats

Attribute	Treatment		
	CON	T_1	T_2
Blood metabolites			
PCV (%)	34.90	35.32	35.22
Hb (gdL^{-1})	9.32	9.25	9.25
Serum glucose** (mg dL^{-1})	62.91[a]	69.20[b]	73.41[c]
Total protein** (g dL^{-1})	6.56[a]	6.59[a]	6.92[b]
Albumin (g dL^{-1})	3.62	3.63	3.67
Globulin (g dL^{-1})	2.87	2.95	3.29
Albumin: globulin ratio	1.27	1.27	1.15
Serum mineral concentration			
Ca (mg/100 mL)	9.41	10.16	10.34
P (mg/100 mL)	3.96	3.95	4.57
Cu** (µg/100 mL)	69.04[b]	83.80[c]	63.27[a]
Fe (µg/100 mL)	347.67	348.97	343.00
Zn** (µg/100 mL)	172[a]	227.38[c]	185.33[b]
Mn (µg/100 mL)	94.78	98.15	91.74

[a,b,c]Means with different superscripts within a row differ significantly (P<0.01)

(*Sargassum wightii*) at 20% level in concentrate mixture. However, Chicco *et al.* (1973) reported that plasma Ca was greater (P<0.05) with increased dietary Ca from 0.19 to 0.43% in sheep. Regarding the serum P level, the result are in agreement with the findings of earlier workers (Alfaro *et al.,* 1988). Alfaro *et al.* (1988) reported that dietary Ca levels varying from 0.17 to 2.35% had no effect on serum inorganic P concentrations in dairy calves.

Serum Cu concentration was higher (P<0.01) in group T_1 followed by groups CON and T_2. However, Stevens *et al.* (1970) observed that neither the ratio of Ca: P (1.5:1.0 to 3.0:1.0) nor the level of P (0.32 to 0.48%) appeared to affect the blood serum concentration of Cu. Serum Zn level was higher (P<0.01) in group T_1 followed by groups T_2 and CON. Zn concentration in plasma increased in animals fed mineral mixture in diet and the results of the present study were in agreement with the findings of earlier workers (Lall and Prasad, 1990; Ashutosh and Singh, 2007). This phenomenon might be due to high bioavailability of seaweed minerals (Chojnacka, 2008).

Therefore, incorporation of brown seaweed at 4 and 8% levels in concentrate mixture did not affect blood biochemical parameters except that the concentration of serum glucose and protein was higher in group T_2. Supplementation of brown seaweed (4% in concentrate mixture) enhanced serum Cu and Zn levels.

ACKNOWLEDGEMENTS

The authors are thankful to Director, IVRI, Izatnagar, for providing necessary facilities to complete this study. The financial assistance provided to first author by ICAR, New Delhi is duly acknowledged.

REFERENCES

Alfaro, E., Neathery, M.W., Miller, W.J., Crowe, C.T. and Black, D.M. 1988. Influence of a wide range of macro elements and microelements in dairy calves. *J. Dairy Sci.* 71: 1295-1300.

Archer, G.S., Friend, T.H., Caldwell, D., Ameiss, K. and Krawczel, P.D. 2007. Effect of seaweed *Ascophyllum nodosum* on lambs during forced walking and transport. *J. Anim. Sci*. 85: 225-232.

Ashutosh, M. and Singh, M. 2007. Blood plasma minerals, enzymes and hormonal profile of mastitic cows.

Indian J. Dairy Sci. 6: 417-421.

Braun, U., Heusmann, B., Camenzind, D. and Haessig, M. 2007. Comparison in blood glucose concentrations after administration of a glucose solution *via* the jugular vein and portal vein in cows. *J. Vet. Med*. 54: 398-400.

Burtin, P. 2003. Nutritional value of seaweeds. *Electron J. Environ Agric. Food Chem*. 2: 498-503.

Chicco, C.F., Ammerman, C.B., Feaster, J.P. and Duvant, B.G. 1973. Nutritional interrelationship of dietary calcium, phosphorus and magnesium in sheep. *J. Anim. Sci*. 36: 986.

Chojnacka, K. 2008. Bioabsorption and bioaccumulation of microelements by *Riccia fluitans* in single and multi-metal system, *Bioresour. Technol*. 98: 2919-2925.

Chojnacka, K., Saeid, A., Witkowska, Z. and Tuhy, L. 2012. Biologically active compounds in seaweed extracts - the prospects for the application. *The Open Conf. Proc.* J. 3: 20-28.

El-Banna. S.G. 2003. Sea algae supplementation of Baladi rabbits diet and its implication on certain biochemical parameters. *Pesticide Control Environ*. Sci. 11: 81-96.

Ito, K. and Hori, K. 1989. Seaweeds: Chemical composition and potential uses. *Food Rev. Int.* 5: 101-144.

Kaneko, J.J. 1997. Serum proteins and the dysproteinemias. *Clinical Biochemistry of Domestic Animals*. 5th edn. Academic Press, London. pp 117-138.

Kannan, G., Terrill, T.H., Kouakou, B. and Galipalli, S. 2007. Blood metabolite changes and live weight loss following brown seaweed extract supplementation in goats subjected to stress. *Small Rumin. Res*. 73: 228-234.

Kaufmann, S., Wolfram, G., Delange, F. and Rambeck, W.A. 1998. Iodine supplementation of laying hen feed: a supplementary measure to eliminate iodine deficiency in humans. 37: 288-293.

Lall, D. and Prasad, T. 1990. Effect of certain calcium supplements on blood calcium and phosphorus levels. *Indian J. Anim. Nutr*. 7:1-6.

Makkar, H. P. S., Tran, G., Heuze, V., Giger-Reverdin, S., Lessire, M., Lebas, F. and Ankers, P. 2016. Seaweeds for livestock diets: A review. *Anim. Feed Sci. Technol.* 212: 1-11.

NRC. 2001. *Nutrient Requirements of Goats*. 7th rev. edn. Natl. Acad. Press, Washington, DC., USA.

Nogueira, R.B., Tomaz, A.C.A., Pessoa, D.R., Xavier, A.L., Pita, J.C.L., Sobral, M.V., Pontes, M L., Pessoa, H.L., Diniz, M.F., Miranda, G.E.C. and Vieira, M.A.R. 2017. Brown algae (*Padina sanctae-crucis*) Borgesen: A potential nutraceutical. *Marine Drugs* 15: 251.

Okab, A.B., Smara, E.M., Abdoun, K.A., Rafay, J., Ondruska, L., Parkanyi, V., Pivko, J., Ayoub, M. A., Al-Haidary, A.A., Alijumaah, R.S. and Peter, M. 2003. Effects of dietary seaweed (*Ulva lactuca*) supplementation on the reproductive performance of buck and doe rabbits. *J. Appl. Anim. Res.* 41:347-355.

Singh, B.K., Chopra, R.C., Rai, S.N., Verma, M.P. and Mohanta, R.K. 2017. Nutritional evaluation of seaweed on nutrient digestibility, nitrogen balance, milk production and composition in Sahiwal cows. *Proc. Natl. Acad. Sci., India Section B: Biol. Sci.* 87: 437-443.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* 8th edn. Iowa State University Press, Ames, Iowa, USA.

Steevens, B.J., Bush, L.J. and Williams, E.J. 1970. Effect of varying amounts of calcium and phosphorus in rations for dairy cows. *J. Dairy Sci*. 5: 655-660.

Urbano, M.G. and Goni, I. 2002. Bioavailability of nutrients in rats fed on edible seaweeds, nori (*Porphyra tenera*) and wakame (*Undaria pinnatifida*) as a source of dietary fibre. *Food Chem.* 76: 281-286.

Venkateswaran, K. 2018. *Effect of Replacement of Mineral Mixture by Calcium Enriched Brown Seaweed (Turbinaria conoides) on Performance of Crossbred Calves.* M.V.Sc. Thesis, IVRI, Izatnagar-243122, India.

Received on 10-01-2019 and accepted on 01-02-2019

Guidelines for author (s)

Indian Journal of Animal Nutrition (IJAN)

Submission of manuscripts

Manuscripts should be written so that these are intelligible to professional readers. The Editor or the Publisher reserves the right to modify typescripts to eliminate ambiguity, repetition and improve communication between author (s) and readers. If extensive alterations are required, the manuscript will be returned to the authors for revision.

Types of articles

The journal accepts original research papers, review papers, short communications and book reviews.

Covering letter

Manuscripts are accepted for publication in the Journal with the understanding that the contents have not been published or submitted for publication elsewhere. This must be stated in the covering letter. Any experiments involving animals must be demonstrated to be ethically acceptable for animal usage in research.

Acceptance

The acceptance criteria for all manuscripts are the quality and originality of the research and its significance to our readership. Except where otherwise stated, manuscripts are peer reviewed by one/two anonymous reviewers. Final acceptance or rejection rests with the Editorial Board, the Chief Editor being the final authority.

Submission

The original manuscript should be submitted online at **http://epubs.icar.org.in/ejournal/index.php/IJAN/about/submissions#onlineSubmissions**

For further details, please contact

Dr. Asit Das
Chief Editor, Indian Journal Animal Nutrition
Animal Nutrition Division
National Dairy Research Institute Karnal-132001
Haryana, India
E-mail: **editoransi@gmail.com**

If a manuscript is returned to the author for revision, it must be resubmitted within three weeks. Please do not forget to mention the manuscript number in all correspondences.

Main format

Submission should be typed in double space using MS word format (Windows 2007). The top, bottom and side margins should be 30 mm All the pages should be numbered consecutively in the bottom of right-hand corner beginning with the title page.

Style

The Journal uses spellings according to the latest edition of Oxford or Chamber's Dictionary. All measurements must be given in SI units. Abbreviations should be used sparingly and only where they ease the reader's task by reducing repetition of long technical terms. Initially, use the word in full followed by the abbreviation in parentheses. Thereafter, use the abbreviation. Upon its first use in the title, abstract and text, the common name of a species should be followed by the scientific name (Genus, species and authority) in parentheses. However, for well-known species, the scientific name may be omitted from the article title. If no common name exists in English, the scientific name should only be used. At the first mention of a chemical substance, give the generic name only. The words like *in vivo, in vitro, et al., ad lib.* etc. should be in italics. Trade names should not be used or avoided. Drugs should be referred to by their generic names rather than brand names.

Parts of the manuscript

Manuscripts should be presented in the following order: (i) TITLE PAGE, (ii) ABSTRACT and Key words (iii) INTRODUCTION (iv) MATERIALS AND METHODS (v) RESULTS AND DISCUSSION (vi) CONCLUSION (vii) ACKNOWLEDGEMENT (s) if any (viii) REFERENCES (ix) TABLES (each table complete with title and footnotes) and (viii) FIGURE LEGENDS and FIGURES. Except Title, all the major heads may be typed in CAPITAL LETTERS.

Title page

The title page should contain (i) the title of the paper **MAY BE TYPED IN TITLE CASE** (Capitalise each word except helping verbs) **along with SHORT TITLE** (ii) the full names of the authors (iii) the addresses of the institutions where the work was carried out together with (iv) the full postal and **e-mail address** plus facsimile and telephone numbers of the author to whom correspondence should be made. The title should be short, informative and contain the major key words. The title should be as: **Feed Intake, Growth and Blood Parameters in Sahiwal Female Calves Varying in Residual Feed Intake**

Abstract and key words

Articles must have a structured abstract that states in within 300 words, the purpose, basic procedures, main findings and principal conclusions of the study. Abstract

should not contain abbreviations or references. Five or fewer key words (for the purpose of indexing) should be given below the abstract.

References

In the text, give the author's name followed by the year in parentheses: Sharma (2000). If there are two authors use 'and': Sharma and Mudgal (2001); but if cited within parentheses: (Sharma and Mudgal, 2001). When reference is made to a work by three or more authors, the first name followed by *et al.* should be used: Thakur *et al.* (2002). In the list, references should be listed in alphabetical order. Cite the name of all authors. Reference to unpublished data and personal communications should not appear in the list but should be cited in the text only (*e.g.* Smith, A., 2000, unpublished data). The latest relevant references should only be cited.

Books and Articles within Edited Books

AOAC. 2005. *Official Methods of Analysis.* 18th edn. Association of Official Analytical Chemists. Washington, D.C., USA.

NRC. 2001. *Nutrient Requirements of Dairy Cattle.* 7th rev. edn. National Academiey of Sciences. National Academy Press, Washington, D.C., USA.

Handbooks, Technical Bulletins, Theses and Dissertations

Goering, H.K. and Van Soest, P.J. 1970. *Forage Fiber Analyses (Apparatus, Reagents, Procedures and Some Applications).* Agric. Handbook No. 379. ARS-USDA, Washington, D.C., USA.

Kumar, A. 2011. *Efficacy of Pentasulphate Mixture and Arsenic in Alleviating Chronic Selenosis and Expression of Glutathione Peroxidase Genes in Buffaloes.* M.V.Sc. Dissertation. ICAR-National Dairy Research Institute (Deemed University), Karnal-132001, Haryana, India.

Journal Articles and Abstracts

Chander Datt, Datta, M. and Singh, N.P. 2008. Assessment of fodder quality of leaves of multipurpose trees in subtropical humid climate of India. *J. Forestry Res.* 19: 209-214.

Conference Proceedings

Akash Mishra, Chander Datt, Sawati Shivani, Veena Mani, Goutam Kaul, A.K. Mohanty and Avinesh Sharma. 2018. Effect of supplementation of nano zinc on *in vitro* rumen fermentation parameters. In: *"Proc. XVII Biennial Animal Nutrition Conference on Nutritional Challenges for Raising Animal Productivity to Improve Farm Economy"* (Chander Datt, A.K. Tyagi, H.H. Savsani, Nitin Tyagi, Sachin Kumar, J.A. Chavda, Raman Malik and N.K. Ribadiya; eds.) held at Junagadh Agricultural University, Junagadh, Gujarat from Feb. 1-3, 2018. p 71.

Electronic Publications

FDA. 2001. Effect of the use of antimicrobials in food-producing animals on pathogen load: Systematic review of the published literature. http:// www.fda.gov/cvm/ antimicrobial/PathRpt. PDF, Accessed Dec.14, 2001.

Faciola, A.P. and Broderick, G.A. 2013. Effects of feeding lauric acid on ruminal protozoa numbers, fermentation, digestion and on milk production in dairy cows. *J. Anim. Sci.* DOI: 10.2527/jas.2012-5169.

Tables

Tables should be self-contained and should not duplicate the information contained in the text. Tables should be numbered consecutively in Arabic numerals. Each table should be presented on a separate sheet of A4 paper with a comprehensive but concise legend above the table. Tables should be brief with units of measurement in parentheses; all abbreviations should be defined in footnotes. Footnote symbols: 1, 2, 3, should be used (in that order) and *, ** should be reserved for P-values. The table and its legends/footnotes should be understandable without reference to the text.

Figures

All illustrations (line drawings and photographs) are classified as figures. Figures should be cited in consecutive order in the text. Figures should be sized to fit within the column (82 mm), intermediate (110 mm) or the full text width (17 mm). Graphics should be prepared using applications capable of generating high resolution, i.e. GIF, TIFF, JPEG or PPT format before pasting in the MS word manuscript file.

Figure legends

Legends should be self-explanatory and typed on a separate sheet. The legend should incorporate definitions of any symbols used and all abbreviations and units of measurement should be explained so that the figure and its legend is understandable without reference to the text. (Authors must provide a letter stating that copyright authorization has been obtained if figures have been reproduced from another source).

New office bearers and CEC members of Animal Nutrition Society of India

Dr. R.S. Bhatt, Returning Officer and Principal Scientist, ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan declared the results of elections for office bearers and CEC members of ANSI as given in the list.

Post	Name
President	**A K Tyagi,** Karnal, Haryana
Secretary	**Udeybir Singh Chahal,** Ludhiana, Punjab
Vice President, North Zone	**Shiv Vani Katoch,** Palampur, Himachal Pradesh
Vice President, South Zone	**MVAN Suryanarayana,** Tirupati Andhra Pradesh
Vice President, West Zone	**Harish Savsani,** Junagarh, Gujarat
Vice President, East Zone	**Pankaj Kumar Singh,** Patna, Bihar
Vice President, Central Zone	**S K Verma,** Meerut, Uttar Pradesh
Chief Editor	**Asit Das,** Karnal, Haryana
Treasurer	**Amrit Pal Singh Sethi,** Ludhiana, Punjab
CEC Members	**Ahmed Haider Ali,** Srinagar, Jammu & Kashmir **Amlan Kumar Patra,** Kolkata, West Bengal **Jaspal Singh Lamba,** Ludhiana, Punjab **Jaswinder Singh,** Ludhiana, Punjab **Kiran Kumari Mishra,** Durg, Chhattisgarh **K.S.N. Prasad,** Bengaluru, Karnataka **Madhu Mohini,** Karnal, Haryana **Nitin Tyagi,** Karnal, Haryana **Pradeep Kumar Malik,** Bengaluru, Karnataka **Rajesh Nehra,** Bikaner, Rajasthan **Sanjay Kumar,** Patna, Bihar **Sanjay Kumar Attri,** Pantnagar Uttrakhand **Sudarshan Singh Thakur,** Karnal, Haryana **Vijay Kumar Sharma,** Jammu, Jammu & Kashmir **Vinod Kumar Paswan,** Banaras, Uttar Pradesh

OBITUARY

Dr. Kamal Kishor Singhal passed away on Nov. 28, 2018

Dr. K.K. Singhal, Ex- Principal Scientist & Head, Department of Animal Nutrition, ICAR- National Dairy Research Institute, Karnal, Haryana left for heavenly abode on Nov. 28, 2018

Dr. K.K. Singhal was born on Dec. 28, 1947 in Manglour town, Haridwar, Uttar Pradesh. He got his Ph.D. degree in 1979 from NDRI, Karnal and later joined Agricultural Research Service as Scientist S-l at the same institute in 1977 where he served in various positions and was selected as Head of Dairy Cattle Nutrition Division (2003-08). He worked in the area of utilization of agro-industrial byproducts and their processing as complete feed, dietary levels of NPN in ruminant's diet and their influence on productivity of animals, detoxification and metabolism of glucosinolates in ruminants, total emission of methane from Indian ruminant animals and dietary manipulation for its mitigation besides the evaluation of transgenic cottonseed for the feeding of lactating cows. He was conferred with Rafi Ahmed Kidwai Award (1980-81), Dr. Rajendra Prashad Award (1994-96) by ICAR, Certificate of Merit (1989- 91) CLFMA, India, Best Research Paper award (1996) by IDA, New Delhi and Dr. S.P. Arora Award (2009) by Animal Nutrition Society of India besides several other awards by NDRI for his technical articles in Hindi. He published more than 100 research papers in national and international peer reviewed journals, 4 text books including Dictionary of Animal Nutrition besides several bulletins, book chapters, review papers and technical articles. He taught several M.Sc and Ph.D. courses till his superannuation and guided 6 Ph.D and several M.V.Sc. scholars. He was the Chief Editor of Indian Journal of Animal Nutrition (1994- 2000). During his 6 years tenure as incharge, Hindi cell, NDRI, he was bestowed ICAR award for best institution.

The Animal Nutrition Society of India conveys the heartfelt condolences to the bereaved family and prays to almighy god that his soul may rest in peace.

(ANSI Family)

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

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

CONTENTS

Printed and published by Dr. Chander Datt, Chief Editor, Indian Journal of Animal Nutrition, on behalf of Animal Nutrition Society of India at M.M. Printing Press, 202-L, Model Town, Karnal. M : 94160-72992