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Nutrition and Feeding of Mithun (*Bos frontalis*) in Hill Livestock Farming System

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ABSTRACT : Mithun (*Bos frontalis*) is an important animal of North-Eastern hilly region of India. It plays an important role in economical, social and cultural life of the tribal people. This animal is reared under free grazing condition primarily as a meat animal. However, as per the World Conservation Union, this species is vulnerable to extinction. Nutrition has a big role to play not only for better productive and reproductive performance of animals but also for conservation of this species through scientific utilization of natural resources. A lot of information on basic and applied nutrition of mithun has been generated in the National Research Centre on Mithun, Nagaland. Mithun husbandry is linked with some age-old social values and ethics. The feelings of the tribal people need to be respected while motivating them for a new system of mithun husbandry. Once population of mithun increases sufficiently with scientific rearing and feeding, it will be easier to convince livestock farmers to rear mithun for economic and social gains.

Key Words : Mithun, *Bos frontalis*, Feeding

INTRODUCTION

Mithun (*Bos frontalis*) is considered to be the domesticated form of wild gaur (*Bos gaurus*). This unique bovine species is believed to be domesticated more than 8000 years ago and is mainly available in the four North-Eastern hilly states of India i.e. Arunachal Pradesh, Nagaland, Mizoram and Manipur. Besides, mithun is also available in Jammu and Kashmir. According to the 2003 livestock census, mithun population in India is 0.28 million out of which 1,92,000 animals are available in Arunachal Pradesh followed by 40,000 in Nagaland, 24,000 in Jammu and Kashmir, 20,000 in Manipur and 2,000 in Mizoram. Mithun is an extremely efficient grazer on steep hilly slopes compared to other animals.

Mithun plays an important role in economical, social and cultural life of the tribal people of North-Eastern hill region of India. It is primarily reared as a meat animal and is highly preferred among the tribal people of North-Eastern India. However, as per the world Conservation Union ((IUCN, 2002), this species is vulnerable to extinction. So maximum care needs to be taken for conservation and improvement of this species.

Feeding habits of mithun

This animal is reared exclusively under free grazing condition. Mithun basically thrives on the jungle forages, tree fodders, shrubs, herbs and other natural vegetations. It prefers to browse and move around the forest in search of selective forages. Farmers do not provide any additional feeding. However, they occasionally provide common salt, especially at the time of restraining for some purposes. The owner of the animals sometimes produces a sound out of the musical instrument prepared by horn and hearing the sound, the mithun comes from jungle in search of common salt.

In the hilly areas of NE region, leaching of minerals by rain water is a common phenomenon especially during rainy season. So soils are deficient in some important mineral elements reducing the level of minerals in the vegetation. Salt licking behavior by the animals is a way to meet the requirement of minerals.

Tree leaves as cheap source of nutrients

The North East India is covered with forests. The tree foliages are available in plenty. Tree forages not only provide a cheap source of nitrogen, energy and micronutrients but also have many other advantages like their laxative influence on the alimentary system, low degradability of nitrogen in the rumen, and above all the scope of adding variety to the diet. However the presence of antinutritional factors like tannin in most of the leaves limit their use as sole animal fodder. Pal and Bujarbaruah (1999a) analysed different tree leaves and grasses consumed by mithun and compared in terms of chemical composition. The DM content of the grasses varied between 13.28 and 47.62, ash between 8.60 and 13.80, CP between 50.60 and 12.81, EE between 1.10 and 3.08 and total carbohydrate between 72.92 and 83.43 percent respectively. The corresponding values for the tree leaves were 20.06 to 47.46, 3.0 to 18.86, 9.83 to 21.00, 1.44 to 6.56 and 61.10 to 84.70 percent respectively. Fibre components of the grasses and tree leaves ranged from: NDF, 38.92 to 76.06 and 37.10 to 58.86; ADF, 25.94 to 47.94 and 17.21 to 41.64 and hemicellulose from 12.98 to 34.26 and 5.13 to 28.98 percent respectively. It was observed that ash and fibre fractions were comparatively higher in grasses than tree leaves whereas CP was higher in tree leaves than grasses. Prakash, (2006a) also analysed about 200 species of tree leaves/shrubs collected from different pockets of NEH region and found *Melia azadiracta, Adenosacme longifolia, Trema orientalis, Ficus hookeri, Macaranga denticulata* to be promising in this region. Tree leaves and shrubs contain more protein compared to cultivated fodders. The list of some of the tree leaves and shrubs and their chemical composition has been given in table 1. (Prakash et al. 2008a)

In sacco degradability of selected forest based foliages has been determined in rumen fistulated mithun using nylon bag technique. As per the findings *Thysanolaena agrostis, Emblica offencinalis, Ficus hirta and Jasminum* sp could be a very good source of energy as the effective DM degradability was higher. Similarly *Dacynia indica, Ficus infectoria* and *Curculigo recusvata* could be a medium source of by pass protein as the ruminal degradation of CP was lower. (Prakash et al., 2006b)

Tree foliage is natural source of bypass protein in the diet of ruminants. They contain certain endogenous secondary plant compounds to bind with protein and protect them from microbial attack. The condensed tannin present in tree leaves is used as organic protector of proteins. When herbage containing condensed tannin is masticated, condensed tannin, protein complexes are formed, these are stable over the pH range of 3.5-7 but dissociate in the abomasum and anterior duodenum. This protects the protein from microbial hydrolysis and deamination in the rumen and increases the proportion of dietary amino acids available for postruminal absorption, since the tannin protein complex is assumed to dissociate at low pH of abomasum. Condensed tannins are claimed to have the potential to modify rumen fermentation. However higher levels of condensed tannins are reported to negatively affect rumen fermentation. Jones (1994) summarized the effects of supplementation with leucaena foliage to dairy cows grazing pasture. In the mithun the effect of condensed tannin on the performance of animals is yet to be found out.

Effect of tree leaves supplementation on mithun performance

Tree leaves/shrubs feeding are a common practice in hilly states of NE region. This system of feeding is not only practical but also appears to be somewhat scientific. Mithun calves (12), weighing 167-177kg were divided into 2 groups and offered adlibitum mixed tree forages and 2 different levels of concentrate (2 and 4kg/day/animal) for 5 weeks. Feed intake and growth rate were significantly increased with increase in concentrate level in the rations. A body weight gain of 376g/day and DMI of 3.67 kg/100 kg body wt were recorded after feeding 4 kg of concentrate mixture and a body weight gain of 260g/day and DMI of 2.70 kg/100 kg body wt were recorded after feeding 2 kg of

Table1. Nutrient composition (g kg^{-1} DM) of individual forest-based foliages (Prakash et al, 2008a)

Name of the fodders Local	Botanical	DM	CP	EE	NDF	ADF	ADL	Ash	CT
Tao	*Ficus roxburghii*	267	178	23.	397	287	74	92	25
Taachin	*Ficus racemosa*	351	151	23	489	330	82	102	30
Nyeihi	*Ficus hirta*	236	156	18	453	322	96	62	31
Aa-hate	*Dendrocalamus hamiltonii*	302	162	16	520	426	90	71	68
Taan yaan	*Pouzolzia sanguinea*	356	146	21	460	312	132	91	41
Hara	*Macaranga denticulata*	378	139	37	531	387	146	56	53
Dae-dasa	*Melastomia sp.*	276	137	20	530	321	123	48	56
Rudie	*Phegopteris auritia*	231	190	17	402	273	69	90	63
Tayir	*Litsea cintreta*	225	201	54	390	269	72	70	45
Perelough	*Sida acuta*	270	149	31	551	382	129	91	27
Yal	*Trema orientalis*	369	106	14	573	426	162	63	64
Gansap	*Crassocephalum crepediodes*	375	142	16	480	317	103	72	53
Remter	*Mastersia assamica*	219	210	20	401	303	89	126	19
Yare	*Piper peticellosum*	238	193	19	386	246	101	74	22
Nik-nare	*Oreochnide integrifolia*	267	164	34	456	316	116	57	37
Donyibubch	*Lenia indica*	310	162	27	487	347	162	71	43
Pahe	*Setaria palmifolia*	196	68.1	21	619	460	149	81	67
Ekembin	*Polygonum runcinatum*	239	102	18	517	369	120	46	60
Leanyetoku	*Ficus fistulosa*	347	133	15	522	384	164	51	19
Gahorisopa	*Magnolia pterocarpa*	398	115	17	556	401	97	61	44
Koplopya	*Daubanga grandiflora*	271	156	23	456	327	123	92	22
Byake	*Solanum kurzii*	236	257	31	374	236	97	89	20
Chuchum	*Ficus sp.*	301	153	22	483	359	132	76	30
Donyichurd	*Mussanda roxburghii*	330	99.7	23	589	435	169	103	43
Tale	*Wallichia densiflora*	254	69.7	31	623	497	174	73	37
Telpep	*Hedychium spicatum*	297	115	28	461	330	121	69	12
Jehea	*Thysaloena sp.*	314	51.3	17	509	371	102	70	32
Echintayin	*Costus specious*	356	119	37	469	310	99	65	21
Hujhobup	*Polygonum sp.*	410	121	12	513	376	123	46	09
Tatumnalu	*Pilea glaberrima*	323	181	24	423	267	108	73	19
Paph	*Hedychium flavum*	291	130	22	536	321	164	126	62
Kuto	*Ficus sp.*	327	169	40	503	337	98	103	54
Poahibu	*Thumbergia*	278	139	30	486	289	145	68	12
Baum	*Angiopetris evecta*	273	157	21	450	278	96	51	23
Phapumlalu	*Sterculia villosa*	327	144	10	479	312	99	63	32
Techir	*Pinnaga gracilis*	246	118	25	598	427	146	49	57
Puprarninch	*Saurauia roxyburglis*	319	112	23	621	460	103	41	32
Tago	*Brassaiopsis hainla*	264	89.5	18	679	482	147	79	58
	Mean	291	142	24	499	350	119	74	38
	S.E.	11.2	6.7	1.4	11.9	10.8	4.8	3.4	2.8

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre, ADF, acid detergent fibre; ADL, acid detergent lignin; CT, condensed tannin

concentrate mixture. The percentage of nutrients except CP, EE and ADF was improved on increase in supplementation of concentrate (Pal et al., 2003a). The blood heamoglobin (g %), glucose (mg/dl), blood urea nitrogen (mg/dl), total protein and albumin: globulin ratio were 11.40, 58.70, 2.73, 6.7 and 1:1.03 respectively on tree leaves based ration. Performance of mithun on other feeding system like napier + concentrate and paddy straw+ concentrate has been assessed. Performance was highest in napier based feeding system with 500g ADG. (Pal et al., 2001) and was lowest in straw based feeding system.

In another study(Das et al. 2008a), performance of growing mithun weighing about 130 kg was evaluated on mixed tree leaves, paddy straw and concentrate based ration for 5 months. The growth rate of 608g for male and 554g for female was recorded after feeding tree leaves and straw based ration to growing mithun. The tree leaves consisted of temechiedie (*Ficus hirta*), Pedu (*Debrogesia longifolia*), thenha (*Litsea* sps) and thumero (*Legroestromea spaciosa*). DMI/100kg body weight was 3.23 kg for male and 2.92 kg for female during the experimental period. So mixed tree leaves and straw based diet can be a good and practical method for feeding of mithun in captivity.

Systematic work has been done on feeding of tree leaves/shrubs which are available in plenty and are also relished by the mithun. *Lagerstroemia speciosa* (Thumero) is one of the important tree foliage having good nutritive value (8 %DCP and 48 %TDN). It is liked by mithun under natural browsing condition. Inclusion of *Lagerstroemia speciosa* as green foliage in total mixed ration (TMR) showed higher daily body weight gain, dry matter (DM) intake and feed conversion ratio in growing mithun compared to straw based total mixed ration. There was an improved apparent digestibility coefficient of DM, crude protein, ether extract, crude fibre, nitrogen free extract. *Lagerstroemia speciosa* tree leaves can be incorporated successfully up to 30% (on DM basis) in TMR for feeding of growing mithun. (Prakash et al., 2008b). Similarly *Ficus hirta* and *Borrena hirticulata* are other important foliages in the NEH region and can be incorporated in the diet of mithun as total mixed ration. (Prakash et al., 2006c). Dhali et al. (2007) used cherry (*Pranus cerasoides*) and tophala (*Borrena hirticulata)* leaves in different proportions and reported that cherry leaves and tophala leaves in 1:1 ratio along with concentrate mixture in the total mixed ration(TMR) had shown better performance in mithun. However, feeding of mixed tree leaves was found to be practical and scientific instead of using one or two tree leaves. It also provides a variety to the diet. Prakash et al. (2005) used mixed tree leaves in total mixed ration and found that mixed tree leaves could be incorporated up to 50% level in total mixed ration.

Study of feed digestibility is an important parameter in animals. But digestibility determination by conventional method is difficult in mithun as it is a free grazing or semi-domesticated animal. Determining the nutrient intake and digestibility coefficients of nutrients is difficult to apply on them. Therefore, an alternative method to determine the same in mithun is essential. Keeping this in view, 2 indicator methods i.e. acid insoluble ash and lignin were studied and compared with the conventional method in order to ascertain the efficacy of indicator methods in determining the nutrient intake and digestibility coefficients of nutrients in mithun. Results obtained on nutrient intake and digestibility coefficients of nutrients under conventional and indicator methods did not indicate any significant difference. (Pal and Bujarbaruah, 1999b)

Performance of mithun vs cattle:

Mithun is mainly reared under free grazing condition and meat of this animal is very much preferred by tribal. Mithun meat was analyzed for muscle, liver, kidney, skin and blood and as per the findings highest moisture (84.85%) and fat contents (8.70%) were recorded in the kidney while the skin recorded a highest level of CP (27.89%) on fresh basis. The muscles contained 17.90% CP on fresh basis. On dry basis, 96.54, 89.40, 84.51, 76.56 and 72.54% CP was recorded in skin, blood, muscle, kidney and liver respectively (Pal, 2000b). The production of milk is very limited only about 1 to 1.5 liter. But mithun milk has higher fat (8.85%), SNF (11.48%) and total solid (20.33%) compared to other domestic animals (Mondal et al., 2001). However, Mech et al. (2007) reported the average fat % of 10.8 in mithun milk and it was higher than Yak milk (Das et al.,1998). This unique species has the potentiality to be explored for its milk production. The Haemato-biochemical profile of mithun, cattle and mithun x cattle were compared on tree leaves based ration. For all parameters like haemoglobin, PCV, TEC, TLC, glucose, total protein and albumin, higher values were recorded in mithun followed by mithun x cattle and cattle (Rajkhowa et al., 2002).

The comparative feed nutrient utilization was studied in mithun and cattle calves on green forage based diet. Total nutrient intake per unit body weight was lower in mithun than that of cattle calves. Dry matter and crude protein intake in mithun calves were 2.52 and 0.188 and 3.26 and 0.202 kg/100 kg body weight in cattle calves. The study indicated that mithun could utilize the nutrients more efficiently than cattle (Pal et al., 2003b). Comparative study of local cattle (Tho Tho) and mithun of average 1.5 year of age were also conducted with grass (*Borrena hirticulata*) and paddy straw based ration. Feeding experiment was continued for 24 weeks of age. Mithun attained 527g average daily gain whereas, Tho tho cattle attained 477g average daily gain. Dry matter (DM) consumption per day was 6.25 kg for mithun and 5.91 kg for Tho Tho cattle. This shows that growth rate of mithun is higher than local cattle and total dry matter (DM) intake is also more in mithun than Tho Tho cattle (Das et al. 2008b)

Vegetative propagation of tree foliages

There is large-scale deforestation and alteration of habitat of animals because of urbanization, shifting cultivation, jungle fire etc. The availability of tree leaves/shrubs is also not uniform throughout the year. In this situation propagation of fodder tree in NEH region like Nagaland, Manipur, Arunachal Pradesh and Mizoram is the best alternative for making available of sufficient quantity of tree leaves for the animals. Though seed is the main method for multiplication of fodder tree species under natural condition, propagation through vegetative means gives an opportunity to shorten the period of multiplication. Raising planting stock by vegetative propagation, especially by stem cuttings is the possible means in the process of fast multiplication. Plant growth hormones like auxins, cytokinins and combination of both have been tried.

Stem cuttings transplanted in polythene bags and kept under green house for sprouting

The propagation study at NRC on Mithun, Nagaland by using higher dosage of auxins (Indole acetic acid IAA @ 250 ppm) on good quality fodder trees revealed encouraging trend with highest survival percentage, increased length and number of sprouts per stem cuttings (Prakash et al., 2007).The selected fodder tree species (*Ficus hirta, Ficus roxburghii*, *Lagerstroemia speciosa, Trema orientalis, Ficus hookeri)* can grow meaningfully through stem cuttings treated with IAA which can provide better option for multiplication in degraded pasture land of low carrying capacity.

Scope for forage resource development

There is vast scope to fit forage crops in the agricultural and non agricultural land use systems in mithun inhabited states. Rice is the major crop cultivated in mithun inhabited states and rice straw is the major crop residue available. Due to lack of awareness and practice of feeding rice straw to the mithuns, farmers allow rice straw to be decomposed in the field with a notion to increase the soil fertility. This can be utilized as a source of roughage for mithun. However, among annual forage crops, oat can be grown where one or two irrigations are made available from perennial or seasonal water springs. The maize, cowpea and soybean can be successfully grown during pre-kharif and kharif season. They produce an average 200-500 q/ha green forage. The subtropical and tropical grasses grow well under the humid agro-climatic conditions of this region. Most of these grasses produce forage during April to October. Among these grasses, a multipurpose plant, *Thysanolaena agortis/ T. maxima* (Broom grass) remains green during the lean period (dry winter months). This grass produces spikes for broom, leaves for fodder and stem for fuel or pulp material for coarse quality papers. The Napier cultivation was also found to be high yielding in Nagaland condition. Total green forage biomass production and nutrients availability was more from cultivated napier than natural grass and so cultivation of napier grass could be adopted on the hillock of NEH region to mitigate the balance between availability and nutrients requirement of mithun (Pal and Bujarbaruah, 2002).

Shifting cultivation commonly known as Jhuming is one of the most ancient systems of

farming in this region. In this system soil nitrogen concentrations declines sharply depending on the intensity of burn which is low in smaller shifting cultivation cycles. Available phosphorus also declines significantly. Slash burning also causes a decrease in the level of soil organic matter through oxidation. These pressures are leading to decrease in production cycles, increase in land and forest degradation; the best top soils will be washed away and silt will be deposited in the valleys. It is important now to wean the hill farmers away from Jhum cultivation and to direct them towards other productive activities. Experiments conducted at Shillong have shown that in hilly areas, the bottom third of the slope can be used for agricultural crops on terraces, irrigated from low dams; the middle third can be used for fruit trees and cash crops such as coffee, black pepper, big cardamom etc., planted along the contours; while the upper third should be used for forestry and fodder crops. Introduction of three components simultaneously agronomy, forestry and animal husbandry will help minimize soil erosion, conserve moisture, increase infiltration, decrease evaporation and balance nutrient status. This could form a basis for the planning of the land-use pattern in programmes for shifting cultivation control and effective utilization of land.

Feeding of mithun

According to Yadav and Verma(1996) the forest grasses and mixed forages are poor in nutritive value and could not meet the requirement in stall fed animals. The DCP and TDN requirements of the growing calves were met up to the extend of 89.16 and 79% respectively, in stall feeding under confinement. In the natural condition the animals meet their requirement due to the availability of different forage resources in the large areas of forest by traveling in search of good forages. Therefore the animals in the natural environment meet their nutritional requirement, though their growth varies across different seasons. (Gupta et al., 1999). As a recommendation, during flush season when abundant fodders are available in forest, salt and mineral mixture together may be fed additionally to the animals to avoid mineral deficiency. During lean season, when availability of jungle fodder goes down, the additional concentrate mixture (15% DCP and 70% TDN) fortified with salt and mineral mixture may be fed at the rate of 1 to 2 kg per animal daily up to 2 years and 2 to 4 kg per animal daily above 2 years may be offered to maintain optimum performances.(Dhali and Rajkhowa, 2006). For lactating mithun as it produces less quantity of milk, no other additional feeding is required. However, no scientific information is available in milk production by mithun through fortified feeding. In free range mithun these feed supplements may be provided to the animals in the shed constructed in strategic location in grazing area. For animals under semi intensive system the feed supplements may be provided in the shed in late evening or early morning when animals are tied.

But due to deforestation, urbanization, shifting cultivation, intensive inbreeding, disease problems etc. the mithun are under severe threat. So mithun may be fed in semidomestic or domestic condition along with concentrate mixture.

Water metabolism in mithun

Normal average water consumption of adult mithun irrespective of sex was recorded to be 0.31± 0.017 lit/W kg $^{0.75}$/day. It was also observed that female mithun consumed more water than male mithun which could be due to relatively higher requirement of water in female mithun for some special physiological functions. In the two systems of feeding i.e. free grazing and free grazing with concentrate, water consumption was observed to be higher in the latter that was suggestive of the fact that consumption of dry feed increased the water requirement. Again in another study it was observed that drinking and total water intake of growing mithun calves were higher during pre-monsoon season (April to May) compared to monsoon (June to September), post-monsoon (November to December) and winter (January to March) season. However feed water intake was observed to be comparatively higher in post-monsoon season though the feed DM intake was almost similar. This could be due to a higher percentage of moisture in tree leaves during the months of November and December. Total water intake was also observed to be higher in summer season. A digestion trial of 7 days duration was conducted in the calves to observe the utilization of water in mithun. Daily intake of feed and water and faeces voided were recorded to ascertain feed intake, water intake, faecal water loss, metabolic water and daily water turn over in the body. It was found from the results that the faecal water loss was 4.08 ± 0.30% of their body weight. Metabolic water and daily water turn over

in the body were recorded to be 1.635± 0.096 and 7.92 ±0.18 lit respectively (Pal, 2000b). Similarly the drinking water intake increased significantly in mithun with the increased supplementation of dry concentrate feed, but total feed water intake did not differ with the increased concentrate supplementation (Pal et al., 2007)

For mithun the optimum drinking water requirement is approximately 9% and 12% of body weight during winter and summer respectively.

Mineral nutrition of mithun

Minerals play a vital role in productive and reproductive performance in animals. Deficiency of minerals may affect the digestive, physiological and biosynthetic processes of the body and as a consequence affect the animal performance. In many parts of India, animal productivity has become limited because of deficiency or imbalances of both macro and micro-minerals.

Most of the foliages (Table-2) contained Ca more than 1%, K more than 1% and Mg more than 0.17% but Na content was low in all selected foliages. P was mostly within the normal required range of 0.12–0.40%. Cu, Fe, Mn and Zn values were 10.8, 215, 88 and 93 mg/kg respectively on DM basis. (Prakash et al., 2007). High Ca content of the foliages can be useful for other high yielding ruminants like cattle and buffaloes to prevent them from milk fever. Higher Ca and Mg concentration in collected foliages could be due to relatively higher uptake of these elements from coarse textured soils with low cation exchange ability. Na was deficient in almost all the foliages studied except *Polygonum runcinatum* (0.061%) which was within the normal requirement range. Na deficiency in all the foliages could be the reason why, the mithun shows the salt hunger behavior that are reared in natural habitat in contrast to those kept under confined condition. The concentration of Ca was higher than P, it is likely that Ca: P ratio enhances as the foliages are the sole feeding source for mithun, which could create some problem with Ca: P and vitamin D metabolism.

Plasma levels of different macro and micro minerals were analysed in both growing and adult mithun reared under semi-intensive system. Plasma samples were collected from adult mithun maintained on free grazing conditions and offered concentrate mixture fortified with mineral mixture and salt. Calves were allowed to suck their mother and offered concentrate mixture fortified with mineral mixture and salt from 3 months of age onwards. Plasma content (%) of Ca and P were found higher in animals of 6 months to 2 years of age but the variation was not significant. It was again observed that plasma content of Ca, P, Mg, Fe, Cu, Zn of all these animals were higher in male than female but the variation was not significant (Dhali et al., 2004)

Some of the advanced technologies for mithun

1. Feeding of urea treated rice straw and concentrate based feed blocks to mithun

Rice (*Oryza sativa*) is a major crop cultivated in mithun inhabited areas and paddy straw is abundantly available in this region. Due to lack of awareness for feeding paddy straw to ruminants in hilly areas, is leading to decomposition of paddy straw in the field itself. Feed blocks were made successfully by incorporating 4% urea treated paddy straw up to 70% in complete ration. The digestion cum metabolic study revealed the higher nutritive value (DCP 8.5%, TDN 63%) in animals fed with ration containing 70% paddy straw. (Sinha et al., 2007)

Fig 4: Urea treated rice straw based feed blocks are being fed to the experimental animals.

2. Feeding of yeast

Feeding of yeast (*Saccharomyces cerevisiae*) in cattle for improving growth and feed efficiency is well documented (Dutta and Kundu, 2005). The Yeast can also be fed to mithun in free range and semi-domesticated condition for economizing the production and also for improving the overall health status of animals. In one of the experiments at NRC on mithun, Nagaland, male mithun calves were fed with 5 to 6 g of dried Bakers yeast (*Sacharomyces cerevisiae*) containing 20 billion CFU / g of jungle

Table 2. Minerals concentration in forest based foliages collected from various pockets of mithun inhabited area of Arunachal Pradesh (Prakash et al., 2007)

Name of the forest-based foliages		Macro minerals (% DM basis)					Micro minerals (ppm)			
Local Name	Botanical	Ca	P	Mg	Na	K	Cu	Fe	Mn	Zn
1 Tao	*Ficus roxburghii*	2.31	0.18	0.82	0.040	2.11	7.70	460	102	63.1
2 Taachin	*Ficus racemosa*	3.10	0.40	0.55	0.031	1.93	15.1	183	110	79.3
3 Nyeihi	*Ficus hirta*	2.08	0.24	0.31	0.026	1.83	7.35	210	144	51.6
4 Aa-hate	*Dendrocalamus hamiltonii*	2.70	0.16	0.45	0.028	1.95	12.3	461	74.2	23.6
5 Taan yaan	*Pouzolzia sanguinea*	2.12	0.22	0.74	0.029	2.73	11.7	218	112	35.8
6 Hara	*Macaranga denticulata*	1.98	0.27	0.63	0.041	0.71	10.8	310	117	72.9
7 Dae-dasa	*Melastomia sp.*	2.36	0.23	0.50	0.027	1.35	5.70	361	123	34.0
8 Rudie	*Phegopteris auritia*	2.07	0.20	0.83	0.011	2.11	6.31	231	84.0	211
9 Tayir	*Litsea cintreta*	2.91	0.33	0.32	0.026	1.94	14.6	219	91.4	35.9
10 Perelough	*Sida acuta*	2.06	0.21	0.28	0.015	2.13	12.3	195	102	48.7
11 Yal	*Trema orientalis*	3.41	0.38	0.70	0.025	1.87	11.0	282	38.7	430
12 Gansap	*Crassocephalum crepidiodes*	1.07	0.17	0.72	0.036	2.21	7.91	179	42.1	127
13 Remter	*Mastersia assamica*	2.11	0.23	0.34	0.034	3.10	6.34	163	112	163
14 Yare	*Piper peticellosum*	1.07	0.27	0.18	0.017	2.47	7.88	217	156	125
15 Nik-nare	*Oreochnide integrifolia*	1.23	0.31	0.34	0.036	2.27	9.62	121	60.8	71.0
16 Donyibubch	*Lenia indica*	1.75	0.14	0.69	0.027	1.91	7.31	179	76.0	61.4
17 Pahe	*Setaria palmifolia*	1.35	0.23	0.24	0.012	1.67	11.3	210	89.7	48.9
18 Ekembin	*Polygonum runcinatum*	1.60	0.31	0.37	0.061	2.18	14.6	186	103	121
19 Leanyetoku	*Ficus fistulosa*	2.11	0.34	0.54	0.007	2.39	11.3	172	57.1	34.8
20 Gahorisopa	*Magnolia pterocarpa*	3.14	0.12	0.57	0.026	2.38	10.4	137	67.0	47.6
21 Koplopya	*Daubanga grandiflora*	2.79	0.24	0.81	0.200	2.70	19.6	119	107	55.2
22 Byake	*Solanum kurzii*	2.91	0.31	0.50	0.038	2.37	18.2	210	120	107
23 Chuchum	*Ficus.sp.*	2.35	0.27	0.37	0.040	2.86	11.3	213	62.6	111
24 Donyichurd	*Mussanda roxburghii*	2.02	0.30	0.71	0.020	2.52	7.25	281	123	108
25 Tale	*Wallichia densiflora*	1.78	0.19	0.64	0.008	2.04	11.8	211	91.4	113
26 Telpep	*Hedychium spicatum*	2.34	0.23	0.52	0.050	2.66	13.6	186	180	123
27 Jehea	*Thysaloena sp.*	1.75	0.31	0.84	0.050	2.30	10.3	133	74.0	107
28 Echintayin	*Costus specious*	1.64	0.24	0.34	0.020	2.76	14.2	128	41.9	116
29 Hujhobup	*Polygonum sp.*	2.12	0.28	0.17	0.040	2.68	8.64	148	109	76
30 Tatumnalu	*Pilea glaberrima*	1.94	0.20	0.30	0.036	2.08	10.5	214	81.1	113
31 Paph	*Hedychium flavum*	2.19	0.34	0.27	0.047	1.17	13.5	181	52.0	81.0
32 Kuto	*Ficus sp.*	2.79	0.23	0.39	0.010	1.58	7.61	182	82.4	73.7
33 Poahibu	*Thumbergia*	3.10	0.18	0.34	0.031	1.61	18.6	163	44.6	46.8.
34 Baum	*Angiopetris evecta*	2.33	0.16	0.78	0.024	2.05	9.61	175	34.1	35.4
35 Phapumlalu	*Sterculia villosa*	2.71	0.30	0.17	0.035	1.82	7.26	257	32.3	41.5
36 Techir	*Pinnaga gracilis*	2.04	0.28	0.23	0.010	1.36	6.21	237	76.0	127
37 Puprarninch	*Saurauia roxyburglis*	2.94	0.19	0.34	0.024	1.35	10.3	169	61.4	104
38 Tago	*Brassaiopsis hainla*	1.93	0.24	0.81	0.010	2.58	12.2	267	69.5	63.7
	Mean	2.22	0.25	0.49	0.033	2.10	10.8	215	87.7	93.1
	S.E.	0.09	0.01	0.035	0.005	0.083	0.57	12.7	5.6	11.5

grass(*Borrena hirticulata*) based diet. The experiment was continued for 16 weeks of age. During this period the animals of control group attained 544g ADG while the animals of treated group attained 651g ADG. DM consumption per day was 6.65 kg for control group and 7.25 kg for treated group. The FCR was improved in animals fed with yeast. (Das et al., 2008c)

c. Rumen manipulation by genetic technique:

Mithun inhabited areas are quite different and isolated compared to the inhabited areas of other domestic animals species. Again, mithun adapt them to the environment in which the availability of feeds is limited in some part of the year. It may be hypothesized that the mithun rumen possibly harbours some bacteria that are genetically different and may be more efficient compared to other domesticated and wild ruminant species.

REFERENCES

Das, D. N., B.C.Das, M.Sarkar, T.K. Mohanty, D.B. Mondal, B.P.Singh, R.N. Pal, and S. Ray. 1998. Lactation performance of Yak (*Poephagus grunniens*), a field study. Indian Vet. J. 75: 926-927

Das, K. C., B. Prakash, and C. Rajkhowa. 2008a. Performance of growing mithun (*Bos frontalis*) on mixed tree leaves and straw based ration. Annual Report, National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Das, K. C., B. Prakash, and C. Rajkhowa. 2008b. Comparative studies on Mithun (*Bos frontalis*) & local cattle (Tho Tho) on grass and paddy straw based diet, Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Das, K. C., B. Prakash, and C. Rajkhow. 2008c. Effect of feeding Bakers yeast (*Sacharomyces cerevisiae*) in mithun on roughage based diet. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Dhali, A., M. Mondal, A. Mech, B.C. Sharma, and C. Rajkhowa. 2004. Levels of macro and micro minerals in Mithun plasma. Annual Report. 2003-04. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Dhali, A., D.T. Pal, A. Mech, S.K. Mondal, and C. Rajkhowa. 2007. Growth performance and nutrient utilization in growing Mithun (*Bos frontalis*) fed on concentrate and forage based total mixed ration. Ind. J. Anim. Sci. 77:1041-1044

Dhali, A., and C. Rajkhowa, 2006. Mithun: A promishing component for the livestock production system in the North Eastern Hill Region, published by NRC on mithun, Jharnapani, Medziphema, Nagaland.

Gupta, S.C., N. Gupta, and A. E. Nivsarkar. 1999. Mithun: a bovine of Indian origin. Directorate of Information and Publications of Agriculture. Indian Council of Agricultural Research, New Delhi (India) IUCN. 2002. The International Union for the Conservation of Nature and Natural Resources.

IUCN. 2002. Red list of threatend species, www. iucnredlist.org.

Jones, R.M.1994. In: Forage Tree legume in tropical Agriculture, pp 232-244 (R.C. Gutteridge and H.M. Shelton, editors) Wallingford, Oxford: CAB international.

Mech, A., A. Dhali, and C. Rajkhowa. 2007. Diurnal variation of milk constituents and milk yield in Mithun. Indian Vet. J. 84:1095-1096

Mondal, S. K., D.T. Pal, G. Singh, and K.M. Bujarbaruah. 2001. Physio-chemical properties of mithun milk. Indian J. Anim. Sci. **71**:1066

Pal, D.T. 2000a. Chemical composition of different body tissues of Mithun. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Pal, D.T. 2000b. Water metabolism in Mithun. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Pal, D.T. and K.M. Bujarbaruah. 1999a Feed resources of Mithun in North Eastern Hill Region. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Pal, D.T. and K.M. Bujarbaruah.1999b Comparison between indicator and conventional methods for determination of nutrient intake and digestibility coefficients in Mithun. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Pal, D. T. and K. M. Bujarbaruah. 2002. Comparative studies on production potential of natural and cultivated grasses and their nutrient utilization in mithun. Indian J. Anim. Sci. 72:1001-1003

Pal, D.T., A. Dhali, C. Rajkhowa, S.K. Mondal, K.Vupru and K.M. Bujarbaruah. 2007. Effect of dry feed supplementation on water intake and feed and water utilization in growing mithun (*Bos frontalis*) calves. Indian J. Anim. Sci. 77**:** 1045-1047

Pal, D.T., M. Dutta, and K.M. Bujarbaruah. 2003a. Level of concentrate allocation and its effect on growth rate and feed utilization in mithun calves. Indian J. Anim. Sci. 73: 90-93

Pal, D.T., M. Dutta, and K.M. Bujarbaruah. 2003b Comparative studies on feed utilization by Mithun and cattle calves. Indian J. Anim. Sci. 73: 102-103

Pal, D.T., G Singh, and K.M. Bujarbaruah, 2001. Performance of Mithun calves on napier grass based feeding. Ind. J. Anim. Sci. 71:712-13

Prakash, B. 2006a. A comprehensive study on survey, nutritional evaluation and development of improved agronomic practices for year round availability of green fodder for Mithun *(Bos frontalis)* under north eastertn hill region of India. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Prakash, B., K.C. Das, A. Dhali, S.S. Rathore, A. Meck, M. Sangtham, I. Walling, and C. Rajkhowa. 2007. Estimation of macro and micro-minerals in some of the important foliages of Medziphema area. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Prakash, B., A. Dhali, K.C. Das, A. Mech, S. S. Rathore, and C. Rajkhowa. 2008a. Nutrient Composition and In Situ Degradability of Forest Foliages Relished by Mithun(*Bos frontalis*). Animal Nutrition and Feed Technology (communicated).

Prakash, B., A. Dhali, M. Mondal, M. Sangtam, K. Khate, S. S. Rathore, and C. Rajkhowa. 2008b. Effect of feeding Lagerstroemia speciosa and conventional fodders based rations on nutrient utilisation, ruminal metabolites and body weight gain in Mithun (*Bos frontalis*). Journal of Animal Physiology and Animal Nutrition (In press: DOI: 10.1111/j.1439-0396.2007.00753.x).

Prakash, B., M. Mondal, S. Rajkhowa, A Dhali, and C. Rajkhowa. 2006c. Feeding of tree leaves based total mixed ration to Mithun(*Bos frontalis*) and effect on nutrient utilization, growth and rumen fermentation. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland

Prakash, B., M. Mondal, S. Rajkhowa, and C. Rajkhowa. 2005. Nutritional evaluation of tree lopping based ration in Mithun, Indian J.Anim. Nutr. 22 (3): 166-69.

Prakash B., M. Mondal, A. Rongsensusang, K. Hannah and C. Rajkhowa 2006b: Chemical composition and in sacco degradability of forest based fodders of Nagaland state of India in Mithun (*Bos frontalis*). Livestock Research for Rural Development. Volume 18, Article #119. Retrieved January 5, 2008, from http://www.cipav.org.co/lrrd/lrrd18/8/prak18119.htm

Prakash, B., S.S. Rathore, K.C Das, A. Dhali, K. Khate, M. Sangtham, , I Walling, and C. Rajkhowa. 2007 vegetative propagation to standardize the propagation technique of selected foliages under green house. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Rajkhowa, S., K.M. Bujarbaruah, C. Rajkhowa, R. Zuyei, and M. Dutta. 2002. Comparative study on blood profile of Mithun, cattle and mithun x cattle. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Sinha, D., B. Prakash, B.N. Neog, K.K. Baruah, and C. Rajkhowa. 2007. Studies on nutrient utilization and nitrogen balance in adult Mithuns fed on urea treated rice straw and concentrate based feed blocks. Annual Report. National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland.

Yadav, B.P.S. and N.D. Verma, 1996. Studies on feed utilization, growth pattern and milk composition in mithun (*Bos frontalis*). In**:** Animal Genetic Resources Information (FAO/UNEP), 20:9-15.

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Total Gastro-Intestinal Tract Survivability of Viable Probiotics in Crossbred Calves and their Evaluation under In Vitro System

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ABSTRACT : Six promising probiotic cultures namely *Saccharomyces cerevisiae*-Strain-B (SC-B), *S. cerevisiae*-Strain-522 (SC-522), *Lactobacillus acidophilus* (LA), *L. plantarum* (LP), *Lactococcus lactis* (LL) and *Enterococcus faecium* (EF) were supplemented individually to crossbred calves (7-8 months old) to study the survivability pattern of these viable cultures in the total gastro-intestinal tract during treatment and post-treatment period and also evaluated under in vitro system. Least squares mean (LSM) (Log_{10} cfu/g of faeces) of yeast culture in faeces tended to be higher in treatment period (2.919) than control (no probiotics) period (2.791) during *S. cerevisiae*-B feeding. But the difference was statistically similar in pre-treatment, treatment and post treatment periods. In the treatment group of *S. cerevisiae*-522, similar trend was observed as in SC-B fed group. Acid forming bacilli counts (LSM of Log_{10} cfu/g faeces) in faeces were statistically higher ($P<0.01$) during treatment period (6.365) as compared to pretreatment control (6.092) and post treatment periods (6.214) in LA group. LSM values (Log_{10} cfu/g faeces) of faecal acid producing cocci were similar in three periods, i.e., control (5.950), treatment period (6.055) as well as post treatment period (5.807) in *L. lactis* (LL) supplemented calves. Whereas, in EF supplemented group LSM value was marginally higher in treatment period (6.257) as compared to control (6.070) period. But during post treatment period the counts were significantly less ($P<0.01$) than those during control and treatment period. In vitro DM degradability was significantly higher ($P<0.01$) in all probiotics supplemented groups; which in turn increased ($P<0.05$) total gas production (ml/0.5 g substrate with roughage: concentrate=70: 30) under in vitro system over no probiotics control bottle. However, total VFA concentration was lower ($P<0.05$) in LP and LL added bottles as compared to control. The values in other probiotics supplemented bottles were similar with that of control. Acetate to propionate ratio in the incubation medium was not altered due to addition of above cultures except LL supplemented group. It may be inferred that above yeast cultures (SC-B and SC-522), *L. acidophilus* and *E. faecium* remained viable in the total GI tract.

Key words : Probiotics survivability, Calves, in vitro Degradability

INTRODUCTION

Ruminant productivity may be enhanced by manipulating the rumen environment, which provides impetus in cellulolysis and proteo-anabolic processes. One of the most exciting and useful materials for supplementing the animal feed is living microflora called probiotics or direct fed microbials (DFM). Feeding viable cells of *L. acidophilus* to young dairy calves was shown to reduce the incidence of diarrhoea (Bechman et al., 1977) and increase the numbers of lactobacilli and reduce coliform counts in faeces (Bruce et al., 1979).

The use of probiotics culture in large and small ruminants has been appreciated for the improvement in feed intake (Adams et al., 1995) and enhancement in weight gain and feed conversion efficiency in meat producing animals (Hughes, 1988). *Saccharomyces cerevisiae* has potential to improve the milk production in dairy cows (Adams et al., 1995). In contrast of earlier concept, the

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yeast and some lactic acid bacteria may survive and multiply in the anaerobic environment of rumen and intestine. *S. cerevisiae* may multiply and exhibit growth in the rumen or in rumen simulating continuous cultures and confers beneficial effects on cellulolysis and productive trait of the host animal (Dawson and Newman, 1988; Harris and Lobo, 1988). Dawson et al. (1990) also observed the growth of yeast and lactobacilli in the rumen, whereas streptococcus remained constant when the steers fed with mixed microbes (yeast, lactobacilli and streptococci). In another study by Newbold et al. (1990) it was observed that there were increased counts of yeasts in rumen fluid 1 h after yeast culture addition. Viable yeast persisted in the duodenum and ileum of treated animals, at values 6.5 and 6.8 times higher than control. Earlier Arambel and Tung (1987) concluded that *Saccharomyces cerevisiae* was unable to maintain a productive population within the rumen ecosystem.

The viability of yeast cells in the rumen has been considered crucial because El Hassan et al. (1993) reported that yeast cultures need to be both viable and metabolically active to have a full stimulatory effect on ruminal fermentation. The positive responses of viable yeast cultures may be due to decreased ruminal ammonia concentration (Harris and Lobo, 1988); altered VFA production (Stein et al. 2006), increased concentration of anaerobic bacteria, more specifically cellulolytic bacteria (Dawson et al. 1990), stimulated rumen fermentation (Lila et al. 2004) and altered digestive pattern (Chademana and Offer, 1990).

Therefore, present study was conducted to evaluate the total gastro-intestinal tract survivability pattern of different promising probiotic cultures (yeast and lactic acid bacteria) in cross bred calves and their potential to stimulate rumen fermentation in vitro.

MATERIALS AND METHODS

Six promising probiotic cultures were obtained from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal (India) as listed below:-

1. *Saccharomyces cerevisiae*-Strain-B (SC-B) (NCDC-47)
2. *S. cerevisiae*-Strain-522 (SC-522) (NCDC-45)
3. *Lactobacillus acidophilus* (LA) (NCDC-15)
4. *L. plantarum* (LP) (NCDC-25)
5. *Lactococcus lactis* (LL) (NCDC-94)
6. *Enterococcus faecium* (EF) (NCDC-124)

These cultures were maintained using malt extract agar for yeast, MRS agar for lactobacilli, and LYPA agar medium for *Lactococcus* and *Enterococcus* species (Dutta, 1998). The same viable cultures were used for survivability of these cultures in the total gastro-intestinal tract and their evaluation under in vitro rumen fermentation.

Survivability study

Six crossbred (Tharparker x Holstein Friesian) calves (age 7-8 months) were randomly divided equally into two groups, namely SC-B and SC-522 based on their body weight. Animals of both groups were offered 1.5 kg concentrate mixture plus maize (*Zea maize*) fodder adlibitum for 20 days. Faecal samples (about 50g) were collected from rectum for next three consecutive days at 9 AM and cultured in malt extract agar plate to take observation on naturally occurring yeast present in the faeces (control). Subsequently, *S. cerevisiae*-strain B (SC-B) and *S. cerevisiae*-strain 522 (SC-522) were given @ 1.08 x 10^9 cfu/kg of concentrate mixture/ animal for 5 days in SC-B and SC-522 groups respectively. Yeast suspension was mixed in about 250g concentrate mixture and offered to each animal at 9 AM followed by remaining amount of concentrate. The faecal observations during yeast feeding period were recorded for 5 days. After 5 days of yeast feeding, faecal samples were also collected from rectum for next 3 days to observe the post-treatment effect on survivability of yeast.

The faecal samples were thoroughly mixed under aseptic condition and one gram representative sample was taken and diluted with sterile saline water in the ratio of 1:50 and 1:100. After thoroughly mixing, 0.5 ml of diluted sample was added in the culture plate, and incubated at 25°C±1°C for 36 to 40h. Viable colonies per gram of faeces were calculated based on the yeast colonies developed in the agar plates (Dutta, 1998). Same set of animals were used for survivability study of LA and LP; LL and EF with feeding schedule of 1.5 kg concentrate mixture and ad lib oat fodder with proper rest period. Supplementation of LA and LP; and LL and EF were done similarly to that of yeast culture and faeces were diluted with sterile saline water in the ratio of 1:10^5. After thorough mixing of each faecal sample with normal saline, 0.5 ml of diluted sample

was added in the respective culture plates. Then they were incubated at 37°±1°C for 24h. The number of colonies was counted and viable colonies per gram of faeces were calculated by multiplying the dilution factor. Litmus milk test was conducted to confirm the acid forming bacilli or cocci isolated from faeces. It was observed that there was curd formation and development of red colour in the tubes within 18 to 30h of incubation when added with faecal isolates from MRS and LYPA agar plate; whereas in the control tubes there was no curd formation and blue litmus colour remained unchanged. Colonies in the respective agar plate were smeared and stained following standard procedure. The slide was examined under compound microscope for further confirmation of yeast, acid forming bacilli or cocci isolated from faecal cultures (Dutta, 1998).

In vitro evaluation

Above six probiotic species were supplemented in the in vitro system using oven dried substrate (roughage:concentate=70:30). Two rumen fistulated crossbred calves were fed with concentrate mixture 2.5 kg/head plus ad lib roughage comprising maize fodder and wheat straw and the animals were allowed for free access to water. These animals served as donor for rumen liquor. In each flask (capacity 100ml) 0.5g (DM) of substrate (oat hay 42: wheat straw 28: concentrate 30), 10 ml strained rumen liquor (SRL) and 40 ml McDougall's buffer (McDougall's, 1948) were added. Concentrate mixture was prepared with maize 40, groundnut cake 25, mustard cake 10, wheat bran 22, mineral mixture 2 and common salt 1 percent. The culture solutions were diluted with sterilized distilled water to have 10^9 cfu/ml of solution before the trial. Before the in vitro trial the viable cells in the preserved cultures were counted following standard plate count procedure. A total dose 10^9 cfu live microbial culture was added in each flask of the respective treatment except control (C). The bottles were then sealed properly after flushing with CO_2. The samples were incubated for 48h at 39°C ±1°C.

The DM was estimated according to AOAC (1984) in the samples of substrates and residues. Total gas production (ml) was measured at 4h interval up to 48h using pressure transducer (Bailey and Mackey ltd., UK). The pressure created in the bottle was measured by transducer and sucked to zero by 50 ml syringe. The reading shown in the syringe was the gas (ml) produced at that hour. Total volatile fatty acid (TVFA) concentration in incubation medium was estimated according to Barnet and Reid (1957). The TVFA was fractioned in accordance with the method of Ervin et al. (1961) using Nucon gas chromatograph series 5500. Statistical analysis of data in randomized block design (RBD) for in vitro experiment and least squares analysis for survivability study was done following the method of Snedecor and Cochran (1980). Using the data of gas production at 4 hour intervals up to 48 h regression equation with R^2 value was developed for each treatment with the help of MS-Excel programme.

RESULTS AND DISCUSSION

Proximate composition of all feeds fed to the experimental animals during survivability study are presented in Table 1. DMI, OMI and CPI per 100 kg and per kg $W^{0.75}$ was similar ($P>0.05$) in two groups supplmented with either two strains of *S. cerevisiae* (SC-B and SC-522) or two species of Lactobacilli (LA and LP) or Lactococci and Streptococci (LL

Table 1. Chemical composition of feedstuffs fed during survivability study of probiotics (% DM basis)

Parameter	SC-B and SC-522 feeding		LA and LP feeding		LL and EF Feeding	
	Concentrate mixture	Maize fodder	Concentrate mixture	Oat fodder	Concentrate mixture	Oat fodder
OM	89.89	84.90	89.95	89.03	89.88	91.23
CP	20.65	11.69	21.12	12.57	20.66	7.56
CF	8.45	25.16	8.32	27.05	8.18	29.97
EE	4.41	1.88	4.38	3.02	4.45	2.57
NFE	56.38	46.17	56.13	46.39	56.59	51.13
Ash	10.11	15.10	10.05	10.97	10.12	8.57

Table 2. Voluntary nutrient intake during survivability study of Probiotics in calves

Parameter	SC-B	SC-522	Sig.	LA	LP	Sig.	LL	EF	Sig.
DM Intake									
Total (kg)	4.06±0.02	3.88±0.11	N S	3.18±0.04	3.15±0.06	NS	4.79±0.02	4.76±0.07	NS
kg/100 kg BW	3.67±0.25	3.44±0.04	NS	2.48±0.08	2.52±0.15	N S	3.63±0.15	3.68±0.29	NS
g/kgW$^{0.75}$	119.09±6.31	112.21±1.71	NS	83.46±1.81	84.10±3.67	NS	122.92±3.79	120.23±3.93	NS
OM Intake									
Total (kg)	3.52±0.02	3.36±0.09	NS	2.82±0.04	2.72±0.05	NS	4.34±0.02	4.31±0.07	NS
g/kgW$^{0.75}$	103.02±5.45	97.11±1.46	NS	74.10±1.61	72.64±3.14	NS	11.35±3.43	108.89±3.59	NS
CP Intake									
Total(g)	598.42±2.32	578.20±11.67	NS	591.88±1.92	586.59±2.64	NS	553.10±0.79	551.45±3.27	NS
g/kgW$^{0.75}$	17.52±0.90	16.59±0.26	NS	15.54±0.41	15.69±0.81	NS	14.18±0.46	13.93±0.39	NS

and LP) (Table 2). DMI (g)/W$^{0.75}$ kg were 119.09 and 112.21 g in SC-B and SC-522 fed groups. The values were 83.46 and 84.10 g in LA and LP groups and 122.92 and 120.23 in *L. lactis* (LL) and *E. faecium* (EF) treated groups respectively. The nutrient intake in all groups was almost comparable with NRC (1989) standard.

Survivability of probiotics

Least squares mean (LSM) (Log $_{10}$ cfu/g of faeces) of faecal yeast culture tended to be higher in treatment period (2.919) than control period (2.791) during *S .cerevisiae*-B feeding (Table 3). But the difference was statistically similar in pretreatment, treatment and post treatment periods. In the treatment group of *S. cerevisiae*-522, similar trend was observed as in *S. cerevisiae*-B fed group.

The results indicated that a fraction of yeast which was fed to the animals remained viable and appeared in the faeces in viable state, during treatment period. Probably yeast fed to the animals could not multiply for long period in GI tract and hence, faecal yeast counts in the post-treatment period were similar with that of control. Yeast counts during treatment period in SC-B and SC-522 were statistically similar.

The results corroborated the findings of Newbold et al. (1990) who have recorded increased counts of yeasts in rumen fluid, 1h after yeast culture addition. Number then decreased by 61% after 6 h, but remained higher by two orders of magnitude than that of control. Viable yeast persisted in the duodenum and ileum of experimental animals, at values 6.5 and 6.8 times higher than control

Table 3. Presence of probiotics in faeces during treatment and post-treatment period (least squares mean of Log_{10} cfu/g faeces)

Parameter	Yeast feeding		Lactobacilli feeding		Streptococci feeding	
	SC-B	SC-522	LA	LP	LL	EF
Control	2.791±0.072	2.752±0.115	6.092±0.057^{a}	6.176±0.047	5.953±0.105	6.070±0.133^{b}
Treatment effect	2.919±0.056	2.944±0.089	6.365±0.044^{b}	6.147±0.036	6.055±0.081	6.257±0.103^{b}
Post-treatment effect	2.803±0.072	2.892±0.115	6.214±0.057^{ab}	6.258±0.047	5.807±0.105	5.666±0.133^{a}
Overall effect	2.838±0.039	2.863±0.062	6.224±0.031	6.193±0.025	5.938±0.056	5.997±0.071
Significance	NS	NS	**	NS	NS	

NS = Non-significant; **P<0.01; means with different superscripts (a, b) within a column differ significantly.

Chart 1: In vitro gas production under single probiotics culture supplementation

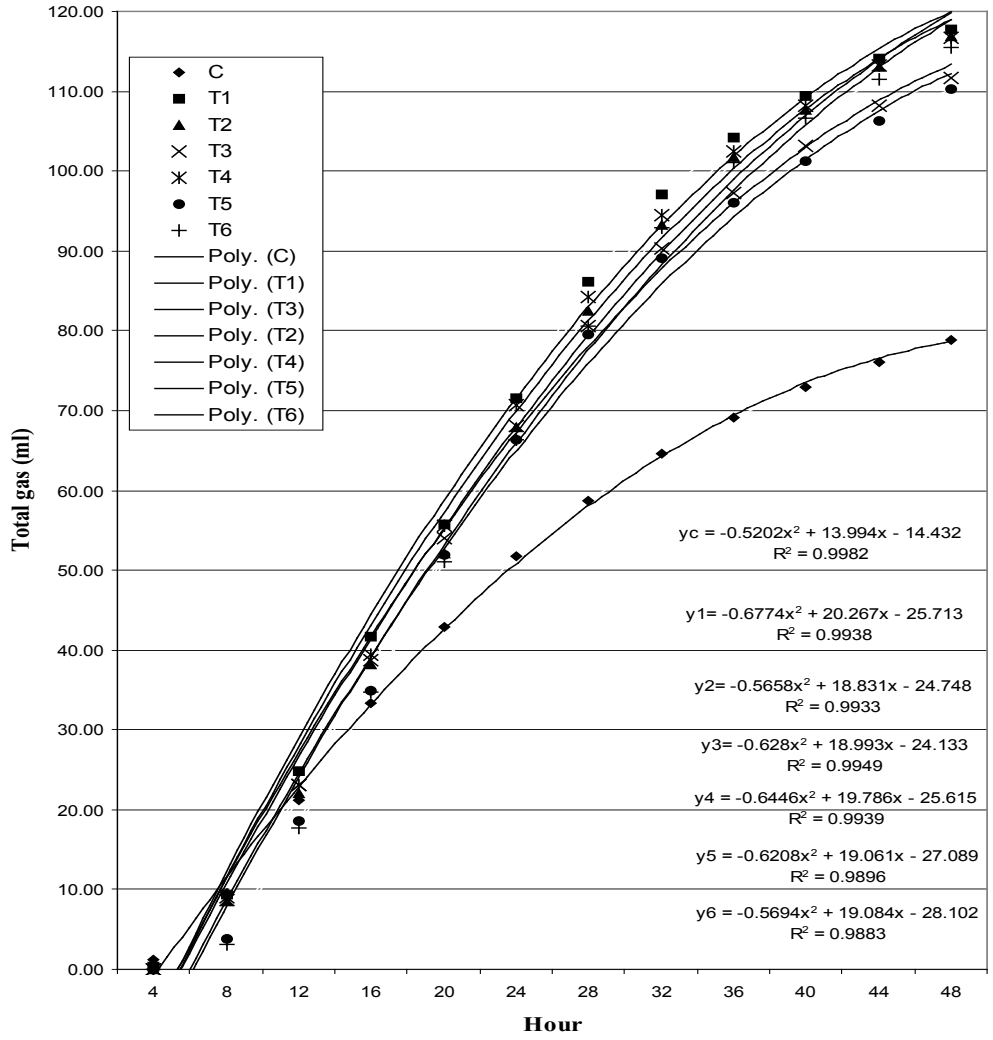

Table 4. Effect of different viable probiotics on in vitro DM degradability, total gas and VFA production

Treatments	IVDMD (%) (ml)	Total gas (48h) (mmol/dl IM¥)	Total VFA (%)	Acetate (%)	Propionate (%)	Butyrate	Acetate/ propionate ratio
Control	68.24±1.36^{a}	79.58±3.73^{a}	7.23±0.03^{cd}	62.55±0.61^{de}	28.59±0.50^{b}	8.86±0.11^{a}	2.21±0.04^{bc}
SC-B	73.52±0.99^{b}	117.67±1.17^{b}	7.02±1.43^{cd}	60.83±0.70^{cde}	27.77±1.24^{ab}	11.39±0.54^{ab}	2.19±0.12^{bc}
SC-522	73.45±0.79^{b}	116.90±0.56^{b}	6.07±0.59^{bc}	62.17±0.43^{de}	26.62±0.26^{ab}	11.22±0.17^{ab}	2.34±0.04^{cd}
LA	74.56±0.62^{b}	109.67±6.57^{b}	6.23±0.71^{bcd}	60.37±1.46^{bcde}	27.51±1.18^{ab}	12.11±0.27 ^{ab}	2.20±0.15^{bc}
LP	73.42±0.89^{b}	114.33±5.76^{b}	5.53±0.10^{ab}	55.56±2.41^{ab}	28.50±0.86^{b}	15.93±1.55^{c}	1.95±0.14^{abc}
LL	74.85±0.38^{b}	110.30±2.45^{b}	4.57±0.36^{a}	64.61±2.94^{e}	24.20±1.73^{a}	11.20±1.21^{ab}	2.69±0.31^{d}
EF	74.29±0.58^{b}	115.57±1.55^{b}	7.43±0.73^{d}	58.32±2.19^{bcd}	26.87±1.72^{ab}	14.48±4.20^{bc}	2.18±0.22^{bc}
Sig.	**	*	*	*	*	*	*

Figures with different superscripts (a,b,c,d,e,f,g,h,i) in the same column differ. Means with different superscripts (a, b, c, d, e) in a column differ significantly, *P<0.05; **P<0.01.

¥ Incubation Medium (IM)

(Newbold et al., 1990). In present study, faecal yeast count remained 50% and 49% higher during treatment period over control in SC-B and SC-522 treatment groups. These observations were consistent with the findings of Dawson and Newman (1988) and McLeod et al. (1990). In Rusitech fermentors also viable cells were present when received the yeast supplement up to 14 hours after feeding (Dawson and Newman, 1987). Kunj et al. (1997) also observed the viability of yeast in ruminal fluid for 24h. Reports of Arambel and Tung (1987) and Harrison et al. (1987) were contrary to these findings.

Acid forming bacilli counts (LSM of Log $_{10}$ cfu/g faeces) were statistically higher ($P<0.01$) during treatment period (6.365) as compared to pre-treatment control (6.092) and post treatment periods (6.214) (Table 3) in LA group. Increase (73%) of acid producing bacilli counts was recorded in LA during treatment period over control period (Table 3) and remained 24% higher during post treatment period. Therefore, it appeared that some portion of lactobacilli passed through the rumen and intestine and were found viable in the faeces. Results of the present study are in agreement with earlier findings. In calves, faecal lactobacilli increased when fed lactobacilli in diet (Abu-Tarboush et al., 1996). In *L. plantarum* fed crossbred calves the faecal acid forming bacilli counts were similar during control, treatment and post treatment periods; hence it seems that *L. plantarum* cell could not pass live to the lower tract. Counts of acid forming bacilli in LA group (6.365) were higher ($P<0.01$) than LP group (6.147) during treatment period. This observation is indicating that *L. acidophilus* is more resistant to the digestive process in calves than that of *L. plantarum.* The results are corroborated by earlier reports of Abu-Tarboush et al. (1996), who have shown that calves fed *L. acidophilus* 27SC had higher faecal lactobacilli counts than calves fed mixed Lactobacilli (*L. acidophilus* and *L. plantarum*).

LSM values (Log_{10} cfu/g faeces) of faecal acid producing cocci were similar in three periods, i.e., control (5.950), treatment period (6.055) as well as post treatment period (5.807) in *L. lactis* (LL) supplemented calves (Table 3). Whereas, in EF supplemented group LSM value was marginally higher in treatment period (6.257) as compared to control (6.070) period. But during post treatment period the counts were significantly less ($P<0.01$) than control and treatment period (Table 3). During treatment period, faecal counts (cfu/g faeces) remained 1.75 times higher over control. Therefore, it may be suggested that *E. faecium* remained partially viable in gastro-intestinal tract.

Havrevoll et al. (1988) found that diarrhoea was less pronounced in calves supplemented with *S. faecium* and less diarrhoea was mainly due to competitive attachment of receptor sites in the intestines between lactic acid bacteria and coliform (Muralidhara et al., 1977). This finding indirectly indicated that a portion of *E. faecium,* which was fed to calves, was passing to the GI tract for competitive attachment. These findings supported our observations indirectly, although the *E. coli* counts were not estimated in the present experiment.

In vitro evaluation

In vitro DM degradability was significantly higher ($P<0.01$) in all probiotics supplemented groups (Table 4); which in turn increased total gas production (ml/0.5 g substrate) under in vitro system over no probiotics control bottle (Chart 1). Polynomial regression equation with R^2 values were developed for each treatment (Chart 1). However, total VFA concentration was lower ($P<0.05$) in LP and LL added bottles as compared to control. The values in other probiotics supplemented bottles were similar with that of control. Acetate concentration (%) was reduced significantly ($P<0.05$) in LP (55.56%) and increased in LL group; whereas, butyrate level was increased ($P<0.05$) in LP and EF groups. Acetate proportion in other treatment group was similar with that of control. Propionate concentration was not influenced by probiotics addition in this study except LL group. Its concentration was decreased ($P<0.05$) in LL treated bottles (24.20%) than control (28.59%). Acetate to propionate ratio were similar in all direct fed microbials supplemented groups with that of control except in LL (2.69), which was significantly ($P<0.05$) higher than control. The results are in agreement with the earlier reports (Chademana and Offer, 1990, Carro et al. 1992 and Newbold et al. 1995).

In the present study, acetate and propionate concentration in the incubation medium remained unaffected due to probiotics addition except LP and LL groups. Recent study (Raeth-Knight et al, 2007) revealed that supplementing mid-lactation cows with DFM products having *L. acidophilus* and *Popionibacteria freudenreichii* did not affect cow performance, diet digestibility or rumen fermentation. Whereas, Lila et al. (2004) reported

that supplementation of *S. cerevisiae* resulted in a quadratic increase of propionate, with quadratic decrease of acetate:propionate under in vitro system. Similarly, enhanced production of propionate and reduction of acetate to propionate was reported by Moloney and Drennan (1994) and Dutta and Kundu (2005) due to DFM addition in either in sacco or in vitro. Supplementing feedlot cattle diets with *E. faecium* increased propionate and decreased butyrate concentrations (Beauchemin, et al., 2003).

It is inferred that acid forming bacilli increased in faeces during LA supplementation period, therefore, could have multiplied in the gastro-intestinal tract and both yeast cultures (SC-B and SC-522) and EF remained viable in the GI tract during treatment period. Although, IVDMD and total gas production increased due to these probiotics addition under in vitro system, but VFA production was mostly unaffected due to these probiotics addition (except LP and LL); however, further study is required to observe the potential of above probiotics under in sacco and in vivo ambience.

REFERENCES

Abu-Tarboush, H. M., M. Y. Al-Saiady, and A. H. K. El-Din. 1996. Evaluation of diet containing Lactobacilli on performance, faecal coliform, and lactobacilli of young dairy calves. Anim. Feed Sci. Technol. 57: 39-49.

Adams, A. L., Jr. B. Harris, H. H. Van Horn, and C. J. Wilcox. 1995. Effects of varying forage types on milk production responses to whole cottonseed, tallow and yeast. J. Dairy Sci. 78: 573-581.

AOAC. 1984. Official Methods of Analysis. 14th ed. Assoc. Off. Anal. Chem. Washington, D.C.

Arambel, M. J., and R. S. Tung. 1987. 19th Biennial Conference on Rumen Function. Chicago, Illinois.

Barnet, A. J. G., and R. L. Reid. 1957. Studies on production of volatile fatty acids from grass by rumen liquor in artificial rumen, TVFA production from fresh grass. J. Agric. Sci. 48: 315.

Beauchemin, K. A., W. Z. Yang, D. P. Morgavi, G. R. Ghorbani, W. Kautz, and J. A. Z. Leedle. 2003. Effects of bacterial direct fed microbials and yeast on site and extend of digestion, blood chemistry and subclinical ruminal acidosis in feedlot cattle. J. Anim. Sci. 81:1628-1640.

Bechman, T. J., J. V. Chambers, and M. D. Cunningham. 1977. Influence of *L. acidophilus* on performance of young dairy calves. J. Dairy Sci. 1:74. (Abstr.).

Bruce, B. B., S. E. Gilliland, L. J. Bush, and T. E. Staley. 1979. Influence of feeding cells of *Lactobacillus acidophilus* on the faecal flora of young dairy calves. Oklahoma Anim. Sci. Res. Report, Stillwater, U.K., pp.207.

Chademana, I., and N. W. Offer. 1990. The effect of dietary inclusion of yeast culture on digestion in the sheep. Anim. Prod. 50: 483-489.

Carro, M. D., P. Libzien, and K. Rohr. 1992. Influence of yeast culture on the in vitro fermentation (Rusitech) of diets containing variable proportions of concentrates. Anim. Feed Sci. Technol. 37: 209-220.

Dawson, K. A., and K. E. Newman. 1987. Effects of yeast culture supplements on the growth and activities of rumen bacteria in continuous culture. J. Anim. Sci. 65 (Suppl. I): 452.

Dawson, K. A., and K. E. Newman, 1988. Fermentations in rumen-simulating continuous cultures receiving probiotic supplements. J. Anim. Sci. 66 (Suppl.I): 500.

Dawson, K. A., K. E. Newman, and J.A. Boiling. 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage-feed ruminal microbial activities. J. Anim. Sci. 68:3392-3398.

Dutta, T. K. 1998. Studies on the potentials of probiotics on in vitro fermentation and productive performance in lactating dairy cows. Ph.D. Thesis, National Dairy Research Institute, Karnal, Haryana (India).

Dutta, T. K. and S. S. Kundu. 2005. In vitro rumen fermentation and gas production as affected by probiotics addition. Indian J. Anim. Sci. 75:817-822.

El-Hassan, S. M., C. J. Newbold, and R. J. Wallace. 1993. The effect of yeast culture on rumen fermentation: growth of the yeast in the rumen and requirement for viable yeast cells. Anim. Prod. 56:463.

Ervin, E. S., G. A. Marco, and E. M. Emergy. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatograph. J. Dairy Sci. 44: 1768.

Harris, Jr. B., and R. Lobo. 1988. Feeding yeast culture to lactating dairy cows. J. Dairy Sci. 71(Suppl. I): 276.

Harrison, G. A., R. W. Hemken, K. A. Dawson, R. J. Harmon, K. E. Newman, and M. C. Morehead. 1987. Yeast culture supplement in diets of lactating cows. II. Effects on liquid dilution rate and apparent digestibility. J. Dairy Sci. 70(Suppl.I): 218.

Havrevoll, O., T. Matre, M. Pestalozzi, K. Storro, and S. Holland. 1988. Probiotics in milk feeds for calves. Proc. Vith World Conf. Anim. Prodn., Helsinki.

Hughes, J. 1988. Effect of a high strength yeast culture in the diet of early weaned calves. Anim. Prod. 46:526.

Kung, Jr. L., E. M. Kreck, R. S. Tung, A. O. Hession, A. C. Sheperd, M. A. Cohen, H. E. Swain, and J. A. Z. Leedle. 1997. Effects of a live yeast culture and enzymes on in vitro ruminal fermentation and milk production of dairy cows. J. Dairy Sci. 80:2045-2051.

Lila, Z. A., N. Mohammed, T. Yasui, Y. Kuokawa, S. Kanda, and H. Itabashi. 2004. Effects of a twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation in vitro. J. Anim. Sci. 82: 1847-1854.

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43: 99.

McLeod, K. R., K. J. Karr, K. A. Dawson, R. E. Tucker, and Jr., G. E. Mitchell. 1990. Rumen fermentation and nitrogen flow in lambs receiving yeast culture and (or) monensin. J. Anim. Sci. (Suppl.1.1):73: 266.

Moloney, A. P., and M. J. Drennan. 1994. The influence of the basal diet on the effects of yeast culture on ruminal fermentation and digestibility in steers. Anim. Feed Sci. Technol. 50: 55-73.

Muralidhara, K., G. G. Sheggeby, P. R. Elliker, D. C. England, and W. E. Sandine. 1977. Effect of feeding lactobacilli on the coliform and lactobacillus flora of intestinal tissues and faeces from pigs. J. Food Protect. 40: 288-295.

Newbold, C. J., P. E. V. Williams, N. McKain, A. Walker, and Wallace, R. J. 1990. The effect of yeast culture on numbers and fermentation in the rumen of sheep. Proc. Nutr. Soc. 49: 47A.

Newbold, C. J., R. J. Wallace, X. B. Chen, and F. M. Mclntosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. J. Anim. Sci. 73: 1811-1818.

NRC, 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Press, Washington, D.C.

Raeth-Knight, M. L., J. G. Linn, and H. G. Jung. 2007. Effect of direct-fed microbials on performance, diet digestibility and rumen characteristics of Holstein dairy cows. J. Dairy Sci. 90: 1802-1809.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. 6th Edition, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.

Stein, D. R., D. T. Allen, E. B. Perry, J. C. Bruner, K. W. Gates, T. G. Rehberger, K. Mertz, D. Jones, and L. J. Spicer. 2006. Effects of feeding propionibacteria to dairy cows on milk yield, milk components and reproduction. J. Dairy Sci. 89: 111-125.

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Nutrient Composition and Phenolic Constituents in some Feed and Fodder Samples from Temperate Regions of Kumaon Himalaya

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ABSTARCT : A total of 57 samples comprising of 15 different types of feeds/fodder, viz. mixed grass hay, barley grass (*Hordeum vulgare*), oat grass (*Avena sativa*), bimalsiya grass (*Pennisetum orientale*), kumeria grass (*Heteropogon contortus*), kikiyu grass (*Pennisetum clandestinum*), mature and immature oak leaves (OL) two types: banj, *(Quercus leucotricophora)*, Kharsun *Q. semicarpifolia*), quiral leaves (*Bahuinia retusa*), poplar (*Populus ciliata*) leaves, maize flour (*Zea mays*), pelleted feed and concentrate mixture were collected from the farmers during the field survey at temperate regions (>1700-2100 m msl) of Kumaon Himalaya. The feed samples were analysed for different nutrient composition and phenolic constituents. The CP content in mixed grass hay ranged from 4.6 to 8.6% and had 81.5% NDF, 55.6% ADF and 10.9% ADL. The bimalsia grass had highest CP (20.9%) followed by kikiyu (10.9%) and kumeria (6.1%) and was also rich in HC (44.8%) and had least ADL (2.8%). The barley and oat grass had CP 5.8 and 16.5 %, respectively. Total Ash was 8.1% in kumeria and 7.6% in mixed grass hay, but had high >50% AIA. The oat had higher P content (0.58%) compared to other grasses and the Ca:P ratio was very narrow (<1.5:1). Ca% was highest in barley grass (0.89) followed by bimalsia (0.71), oat and kumeria (0.62-0.68) and was low in mixed grass hay (0.57) and kikiyu (0.51). The tree leaves were rich in OM (93.0-96.3% vs 88.3-92.4%). The CP content was nearly double in immature oak leaves (19.7-20.7%) than the mature one (9.4-10.1%). In other tree species, the CP content was high in poplar (14.6%) and low in quiral (5.8%). The ADL content was 21.6% in mature Banj leaves and 6.7% in poplar. Quiral leaves had a nearly balanced concentration of Ca (1.93%) and P (0.69%) and the other tree leaves had a very wide ratio (nearly 10:1). The immature oak leaves had nearly equal Ca and P concentration (0.37-0.49%). The concentration (%) of poly-phenolic components, viz. total phenolics (TP), total tannin phenolics, condensed tannins (CT), hydrolysable tannins (HT) and non-tannin phenolics revealed that the TP was 7.06 to 9.81 in different leaves and was highest in immature Kharsun (*Q. semicarpifolia*) (9.61 to 10.01). The immature oak leaves also had higher HT content (5.35 in *Q. leucotricophora* and 7.24 in *Q. semicarpifolia*). CT was highest in mature Banj leaves (3.11) followed by Quiral (2.93), poplar (1.97) and Kharsun (1.65) and was very low in immature oak leaves (0.68-0.88). The CP content was 9.2, 22.7 and 19.7 % in the maize flour, pelleted feed and concentrate mixture, respectively. The pelleted feed ADL content (6.6%) was higher than other two concentrate feeds. The Ca and P content in the concentrate mixture and pelleted feed was nearly at optimal ratio (2:1).

Key words: Nutrients, Phenolics, Feeds and fodder, Kumaon

INTRODUCTION

Livestock development is one of the top priorities in the development agenda of Uttarakhand and dairying has been considered as one of the important dimensions. But, animal productivity is

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quite low mainly due to nutritional inadequacy as one of the principal cause, i.e. scarcity of forages (Singh et al., 2001) farm availability and procurement of cereal crop residues are also limited. There is scanty agriculture land and a short cultivation season for growing cereal grains and utilizing the crop residues for animal feeding, and the cost of transportation from lower altitudes/plains exorbitantly increases its procurement cost. Thus, tree forages and local grass from village upland and forest form an integral part of ruminant feeds for most part of the year in high altitudes of Uttarakhand (Meena et al., 2007). The oak tree leaves as fodder, nevertheless, contributed to livestock feed as much as 19 % in the village at high altitude, where the oak forests are in the vicinity of these villages (Singh et al., 2001). Feed shortage has been a serious problem for livestock farmers throughout the Himalaya Hills. In Uttaranchal the central Indian Himalayas, the shortage of feed and fodder is estimated to be 65 per cent. According to Animal Husbandry Department (AHD, 1998), the overall paucity of green and dry fodder in the Uttaranchal Hills, is 68 and 19 per cent respectively. In this scenario, the feed and fodder resources of the temperate hilly regions needs to be evaluated for nutrient composition and anti-nutrients, for assessing their nutrient availability to livestock.

MATERIALS AND METHODS

The survey area involved seven randomly selected villages located in the temperate zones (from 1700 – 2100m) of Kumaon Himalaya. Different feed and forage samples which were fed to the cattle by the livestock farmers were collected during the field visit for the analysis of their chemical composition. Total 57 samples comprising of 15 different types of feeds/fodder were collected from the farmers that were being fed to their animals. The different feed types were mixed grass hay, barley grass (*Hordeum vulgare*), oat grass (*Avena sativa*), bimalsiya grass (*Pennisetum orientale*), kumeria grass (*Heteropogon contortus*), kikiyu grass (*Pennisetum clandestinum*), mature and immature oak leaves (two types: Banj, *Quercus leucotricophora*; Kharsun, *Q. semicarpifolia*), Quiral leaves (*Bahuinia retusa*), Poplar (*Populus ciliate*) leaves, maize flour (*Zea mays*), pelleted feed and concentrate mixture purchased from the local market.

The feed samples collected were dried in the oven at 60±2 ^{0}C until constant weight (for 48h) and the moisture/dry matter (DM) content of the sample was recorded. The samples were then ground to pass through 2 mm screen and preserved in airtight plastic containers until analyzed.

The ground samples of feed and fodder were analyzed for different proximate constituents, viz. DM, crude protein (CP), ether extract (EE), ash, acid-insoluble ash (AIA) and major minerals, viz. calcium (Ca) and phosphorous (P) as per the methods described in AOAC (1984). The organic matter (OM) and total carbohydrates (TCHO) of the samples were calculated by difference. The fiber constituents, viz. neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed by following the methods described by Van Soest et al. (1991) and the content of hemicellulose (HC) and cellulose (C) were calculated by difference. The samples of tree leaves were further analysed for different phenolic constituents, viz. total phenolics (TP), non-tannin phenolics (NTP) and condensed tannins (CT) as per the methods of Makkar (2000) and the difference between TP and NTP was calculated as total tannin phenolics (TTP) and that of TTP and CT was considered as hydrolysable tannins (HT).

The data on chemical composition are tabulated and the mean±SE was calculated for different feed and fodder samples.

RESULTS AND DISCUSSION

The results on different chemical constituents are expressed as percentage on DM basis and presented in tables 1 to 3.

The feed samples collected during the in-field survey can be grouped into four major groups, viz. mixed grass hay, uncultivated and cultivated grasses, trees leaves and concentrate supplements. The mixed grass hay had a variable CP content (4.6 to 8.6) and the AIA content was more than 50% of TA. It was rich in NDF (81.5%), ADF (55.6%) and ADL (10.9%) as compared to all other grasses. The fodder grass barley had a very less amount of CP (5.8) and was having more DM than oat (18.4 vs 12.4). But, oat was rich in CP (16.5) and the fiber and TCHO content were less compare to barley. Amongst the non-cultivated grasses bimalsia had more CP (20.9) followed by kikiyu (10.9) and kumeria (6.1). The bimalsia grass was also rich in HC (44.8) and had less ADL (2.8). TA was less in kumeria (8.1) and was comparable to mixed grass hay (7.6), but had also high AIA (>50%). The Ca and P content was not

Table 1. Chemical composition of feeds and fodder (% on DM basis) from temperate Kumaon region of Himalaya

Feeds	DM	OM	CP	EE	TCHO	NDF	ADF	ADF	HC	C
Mixed Grass hay (8)	85.4±1.76	92.4±0.17	5.9±0.46	1.80±0.10	84.9±0.52	81.5±1.23	55.6±0.74	10.9±0.57	25.9±1.26	4.7±0.65
Bimalsiya grass (*Pennisetum orientale) (3)*	14.3±0.74	88.3±0.14	20.9±0.14	2.4±0.27	64.9±0.27	75.4±0.18	30.6±0.26	2.8±0.18	44.8±0.18	22.7± 0.17
Kumeria (*Heteropogon contortus) (3)*	20.1±0.89	91.9±0.11	6.1±0.20	1.5±0.08	84.4±0.24	77.4±0.12	48.8±0.25	7.4±0.23	28.6±0.11	1.4±0.20
Kikiyu (*Penesetum clandestinum) (3)*	17.4±0.43	89.8±0.16	10.9±0.48	1.0±0.12	77.9-0.52	73.1±0.59	37.6±0.17	6.3±0.18	35.5±0.66	31.3±0.11
Barley (*Hordreum vulgare) (3)*	18.4±0.67	91.2±0.18	5.8±0.04	1.9±0.05	83.5±0.18	66.2±0.12	41.2±0.33	5.0±0.27	25.0±0.21	36.2±0.60
Oat (grass) (*Avena sativa*) (3)	12.4±1.02	91.9±0.11	16.5±0.64	2.7±0.29	72.7±0.82	53.3±0.24	32.3±0.09	2.9±0.17	21.0±0.15	29.4±0.26
Mature oak leaves (Banj) (*Q. leucotricophora) (8)*	65.4±1.39	96.3±0.13	10.1±0.63	6.0±0.33	80.2±0.60	63.1±1.70	49.1±2.0	21.6±1.98	13.9±1.33	27.6±0.79
Immature oak leaves (Banj) (*Q. leucotricophora) (3)*	35.6±1.81	94.1±0.06	20.7±0.42	2.2±0.55	71.2±0.16	49.4±1.06	30.4±0.46	10.8-0.21	19.0±1.50	19.7±0.48
Mature oak leaves (Kharsun) (*Q. semicarpifolia) (3)*	57.9±1.49	95.6±0.08	9.4±0.05	6.3±0.14	79.9±0.19	55.4±0.19	41.6±0.31	17.2±0.20	13.9±0.22	24.3±0.16
Immature oak leaves (Kharsun) (*Q. semicarpifolia) (3)*	32.3±1.56	94.4±0.07	19.7±0.17	1.8±0.50	72.9±0.73	39.7±0.41	23.9±0.33	8.8±0.68	15.8±0.45	15.1±0.65
Quiral leaves (*Bahuinia retusa) (3)*	33.6±0.62	94.2±0.05	5.8±0.07	6.0-0.11	82.4-0.16	39.7-0.15	31.6±0.34	11.7-0.06	8.2±0.39	20.0±0.28
Poplar (*Populus ciliata) (5)*	59.3±1.67	93.0±0.57	14.6±0.57	6.9±0.29	71.5±1.39	55.7±1.35	27.9±1.14	6.7±0.46	27.8±1.86	21.2±0.59
Maize flour (*Zea mays) (3)*	89.3±0.34	97.8±0.04	9.2±0.06	2.4±0.12	86.2±0.11	10.7±0.27	6.3±1.07	3.8±0.82	4.4±1.01	3.8±1.17
Pelleted Feed (3)	87.4±0.37	89.6±0.12	22.8±0.08	3.00.17	63.8±0.20	27.7±0.45	18.7±0.26	6.6±0.31	8.9±0.19	12.1±0.15
Concentrate mixture (3)	91.3±0.02	92.9±0.07	19.7±0.04	3.8±0.48	69.4±0.45	24.1±0.16	12.3±0.35	3.4±0.33	11.7-0.19	8.9±0.21

ADF, acid detergent fibre; ADL, acid detergent lignin; C, cellulose; CP, crude protein; DM, dry matter; EE, ether extract; HC, hemi-cellulose; NDF, neutral detergent fiber; OM, organic matter; TCHO, total carbohydrates NA, not applicable

Table 2. Mineral composition of feeds and fodder (% on DM basis) at temperate Kumaon region

Name (no)	Ash	AIA	Ca	P
Mixed Grass hay(8)	7.6±0.17	4.76±0.11	0.57±0.02	0.10±0.003
Bimalsiya grass (*Pennisetum orientale)(3)*	11.7±0.14	2.71±0.05	0.71±0.01	0.29±0.00
Kumeria grass (*Heteropogon contortus) (3)*	8.1±0.11	4.45±0.02	0.65±0.02	0.12±0.00
Kikiyu grass (*Penesetum clandestinum) (3)*	10.2±0.16	1.93±0.04	0.51±0.01	0.22±0.00
Barley grass(*Hordreum vulgare) (3)*	8.8±0.18	3.05±0.02	0.89±0.01	0.27±0.00
Oat (green) (*Avena sativa) (3)*	8.1±0.11	1.25±0.03	0.66±0.01	0.58±0.00
Mature oak leaves (Banj) (*Q. leucotricophora) (8)*	3.7±0.13	0.31±0.02	1.10±0.04	0.07±0.002
Immature oak leaves (Banj)(*Q. leucotricophora) (3)*	5.9±0.06	2.69±0.03	0.37±0.01	0.42±0.00
Mature oak leaves (Kharsun) (*Q. semicarpifolia) (3)*	4.4±0.08	0.07±0.02	1.01±0.00	0.12-0.00
Immature oak leaves(Kharsun) (*Q. semicarpifolia) (3)*	8.6±0.07	2.54±0.03	0.49±0.01	0.37±0.00
Quiral leaves (*Bahuinia retusa)*	5.8±0.06	0.11±0.00	1.93±0.01	0.69±0.00
Poplar (*Populus ciliata) (5)*	7.0±0.45	1.32±0.0.15	1.53±0.15	0.17±0.05
Maize flour (*Zea mays) (3)*	2.2±0.04	0.02±0.00	0.24±0.02	0.38±0.00
Pelleted Feed*(3)*	10.4-0.12	2.28±0.01	0.54±0.01	0.28±0.00
Concentrate mixture *(3)*	7.1±0.07	3.98±0.01	1.05±0.01	0.49±0.00

TA, total ash; AIA, acid insoluble ash; Ca, calcium; P, phosphorous NA, not applicable

balanced (2:1 ratio) in any of the grass types, but was having a higher P content (0.58) in oat compared to other grasses and the ratio is very narrow (<1.5:1). The mixed grass hay and kumeria had a very low P content (0.09-0.13). The barley grass had higher Ca (0.89) followed by bimalsia (0.71), oat and kumeria (0.62-0.68) and was still lower in mixed grass hay (0.57) and kikiyu grass (0.51). The conventional feed ingredients are extensively analysed and tabulated in various feeding standards and data on Indian feeds and fodders are tabulated in ICAR Bulletins (Sen et al., 1978; Ranjhan, 1991). The percentage composition of proximate, fiber and mineral constituents of mixed grass hay, oat and barley grass, maize flour observed in the present study fall well within the range described in these documents.

The tree leaves (oak, quiral and poplar) had more OM content than grasses (93.0-96.3 vs 88.3-92.4). The CP content was nearly double in immature oak leaves (19.7-20.7). In other tree leaves, the CP content was high in poplar (14.6) and low in quiral (5.8). The EE content was also higher in tree leaves (6.0-6.9) than grasses (1.0-2.7) and it was also higher in mature leaves than immature oak leaves (1.8-2.2). Between the two types of oak leaves banj contained higher ADF and ADL than kharsun both in mature and immature leaves collected from the field. The ADL content was as high as 21.6% in mature banj leaves and it was low in poplar (6.7). The TA and AIA content were higher in both immature oak leaves and the mature leaves had a very low AIA content amongst all the fodders collected from the field (0.07-0.31). The Ca concentration was approximately ten times higher than P except in quiral leaves which had a nearly balanced concentration of Ca (1.93) and P (0.69). The immature oak leaves had nearly equal Ca and P concentration (0.37-0.49). The poly-phenolic components, viz. TP, TTP, CT, HT and NTP in tree leaves are given in table 3. The TP contents were 6.64 to 9.81% in different leaves showing the highest

Table 3. Poly-phenolic components of tree leaves (% on DM basis) at temperate Kumaon region

Feeds		No.	TP	TTP	CT	HT	NTP
Common name	Botanical name						
Mature oak leaves(Banj)	*Q. leucotricophora*	8	7.06±0.19	6.42±0.20	3.11±0.11	3.31-0.15	0.64±0.03
Immature oak leaves (Banj)	*Q. leucotricophora*	3	6.74±0.58	6.03±0.63	0.68±0.09	5.35±0.54	0.71±0.06
Mature oak leaves (Kharsun)	*Q. semicarpifolia*	3	8.40±0.23	7.72±0.29	1.65±0.11	6.06±0.40	0.68±0.06
Immature oak leaves Kharsun)	*Q. semicarpifolia*	3	9.81±0.11	8.12±0.22	0.88±0.11	7.24±0.11	1.69±0.11
Quiral leaves	*Bahuinia retusa*	3	7.12±0.11	6.69±0.09	2.93±0.03	3.76±0.06	0.43±0.02
Poplar	*Populus ciliata*	5	6.64±0.11	4.86±0.06	1.97±0.05	2.89±0.07	1.78±0.04

TP, total phenolics; TTP, total tannin phenolics; CT, condensed tannins; HT, hydrolysable tannins; NTP, non-tannin phenolics

concentration in immature kharsun (*Q. semicarpifolia*) leaves (9.61 to 10.01). The immature oak leaves also had higher HT content (5.35%) in *Q. leucotricophora* and 7.24% in *Q. semicarpifolia*. The CT was highest in mature banj leaves (3.11%) followed by quiral (2.93), poplar (1.97) and kharsun (1.65) and was very low in immature oak leaves (0.68-0.88). The chemical constituents of unconventional feeds are generally difficult to compare. The literature on nutrient composition and tannin constituents of oak leaves (Lohan et al., 1980; 1983; Makkar and Singh, 1991; Singh et al., 1999; Sharma, 2002) and the observed values for mature and immature oak leaves are very much comparable. Some more literature was available on tannin content of oak leaves outside India (Fenny, 1970; Martin and Martin, 1983). The literature on chemical composition of quiral and poplar tree leaves and the grasses (bimalsiya, kumeria and kikiyu) are inadequate for a direct comparison. However, some other species of poplar had CP 12-17% depending on season and variety (Negi, 1979). Similarly, other bahuinia species had CP higher than the present variety (12.8-15.9 vs 5.5-5.8 %) (Pal et al., 1979).

The concentrate feeds had a variable OM ranging from 89.6 (pelleted feed) to 97.8 (maize flour). The CP content was low in the grain (maize flour, 9.2) and was 22.7 in pelleted feed and 19.7 in the concentrate mixture. The fiber content was lower (NDF, 10.7-27.7) in concentrate feeds compared to all other feed types collected from the field, but the pelleted feed had ADL 6.6 compared to 3.4-3.8 in the other two concentrate feeds. The maize flour contained the minimum TA (2.2) with a very minimal AIA (0.2). Both the Ca and P content were higher in the concentrate mixture with a nearly optimal ratio (2:1). The pelleted feed also had optimal Ca and P ratio but the level was lower than concentrate mixture (0.54 vs. 1.05 for Ca and 0.28 vs. 0.49 for P). Contrary to this, the P content was higher than Ca in the maize flour (0.38 vs. 0.28). The chemical composition of concentrate mixture and pelleted feed is generally dependent on ingredient composition. However, considering the concentration of CP, fiber constituents, ash, acid insoluble ash, Ca and P observed in the field samples of the pelleted feed the lignin and AIA ash content appeared to be higher.

REFERENCES

A.O.A.C. 1984. Official Methods of Analysis. Assoc. of Off. Anal. Chem., 13th ed. Arlington, Virginia. U. S .A.

AHD. 1998. Animal Husbandry Department Livestock Census Report. Lucknow: Animal Husbandry Department, Government of U.P.

Fenny, P. 1970. Seasonal changes in oak leaves tannins and nutrients as a course of spring feeding by winter month caterpillars. Ecology. 51: 565-581.

Lohan, O.P., D. Lall, and S.S. Negi. 1983. Partitioning of total tannins in some tree fodders in

condensed and hydrolysable forms. Indian J. Anim. Sci. 53: 1333-1335.

Lohan, O.P., D. Lall, R.N. Pal, and S.S. Negi. 1980. Studies on tannins in tree leaves. Indian J. Anim. Sci. 50: 881-883.

Makkar, H.P.S. 2000. Quantification of Tannins in Tree Foliage – A Laboratory Manual. FAO/ IAEA Working Document, Vienna, Austria.

Makkar, H.P.S. and B. Singh. 1991. Distribution of CT (proanthocyanidins) in various fibre fractions in young and mature leaves of some oak species. Anim. Feed Sci. Technol. 32: 253-260.

Martin, J. S., and M. M. Martin. 1983. Tannin assays in ecological studies. J. Chem. Ecol. 9: 255-294.

Meena, H. R., H. Ram, S. K. Singh, R. K. Mahapatra, A. Sahoo, and T. J. Rasool. 2007. Animal husbandry practices at high altitude (>6000 feet) in Kumaon region of Uttarakhand, India. Livestock Res Rural Devt. 19: paper 163.

Negi, S. S. 1979. Poplars as feed for animals. Reprinted from Symposium Proceedings. Pp 167-169.

Pal, R.N., K.K. Dogra, L.N. Singh, and S.S. Negi. 1979. Chemical composition of some fodder trees in Himachal pradesh. Forage Res. 5:109-115.

Ranjhan, S.K. 1991. Chemical composition and nutritive value of Indian feeds and feeding of farm animals. ICAR Publication, New Delhi.

Sen, K.C., S.N. Ray, and S.K. Ranjhan. 1978. Chemical Composition of Cattle Feeds and Feeding of Farm Animals. ICAR, New Delhi.

Sharma, R.K. 2002. Degradation kinetics and nutrient utilization of tannin rich top feeds. Ph. D Thesis, Indian Veterinary Research Institute, Izatnagar,

Singh, P., A.K. Verma, R.S. Dass, and U.R. Mehra. 1999. Performance of Pashmina kid goats fed oak (*Quercus semecarpifolia*) leaves supplemented with a urea molasses mineral block. Small Rumin. Res. 31: 239-244.

Singh, V., Tulachan and Tejpratap. 2001. Smallholder dairy farms in the mixed mountain farming systems: A case of the Uttaranchal Hills. Indian Journal of Anim. Sci. 71: 975-984.

Van Soest, P. J., J. B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3587.

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Bacterial Biomass as Feed for Rearing of *Penaeus indicus* (H. Milne Edwards) Larvae

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ABSTRACT : Two experiments were conducted to evaluate the role of bacteria as food source for *Penaeus indicus* larvae. In the first experiment species of *Bacillus, Micrococcus and Pseudomonas*, isolated from prawn culture ponds, were fed to *P. indicus* larvae (N) Z-1 as partial substitute along with Chaetoceros fed as exclusive feed to *P. indicus* larvae (N) Z-1 and growth and development monitored over a period of 10 days. In the second experiment the same bacterial isolates were fed as exclusive feed to *P. indicus* larvae (N) Z-1 and growth and development monitored over a period of 10 days. High survival rates ranging from 64-70% were obtained for *Bacillus* BTM 01 and BTM 05 and *Micrococcus* sp BTM 12 upon 50% supplementation ($P<0.05$) as compared to 40% survival on the Chaetoceros fed control. Feeding *P. indicus* larvae exclusively with bacterial biomass resulted in survival rates of less than 20%. Complete mortality was also observed in the case of *Bacillus* BTM 01 and BTM 05, and *Pseudomonas* BTM 25, even before larvae could metamorphose on to the PL – 1 stage. The starved controls survived only up to the third and fourth day in both the treatments. Results suggest that bacterial biomass could be used as a partial substitute for micro - algal feed during the vulnerable larval stages of penaeid shrimp.

Key words : Bacteria, Feed, *P. indicus*, larval development.

INTRODUCTION

The culture of marine shrimp has shown an exceptional increase worldwide, mainly due to improvement of methods and techniques related to hatching and rearing of larvae (Barnabe, 1990). However, the most critical factor in the industrial farming of shrimp is the reliable availability of quality larvae and larviculture industry of marine shrimp is valued at several hundred million US $ annually for the production of more than 50 billion shrimp post larvae. The key factor in this production of penaeid larvae is the provision of appropriate feed for better growth and survival (Cook and Murphy, 1966, 1969; Aquacop, 1977; Mock et al., 1980). During the early stages, protein molecules larger than a colloid cannot be absorbed through the gut epithelium of the digestive tract because of the lack of adequate enzyme (Tanaka, 1975; Watanabe, 1986). Although small zooplankton, which have a large amount of colloidal protein in its plasma, and microalgae (Chaetoceros, Tetraselmis etc) are indispensable, their continuous availability is questionable , their culture is technically demanding and expensive, representing 30-50% of the hatchery operating costs. Further attempts on replacement feeds have resulted in microparticulate and tissue suspension feeds, but these too have their limitations.

Bacteria accounting for over 80% of the total biosurface in seawater form part of the food chain for many organisms. Bacteriovory is reported to be widespread among many marine larvae (Azam et al., 1984). Bacteria are also used as a diet supplement to zooplankton and in the artificial diets fed to higher tropic levels (Maeda, 1986). Artemia was reared exclusively on a diet of bacteria, Flexibacter sp (Intriago and Jones, 1993), while Brachionus plicatilis was reared using bacteria (Yasuda and Taga, 1980). More recently, studies have tested the

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use of microorganisms in the control of microbial infections in rearing larvae with promising results (Maeda, 1989: Moriarty, 1990: Nogami and Maeda, 1992: Maeda, 1994 : Austin et al., 1995, Gil, 1995). The addition of selected bacteria (Probiotics) in tanks or ponds may control deleterious forms through (a) competitive exclusion of pathogenic bacteria; (b) enhanced nutrition of larvae by supplying essential enzymes; and (c) production of antibiotic substances that inhibit the growth of undesired cells (Garriques and Arevalo, 1995).

Apart from the properties listed above, bacteria can also act as a food source for larvae either by direct consumption, or by feeling aquatic and benthic food webs in aquaculture systems. Such features have been well documented for natural ecosystems, following the seminal papers of Pomeroy (1974) and Azam et al., (1983) who showed the importance of microorganisms in the transfer of matter and energy through aquatic food chains. However, few studies have been conducted in order to evaluate the importance of microorganisms as food source for marine larvae (Moriarty et al., 1983; Maeda, 1989; Moriarty, 1990; Douillet and Langdon, 1994; Moriarty, 1997). In fact, very little effort has been made towards the use of bacterial biomass, as a sole larval feed and little is known of their basic characteristics. In this context we evaluated the potential of marine bacterial biomass, as a feed source, and its effect on growth and metamorphosis in the larvae of the Indian white prawn *P. indicus*.

MATERIALS AND METHODS

Bacteria

Heterotrophic bacteria used in the feeding experiments were isolated from the water samples of the prawn culture pond from a near by prawn farm. Water samples were collected aseptically, in presterilized sample bottles, transported in iced condition to the laboratory and processed for bacteriological analyses immediately. After serial dilution, the samples were plated on ZoBell's Marine 226E agar medium employing pour plate technique and incubated at room temperature (28 ± 2^0C) for three to five days. Single cell colonies, developed on the agar were isolated employing random technique and subcultured on ZoBell's Marine agar slants. All the isolates were identified upto their generic level based on their morphological, physiological and biochemical characteristics (Kreig and Holt, 1984). Potential strains, for use as feed, were selected based on their ability to grow rapidly and yield higher biomass in ZoBell's marine broth. Growth of bacteria was determined in terms of turbidity in the culture broth, by measuring the absorbance at 660nm in a UV-Visible spectrophotometer (Shimadzu) and was expressed as optical density (OD).

Production of bacterial biomass

Bacterial biomass for use as feed was prepared using the same medium mentioned above. Initially the selected bacteria were grown aseptically in 10 ml of the broth for 18h, at room temperature (28 ± 2^0C). In the second stage 100ml of broth was inoculated using the preculture as inoculum (1%v/v) and incubated on a rotary shaker at 150 rpm, for 18h, at room temperature (28 ± 2^0C). The cells were harvested by centrifugation (Kubota 6700 model) at 10,000 rpm at 4^0C for 15 min. and adjusted to a final conc. of 10^8 cells/ml using sterile physiological saline (0.85% Nacl)after repeated washing with the same. In the third stage one liter of the broth was inoculated using the prepared cell suspension at 1% (v/v) and incubated on a rotary shaker at 150 rpm, for 18h at room temperature (28 ± 2^0C). Later the cells were harvested by centrifugation (Kubota 6700 model) at 10,000 rpm at 4^0C for 15 min and adjusted to a final concentration of 10^8 cells/ml using sterile physiological saline (0.85% Nacl) after repeated washing with the same. The prepared cell suspension was used fresh for the feeding trials.

Feeding trials with *P. indicus* larva

Larvae of *P. indicus* (N) Z-1 belonging to the same brood stock, obtained from the Central institute for Brackish water Aquaculture (CIBA), Narakkal, Cochin, India, were used for the study. They were maintained at a density of 120 nauplii /Lit in one liter glass beakers containing filtered seawater (20 micron filter) having a salinity of 34 ± 1 at 28 ± 2^0C and pH 7.80 ± 2^0C. Continuous but low aeration was provided in each beaker with the help of sterile air stones. Two different treatments were adopted for each bacterium in the beakers. One set of treatment included exclusive feeding of bacteria and the second set of treatment included partial supplementation of bacteria to Chaetoceros at a 1:1 ratio. A starved control, comprising of unfed larvae, and an experimental control, comprising of larvae fed exclusively on Chaetoceros, were also

maintained under identical experimental conditions. The diatoms used in the study were supplied by Central Institute of Brackish water Aquaculture, Cochin, India. Feeding was carried out once a day and excepting the starved controls, larvae of all treatments were fed daily with the bacteria/ diatom, at a final cell concentration of 10^5 cells/ ml. Cell density was monitored by cell counts. The multiplication of cells during the course of the experiment was checked with controls consisting of microbial biomass alone and corrections were made wherever necessary. The rearing medium was changed everyday, and all larvae were counted and staged under a microscope. The experiment was terminated on the tenth day (PL-2 stage).

Biochemical analyses

All the selected bacterial isolates were characterised for their biochemical characteristics. 5 ml of the mid log-phase culture, grown in ZoBell's marine broth described above, was centrifuged at 10000 rpm, at 4^0C for 15 min. and the pellet was washed with physiological saline. To the pellet 0.25 ml of a mixture containing 10% SDS and 1% mercaptoethanol was added to lyse the cells and vortexed vigorously for 15 min. The prepared cell lysate was analysed for protein (Lowry et al., 1951), lipid (Folch et al., 1957), reducing sugars (Miller 1959), and dry matter (AOAC 1990). All treatments were carried out in triplicate and Analysis of variance (ANOVA) was carried out for all experimental data.

RESULTS

From among the isolates obtained, five strains which showed good growth and enhanced protein yield were selected for further studies. They were identified as species of *Bacillus* BTM 01 and BTM 05, *P seudomonas* BTM 25 and *Micrococcus* BTM 12 and BTM 14. The biochemical characteristics and the generation time of these strains are given in table 1. *Pseudomonas* BTM 25 recorded the highest protein content (7.6 mg/ml) followed by *Micrococcus* BTM-12 (6.82 mg/ml), whereas *Bacillus* BTM 01 showed the lowest protein content (3.68 mg/ml).

Dry matter content of the strains ranged between 18.63% (*Bacillus* BTM 05) to 22.4% (*Pseudomonas* BTM 25). Lipid content varied between 1.04 mg/ml to 1.26 mg/ml for four of the strains except for *Bacillus* BTM 05, (0.98 mg/ml). *Pseudomonas* sp BTM 25, *Bacillus* BTM 01 and both *Micrococcus* sp BTM 12 and BTM 14 recorded respectively 393,383, and 392 mg/ml of reducing sugar content. *Micrococcus* sp BTM 12 showed the longest generation time (85 min.) while *Pseudomonas* BTM 25 showed comparatively the shortest generation time (36 min).

Results for feeding *P.indicus* larvae with 5 strains of bacterial biomass as a partial supplement

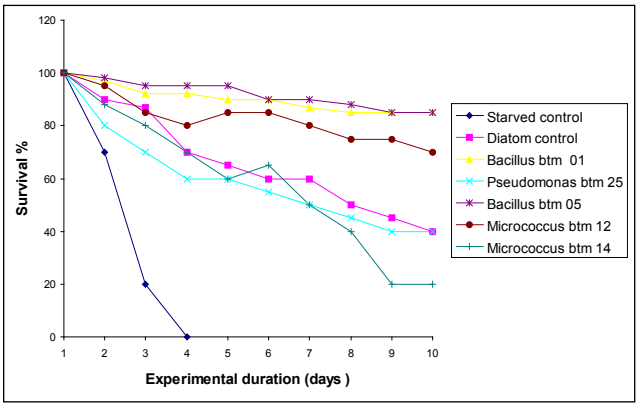

Fig.1 Survival of *P.indicus* larvae fed with partial supplementation of bacterial biomass along with *Chaetoceros*

at a ratio of 1:1 with microalgae are given in (Fig.1). Both *Bacillus* sp BTM 01 and BTM 05 and *Micrococcus* BTM 12 promoted high survival rates of the larvae ranging from 64 to 70% compared to the 40% survival observed in the Chaetoceros fed control group ($P<0.05$). Whereas *Pseudomonas* sp BTM 25 and *Micrococcus* BTM 14 contributed to low survival rates (37% and 12% respectively) upon substitution. Feeding *P.indicus* larvae exclusively with bacterial biomass resulted in survival rates of less than 20% (Fig 2). Complete mortality was also observed in the case of *Bacillus* BTM 01 and BTM 05, and *Pseudomonas* BTM 25, even before larvae could metamorphose on to the PL–1 stage. The starved controls survived only upto the third and fourth day in the two treatments.

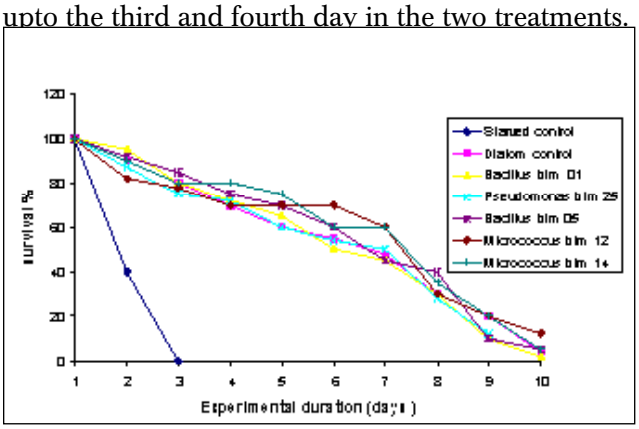

Fig.2 Survival of *P.indicus* larvae fed exclusively with bacteria biomass.

Table 1. The Biochemical characteristics of the selected bacteria used as feed for *Penaeus indicus*

Variable	Bacteria				
	Bacillus **BTM 01**	***Bacillus*** **BTM 05**	***Pseudomonas*** **BTM 25**	***Micrococcus*** **BTM 12**	***Micrococcus*** **BTM 14**
Generation Time (min.)	40	56	36	85	70
Dry matter (%)	19.46	18.63	22.40	18.92	20.46
Protein (mg/ml)	3.68	4.20	7.60	6.82	5.10
Lipid (mg/ml)	1.11	0.98	1.26	1.04	1.21
Reducing Sugars (mg/ml)	393.40	409.60	410.10	383.13	391.80
Total Nucleic Acids(mg/ml)	0.025	0.028	0.031	0.026	0.020

All values are averages of five individual estimations

Best development of larvae was observed on feeding with 50% of *Bacillus* BTM 01($P<0.05$). Although metamorphosis was good with *Bacillus* BTM 05 initially, it declined during the subsequent zoea stage (Fig.3) and was at comparable levels to that of *Bacillus* BTM 01. Substitution with 50% biomass of *Micrococcus* BTM 12 gave results identical to that of

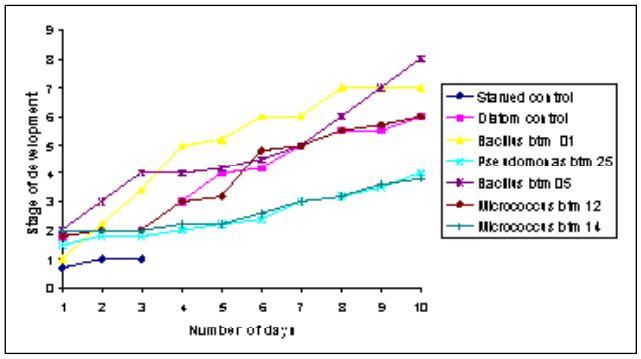

Fig.3 Development of *P.indicus* larvae fed with partial supplementation of bacterial biomass along with *Chaetoceros.*

the diatom fed control, whereas both *Pseudomonas* sp BTM 25 and *Micrococcus* BTM 14 supported, relatively, poor metamorphosis in larvae that survived on these groups of biomass as partial supplement.

Metamorphosis in the group of larvae fed exclusively with *Micrococcus* BTM 12, though initially was good, and declined later with advancement in growth stage (Fig.4). Complete feeding with *Bacillus* (BTM 01 and BTM 05) and *Pseudomonas* BTM 25 yielded poor development with total mortality at the protozoea-2 stage. Nevertheless no pathogenic conditions were observed in any of the experimental larvae during the course of experiments.

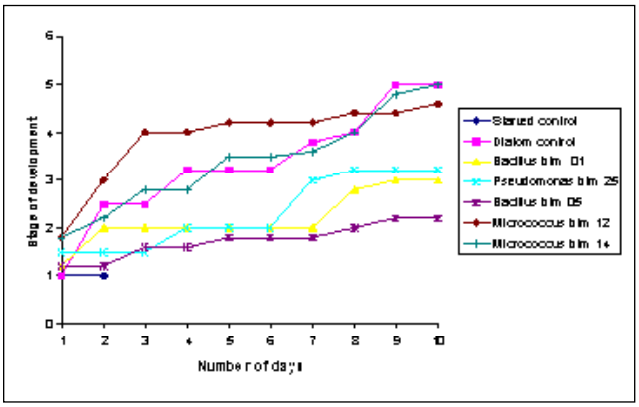

Fig.4 Development of *P.indicus* larvae fed exclusively with bacterial biomass

DISCUSSION

The most vulnerable stage in forming are the early larval stages where survival rates are very low due to non availability of proper nutritious feeds especially in the semi-intensive & intensive forming systems. Though the importance of probiotics in terrestrial animals is well recognized their relevance to aquatic organisms is still not clear (Gatesoupe, 1999) as the latter are quite different from land animals for whom the probiotic concept was developed. Live-bearing endotherms undergo embryonic development within an amnion, while the larvae of fish and shellfish are released into the external environment at a very early ontogenetic stage where they are exposed to all types of micro flora present in it. . The importance of microbial flora in the digestive process varies considerably and bacteria in the digestive tract can be a source of food, vitamins, or perhaps produce digestive enzyme (Ceccaldi, 1997).

The highest number of bacteria in the gut of shrimp were present when the shrimps are in zoea stage (Yasuda & Kitao, 1980). The numbers then decreased with age of shrimp from mysis to post-larval stage (1980) concluding that this could be due to the active consumption of bacterial cells from the medium by zoea, stage larvae. From mysis stage onward, the larval start to feed on larger organisms. This indeed is a pointer to the fact that probiotic organisms can be introduced into the larval stages of shrimp culture with beneficial effects.

Growth during larval stages of shrimp is assessed based upon survival and metamorphosis. Larvae maintained on 50% bacterial biomass, particularly *Bacillus* BTM 01 and BTM 05, showed superior development as compared to the Chaetoceros control on day nine. Even in animals, maintained exclusively on bacterial biomass, inspite of a relatively poor development compared to the control larvae fed on microalgae, the larvae survived well past the mysis stage and the duration of days for development was longer. Our results are in agreement with an earlier observation made for *P.monodon* larvae (Mohamed, 1996) fed with species of *Micrococcus* and *Pseudomonas* which gave better survival and metamorphic rates compared to larvae fed upon with the conventional diet of microalgae.

Though bacteria represent an important food source, due to it higher N and P contents, their small size (0.5 – 1.5µm length) may be a problem, since it is hardly retained by the feeding apparatus of the larvae. To be properly consumed, bacteria must form aggregates or be attached to particles (Conover, 1982). Moreover, not all bacterial macro consumers are produced by these microorganisms (Moriarty, 1990). Feeding *P.indicus* larvae with exclusive bacterial biomass attributed to poor survival (<20%) compared to partial supplementation along with Chaetoceros. The inability of bacterial biomass to support survival and growth when fed exclusively without any supplementation, can be attributed to certain inherent limiting dietary factors, as most bacteria lack polyunsaturated fatty acids (PUFAS), sterols and certain amino acids.

Maeda and Liao (1992) first isolated a strain "PM=4" (subsequently identified as *Thalassobacter utlis*) from the rearing water of larval *Penaeus monodon* for use as a biocontrol agent. This strain increased the survival rate fo the larvae of *P. monodon* and the swimming crab *Porturnus triberculatus* and repressed the growth of Vibrio anguillarum (Maeda et al., 1997); Nogami et al., 1997). Mohamed (1996) used several strains of heterotrophic bacteria as feed for P. monodon larvae and found that a strain of *Pseudomonas* increased the percentage survival and a strain of *Micrococcus* increased the metamorphic rate to PL-1 stage. Haryanti et al., (1998) reported the increased survival of *P. monodon* larvae on rearing with a strain "BY-9"which also inhibited the growth of *V.Harveyi.*

In Ecuadorian shrimp hatcheries Griffith (1995) reported the control of *Vibrio parahaemolyticus* associated outbreak of vibriosis through artificially increasing the proportion of *V. alginolyticus* in the rearing medium. Hatchery down time was reduced from approximately 7 days per month to less than 21 days annually, while production volumes increased by 35% and overall antibiotic use was decreased by 94% between 1991 and 1994. Further, Griffith (1995) found that the survival, production, feed conversion and growth rates in the farm were not negatively affected by the use probiotic fed larvae, on the contrary, they were even improved by their application.

In another recent study by Rengpipat et al. (1998) *Bacillus* S11 bacterium isolated from tiger shrimp habitats in Thailand was added to shrimp feed in three forms: fresh cells, fresh cells in normal saline and lyophilized form. After a 100-day feeding trial with probiotic supplemented and non-supplemented (control) feeds, *P. monodon* (from PL 30) exhibited significant difference ($p<0.05$) in growth, survival and external appearance between probiotic and control groups. There was no significant difference among the three treatment forms. After challenging the shrimps with a shrimp pathogen, *Vibrio harveyi*, by immersion for 10 days, all probiotic treatment groups has 100% survival, whereas the control group had only 26% survival. The main bacterial flora in control group shrimp guts was Vibrio spp., while those in all treatment groups were mostly *Bacillus* S11. This kind of bacterial species replacement was also observed in the rearing medium and faeces. However, whether the *Bacillus* S11 was able to colonise the gut even after stopping the probiotic feeding was not investigated.

The biochemical characteristics observed for the selected strains tested in the present study evidence the probable nutritive quality of the bacteria in terms of their protein content. Inspite of the nucleic acid content (0.025-0.028 mg/ml) of these bacteria they hold promise as nutritive diet for

the larvae. It may also be noted that these strains of bacteria were originally isolated from prawn culture pond where they might play a beneficial role, such as feed. Even at conditions of poor development and low level of survival no clinical symptoms of any disease was observed thus indicating that these bacteria are not pathogens. Our study thus clearly demonstrates that microorganisms can represent an important food source for *P. indicus* larvae. It was shown that the larvae could survive longer by feeding only on bacteria, although much better survival and growth were obtained when larger microorganisms (*Chaetoceros*) were included in the diet.

Conclusion

Though bacteria have higher conversion efficiencies, faster rates and higher protein contents as compared to yeasts which have advantages of lower nucleic acid content and ease of separation, very little is known on their utilization in penaeid larval nutrition. The present study demonstrates that a diet consisting of bacterial biomass can be successfully used in larviculture of shrimp, as a partial replacement for the conventional microalgae feed in complete live feed rations. However further studies on the exact mass transfer of biochemical constituents of bacteria in larvae during growth and development, their energetics and impact is warranted towards commercial application.

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REFERENCES

A.O.A.C 1990. Official methods of analysis of AOAC. Vol I, 15th edn., K.Helrich (ed). Association of Analytical Chemists, Inc., Arlington, VA 684pp

Aquacop 1977. Elevage larvaire de penaeides en milieu tropical groupe detravail Aquaculture ICES.10-13 mai, Brest, france. Acetes de Colloques du CNEXO 4: 179-191

Azam,F., J.WAmmerman, J.A Fuhram, A Hagstrom, 1984. Role of bacteria in polluted marine ecosystems. In: H.H White (ed). Concepts in marine pollution measurement, Univ. of Maryland. 431-441p

Cook, H.L and M.A. Murphy, 1966. Rearing penaeid shrimp from eggs to postlarvae. Proc.12th ann. Conf. S.E. ASS. Game & Fish Comm., 19:283 288.

Folch, J. , M.Less, GH. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226 : 497 - 509.

Hino A, 1993. present culture systems of the rotifer (*Brachionus plicatilis*) and the function of microorganisms. TML Conference Proceeding.3:51-59.

Intriago, P. and D.A. Jones, 1993. Bacteria a food for Artemia.Aquaculture,113: 115-127

Kreig, R.N. and G.J.Holt,1984. Bergys Manual of Systematic Bacteriology Vol. I, 8th edn., Williams and Wilkins Co., Baltimore.

Lowry, O. H., N.J. Rosedorough, A.L. Farr and A.I. Randall, 1951. Protein measurement with Folin Phenol reagent. J. Biol. Chem. 193:265-275.

Maeda, M.,1986. Microorganisms and protozoa as food in mariculture. Prog. Oceanogr. 21:201-206.

Miller G.L,1959. Use of dinitrosalicyclic acid reagent for determination of reducing Sugars.Anal. Chem. 31:426-428.

Mock, L.R., D.B.Revera and C.T.Fontaine, 1980. The larval culture of *Penaeus stylirostris* using modifications of the Galveston. Laboratory technique. Proc. World Maricul. Soc., 11:102-117

Mohamed, K.S., 1996. Heterotrophic marine bacteria as supplementary feed for larval *Penaeus monodon*. NAGA The Iclarm Quarterly. 17:23-26

Sorgeloos, P, and P.Leger 1992. Improved larviculture out puts of marine fish shrimp and prawn. J world Aquacul Soc., 23; 251-263

Tacon, A.G.J, 1993. Feed ingredients for warm water fish: fish meal and other processed feedstuffs. FAO Fisheries Circular No.856.Rome, FAO, 64p

Tanaka, M, 1975. Digestive channels of juveniles. In: Feeding and growth of juvenile fishes. Fisheries series 8, Kouseishs-Kouseikakau, Tokyo

Watanabe , Y.C. 1986. Digestion and absorption in fish larvae. In: Fish and Nutrition, Fisheries series 54, Kouseishs-Kouseikaku, Tokyo.

Yasuda, K. and N. Taga%1980. Culture of *Brachionis plicatilis* (Muller) using bacteria as food. Bull. Jap. Soc. Sci. Fish.46: 933-939.

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Effect of Different Levels of Rumen Degradable and Undegradable Proteins on Milk Production and Composition in Crossbred Cows

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ABSTRACT : A study was conducted to observe the effect of three levels of rumen degradable and undregradable proteins (RDP: UDP-70:30, 60:40 and 50:50) in isonitrogenous and isocaloric ration to observe the changes in quality and quantity of milk in medium producing (10-15L milk/d) crossbred cows. Fourteen cows within 40 days of calving were divided into three groups (5 each in groups I and II, and 4 in group III) based on similar milk production. Body weight changes were 21.01, 25.9 and 26.9 kg, in groups I, II and III respectively. Average DMI was similar with values of 11.24, 11.18 and 11.39 kg/d in groups I, II and III, respectively. Nutrient intake was similar in all the groups except RDP and UDP. RDP intake was reduced significantly ($P<0.05$) and UDP intake increased (150 to 200 percent) significantly ($P<0.01$) in groups II and III than in group I. Higher but not significant feed conversion ratio and gross protein utilization was observed in later two groups than in group I. Average daily milk yield (kg/d) was 13.14 in group II and 12.74 in group III, which was higher than in group I (10.88, $P<0.01$). However, No significant difference in milk production parameters was observed between group II and III. Fat, protein, total solid and solid not fat (SNF) percent were similar in three groups. It was concluded that the feeding of bypass protein (RDP and UDP ratio 60:40) is beneficial in increasing the milk yield without affecting composition of milk of medium producing crossbred cows.

Key words: RDP/ UDP level, Milk production, Crossbred cows.

INTRODUCTION

A number of technologies to increase milk production, experimental trials i.e. cross breeding, dietary manipulations and /or supplementations, health care, application of biotechnology etc, have been suggested. Protection of protein may be one of the most reliable, easier and economical propositions to the farmers. This requires less input and gives high return. Chaturvedi and Walli (2001) observed higher FCM yield on feeding higher levels of protected protein to the crossbred cows. Recently, Garg et al. (2005) reported that when the local cows, crossbred cows and buffaloes were fed bypass protein under field conditions, milk yield increased by 24, 5 and 12%, respectively. Ravikumar et al. (2006) reported that the use of high UDP in the diet of dairy cows increased milk production without affecting the feed intake, body weight changes or blood parameters. Mieso Guru et al. (2006) also observed that the inclusion of high UDP to the diet of crossbred goats improved milk yield, fat corrected milk without affecting composition of milk. In present experiment it was tried to evaluate the effect of adding higher levels of undegradable protein in the early lactation ration, on nutrient intake, milk yield and its composition in crossbred cows.

MATERIALS AND METHODS

Fourteen lactating crossbred cows (Karan Fries and Karan Swiss) in their early lactation were selected from NDRI, Karnal herd. The animals were quite healthy and their calves were weaned soon after birth. The animals were randomly divided into 3 groups (5 animals in groups I and II each and 4 animals in group III) based on milk yield. The groups

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were fed diets with RDP: UDP ratio of 70:30, 60:40 and 50:50 for group I, II and III, respectively. The composition of the concentrate mixture is detailed in Table 1. The mean CP and DM was 20.08 and 9.36 percent in maize fodder. Requirements of the animals were met as per NRC, 1989. Maize fodder was analyzed for DM and CP fortnightly and the average value is presented. Watering was done ad lib thrice a day and the study was conducted upto successful conception of the animals.

DM intake was recorded fortnightly with the DM of the feed offered and residue left consecutively for 2 days. The cows were weighed on every 15th day for consecutive 2 days in the morning before offering any feed or water. In the mid of the experimental period, a digestion trial was conducted for 6 d involving collection of feed offered, residues left and fecal samples which were pooled daily. The intake and digestibility of DM, CP, EE, CF, NFE and NDF were determined. Machine milking was practiced thrice a day (6.00 am, 12.00 noon and 6.00 pm). Daily milk yield (MY) was recorded for individual animals at each milking time by using a spring balance with the capacity to weigh upto 10 kg with an accuracy of 0.10 kg. The FCM (4% fat) was calculated on the basis of the formula given below.

4% FCM (kg) = 0.4 x MY (kg/d) + 15 x MY (kg/d) x fat (%)/ 100.

Milk samples from individual animals were collected and analyzed for milk composition at fortnightly interval throughout the experimental period. The samples collected from three milkings were pooled together which represented the milk of that animal on that particular day. Representative samples were drawn in a clean, dry plastic bottle and analyzed for fat, total protein, total solid, solid not fat as per BIS (1961). Statistical analysis was done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Nutrient intake

Average body weight and nutrient intake during the digestibility trial is shown in Table 2. The total DMI of the various groups were 10.23, 10.04 and 10.53 kg/ d whereas the DMI 100 kg body weight was 2.76, 2.81 and 2.79 kg and the DMI / kg W $^{0.75}$ was 117.4, 117.2 and 120.7g in groups I, II and III, respectively, and the differences between the groups were statistically non significant. The DM digestibility (%) was 61.27, 59.53 and 62.90 in groups I, II and III, respectively. OM digestibility was 61.24, 57.55, and 60.82 percent in the groups I, II and III, respectively. CP digestibility was 56.80, 62.60 and 57.59 percent, respectively in the groups I, II and III. Ether extract digestibility was 64.18 in group I, 68.36 in group II and 65.88 percent in the group III. Total carbohydrate digestibility was

Table 1. Ingredients (%) in the concentrate mixtures fed to different groups of milch cattle

Ingredients (%) RDP: UDP ratio	Group I (70:30)	Group II (60:40)	Group III (50:50)
Maize	33.00	28.05	26.45
Groundnut cake	21.00	17.40	12.60
Mustard cake	12.00	10.20	7.80
FA treated mustard cake	—	15.00	30.00
Wheat bran	20.00	17.00	13.00
Deoiled rice bran	11.00	9.35	7.15
Common Salt	1.00	1.00	1.00
Mineral mixture	2.00	2.00	2.00
Crude protein (%)	20.85	22.50	23.35
Total digestible nutrients (%)*	70.37	69.28	70.45
Rumen degradable protein (%)	68.50	60.50	52.10
Rumen undegradable protein (%)	31.50	39.50	47.90

* Calculated table values.

Table 2. Effect of various levels of RDP and UDP on nutrient intake during digestibility trial

Attributes	Group I	Group II	Group III	Pooled SE
Body weight (kg)	390	379	402	18.38
Metabolic bodyweight (kg)	87.6	85.9	89. 7	3.11
Dry matter intake				
Concentrate (kg/d)	4.20	4.00	3.90	0.12
Roughage (kg/d)	6.03	6.08	6.43	0.41
Total (kg/d)	10.23	10.04	10.53	0.41
DMI (kg/ 100kg BW)	2.76	2.81	2.79	0.10
DMI g/ kg $W^{0.75}$	117.4	117.0	120.7	3.91
Crude protein intake				
Concentrate(kg/d)	0.88	0.88	0.91	0.02
Roughage (kg/d)	0.64	0.65	0.69	0.04
Total (g /d)	1518	1548	1589	43.63
CPI (g/ 100 kg BW)	394	408	410	17.14
CPI (g/ kg $W^{0.75}$)	17.42	18.01	18.41	0.48
Total RDP intake (g /d)*	1063^{a}	929^{b}	824^{c}	21.62
Total UDP intake (g /d)**	455^{a}	619^{b}	824^{c}	17.26
TDN intake				
TDNI (kg/d)	7.34	7.37	7.88	0.25
TDNI(kg/ 100 kg BW)	1.90	1.94	1.97	0.07
TDNI (g/ kg $W^{0.75}$)	84.18	85.68	87.88	2.48
ME intake (Mcal/d)	26.41	26.52	28.35	1.44

Different superscripts in the same row indicate significant difference (*- $P<0.05$, ** - $P<0.01$

62.34, 60.16 and 65.59 per cent in the groups I, II and III, respectively. NDF digestibility was 60.09, 59.02 and 63.09 percent in the groups I, II and III, respectively. These observations were statistically similar for all the nutrients. Similar observations have been reported by Chaturvedi and Walli (2000) and Winsryg et al. (1991).

Total crude protein intake was 1518, 1548 and 1589 g/d in groups I, II and III, respectively. The CP intake (100 kg body weight) was 394, 408 and 410g, respectively in the groups I, II and III. Similarly, CP intake (g/ kg $W^{0.75}$) during the digestibility trial was 17.42, 18.01 and 18.41, respectively, in three groups. These observations were also similar statistically. RDP intake was 1062.71, 928.67 and 824.35 g/ d in groups I, II and III, respectively. These values were significant at 5% level. The group I animals were given high RDP in the ration (70% of CP) and had high intake (1062.71 g/d) of RDP and when it was reduced to 60% and 50% in the ration, the intake was decreased to 928.67 and 824.35 g/ d in groups II and III, respectively. Total UDP intake of groups I, II and III was 455.45, 619.11 and 824.35 g/ d, respectively. Addition of higher UDP in the ration increased the UDP intake (150 and 200 percent) significantly ($P<0.01$). TDN intake during the digestibility trial was 7.34, 7.37 and 7.88 kg/d. TDN intake / 100 kg body wt was 1.90, 1.94 and 1.97 kg/d. TDN intake (g/kg $W^{0.75}$) was 84.18, 85.68 and 87.88 in groups I, II and III, respectively. The observations were statistically similar between the groups. ME intake (Mcal/d) was also statistically similar between the groups and it was 26.41, 26.52 and 28.35 (Mcal/d) in groups I, II and III, respectively.

Table 3. Efficiency of nutrient utilization for various groups fed different levels of RDP and UDP in the ration

Attributes	Group I	Group II	Group III	Pooled SE
Feed conversion efficiency (DM consumed/ kg milk yield)	0.90	0.73	0.84	0.06
Gross energetic efficiency (%)	29.41	35.56	31.28	2.16
Gross protein utilization (%)	28.90	35.40	30.76	2.32
CP intake (g)/ kg milk yield	134.0	112.2	112.2	7.00
RDP intake (g)/ kg milk yield**	93.80 [b]	67.30[a]	64.0[a]	5.08
UDP intake (g) / kg milk yield**	40.20 [a]	44.90[a]	64.0 [b]	3.26
TDN intake (kg)/kg milk yield	0.65	0.55	0.53	0.03

Different superscripts in the same row indicate significant difference
(** - P<0.01)

Table 4. Effect of different levels of RDP and UDP on body weight changes, DMI, milk yield and its composition in crossbred cows

Attributes	Group I	Group II	Group III	Pooled SE
Body wt. gain (kg)	21.08	25.96	26.90	2.53
DMI (kg/d)	11.24	11.18	11.39	0.61
Milk yield (kg/d)*	10.88[a]	13.19[b]	12.74[b]	0.60
FCM yield (kg/d)**	10.45[a]	12.65 [b]	12.32[b]	0.57
Fat (%)	4.00	3.74	3.80	0.25
Protein (%)	3.86	3.89	3.89	0.15
Total solids (%)	12.20	11.88	12.35	0.69
SNF (%)	8.30	8.14	8.55	0.46

* P<0.01, ** P<0.05

Efficiency of nutrient utilization

Efficiency of nutrient utilization by various groups is presented in Table 3. Feed conversion efficiency (DM consumed/ kg milk yield) was best (0.73) in group II, followed by group III (0.84) and group I (0.90). Gross energetic efficiency (%) was highest (35.56) in group II, followed by group III (31.28) and group I (29.41). Gross protein utilization (%) of groups I, II and III was 28.90, 35.40 and 30.76, respectively. Higher UDP in the ration increased the efficiency of nutrient utilization for production purposes as the changes in body weight were similar in all the groups.

CP intake was 134, 112 and 112g/ kg of milk production in groups I, II and III respectively. Though the values were statistically similar but there was a trend (P=0.2) to spend less amount of CP in groups II and III for production of 1kg of milk. The RDP intake (g/ kg milk yield) was 93.8, 67.3 and 64.0 in groups I, II and III, respectively. The intake was similar in groups II and III, whereas in the group I it was significantly (P<0.01) higher. The UDP intake (g/ kg milk yield) was similar in groups I and II (40.20 and 44.90) but significantly (P<0.01) lower than that in group III. TDN intake (kg/kg milk production) was 0.65, 0.55 and 0.53 in groups I, II and III, respectively. The values were statistically

similar but in groups II and III production of 1 kg milk required less amount of energy.

Body weight changes and feed intake

Average body weight of the groups changed in a similar fashion and the increase was 21.08, 25.96 and 26.90 kg, respectively in groups I, II and III. The values were similar in all the groups. Daily dry matter intake in the groups I, II and III were 11.24, 11.18 and 11.39 kg/d in groups I, II and III, respectively. The present observations are in concurrence with the findings of Chaturvedi and Walli (2000). Robinson et al. (1991) also could not find any significant effect on DM intake per unit body weight when isonitrogenous diets varying RDP/UDP ratio were fed to the lactating cows from early to mid lactation.

Milk production and its composition

The average daily milk yield was 10.88, 13.19 and 12.74 kg in groups I, II and III, respectively ($P<0.01$). In groups II and III, with increasing UDP to RDP ratio, milk yield did not increase in comparison to group I. The milk production was increased by 17.51% and 14.60% in groups II and III, respectively. The FCM yield was 10.45, 12.65 and 12.32 kg/d in groups I, II and III, respectively. The values observed in the experiment were statistically significant between the groups ($P<0.05$). The average milk fat percent was 4.00, 3.74 and 3.80, in groups I, II and III, respectively. Mean milk protein was similar with 3.86, 3.89 and 3.89 percent, respectively in 3 groups and with the addition of higher UDP to the ration, no significant effect on protein percent was observed. Total solids percent in milk was also similar. The solid not fat (SNF) content of milk did not differ significantly between the groups due to addition of higher UDP to the ration. Chatterjee and Walli (2003) also reported similar observations on feeding FA treated mustard cakes to medium producing buffaloes with good economic return. Akbar et al. (1999) observed higher milk production in low producing buffaloes when fed with FA treated concentrates. Chaturvedi and Walli (2001) observed higher FCM yield with higher UDP level in the diet but without any effect on milk yield and SCM yield. Garg et al. (2002) observed that supplementation of protected sunflower meal increased milk yield, fat and protein per cent in the crossbred cows. Recently, Meiso Guru et al. (2006) reported that higher UDP in the ration of crossbred goats increased FCM yield. Ravi Kumar et al. (2006) reported similar observations in crossbred cows. Garg et al. (2005) reported that when farmers used bypass protein for feeding of lactating local cows, crossbred cows and buffaloes, 24, 5 and 12% higher milk yield was observed, respectively. Mishra and Rai (1996) did not observe any effect on milk composition with increasing UDP to the ration with low CP. Blauwiekel and Kinacid (1986) showed that the higher CP level with high soluble protein did not affect fat, protein or lactose per cent in milk. They observed that high CP group produced more milk ($P<0.05$). On alfalfa based diets, Dhiman et al. (1993), conducted a series of experiments and observed that infusion of casein had beneficial effect over infusion of propylene glycol in early lactating cows. They concluded that infusion of protein increased milk yield and protein in milk. They also concluded that the high CP content in alfalfa silage did not meet the requirement of undegradable protein supply and supply of fermentable carbohydrate will enhance the microbial protein production. Hristov et al. (2004) observed that high RDP diet fed to lactating cows was not used efficiently, rather it was lost through urine and it was decreased the efficiency of conversion of dietary N into milk protein. It was concluded that the level of UDP when increased from 30 to 40% and more, the milk production increased in medium producing crossbred cows without affecting its composition.

REFERENCES

Akbar, M.A., R.K. Kumar and N. Singh. 1999. Effect of feeding bypass protein with or without biopromotors on milk production and certain rumen and blood metabolites in lactating buffaloes. Indian J. Anim. Sci. 69: 967-71.

BIS. 1961. Bureau of Indian Standard. Method of test for dairy industry: Part 2: Chemical analysis of milk.

Blauwiekel, R., and R.L Kincaid. 1986. Effect of crude protein and solubility on performance and blood constituents of dairy cows. J. Dairy Sci. 69: 2091-98.

Chatterjee, A., and T.K. Walli. 2003. Effect of feeding formaldehyde treated mustard cake as bypass protein on milk yield, milk composition in Murrah buffaloes. Indian. J. Dairy Sci. 56: 299-306.

Chaturvedi, O.H. and T.K. Walli. 2000. Effect of feeding graded levels of by pass protein on nutrient utilization, nutrient partitioning and on performance of lactating crossbred cows. Indian J. Dairy Sci. 53: 1-10.

Chaturvedi, O.H. and T.K Walli. 2001. Effect of feeding graded levels of undegraded dietary protein on voluntary intake, milk production and economic return in early lactating crossbred cows. Asian Aust. J. Anim. Sci. 14: 1118-1124.

Dhiman, T. R., C. Cadorniga and L. D Satter. 1993. Protein and energy supplementation of high alfalfa silage diets during early lactation. J. Dairy Sci. 76: 1945-59.

Garg, M.R., P.L Sherasia. B. M. Bhanderi, S. K.Gulati, T.W. Scott, and P.S George. 2005. Economic evaluation of feeding bypass protein feed on milch animals in Vadodara District of Gujarat. Indian J. Dairy Sci. 58: 420-425.

Garg, M.R, P.L. Sherasia, B. M., Bhanderi S.K. Gulati and T.W Scott. 2002. Effect of feeding rumen protected nutrients on milk production in crossbred cows. Indian. J. Anim. Nutri. 19: 191-198.

Hristov, A.N, R.P. Etter, J.K. Ropp, and K.L Grandeen. 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J. Anim. Sci. 82: 3219-29.

Mieso-Guru, Shiv Prasad and M.L. Kamboj. 2006. Effect of energy and bypass protein supplimentation on production performance of lactating crossbred goats. Indian. J. Dairy Sci. 59: 92-94

Mishra, S. and S.N. Rai. 1996. Effect of different RDP and UDP ratios on voluntary intake, milk production and feed conversion efficiency in lactating goats. Small Ruminants Res. 20: 31-38.

NRC, 1989. National Research Council, Nutrient requirements of dairy cattle. 6th Nov. Ed. National Academic Sci., Washington, DC, USA.

Ravikumar, M., D.P. Tiwari, Anil Kumar, and N. Gupta. 2006. Effect of dietary undegradable protein level and plane of nutrition on milk yield and serum biochemical constituents in crossbred cows. Indian J. Anim. Sci. 76: 733-36.

Robinson, P.H., R. E. Mcqueen and P.L. Burgess. 1991. Influence of rumen degradable protein levels on feed intake and milk production of dairy cows. J. Dairy. Sci., 74: 1623-31.

Snedecor, G.W. and W.G. Cochran. 1994. Statistical methods. 8th ed. Oxford and IBH Publishing Company, Kolkata, India.

Winsryg, M.D., M.J. Arambel, and J.L. Walters. 1991. The effect of protein degradability on milk composition and production in somatotropin injected cows. J. Dairy. Sci. 74: 1648-53.

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Methane Emission as Affected by Dietary Supplementation of Raw and Roasted Fenugreek seeds in Cattle

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ABSTRACT : Eighteen crossbred steers (2.0- 2.5 years), divided in 3 groups of 6 each, were fed on a diet containing wheat straw and concentrate mixture in the ratio of 60: 40 as per their nutritional requirements. Diets of groups II and III were supplemented with 100 g raw and roasted ground fenugreek seeds (FS) powder, respectively. The dry matter intake and digestibility of the ration were similar among the three groups. Supplementation of raw as well as roasted FS powder, reduced ($P < 0.01$) the protozoal population in rumen liquor (14.58± 0.85 to 4.61± 0.76). However, TVFA concentration was increased ($P < 0.01$) in group III in comparison to groups I and II. Total anaerobic as well as proteolytic bacteria did not vary among the groups. Lower ($P > 0.05$) methane production (g/d) in group II (83.56 ± 11.73) and group III (98.21 ± 9.73) than that in group I (109.31 ± 10.59) revealed that supplementation of 2 % raw and roasted FS reduced methane emission without affecting the palatability as well as nutrient digestibility of ration due to the decrease in protozoal population in the rumen. However, roasting of FS did not reveal any additional advantage.

Key words: Methane emission, Fenugreek seeds, Protozoal population, Cattle

INTRODUCTION

For improving the palatability of crop residue based ration and also to mitigate methane emission from animals, several methods such as, improvement in the feed quality by providing concentrate, green fodder and also by using feed additives (Barman et al., 2001) were attempted with limited success. Ruminal protozoa harbor methanogens and facilitate hydrogen transfer for the production of methane (Stumm et al., 1982). Therefore, controlling the ruminal protozoal population through dietary means may be a good strategy for mitigating the methane emission. Hess et al. (2003) reported reduced methane emission following the dietary supplementation of saponins from naturally occurring resources. Fenugreek (*Trigonella foenum-graecum*) seeds, a herb being supplemented traditionally in the ration of lactating cows to enhance the voluntary intake and milk production (Tomar et al., 1996), have appreciable level of saponins (Dawidar et al., 1973). Roasting of FS enhances its flavour, hence roasted FS may be more effective in enhancing the palatability of crop residue based ration, however, effect of roasting on saponin content is not known. Present study was therefore, undertaken to investigate the effect of dietary supplementation of raw and roasted FS powder on voluntary feed intake, dry matter digestion and methane production in cattle.

MATERIALS AND METHODS

Eighteen crossbred steers (2 to 2½ years age, 240-300 kg b.wt.) were randomly divided into three groups of six animals each. The group I animals were fed on control diet of wheat straw and concentrate in the ratio of 60:40 as per their nutritional requirements (NRC, 2001). Animals in group II and III were fed on control diet plus 100 g raw and roasted fenugreek seed powder, respectively. Concentrate mixture (Maize-33, GNC-21, wheat bran-20, rice bran-11, mustard cake-12, common salt-1 and mineral mixture-2 parts) and the wheat straw in the ratio of 40:60 were fed at 9.00 A.M. individually. Fresh and clean water was

provided ad lib twice daily at 10.00 A.M. and 4.00 P.M. throughout the experimental period.

After adaptation of animals on experimental diets for 21 days, a digestibility trial of 7 days duration was conducted. Daily consumption of wheat straw and concentrate was recorded for individual animals. Samples of feeds were analyzed for proximate principles (AOAC, 1984) and NDF (Goering and Van Soast, 1970). Samples of feeds, residue left-over and faeces were analyzed for their DM content, during the metabolic trial period.

In vivo methane production from steers was measured using SF_6 (sulphur hexafluoride) tracer technique in their normal environment (Johnson et al., 1994). A small permeation tube (2"long with 3/16"hole to a depth of about 1"), which was filled with SF_6 gas and made to emit tracer gas (SF_6) at a constant and known rate, was placed in the rumen of each animal through their mouth. Before final collection of gas in a air evacuated canister, fitted on the neck of each animal, animals were trained to adjust with the fittings for 3 days to avoid the sudden change in the feed intake and to make them comfortable with attachments. The SF_6 and methane emitted were collected through a capillary tube ending just above the nostril of animal into the canister for 24 h and thereafter it was replaced daily with a new evacuated canister for 4 days. The ratio of CH_4 to SF_6 in the sample was determined using gas chromatograph (Nucon model 5500) fitted with flame ionization detector and a stainless column filled with Porapak N as a stationary phase for methane and electron capture detector with molecular sieve 5A column for SF_6 and using the standard CH_4 (35 ppm) and SF_6 (136.2 ppt) gases. The methane emission rate [Q (CH_4)] was calculated from the ratio of CH_4 and SF_6 in the collected gas and known release rate of SF_6 (Q SF_6). Background concentration level [(CH_4) b] was subtracted from methane concentration in the canister [(CH_4)y].

Calculation :

$$Q\ CH_4 = \frac{Q\ SF_6 \times Conc.\ [(CH_4)y - (CH_4)b]}{Conc.\ of\ SF_6}$$

After completing the methane emission study, rumen liquor samples were collected through mouth tube from each animal before feeding. In rumen liquor samples, concentration of TVFA (Barnett and Reid, 1957) , NH_3 -N (Conway, 1962) and protozoal counts (Langer et al., 1968) were estimated besides enumerating the live counts of total (Hungate, 1957) and proteolytic bacteria (Abou-Akkada and Blackburn, 1963). The data were analyzed statistically according to the method of Snedecor and Cochran (1986).

RESULTS AND DISCUSSION

Chemical composition of different feeds have been presented in Table1. Average body weights and DM intake through concentrate and wheat straw in different groups were similar (Table 2). The total DMI ranged from 5.06 ± 0.14 to 5.25 ± 0.16 in the three groups and the variation among the groups was not significant. Similarly, DMI per 100 kg BW and OMI/ 100 kg BW also did not differ significantly among the groups. Lu and Jorgensen (1987) and Valdez et al. (1985) also reported similar results following the dietary supplementation of

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

Parameters	Wheat straw	Concentrate Mixture	Raw fenugreek seeds	Roasted fenugreek seeds
OM	93.05	92.62	96.85	96.69
CP	3.55	18.51	22.73	22.35
EE	3.10	7.28	5.38	6.64
CF	38.16	8.98	9.56	9.74
Total ash	6.95	7.39	3.15	3.31
NFE	40.17	54.10	50.28	52.22
NDF	75.53	28.54	45.55	45.20

Table 2. DM and energy intake in different groups

Parameter	Group I	Group II	Group III
Body weight (kg)	271.16 ± 11.24	276.33 ± 8.42	279 ± 11.13
Dry Matter Intake (kg/d)			
Concentrate mixture	2.08 ± 0.05	2.03 ± 0.06	2.05 ± 0.06
Wheat straw	2.90 ± 0.08	2.86 ± 0.08	2.89 ± 0.09
Fenugreek seed	-	0.10	0.10
Total	5.06 ± 0.14	5.18 ± 0.14	5.25 ± 0.16
DMI/100 kg b.wt (kg)	1.83 ± 0.38	1.84 ± 0.33	1.85 ± 0.02
OMI (kg/d)	4.62 ± 0.13	4.71 ± 0.10	4.70 ± 0.15
GEI (M cal/kg)	21.17± 0.12	21.27 ± 0.1	21.49 ± 0.12
DEI (M cal/kg)	12.66 ± 0.11	12.83 ± 0.08	12.95 ± 0.12
MEI (M cal/kg)	10.28 ± 0.07	10.41 ± 0.08	10.51 ± 0.12
DM Digestibility (%)	65.11 ± 0.79	65.68 ± 1.24	65.41 ± 1.38

saponin and sarsaponin, respectively. The results showed that neither saponin nor flavour of fenugreek seed powder, irrespective of its processing, affected voluntary feed intake in crossbred steers. Similar to these results, Sahin et al. (2003) also did not observe any effect on feed intake and its digestibility following the dietary supplementation of various levels of fenugreek seeds in the ration of lambs.

Rumen fermentation pattern

The mean values of rumen fermentation parameters are given in Table 3. The NH_3-N (mg/100 ml) was higher ($P < 0.05$) in control group as compared to group II and III, in spite of the supplementation of additional CP @ 2.2 g/day, respectively. However, variation between groups II and III was not significant. Similar reduction was recorded earlier following the supplementation of Yucca saponin (Grobner et al., 1982) and sarsaponin (Hristov et al., 1999). From in vitro studies, Lila et al. (2003) reported decreased ammonia nitrogen concentration following the supplementation of 1.2 to 3.2 g/l saponin in the diet. Headon et al. (1991) explained that glyco-component of Yucca extract binds with ammonia and saponin fraction may affect ammonia concentration indirectly via their toxicity to rumen ciliate protozoa or the inhibitory action of sarsaponin on urease activity (Hussain and Cheeke, 1995).

Mean values for TVFA concentration (meq/100 ml) increased linearly ($P<0.01$) with the supplementation of fenugreek seed powder particularly with roasted ones (Table 3). Klita et al. (1996) also reported that TVFA concentration improved by supplementation of alfalfa root saponins. The increase in TVFA concentration in rumen liquor by saponin may be attributed to increased microbial

Table 3. Effect of raw and roasted fenugreek seed powder supplementation on rumen fermentation

Parameters	Group I	Group II	Group III
TVFA **(meq/100 ml)	7.03^a ± 0.13	8.65^a ± 0.38	12.30^a ± 0.62
NH_3N* (mg/100ml)	14.53^a ± 0.60	11.55^b ± 0.20	11.90^b ± 0.00
Protozoa **(× 10^4 /ml)	14.58^a ± 0.85	8.42^b ± 0.60	4.61^c ± 0.76
Total anaerobic bacteria (× 10^9/ml)	25.33 ± 2.17	35.00 ± 4.04	27.83 ± 4.97
Proteolytic bacteria (×10^5/ml)	10.33 ± 0.33	12.53 ± 0.73	16.33 ± 3.84

Figures bearing different superscripts in a row differ significantly.
* $P < 0.05$, ** $P < 0.01$

population due to defaunating action of saponin. However, the protozoal population decreased ($P < 0.01$) due to the supplementation of raw fenugreek seed powder, which was further decreased when roasted fenugreek seeds were supplemented (Table 3). A negative relationship between protozoal count and dietary saponin levels was recorded by Lu and Jorgensen (1987) on both roughage as well as concentrate based diets as a result of precipitation of saponin and sterol in cell membranes of protozoa. Similar reduction in protozoal counts in rumen fluid also reported on dietary inclusion of saponin from Yucca (Valdez et al., 1985; Hristov et al., 1999), *Quillaja saponin* (Makkar and Becker, 1997) and from saponin rich plants or fruits (Navas-Camacho et al., 1993; Klita et al., 1996; Hess et al., 2003). These results revealed that roasting of FS did not affect their saponin content, rather enhanced its efficacy in controlling the protozoal population.

Total anaerobic bacterial number (x 10^9/ml) was higher in group II (35.07 ± 4.04) than in group I (25.33 ± 2.17), though the difference was not significant. Similarly, population of proteolytic bacteria (x 10^5/ml) did not differ among the groups. However, there was improvement in proteolytic bacterial numbers, which was more pronounced in group III. Saponin influenced both rumen bacterial species composition and number through inhibition or a selective enhancement of growth of individual species. An increase of rumen fluid bacterial counts by sarsaponin was also recorded (in vitro) by Valdez et al. (1985). The reason for increase was attributed to the reduction in the number of protozoa (Klita et al., 1996).

Methane production

Average methane production (g/day) was 23% lower in group II (83.56 ± 11.73) and 10% lower in group III (98.21 ± 9.73) than in group I (109.31 ± 10.59), however, variation among the groups was not significant (Table 4). Almost similar results were seen when the values were expressed as per kg DM and DDM intake which may be because neither the energy intake nor digestibility of the feeds varied significantly among the groups.

The energy loss through methane expressed as percentage of GE, DE and ME also indicated same trend as that of total methane production. The loss was less in case of raw fenugreek supplemented group. The percentage of GE lost as methane was 6.64 ± 0.84, 4.90 ± 0.77 and 6.04 ± 0.47 in groups I, II and III, respectively. The methane energy loss as percent of GEI in raw and roasted fenugreek group was less by 26.20 and 9.0% respectively. Methane energy as % of DE and ME ranged from 10.57 to 7.88 and 12.42 to 9.28 respectively, indicating a decrease in experimental groups.

Hess et al. (2003) found that saponin rich fruits *Sapindus saponania*, *Enterolobium cyclocarpum* and *Pithecellobium saman* decreased methane production in vitro. The decrease in methane production was

Table 4. Effect of differently processed fenugreek seed powder supplementation on methane emission (g/d) and its relationship with nutrients intake

Parameters	Group I	Group II	Group III
CH_4 production (g/d)	109.31 ± 10.59	83.56 ± 11.73	98.21 ± 9.73
CH_4 Energy (Mcal/d)	1458.24 ± 41.31	1114.69± 56.52	1310.08± 63.05
CH_4 emission g per kg intake			
DM	21.19 ± 3.09	15.67 ± 2.44	18.89 ± 1.46
OM	23.78 ± 2.91	17.04 ± 2.06	19.67 ± 1.29
DDM	32.29 ± 5.16	23.46 ± 4.06	28.327 ± 2.38
CH_4 energy loss as percentage of			
GE	6.64 ± 0.64	4.9 ± 0.77	6.04 ± 0.47
DE	10.57 ± 1.34	7.88 ± 1.22	9.54 ± 0.73
ME	12.42 ± 1.57	9.28 ± 1.44	11.23 ± 0.86

Methane energy = methane g X 13.34 K cal (Ranjhan, S.K. 1986)

supported by the decrease in protozoal count (Ushida et al., 1997), increase in TVFA concentration and increase in total anaerobic bacteria and change in type of microbes (Barman et al., 2001). It might be due to the inhibition of hydrogen producing cellulolytic bacteria (Wang et al., 2000) having association with protozoa (Stumm and Zwart, 1986) or other bacteria that use pyruvate ferredoxin oxidoreductase to metabolise pyruvate to acetyl CoA (Lila et al., 2003). Hence, the difference in the treatments is more due to alterations at rumen microbial level, so detail studies are required to get the information about actual mode of action of fenugreek seeds.

The results of this study indicated a positive effect of fenugreek seeds supplementation at 2% of total diet on rumen fermentation by decreasing ammonia level and protozoal counts in rumen thereby reduced methane emission, which increased energy availability in the form of TVFAs in the crossbred steers, however, roasting of fenugreek seeds did not yield any additional benefit.

REFERENCES

Abou-Akkada, A.R., and T.H. Blackburn. 1963. Medium for proteolytic rumen bacteria. Methods in Microbiol. 3B: 40-141.

AOAC. 1984. Official Methods of Analysis (14th Edn.) Association of official analytical Chemists. Washington, D.C.

Barman, K., Madhu Mohini, and K.K. Singhal. 2001. Effect of supplementation of rumensin and level of roughage on methane production. Indian J. Anim. Nutr. 18: 325-329.

Barnett, A.J.G., and R.L. Reid. 1957. Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen.1. The volatile fatty acid production from fresh grasses. J. Agri. Sci. Camb. 48: 315.

Conway, E.J. 1962. Microdiffusion Analysis and Volumetric Error, 5th edn. Crossby Lockwood & Sons Ltd., London.

Dawidar, A.M., A.A. Saleh, and S.L. Elmotei. 1973. Steroid sapogenin constituents of fenugreek seeds. Planta Medica. 24: 367-370.

Goering, H.K., and P.J. Van Soest.1970. Forage Fibre Analysis (Apparatus, Reagents, procedures and some applications) ARS US Deptt. Handbook No. 379, Washington, D.C.

Grobner, M.A., D.E. Johnson, S.R. Goodall, and D.A. Benz. 1982. Sarsaponin effects on in vitro continuous flow fermentation of a high grain diet. In Proc. West. Sect. Am. Soc. Anim. Sci. 33: 64-65.

Headon, D.R., K. Buggle, A. Nelson, and G. Killeen. 1991. Glycofractions of the Yucca plant and their role in ammonia control. Biotechnology in the Feed Industry. T.P. Lyons (ed.), Alltech Inc, Nicholas Ville, KY.p.95-108.

Hess, H.D., M..Kreuzer, T.E. Diaz, T.E. Lascano, J.E. Carulla, C.R. Soliva, A. Machmuller. 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. Anim. Feed Sci. & Technol. 109: 79-94.

Hristov, A.N., T.A. McAllister, F.H.V. Herk, K.J. Cheng, C.J. Newbold, and P.R. Cheeke. 1999. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. J. Anim. Sci. 77: 2554-2563.

Hungate, R.E. 1957. Microorganisms in rumen of cattle fed on constant ration. Can.J. Microbiol. 3: 289.

Hussain, I., and P.R Cheeke. 1995. Effect of dietary *Yucca Schidigera* extract on rumen and blood profiles of steers fed on concentrate or roughage based diets. Anim. Feed Sci. and Technol. 51:231-242.

Johnson, K.A., M.T. Huyler, H.H. Westberg, B.K. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using SF_6 tracer technique. Environ. Sci. and Technol. 28:359.

Klita, P.T., G. Mathison, T.W. Fenton, and R.T. Hardin. 1996. Effect of alfalfa root saponins on digestive function in sheep. J. Anim. Sci. 74: 1144-1156.

Langer, P.N., G.S. Sidhu, and I.S. Bhatia. 1968. A study of microbial population in the rumen of buffalo (*Bos bubalis*) and Zebu (*Bos indicus*) on a feeding regime deficient in carbohydrate. Indian J. Vet. Sci. & Anim. Husb. 38: 333.

Lila, Z.A., N.Mohammed, S. Kanda, T. Kamada, and H. Itabashi. 2003. Effect of sarasaponin on Ruminal Fermentation with particular reference to methane production in vitro. J. Dairy Sci. 86: 3330-3336.

Lu, C.D., and N.A. Jorgensen.1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. J. Nutr. 117: 919-927.

Makkar, H.P.S., and Becker. 1997. Degradation of saponin by mixed cultures of rumen microbes. Lett. Appl. Microbiol. 25: 243-245.

Navas-Camacho, A., M.A. Laredo, A. Cuesta, H. Anzola, and J.C. Leon. 1993. Effect of supplementation with a tree legume forage on rumen function. Livestock Res. Rural Dev. 5: 59-73.

NRC. 2001. Nutrient requirements of beef cattle (7th Revised Edn.), National Academy Press, Washington, D.C.

Sahin, A., M. Keskin, and O. Bicer. 2003. Responses of lambs to the dietary inclusion of *Trigonella foenum graecum* L. J. Anim. Vet. Adv. 2: 74-75.

Snedecor, G.W., and W.G. Cochran. 1986. Statistical methods. Oxford and IBH Publishing Co., Calcutta, India.

Stumm, C.K., and K.B. Zwart. 1986. Symbiosis of protozoa with hydrogen utilizing methanogens. Microbiol. Sci. 3: 100-105.

Tomar, K.S., V.P. Singh, and R.S. Yadav. 1996. Effect of feeding Maithy (*Trigonella foenum graecum* L.) and Chandrasoor (*Lepidium sativum* L.) seeds on milk and blood constituents of Murrah buffaloes. Indian J. Anim. Sci. 66: 1192-1193.

Ushida, K., M. Tokura, A. Takenaka, and H.Itabashi. 1997. Ciliate protozoa and ruminal methanogenesis. In: Rumen Microbes and Digestive Physiology in Ruminants. R. Onodera, H. Itabashi, K. Ushida, H. Yano and Y. Sasaki (eds.). Japan Scientific Societies Press, Tokyo and S. Karger AG Basel, Switzerland, pp. 209-220.

Valdez, F.R., L.J, Bush, A.L Goetsch, and F.N. Owens. 1985. Effects of steroidal sapogeninsan Ruminal fermentation and on production of lactating Dairy cows. J. Dairy Sci. 69: 1568-1575.

Wang, Y., T.A. Mc Allister, L.S. Yanke and P.R. Cheeke, 2000. Effect of steroidal saponin from *yucca schidigera* extract on ruminal microbes. J. Appl. Microbiol. 88 : 887-896.

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Effect of Different Feed Ingredients on the Hardness and Chemical Composition of Lick Blocks

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ABSTRACT : The feed ingredients viz. oil seeds: til seed (*Sesamum indicum*), sunflower seed (*Helianthus annus*), lin seed (*Linum usitatissimum*), mustard seed (*Brassica juncea*) and cotton seed (*Gossypium spp*); oil seed cakes/meals: cotton seed cake, groundnut cake (*Arachis hypogaea*), mustard cake, deoiled groundnut cake and soyabean meal (*Glycine max*); cereals: barley (*Hordeum vulgare*), oat (*Avena sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), bajra (*Pennisetum typhoides*) and jowar (*Sorghum vulgare*); cereal byproducts: wheat bran, rice (*Oryza sativa*) polish and deoiled rice bran, and pulses: bakla (*Vicia sativa*), rice bean (*Vigna umbellata*), gram (*Cicer arietinum*), methi (*Trigonella faenumgraecum*), masoor (*Lens culinaris*), urd (*Vigna mungo*), moong (*Vigna radiata*) and guar (*Cyamopsis tetragonaloba*) were ground through a hammer mill fitted with 4mm sieve.The lick blocks were prepared by using the cold process. The level (%) of molasses (40), urea (12), calcium oxide(CaO) (6), mineral mixture (6), phosphoric acid (1) and each feed ingredient (35) were kept constant. The hardness of blocks was measured by using a penetrometer. The hardness of the blocks was significantly ($P<0.05$) higher with cereal byproducts followed by oilseed cakes, pulses, cereals and oil seed. The hardness of the blocks was maximum (16.37 kg/cm^2) with wheat bran but the problem of cracks in the blocks was observed. The CP content of cakes/meal based blocks was significantly ($P<0.05$) higher than other categories of feed ingredients and lowest in cereal based blocks. There was a negative correlation between the hardness and fat content of blocks. The hardness of the blocks was minimum (0.71 kg/cm^2) with oil seeds based blocks. It was concluded that the blocks of desired hardness can be formulated by altering the ingredients composition.

Key words: Feed ingredients, Hardness, Urea Molasses Mineral Blocks

INTRODUCTION

Roughages require adequate energy, protein and mineral supplementation in order to be degraded efficiently by micro flora in the rumen. Since straws and other crop residues are short in nitrogen and available energy, their improvement as animal feed can be achieved through treatments and supplementation of nutrients at the time of feeding. The improvement in the digestibility and intake of crop residue has been

achieved by supplementary feeding with urea-molasses mineral (Mudgal et al., 1981). Intake of urea through licking has several advantages over other methods of feeding urea to the ruminants (Beams, 1963). Feeding of urea molasses mineral blocks prepared by cold process and containing different energy and protein sources encouraged optimal fermentation and microbial protein synthesis in the rumen of buffaloes maintained solely on wheat straw based diet (Sihag et al., 2003). There is a direct relationship between the hardness of lick blocks and their intake by the animal. Hardness is affected by many factors like molasses level, binder level, binder type, urea level and phosphoric acid level (Sihag et al., 1994). Based on this line of action, lick blocks were developed by using cold process (Sihag and Chahal, 1996) keeping the levels of i.e. molasses, calcium oxide, urea, mineral mixture and phosphoric acid constant to study the effect of

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different feed ingredients on the hardness of lick blocks.

MATERIALS AND METHOD

Twenty nine feed ingredients were divided into 5 categories i.e. oil seeds namely til (*Sesamum indicum*), sunflower(*Helianthus annus*), linseed(*Linum usitatissimum*), mustard(*Brassica juncea*) and cotton seed(*Gossypium spp*), oil seed cake (cotton seed cake, ground nut cake(*Arachis hypogaea*), mustard cake, deoiled ground nut cake and soya bean meal(*Glycine max*), cereals: barley(*Hordeum vulgare*), oat(*Avena sativa*), wheat(*Triticum aestivum*), maize(*Zea mays*), bajra (*Pennisetum typhoides*) and jowar(*Sorghum vulgare*), cereal byproducts: wheat bran, rice(*Oryza sativa*) polish and deoiled rice bran and pulses: bakla(*Vicia sativa*), rice bean(*Vigna unbellata*), gram(*Cicer arietinun*), methi(*Trigonella faenumgraecum*), masoor(*Lens culinaris*), urd(*Vigna mungo*), moong(*Vigna radiate)* and guar(*Cyamopsis tetragona*) and ground through a hammer mill having 4mm sieve size. Urea-molasses-mineral block licks were prepared using molasses as a base material, urea as NPN source, CaO as binder, phosphoric acid as a catalyst and mineral mixture as mineral supplement. The level (%) of molasses (40), urea (12), CaO (6), mineral mixture (6) and phosphoric acid (1) were kept constant. The blocks were prepared in triplicate as per the method used by Sihag and Chahal (1996) by using 35 parts of each ingredient in each type of block. Hardness of the blocks was measured by using a penetrometer where force in kg required for the penetration of 2.54 cm of metallic probe having 7.6 cm length, 2.9 cm diameter and cross-sectional area of 3.89 cm^2 was recorded. The samples were analysed for proximate principles (AOAC, 1995). The data were analysed statistically (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Data pertaining to the effects of different feed ingredients on hardness of feed blocks, moisture content, crude protein (CP), crude fibre (CF) and crude fat (EE) are given in Table 1. The hardness of blocks was significantly ($P<0.05$) higher with cereal byproducts (9.31kg/cm^2), followed by oilseed cakes/meals (4.78kg/cm^2), pulse seed (3.01kg/cm^2) cereals (2.85kg/cm^2) and lowest with oil seed (0.71kg/cm^2). Among oil seeds, the hardness was significantly ($P<0.05$) higher with cotton seed (0.98kg/cm^2) and lowest with sunflower seed (0.46kg/cm^2). Among oil seed cakes / meals the hardness was maximum ($P<0.05$) with soyabean meal (9.69kg/cm^2) followed by cotton seed cake (6.48kg/cm^2) and being lowest with sunflower seed cake (0.98kg/cm^2). Among cereals, the hardness was significantly ($P<0.05$) higher with oats (4.82kg/cm^2) and barley (4.60kg/cm^2) compared to other feed ingredients being lowest with Jowar (0.98kg/cm^2), however the difference between barley and oat was non-significant. The hardness of blocks was significantly ($P<0.05$) higher with wheat bran (16.73kg/cm^2) followed by deoiled rice bran (7.50kg/cm^2) and rice polish (3.69kg/cm^2), however the blocks prepared with wheat bran were of poor texture and there was problem of cracking in the blocks. The hardness of blocks with rice polish was significantly ($P<0.05$) lower compared to deoiled rice bran and wheat bran, because of high fat content in the rice polish. Among pulses, the hardness was maximum ($P<0.05$) with bakla followed by moong (4.60kg/cm^2) and lowest with masoor (1.44kg/cm^2).

Average moisture content of oil seed and oilseed cakes/meals based blocks was significantly ($P<0.05$) higher than the pulse seeds, cereals and cereal byproduct based blocks. The average moisture content of cereal byproducts based blocks was significantly ($P<0.05$) lower than other categories of feed ingredients. This might be one of the reasons for significantly ($P<0.05$) higher hardness of cereal byproducts based lick blocks. The findings are in line with the results of Sihag and Chahal (1997), where they reported a negative correlation between hardness of blocks and their moisture content. The effect of different feed ingredients on ultimate CF, CP and EE content of blocks was significant ($P<0.05$). This might be due to different CF, CP and EE content of individual grain/ingredient and different binding capacities. The CP content of oil seed cake/meals based blocks was highest ($P<0.05$) followed by pulses, oilseeds and cereal byproducts based feed blocks, being lowest in cereal based blocks. There was –ve correlation between hardness of blocks and moisture level, and hardness of blocks and ether extract content (Table 2). However, there was a +ve correlation between hardness of the blocks and crude fibre and crude protein content. It was concluded that the hardness of the lick blocks depends upon the feed ingredients used in the manufacturing of urea molasses mineral blocks. The hardness of the blocks was also affected by the CP, CF, EE and moisture content of blocks also.

Table1. Effect of feed ingredients on hardness and proximate principles of lick blocks

Ingredients	Hardness (kg/cm^2)	Moisture %	CF %	CP %	EE %
Oil Seeds					
Til seed	0.61±0.08	13.46±0.74	3.40±0.08	40.37±0.46	17.43±0.32
Cotton seed	0.98±0.04	13.17±0.19	7.79±0.09	43.31±0.22	7.52±0.13
Lin seed	0.91±0.08	13.43±0.29	3.98±0.09	41.62±0.29	9.16±0.15
Mustard seed	0.61±0.08	13.71±0.15	4.10±0.06	39.19±0.19	12.33±0.36
Sun flower seed	0.46±0.08	12.78±0.20	7.42±0.21	40.06±0.09	10.63±0.30
Average	**0.71±0.09**	**13.31±0.14**	**5.34±0.84**	**40.91±0.64**	**11.41±1.52**
		Oil seed cakes			
Cotton seed cake	6.48±0.30	11.61±0.27	11.54±0.14	42.06±0.10	5.24±0.21
Ground nut cake	2.94±0.15	12.94±0.22	3.40±0.10	49.25±0.39	3.87±0.24
Mustard cake	5.65±0.08	13.36±0.11	5.87±0.24	46.19±0.21	2.48±0.25
Deoiled mustard cake	5.42±0.00	13.71±0.17	5.32±0.14	47.19±0.19	0.62±0.09
Deoiled groundnut cake	2.30±0.04	13.11±0.15	4.84±0.08	48.44±0.17	0.25±0.02
Sunflower seed cake	0.98±0.00	13.77±0.20	8.17±0.06	43.62±0.30	5.39±0.28
Soya bean meal	9.69±0.12	13.80±0.12	2.08±0.04	46.75±0.18	0.40±0.02
Average	**4.78±1.03**	**13.18±0.27**	**5.88±1.10**	**46.21±0.90**	**2.61±0.79**
		Cereals			
Barley	4.60±0.23	12.72±0.12	4.12±0.12	39.12±0.42	2.84±0.17
Oats	4.82±0.00	12.75±0.17	5.52±0.13	35.12±0.45	3.76±0.13
Wheat	2.87±0.23	11.27±0.23	1.68±0.08	37.50±0.28	2.48±0.12
Maize	2.19±0.08	11.73±0.21	1.84±0.04	38.19±0.18	3.37±0.14
Bajra	1.66±0.08	12.38±0.18	1.74±0.07	38.37±0.33	2.74±0.15
Jowar	0.98±0.00	13.78±0.13	3.17±0.12	38.56±0.37	1.62±0.07
Average	**2.85±0.58**	**12.43±0.37**	**3.01±0.58**	**37.81±0.53**	**2.80±0.28**
		Cereal by-products			
Wheat bran	16.73±0.00	10.65±0.21	4.94±0.20	40.87±0.21	2.71±0.18
Rice polish	3.69±0.15	12.66±0.22	3.08±0.08	40.81±0.45	5.89±0.14
Deoiled rice bran	7.50±0.19	11.37±0.20	6.43±0.24	38.25±0.86	1.55±0.36
Average	**9.31±3.16**	**11.56±0.48**	**4.01±0.53**	**39.98±0.71**	**3.38±0.62**
		Pulses			
Bakla	5.69±0.11	11.59±0.06	5.93±0.18	42.06±0.20	3.69±0.17
Rice bean	2.60±0.11	10.73±0.27	3.02±0.02	42.12±0.16	3.06±0.07
Gram	3.55±0.08	13.28±0.28	4.57±0.08	40.06±0.18	3.16±0.15
Methi	1.56±0.05	10.07±0.08	3.33±0.15	46.94±0.39	1.87±0.03
Masoor	1.81±0.15	12.35±0.21	3.20±0.05	39.81±0.30	0.76±0.04
Urd	1.44±0.08	13.93±0.17	3.76±0.17	43.94±1.03	4.29±0.18
Moong	2.79±0.15	13.03±0.03	2.91±0.10	46.31±0.43	0.62±0.62
Guar	4.60±0.08	13.52±0.16	4.57±0.07	42.00±0.50	0.84±0.05
Average	**3.01±0.50**	**12.31±0.46**	**3.91±0.35**	**42.91±0.87**	**2.29±0.48**
CD (P<0.05)	**0.35**	**0.67**	**0.37**	**1.12**	**0.54**

Table 2. Correlation of hardness with moisture content, crude fibre, crude protein, ether extract of lick blocks

Attributes	Correlation coefficiant
Hardness/moisture content	-.328
Hardness/crude fiber content	+.132
Hardness/crude protein content	+.046
Hardness/ether extracts content	-.331

REFERENCES

AOAC. 1995. Official Methods of Analysis. Association of Official Analytical Chemists, (16th Ed.) Washington, D.C.

Beams, R.M. 1963. Provision of cattle in a salt/urea/ molasses block. Queensland J. Agric. Anim. Sci. 20: 213-217.

Mudgal, VD., R.R. Khajuria, and K.K. Singhal. 1981. Effect of different levels of urea molasses supplement on nutrient utilization in cows and buffaloes. Indian J. Dairy Sci. 34 :406-409.

Sihag, Z.S., and S.M. Chahal. 1996. Effect of different ingredients on the hardness of urea molasses block licks. Indian J. Anim. Sci. 66 : 1149-1153.

Sihag, Z.S., and S.M. Chahal. 1997. Effect of storage on the stability of urea molasses mineral block (UMMB) licks. Indian J. Anim. Nutr. 14: 31-35.

Sihag, Z.S., S.M. Chahal, and B.S. Punia. 2003. Influence of urea molasses mineral blocks (UMMB) containing different energy and protein sources on nitrogenous metabolites in the rumen of buffaloes. Indian J. Anim. Nutr. 20: 124-130.

Sihag, Z.S., Nand Kishore, S.M. Chahal, and C.S. Rathee. 1994. Effect of different additives on hardness of urea molasses mineral lick blocks. Indian J. Anim. Sci. 64: 198-201.

Snedecor, G.W., and W.G. Cochran. 1994. Statistical Methods. (8th Ed.) The Lowa State University Press, Ames, Lowa, U.S.A.

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Tephrosia purpurea (Sarphonk), a Nutritionally Potential Plant in Grazing Land of Semi Arid Region

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ABSTRACT : The study was conducted to evaluate the effect of season, on chemical composition of *Tephrosia purpurea* and its nutritive value for sheep. *Tephrosia purpurea* was collected from a protected grazing field in March, June, September and December months of the same year. Maximum CP (%) was found in September (19.29) and lowest (11.95)in December. The lignin content ranged from 5.76 to 9.81 % during various seasons of the year. During rainy season, lowest dry matter content (28.25 %) was observed. The plant material was also analysed for Fe, Cu, Mn , Zn and Mg contents which were present in more than critical limits. The DCP and TDN contents on DM basis were 13.33 and 50.95 %, respectively.

Key words: *Tephrosia purpurea,* Nutritive value, Sheep

Semi arid region of India has very large population of small ruminants especially goat and sheep. These animals mainly depend upon grazing and browsing. Grazing land are often barren, unutilized and problematic lands, and solely depend on rain for their moisture content. *Tephrosia purpurea* belong to leguminous family (Sastry and Kavathokar, 1998), perennial in nature and can thrive well in poor eroded sandy soils of semi-arid regions of the Yamuna ravines even in very low rain fall. *Tephrosia purpurea* is also known as wild indigo, fish poison in English and Sarphonk and sharpunkha in Hindi is a much branched erect, perennial herb (Oudhia, 2004) and very well consumed by the small ruminants. It not only enhances the biomass production but also increases quality of the pasture. The present study was conducted to evaluate the feeding value of this plant in different seasons.

Plant samples of *Tephrosia purpurea* were collected from the protected grazing fields in spring, summer, rainy and winter seasons and were chemically analysed after drying (70±5^{0}C) and grinding. Soil of experimental field from which plant material collected was loamy sand in texture and deficient in organic carbon, nitrogen and phosphorus with low water retention capacity. Soil analysis was done as per methods suggested by Singh et al. (1999). For nutritive evaluation, six healthy dry female sheep of Muzaffarnagari breed weighing 28.61±1.3 kg were selected for experimentation and they were offered *Tephrosia purpurea* chaffed fodder ad lib with out any supplementation in September. Preliminary feeding was done for 22 days followed by a metabolism trial of 5 days collection period. During the metabolic trial clean drinking water was offered thrice a day. Proximate composition, NDF, ADF, lignin were analysed as per AOAC (1990). Micro- nutrients were analysed using atomic absorption spectrophotometer.

Maximum biomass production was observed during rainy season both in terms of fresh weight (2.69±1.01 t/ha) as well as dry weight (0.76±0.27 t/ha). There was 10 times increase in fresh biomass production and 4.8 times increase in dry matter production per hectare in rainy season over spring season (Table 1).The proximate composition observed in the present experiment is quite comparable to that of leguminous hays (Rama Prasad et al., 1999). Fe, Cu, Mn, Zn and Mg contents in *Tephrosia purpurea* were higher than critical limit of these elements in forages of semi arid regions (Chaudhary, 2006)

Table 1. Composition of *Tephrosia purpurea* during different months

Parameters	Months of various seasons			
	March	June	September	December
Fresh weight (t/ha)	0.27 ± 0.06	0.75 ± 0.14	2.69 ± 1.01	1.12 ± 0.32
Dry weight (t/ha)	0.16 ± 0.015	0.44 ± 0.15	0.76 ± 0.27	0.53 ± 0.14
Dry matter (%)	59.25 ± 5.50	58.66 ± 10.41	28.25 ± 1.32	44.75 ± 1.70
Chemical composition (%) on DM basis				
Crude protein	12.95 ± 0.85	13.45 ± 0.65	19.29 ± 1.05	11.95±0.38
Ether extract	3.62 ± 0.48	4.49 ± 0.49	3.45 ± 0.35	3.82 ± 0.56
Ash	5.51 ± 1.11	9.65 ± 1.1	6.22 ± 0.15	6.60 ± 0.50
Neutral detergent fiber	64.31 ± 1.98	49.72 ± 1.58	62.97 ± 1.66	64.05 ± 0.13
Acid detergent fiber	50.89 ± 0.015	31.79 ± 0.40	36.14 ± 0.75	31.44 ± 0.54
Acid detergent lignin	9.81 ± 0.71	5.76 ± 0.20	7.92 ± 0.11	8.54 ± 0.42
Hemicellulose	13.42 ± 0.8	17.93 ± 0.5	26.83 ± 0.6	32.61 ± 0.30
Micronutrients content (ppm) on DM basis				
Fe	404.79 ± 36.60	605.33± 181.2	390.28 ± 51.4	-
Cu	15.52 ± 5.71	23.21 ± 8.5	19.40 ± 4.59	-
Mn	50.91 ± 12.15	57.47 ± 4.84	66.27 ± 2.75	-
Zn	38.69 ± 0.13	46.26 ± 1.22	52.87 ±11.52	-
Mg (%)	0.45 ± 0.02	0.52 ± 0.04	0.47 ± 0.05	-
Nutritive value				
Total DMI (g)	-	-	939.93± 51.63	-
DMI (kg)/100kg	-	-	3.28±0.09	-
Total DOMI(g)	-	-	484.14± 24.69	-
DOMI (kg)/100kg	-	-	1.69± 0.04	-
Total TDN intake (g)	-	-	506.25±24.52	-
TDN intake (kg) /100kg	-	-	1.77± 0.04	-
Total DCP (g)	-	-	164.27±2.85	-
DCP/100 kg.	-	-	579.85±26.01	-
TDN(%)	-	-	50.95±0.7	-
DCP (%)	-	-	13.33±0.2	-.

DMI per 100 kg body weight was 3.28 kg and total mean DMI (g/day/animal) was 939.9. Total digestible organic matter intake g/day /animal and per 100 kg body weight were 484.14 g and 1.69 kg respectively. DCP and TDN content of *Tephrosia purpurea* fodder on DM basis were 13.3 and 50.95 percent respectively. DCP and TDN intake per 100 kg body weight were 579.85 g and 1.77 kg respectively. Dry matter, DCP and TDN intake were on higher side than the maintenance requirement of sheep as reported by (Kearl,1982).

Since *Tephrosia purpurea* is a non- traditional feed in semi arid regions of India hence reports on effect of seasonal variation with regards to chemical composition were not available for comparision. Apparently on the basis of chemical composition in general, it appears to be a nutritious feed during September month and it can also fulfill the nutritional requirements for maintenance as well as can support some production requirements of small ruminants in semi arid regions.

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15 th Edn. Association of Official Analytical Chemists, Washington, D.C.

Chaudhary U.B. 2006. Annual report of AICRP on improvement of feed resources and nutrient utilization in raising animal production. CIRG, Makhdoom.

Oudhia, P. 2004. Sarphonk (*Tephrosia purpurea*). http:// www.host.purdue.edu/ new crop/crop fat sheets/sarphonk html.

Kearl, L.C.1982. Nutrient requirements of ruminants in developing countries. Published by International feed stuffs institute Utah Agriculture Experiment station Utah state University logan Utah. pp 45-54.

Rama Prasad, J., Rao, Z. Prabhakara, and Rao, D. Srinivasa. 1999. Evaluation of leguminous based complete rations in sheep. Indian J. Anim. Nutr., 16: 48-52.

Sastry, C.S.T., and K.Y. Kavathokar. 1990. Pages 402-405 in plants for reelamation of waste lands C.S.I.R. New Delhi, India.

Singh Dhyan, P.K. Chhonkar, and R.N. Pandey. 1999. Soil Plant analysis: A Methods Manual, Indian Agricultural Research Institute, New Delhi. pp viii +160.

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Chemical Composition and In-Sacco Dry Matter Degradability of Fodder from Two Cultivars of Sorghum

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ABSTRACT : Local sorghum variety for upper part and whole plant at pre-flowering stage, variety Rio at flowering and dough stage were evaluated for proximate principles, cell wall constituents, in-sacco dry matter degradability and DM degradation kinetics. A significant ($P<0.05$) increase in DM content of whole sorghum plant compared to upper part of the plant in local variety was observed whereas OM content for upper part of local and Rio variety at dough stage was found similar. Increased OM content was observed in variety Rio than pre-flowering whole plant of local variety. The CP content decreased from flowering to dough stage in Rio variety but NDF content for local variety was higher as compared to Rio variety at both stages of maturity. Similarly, ADF, hemicellulose, cellulose and lignin contents were higher in local variety than in Rio variety at both the stages of maturity. Significant ($P<0.05$) decrease in dry matter degradability was observed in Rio variety with advancing stage of maturity. Variety Rio at flowering stage excelled others in studied parameters.

Key words: In sacco degradability, Sorghum fodder and Chemical constituents

INTRODUCTION

The roughages like sorghum, maize, bajra, wheat straw and berseem constitutes the major energy feeds for ruminants in India. Sorghum (*Sorghum bicolor*) is one of the most important drought and heat resistant kharif forage crop grown green fodder. The nutrient content of a fodder varies depending upon the various factors such as climate, type of fodder, variety and stages of harvesting. The latter has a great bearing on the nutritive value, digestibility and voluntary intake of fodder. As the plant matures, it generally tends to decline in nutritive value. Such changes occur due to the altered chemical composition, lignification and decreased proportion of leaves to stem (Van Soest, 1987). In the present study, local sorghum (upper part and whole plant) at pre-flowering stage and variety Rio at flowering and dough stage have been evaluated through their chemical composition and in-sacco studies.

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MATERIALS AND METHODS

The present study was conducted on local sorghum (upper part and whole plant at pre-flowering stage) and variety Rio (flowering, 86-93 days; and dough stage, 110-117 days) to determine their chemical composition and in-sacco dry matter degradability. All samples were analysed for proximate composition (AOAC, 1995) and fibre fractions (Goering and Van Soest, 1970). In- sacco degradability of dry matter was determined by nylon bag technique (Mehrez and Orskov, 1977) in three rumen fistulated crossbred adult (6 years of age) bullocks of about 400 kg body weight, maintained on green sorghum and stover-based diet.

In sacco dry matter degradation of all samples was studied at 0, 3, 6, 9, 12, 18, 24, 36, 48 and 72 hours of incubation periods. All samples were incubated in duplicate at each incubation period in all three fistulated animals. For this, about 5g oven-dried samples of 2.0 mm particle size were kept in nylon bags (17x9cm) with pore size of 40x40µm. Bags were

suspended in the rumen of bullocks with the help of 50cm long cord tied with a heavy iron ring chain piece. After the stipulated time, each bag was removed from the rumen and washed thoroughly under running tap water till the water was colorless. Then bags were transferred to a hot air oven for determining the dry matter at 70±1^{0}C for 48 hours. For estimation of kinetic parameters Orskov model (Orskov and Mc Donald, 1979) was used.The data were analyzed statistically (Snedecor and Cochran, 1970).

RESULTS AND DISCUSSION

DM content of Rio variety at dough stage (110-117 days after sowing) was significantly (P<0.05) higher (35.23%) than other samples Table 1, similarly DM content of whole plant of local variety was significantly (P<0.05) higher (27.89%) than upper part of the plant (22.70%). It could have been due to increased photosynthetic activity of the plant, which led to higher biomass production in leaves (Azim et al., 1989). The value for DM of variety Rio was in agreement with the value reported by Kumar and Singh (1985). The OM content of local variety (upper part) and Rio (dough stage) was similar and not differ significantly between flowering stage (93.65%) and dough stage (94.47%) in variety Rio. Joshi et al. (1985) reported the OM content of four sorghum varieties ranged from 90.5-94.4% which is in agreement with the present findings. Significantly higher (P<0.05) value for CP in upper part of local variety (8.95%) indicates that leaves contain more CP than the whole plant of the sorghum. The CP content was significantly decreased (P<0.05) from flowering stage (7.3%) to dough stage (5.6%) in variety Rio. Similar trend for CP in sorghum fodder was reported by other workers (Manoj, 1993; Randhawa et al. 1988). The NDF in both parts of local variety was similar, whereas The ADF in whole plant of local variety was significantly (P<0.05) higher (43.51%) than others. Variety Rio contained lower ADF than the local one. Similar trends were also reported earlier (Gupta et al. 1983; Firdous and Gilani 2001). Hemicellulose, cellulose and lignin content of local variety was found significantly higher (P<0.05) than variety Rio at both stage. Similar results have been reported earlier also (Kim and Voigtlaender 1985; Gupta et al., 1983; Gabra et al., 1989).

In-sacco disappearance of dry matter of variety Rio were significantly (P<0.05) decreased with maturity, indicative of an inverse relationship between maturity and in-sacco disappearance (Table 2). DM disappearance increased with increase in incubation periods. Negi et al. (1988) and Khazaal et al. (1994) also reported the similar trend in disappearance study. The initial solubility of DM was significantly (P<0.05) higher in variety Rio at flowering stage (20.7%) than others. The DM disappearance for upper part of local variety and variety Rio at flowering stage was comparable (59.4 and 60.4, respectively) followed by whole plant of local variety (55.1%) and Rio at dough stage (46.6%) at 72 h. The DM disappearance of variety Rio at flowering stage and upper part of local variety was significantly (P<0.05) higher than dough stage of Rio and whole plant of local at all incubation periods. DM disappearance of all sorghum samples ranged

Table 1. Chemical composition (% DM basis) of two varieties of sorghum fodder at different stages of growth

Particulars	Local (upper part)	Local (whole plant)	Rio (flowering stage)	Rio (dough stage)
DM	22.70±0.42^{d}	27.89±0.53^{b}	24.65±0.25^{c}	35.23±0.67^{a}
OM	94.28±0.70^{a}	91.69±0.43^{b}	93.65±0.50^{ab}	94.47±0.42^{a}
CP	8.95±0.20^{a}	7.10±0.07^{b}	7.33±0.07^{b}	5.63±0.35^{c}
NDF	74.12±0.44^{a}	72.57±0.42^{a}	63.89±0.30^{c}	67.37±0.48^{b}
ADF	40.51±0.39^{b}	43.51±0.39^{a}	38.08±0.52^{c}	39.54±0.09^{bc}
Hemicellulose	33.62±0.05^{a}	29.06±0.80^{b}	25.84±0.81^{c}	27.83±0.57^{bc}
Cellulose	33.79±0.21^{ab}	35.12±0.65^{a}	31.74±0.30^{c}	33.55±0.01^{b}
Lignin	6.72±0.18^{b}	8.39±0.27^{a}	6.34±0.22^{b}	5.99±0.10^{b}

$^{a, b, c, d}$ Figures bearing different superscripts in a row differ significantly (P<0.05)

from 46.61-60.38 percent at 72 h of incubation period.

Dry matter degradation kinetics and effective degradability of all sorghum sample taken in present study have been presented in Table 3. The value for 'a' was higher in variety Rio at flowering stage (21.19%), whereas 'b' value was higher in local variety than Rio. Degradation rate constant 'c' ranged between 0.0169 in variety Rio at dough stage to 0.034 in upper part of local variety. Effective

Table 2. Dry matter degradability (% DM basis) of two varieties of sorghum fodder at different stages of growth

Incubation periods(hr.)	Local (upper part)	Local (whole plant)	Rio (flowering stage)	Rio (dough stage)
0	12.99±0.40^{b}	10.99±0.26^{c}	20.74±0.42^{a}	13.63±0.17^{b}
3	16.46±0.26^{b}	13.03±0.29^{c}	25.92±0.25^{a}	16.45±0.67^{b}
6	19.05±0.32^{b}	17.25±0.34^{c}	29.14±0.18^{a}	19.67±0.35^{b}
9	24.04±0.28^{b}	19.74±0.42^{d}	30.92±0.20^{a}	21.16±0.20^{c}
12	28.59±0.09^{b}	25.65±0.25^{c}	34.87±0.27^{a}	22.71±0.30^{d}
18	36.33±0.28^{b}	29.30±0.38^{c}	38.57±0.30^{a}	23.81±0.13^{d}
24	43.03±0.27^{a}	36.43±0.36^{b}	43.83±0.20^{a}	29.37±0.19^{c}
36	48.29±0.20^{b}	45.26±0.36^{c}	51.85±0.41^{a}	36.93±0.29^{d}
48	55.14±0.21^{a}	50.42±0.37^{b}	55.79±0.27^{a}	40.62±0.26^{c}
72	59.42±0.26^{a}	55.10±0.40^{b}	60.38±0.41^{a}	46.61±0.29^{c}

$^{a, b, c, d}$ Figures bearing different superscripts in a row differ significantly (P<0.05)

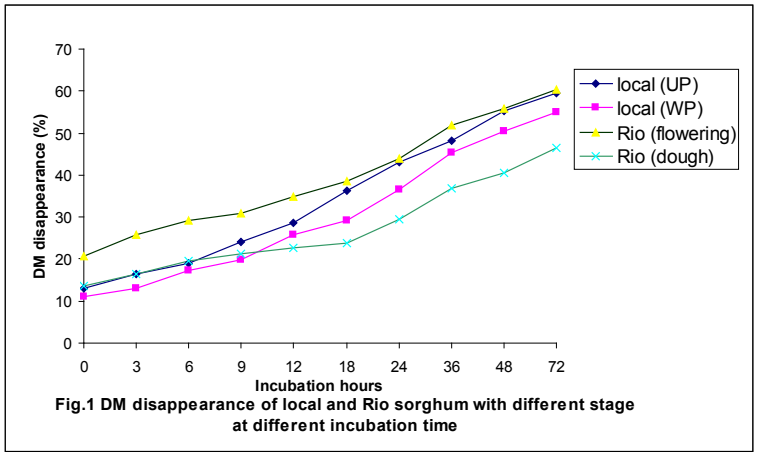

Fig.1 DM disappearance of local and Rio sorghum with different stage at different incubation time

Table 3. Dry matter degradation kinetics and effective degradability (%) of two varieties of sorghum fodder at different stage of growth

Particulars	Local (upper part)	Local (whole plant)	Rio (flowering stage)	Rio (dough stage)
a	11.19±0.57	9.23±0.55	21.19±0.37	14.00±0.46
b	53.58±1.20	53.95±1.55	45.08±0.97	46.90±3.46
c	0.034±0.001	0.0285±0.001	0.02957±0.001	0.0169±0.002
ED (k=0.04)	35.81	31.67	40.35	27.93

degradability was highest (40.35%) in variety Rio at flowering stage and lowest (27.93%) in same variety at dough stage.

It is therefore concluded that variety Rio was found superior than its local counterparts in terms of chemical constituents and dry matter degradability . Variety Rio at flowering stage excelled all others.

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REFERENCES

AOAC. 1995. Official Method of Analysis. 16th ed. Association of official Analytical Chemist. Virginia. 2201-3301, USA.

Azim. A., Z. Naseer, and A. Ali. 1989. Nutritional evaluation of maize fodder at two different vagatative stages. Asian Australian J. Anim. Sci. 2: 27-34.

Firdous, R., and A.H. Gilali. 2001. Changes in chemical composition of sorghum as influenced by growth stage and cultivers. Asian-Aust. J. Anim. Sci. 14: 935-940.

Gabra, M.A., H.M. El-Hosseiny, and A.E.M. Khinizy.1989. Nutritional studies on some local and improved variety of sorghum sown in reclaimed soils. Page 119-124, in Proc.of Third Egyp. Brit. Conf. on Anim., Fish and Poult. Prod., Alexandria, Egypt. Bangor, U.K.; Univ. College of Northwales.

Goering, H.K., and P.J. Van Soest. 1970. Forage fibre analysis (Apparatus, Reagents, Procedures, and Some applications). Agric. Handbook No. 379: ARS-USDA, Washington, DC.

Gupta, B.K., R.V. Singh, and P.D. Mehandiratta. 1983. Comparative evaluation of varieties of sorghum and sudan grass for yield and quality of fodder. J. Res. Punjab Agri. Univ. 20:19-26.

Joshi, D.C., M.Y Khan., and C. Jaganadharan. 1985. Nutritive evaluation of pusa chari, fodder varieties and sources of energy for ruminants. III. Pusa chari 7, 9 (*Sorghum bicolor lin moech*) and 21, 40 (*Sorghum bicolor* x S. *sudanese*). Indian Nutr Dietetics. 22:167.

Khazaal, K., J. Boza, and E.R Orskov. 1994. Assessment of phenolics related anti nutritive effect in mediterranean browse: A comparison between the use of gas production technique with or without insoluble poly vinyl poly pyrrolidone or nylon bag. Anim. Feed Sci. and Tech. 49:133.

Kim, J.G., and G. Voigtlaender. 1985. Study on reserve carbohydrates and net energy for lactation NEL in maize and sorghum. II synthesis and accumulation of cell wall constituents. J. of Korean Society of Grassland Sci. 5:127-135.

Kumar, P and J.P. Singh. 1985. Evaluation of sorghum fodder (Rio) for cattle and buffaloes. Indian J. Nutr. Diete. 22 : 313-316.

Manoj, K. 1993. Availability of various nutrients in different varieties of sorghum as evaluated by nylon bag technique. M.Sc.Ag. Thesis G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Mehrez, A.Z., and E.R. Orskov. 1977. A study of the artificial fibre technique for determining the digestibility of feeds in rumen. J. Agric. Sci. (Camb.) 88: 645-650.

Negi, S.S., B.Singh, and H.P.S. Makkar. 1988. An approach to the determination of rumen degradability of nitrogen in low grade roughages and partition of nitrogen. J.of Agric. Sci.(Camb.) 111: 3187-3194.

Orskov, E.R., and I. Mc Donald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. (Camb). 92: 499-503.

Randhawa, S.S., R.S Gill, S.S Gill, and L.S Hundal. 1988. Effect of feeding green sorghum its silage or hay in milk production in buffaloes. Ind. J. Dairy. Sci. 41: 255-257.

Snedecor, G.W., and W.G. Cochran. 1970. Statistical Method. The Iowa State University Press, Arness Iowa, U.S.A.

Van Soest, P.J. 1987. Nutritional Ecology of the Ruminants. Comstock Publishing Associates: A division of Cornell University Press, Cornell, New York.

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Effects of Feeding Concentrate, Tree Leaves and Straw Based Diet on Growth and Nutrient Utilization in Yak (*Poephagous Grunniens* L.) under Semi-Intensive Rearing System

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ABSTRACT : Effect of feeding concentrate, tree leaves and straw based diet on growth and nutrient digestibility in yak (*Poephagous grunniens* L.) was studied under semi-intensive system of management. Twelve male yaks, (2-3 years of age with initial body weight 135.8±7.38 kg) were fed concentrate mixture containing CP 16.52, EE 5.60, CF 6.31, NFE 64.47, and TA 7.09% (on DM basis) along with tree fodder and paddy straw in the ratio of 3: 2: 4 for a period of one year. The average daily dry matter intake (DMI) was recorded as 60.68±1.23 g/ kg $W^{0.75}$ and digestibility of DM, CP, CF, EE, NFE, ADF, NDF and OM were 57.95±1.30, 43.01±2.99, 43.25±2.60, 61.41±2.43, 68.99±0.84, 54.15±3.29, 28.80±2.26 and 59.75±1.28% respectively. Monthly body weight gain ranged between 7.58±3.29 to 15.17±1.75 kg and final average body weight at the end of the experiment was 216.3±10.37 kg indicating a positive trend of growth. From these findings, it can be concluded that feeding of concentrate, tree leaves-straw based diet supports the nutritional requirements of growth in yaks under semi-intensive system of management in Arunachal Pradesh.

Key words: yak, concentrate feeding, semi-intensive system, growth, nutrient digestibility.

INTRODUCTION

Yak (*Poephagous grunniens* L.) is the livelihood for the agrarian highlanders in Arunachal Pradesh at an altitude on and above 3000 m above MSL where other livestock species can rarely adapt. However, productivity of yak is considerably hampered due to irrational grazing system, unscientific feeding and traditional managemental (Dong and Li, 2003) practices. Imbalances of herbage supply both in quantity and quality (Zhao et al., 1991) also affects nutrition and health. In traditional way of maintaining the yaks, concentrate feeding is very rare and most animals are not given any supplements even during cold season on dry grassland (Long et al., 1999) except for weak or lactating yaks in some occasions (Pal et al., 1994). As a result, growth rate and calving potential is severely affected (Ruijun, 2003) and losses of 80 to 120 percent of live weight have been reported under the traditional farming system (Miller, 1996; Dong and Li, 2003) particularly in the winter months. This is due to herbage deficiency and hence poor grazing. In the present experiment, an attempt was made to elucidate the effect of feeding concentrate, tree leaves and straw based diet on growth and nutrient utilization in yak under semi-intensive system of management in Arunachal Pradesh.

MATERIALS AND METHODS

The study was carried out at National Research Centre on Yak, Indian Council of Agricultural Research, Dirang, Arunachal Pradesh situated at an altitude of about 8500 ft. above MSL. Twelve

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male growing yaks, selected randomly from the yak herd of 2-3 years of age weighing 135.8±7.38 kg. The animals were reared under semi-intensive system of management in well ventilated stall. They were fed a concentrate mixture, containing Organic Matter 92.90%, Crude Protein 16.52%, Ether Extract 5.60%, Crude Fibre 6.31%, Nitrogen Free Extract 64.47%, and Total Ash 7.09% along with paddy straw and tree fodders in the ratio of 3: 2: 4 to ensure DMI of 2.5-3.0 kg/100 kg body weight. The concentrate was divided into two portions and given at 8.00 h in the morning and 13.00 h in the evening. Clean drinking water was made available all the day. The concentrate mixture was provided first followed by straw and tree leaves mixture.

The animals were fed individually and feeding trial was conducted for a period of 12 months. During this period, monthly individual body weights of the yaks were recorded. At the end of experimental feeding, a digestibility trial for 7 days duration was conducted and the quantity of feed intake, residues and faeces voided were recorded and the samples were collected for analysis. All the samples of concentrate, paddy straw and tree fodders, residues and faeces were dried at 100^0c±1 for overnight, ground to pass through a 1 mm screen and stored. Proximate principles were analyzed as per AOAC (1990). Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were estimated according to Goering and Van Soest (1970). Analysis of total nitrogen of feed and faeces or other samples was carried out using Kjeldahl Method (Davidson et al., 1970).

RESULTS AND DISCUSSION

The percent chemical composition of the experimental diet is presented in Table 1. Because of transportation constraints, the yak herdsmen have to face hardship for the conventional concentrate ingredients. However, in the present experiment, ingredients those were easily available in nearby locality have been used for preparing the concentrate mixture. The tree leaves were the mixture of many species. In the present experiment, those tree leaves which were evergreen and available in abundance throughout the year were used for feeding. They were namely, Domkar (*Symplocus racemosa*), Blemkar (*Buddlija asitica*), Maar (*Costanpsis* spp.), Bagger (*Berberis* Spp.) and Metekpa (*Quercus fenestrata*) under family Symplocaceae, Loganiaceae, Fragaceae and Berberiaceae, respectively.

The nutrient intake and growth performance of yaks are presented in Table 2. The average daily DM intake was 1.54±0.04, which was observed to be lower i.e. 1.62±0.10 Kg/100 Kg body weight reported earlier (Chatterjee et. al. (2005) as 1.62±0.10 kg/100 kg body weight for yak fed paddy straw supplemented with concentrate mixture and of 2.24±0.26 kg/100 kg body weight in yaks fed on Salyx tree leaves (Chatterjee et al., 2001). However, Pal (1999) reported a very low value (1.25% of body weight) of DMI in yaks fed on finger millet straw.

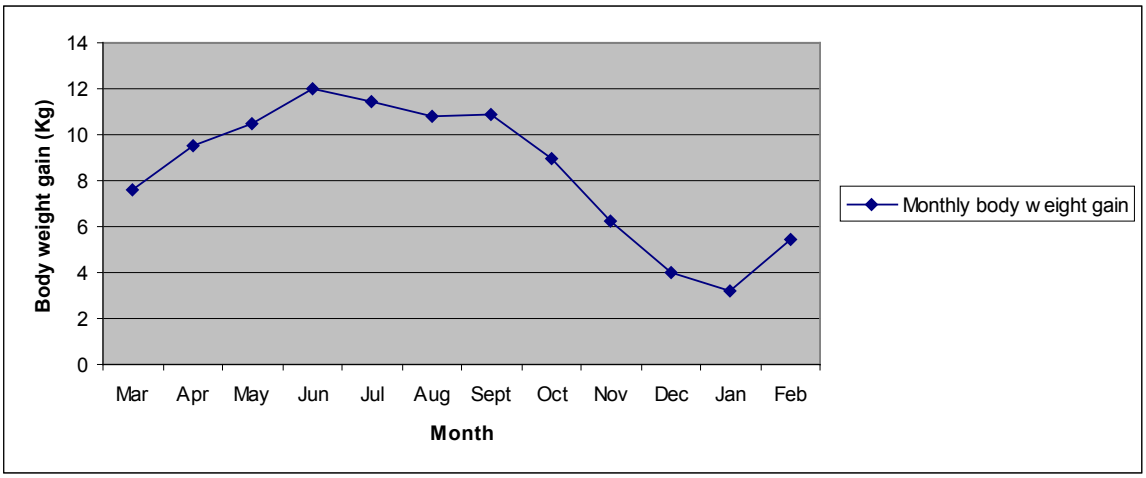
Table 1. Chemical composition of the experimental diet (% dry matter basis) and nutrient digestibility

Chemical composition	(% DM basis)			
Parameter	**Concentrate mixture**	**Tree leaves**	**Paddy straw**	**Nutrient Digestibility (%)**
Dry Matter	86.54	40.69	81.09	57.95±1.30
Crude Protein	16.52	9.27	3.58	43.01±2.99
Ether Extract	5.6	1.58	1.44	43.25±2.60
Crude Fibre	6.31	14.19	31.45	61.41±2.43
Nitrogen Free Extract	64.47	69.04	49.14	68.99±0.84
Acid Detergent Fibre	16.39	32.72	47.06	54.15±3.29
Neutral Detergent Fibre	37.98	43.13	58.15	28.80±2.26
Organic Matter	92.9	94.07	85.62	59.75±1.28

Table 2. Nutrient Intake in yak fed concentrate-tree leaves-straw based diet under semi-intensive system of management

Ingredients	Quantity
Concentrate (g/ kg $W^{0.75}$)	13.94±0.93
Tree fodders (g/ kg $W^{0.75}$)	34.04±0.96
Paddy straw (g/ kg $W^{0.75}$)	12.09±0.44
Total (g/ kg $W^{0.75}$)	60.68±1.23
Overall (kg/ 100kg)	1.54±0.04
DCP (%) of the ration consumed	4.17
TDN (%) of the ration consumed	59.87

Fig. I. Monthly gain in body weight (kg) of yak fed concentrate-tree leaves-straw based diet



The lower overall DMI (g/ kg $W^{0.75}$) in this study be due to incorporation of both tree leaves and paddy straw with concentrate. The overall average body weight increase was 216.3±10.37 kg and body weight gain ranged from 3.17±4.47 to 15.17±1.75 kg during the experiment. However, monthly average body weight gain showed variation (Figure I) which may be due to changes in climatic condition during the year, hampering intakes and utilization of nutrients.

The data on digestibility of nutrients (Table 1) was quite comparable to that reported earlier by Pal (1999) for yak fed finger millet straw. However, digestibility of DM was higher than as reported by Basu et al. (2005) for yaks grazed only on range land in high altitude areas. But, comparatively decreased digestibility of the nutrients than as reported by Chatterjee et al. (2005) may be due to incorporation of tree fodders in the present experiment. There are also reports that high plane of feeding level led to low digestibility in ruminants (Bondi, 1987) due to rapid passage of digesta from the rumen (Balch and Campling, 1962; Chen et al., 1992 and Han et al., 1992).

The study clearly showed that feeding of concentrate-tree leaves-straw based diet enhanced intake and digestibility of nutrients in yak. Rapidly increasing yak population with obvious rangeland degradation increases the gap between feed supply from natural pastures and the animals feed demand and hence likely to predispose malnutrition in the foreseeable future. Thus, the study may provide valuable information regarding usefulness of concentrate feeding by incorporating locally available tree fodders and straw (%DCP 4.17and %TDN 59.87) to support the nutritional requirements and growth of yaks under semi-intensive system of management in Arunachal Pradesh.

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th Edn. Assoc. Off. Anal. Chem. Arlington, VA.

Balch, C.C., and R.C. Campling. 1962. Regulation of voluntary food intake in ruminants. Nutr Abs and Review. 32: 669-686.

Bondi, A.A. 1987. Animal Nutrition, Wiley, NY, USA.

Basu, A., A. Chatterjee, K.K Baruah, and M. Bhattacharya. 2005. Voluntary feed intake and live weight gain in Yak (*Poephagous grunniens* L.) grazed on a high altitude grass land. Indian J. Anim. Sci. 75: 81-83.

Chatterjee, A., A. Basu, and M. Sarkar. 2005. Nutrient utilization in yaks fed paddy straw supplemented with concentrate mixture. Indian Vet. J. 82: 579-680.

Chatterjee, A., A. Basu, and M. Sarkar. 2001. Nutritive value of salyx tree leaves for yak. Indian J. Anim. Nutr. 18: 285-287.

Chen, X.B., G. Grubic, E.R. Orskov, and P. Osuji. 1992. Effect of feeding frequency on diurnal variation in plasma and urinary purine derivatives in steers. Anim Prod. 55: 185-191.

Davidson, J., J. Mathieson, and A.W. Boyne. 1970. The use of Automation in determining nitrogen by Kjeldahl methods with final calculation by computer analyst. 96: 181-183.

Dong, Q.M., and Q.Y. Li. 2003. Distribution and production situation of yaks in the wild world. Qinghai Prataculture. 12: 32-35.

Goering, H.K., and P.G. Van Soest. 1970. Forage Fibre Analysis. Agriculture Handbook. No. 379. US Dept of Agriculture, Washington, DC, USA.

Han, X.T., L.H. Hu, A.Y. Xie, S.J. Liu, and X.C. Bi. 1992. The estimation of energy metabolism in yaks under roughly feeding condition. Qinghai Anim. Husb. and Vet. Sci. 22: 21-22.

Long, R.J., D.G. Zhong, X. Wang, Z.Z. Hu, and S.K. Dong. 1999. Effect of strategic feed supplementation on productive and reproductive performance of yak cows. Pre. Vet. Medi. 38:195-206.

Miller, D.J. 1996. Conservation and managing yak genetic diversity: an introduction. Page 29-31 in Conservation and management of yak genetic diversity – proceedings of a workshop in ICIMOD. D.J. Miller, S.R. Craig, and G.M. Rana, eds. Kathmandu, Nepal.

NRC. 2001. Nutrient Requirements of Domestic Animals. National Academy Press, Washington, DC.

Pal, R.N., S.K. Barari, and A. Basu. 1994. Yak (*Poephagous grunniens* L.) and its type – A field study. Indian J. Anim. Sci. 64: 853-856.

Pal, R.N. 1999. Studies on yak nutrition, Final Project Report, Emeritus Scientist Scheme, National Research Centre on Yak (ICAR), Dirang, Arunachal Pradesh, India.

Ruijun, L. 2003. Yak nutrition – A Scientific basis. Page 389-414 in The Yak (2nd Edn.). G. Wiener, H. Jianlin, and L. Ruijun, Eds, RAP Publication, FAO, Bangkok. Thailand.

Zhao, X.Q., N.L. Pi, and J.H. Feng. 1991. A study on the efficiency of energy utilization and requirement for breeding Tibetan sheep. Alpine Meadow Ecosystem. 3: 97-102.

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Farmer's Reasons for Preference of Dairy Interventions under IVLP

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ABSTRACT : Animal husbandry constitutes about 30% of the country's agricultural output. The study was carried out at two Institute Village Linkage Programme (IVLP) centres namely; NDRI, Karnal representing irrigated agro eco-system and IGFRI, Jhansi, representing rainfed agro eco-system. A total of 150 farmers were selected randomly for this study. Under fodder crops, the improved varieties of berseem were ranked first due to high productivity, more number of cuttings, fodder quality and, availability for a longer period, in both the systems. A number of technological interventions related to feeding, breeding and healthcare were assessed at real farm situation and they were ranked by the farmers. Among feeding interventions, complete feeding and concentrate feeding were most preferred by the farmers of both the systems. Breeding interventions like Artificial Insemination (AI) and bypass protein were found very much useful for the animals. Many unproductive cattle and buffaloes were made productive with the help of bypass protein method. In both the systems, vaccination against major diseases like FMD, BQ and HS was the most preferred intervention among the farmers as this intervention safeguards the animals against many diseases and ultimately lessens the economic loss to the farmers. Farmers felt that it was very difficult to adopt these technologies at their own as they require expert handling. Farmers in both the systems discontinued urea treatment of straw and silage making.

Key Words: Institute Village Linkage Programme (IVLP), Dairy farmers, Animal husbandry, Preference, Reasons, Breeding, Feeding, Healthcare

INTRODUCTION

Animal husbandry and dairy development play a prominent role in the rural economy in supplementing the income of rural households, particularly the landless, small and marginal farmers. Animal husbandry output constitutes about 30 percent of the country's agricultural output. India is endowed with the largest livestock population in the world. It accounts for 57 per cent of the world's buffalo population and 15 per cent of the cattle population. Livestock development has always been a major concern in the Indian economy along with agriculture. The livestock sector registered a steady growth rate of 6.2 per cent during the post independence period from 1951 to 1991 as compared to the growth rate of 3.42 percent in agriculture. The livestock sector contributes 8 percent to India's GDP and the single largest contributor of milk, contributing about 66 percent of the share from animal husbandry sector. This has been possible due to varying extension approaches adopted from 1951 onwards (Tiwari and Singh., 2005).

Since independence, several technologies have been evolved for increasing the production, productivity of animals by improving the adoption behaviour of the farmers (Dwivedi et al., 2004). The technologies generated by the system need to be assessed and refined in order to suit the requirements of farmers with varying bio-physical and socio-economic environment (Singh et al., 2005). A number of dairy interventions had been attempted in the IVLP villages. This paper tries to highlight farmer's preferences towards animal husbandry practices.

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RESEARCH METHODOLOGY

The study was carried out at two IVLP centres namely; NDRI, Karnal representing irrigated agro eco-system situated in Karnal district of Haryana and IGFRI, Jhansi, representing rainfed agro eco-system. These two centres were selected purposively to have more number of interventions on crops and dairying. The ex-post facto research design was applied to study the changes occurred due to project interventions.

Sampling plan

Under irrigated agro eco-system all the five villages namely, Gaurgarh, Amargarh, Sikri, Shekhpura and Gumto of district Karnal, Haryana and all the three adopted villages of IGFRI, Jhansi namely, Algi, Sanora and Garera and their hamlets of district Datia/Shivpuri of MP state were selected purposively. From irrigated agro eco-system a total of 75 farmers i.e. 15 farmers from each village were selected by random sampling technique. Similarly, 75 farmers were selected from the rain fed agro eco-system i.e. 25 farmers from each village. A total of 150 respondents formed the sample size. All the respondents were real beneficiaries of Institute Village Linkage Programme.

Reasons for adoption of fodder production technologies

Improved varieties of berseem, oat, and sorghum and fodder maize were assessed in the study area and it was observed that improved varieties of berseem were ranked first on the parameters like more productivity, more number of cuttings, better fodder quality, availability for a longer period, etc. Improved varieties of sorghum, oat and fodder maize were placed at II, III and IV places, respectively (Table 1).

Similarly, improved fodder varieties were assessed in rain fed agro eco-system and improved variety of berseem was ranked first followed by sorghum, hybrid Napier, and fodder maize (Table 1).

Table 1. Reasons for adoption of improved fodder crop varieties

Irrigated Agro ecosystem

Reasons	Rank			
	Berseem	Oat	Fodder Maize	Sorghum
More productivity	I	IV	III	II
More no. of cuttings	I	II	III	IV
Better fodder quality	I	II	IV	III
Supply of fodder for a longer time	I	III	IV	II
Early maturity	III	IV	I	II
Cultivation during lean period	I	IV	III	II
Overall	I	III	IV	II

Rainfed Agro ecosystem

Reasons	Rank			
	Berseem	Sorghum	Hybrid Napier	Fodder maize
More productivity	I	IV	III	II
More no. of cuttings	I	II	III	IV
Better quality of fodder	I	II	IV	III
Supply of fodder for a longer time	II	III	I	IV
Early maturity	I	III	IV	II
Cultivation during lean period	II	III	I	IV
Overall	I	II	III	IV

Reasons for adoption of dairy interventions

In dairying several technological interventions related to feeding, breeding and healthcare were assessed and ranked.

a. Improved feeding technologies

Among the feeding technologies, complete feed block was found best followed by mineral and vitamin supplementation and bypass protein technology.

In rain fed system, concentrate feeding was the most preferred one. Farmers ranked complete feeding, mineral mixture supplementation and enriched roughage at II, III and IV, respectively (Table 2)

b. Improved breeding technologies

Artificial Insemination and induced lactation were the main technologies assessed under IVLP at real farm situation and were ranked first and second, respectively. The induced lactation was ranked second because it was difficult to be implemented at field level (Table 3). AI was ranked first followed by induced lactation. The reasons put forth for the adoption of breeding technologies were superior progeny, cost of technology, overcome reproductive disorders and rate of success

Table 2. Reasons for adoption of improved feeding technologies

Irrigated agro ecosystem	Rank		
Reasons	**Mineral and Vitamin Supplementation**	**Complete feed block**	**Bypass protein technology**
More yield	III	I	II
More income	III	I	II
Better growth	I	II	III
Good health	I	II	III
Overall	II	I	III

Rainfed agro ecosystem

Reasons	Mineral Mixture Supplementation	Complete feed block	Concentrate	Enrichment of low grade roughages
More yield	III	II	I	IV
More income	III	II	I	IV
Better growth	III	I	II	IV
Overall	III	II	I	IV

Table 3. Reasons for adoption of improved breeding technologies in irrigated agro ecosystem

Reasons	Rank	
	Artificial Insemination	Induced Lactation
Superior progeny	I	II
Overcome reproductive disorders	-	I
Cost of the technology	II	I
Rate of success	I	II
Overall	I	II

Table 4. Reasons for adoption of improved healthcare technologies

Attributes	Rank	
	Irrigated agro system	
Reasons	Mineral and Vitamin Supplementation	Vaccination
Better growth	I	II
Good health	II	I
Less mortality	II	I
Overall	II	I
	Rainfed agro eco–system	
Reasons	Vaccination against FMD, BQ & HS	Deworming and Parasite Control
Good health	I	II
Better growth	I	II
Less mortality	I	II
Less chances of diseases	I	II
Overall	I	II

c. Improved healthcare technologies

Vaccination was the best healthcare technology and preferred because it has direct bearing on health, growth and mortality. While the other technology i.e. mineral and vitamin supplementation has been very successful due to positive impact on the growth of young calves in the study area.

In rain fed agro eco-system, vaccination against FMD, BQ and HS was ranked first followed by deworming and parasite control among the healthcare interventions. Farmers adopted these technologies for good health, better growth, less mortality and less incidence of diseases.

Table 5. Overall preference for dairy interventions

Irrigated agro eco-system Technologies	Rank
Mineral and Vitamin Supplementation	IV
Complete Feeding	III
Bypass protein technology	VI
Artificial Insemination	I
Induced Lactation Technology	V
Vaccination and deworming	II
Rainfed agro eco–system	
Mineral and vitamin supplementation	V
Complete feeding	III
Feeding of concentrate	II
Enrichment of low grade roughages	VI
Parasite (Ecto & Endo) control	IV
Vaccination	I

Table6. Reasons for rejection of urea treatment of straw and silage making

Irrigated agro ecosystem	Rank	
Reasons	**Urea treatment of straw**	
Labour consuming	II	
Tedious	I	
Availability of green fodder	III	
Storage problem	IV	
Poor taste	V	
Rain fed agro ecosystem		
Reasons	**Urea treatment of straw**	**Silage Making**
Labour consuming	II	I
Tedious	II	I
Poor taste	I	II
Availability of green fodder	I	II
Storage problem	I	II
Grazing of animals	I	II
Lack of technical knowledge	II	I
Overall	I	II

Overall Ranking

The overall perception of the farmers about these feeding, breeding and healthcare technologies was very positive but they felt that most of these technologies were difficult to adopt as they require expert handling.

All the technologies related to dairying were ranked and found that AI was the most preferred followed by vaccination and deworming and complete feeding.

From overall ranking in rainfed system (Table5) it could be inferred that among all the interventions on dairying, vaccination was most preferred followed by feeding of concentrate, complete feeding, deworming and parasite control, mineral and vitamin supplementation and enriched low grade roughages.

Reasons for rejection of interventions

Among the dairy interventions (Table 6), urea treatment of straw was rejected in both the systems and silage making was rejected by the farmers of rain fed agro ecosystem because it is labour consuming, tedious, lack of knowledge about the procedure, grazing habit of animals and availability of green fodder in the area.

REFERENCES

Dwivedi, R.N., M. Singh, P. Sharma, S. Pandey, B.S. Meena, M.B. Tamankar, R.K. Sharma, and J.P. Upadhayay. 2004. Nutritive cereal based rain fed agro-ecosystem for Bundelkhand region: Technology Assessment and Refinement through Institute Village Linkage Programme, IGFRI, Jhansi, UP. 2.

Singh, S.K., R.R. Burman, R.G. Chaudhary, and K.K. Singh. 2005. Technology Assessment and Refinement through Institute Village Linkage Programme: A participatory approach in technology assessment and refinement, Indian Institute of Pulse Research, Kanpur, 97.

Tiwari, R., and B.P. Singh. 2005. Extension approaches for livestock development in the context of Globalised Trade, Kurukshetra. 53, 4:10.

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Existing Seasonal Feeding Patterns of Dairy Animals in Jhansi District of Bundelkhand Region

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ABSTRACT : Inadequate nutrition is often considered as the single largest factor responsible for low milk production in India. An exploratory study was conducted in Jhansi district of Bundelkhand region characterized as semi arid, resource poor and undeveloped area, to assess the existing seasonal patterns of feeding practices of dairy animals. A total of 240 farmers was interviewed on a semi-structured interview schedule. It was observed that a combination of stall-feeding and grazing was the most prevalent mode of feeding of dairy animals in the district. Animals were sent for grazing for a period of 5 to 7 h daily. On the basis of mean data, it was observed that wet cows, wet buffaloes and calves were sent for grazing (5.51, 7.02 and 7.01 h) during winter, summer and rainy seasons, respectively. Stall-feeding was practiced twice a day (morning and evening) for all types of animals. Most commonly used dry fodders were wheat straw, barley straw, dried grass and stovers (dried sorghum chari). The amount of green fodder fed to wet cow was 5.71 and 4.81kg per day during winter and rainy seasons, respectively. The lactating animals were fed 1 to 1.50 kg concentrate daily in the form of sani. The farmers of Jhansi district possessed less knowledge about recommended feeding practices of dairy animals.

Key Words: Dairy animal, Feeding, Exploratory study, Grazing, Green fodder, Knowledge

INTRODUCTION

India has emerged as the largest milk producer in the world, but the productivity of dairy animals is still dismally low. The non-descript cows and buffaloes produce around 1 litre of milk per day, while the graded cows and buffaloes produce around 3 litre and crossbred cattle and Murrah33 buffaloes produce between 8-10 litre of milk per day (Pathak and Kundu, 2006). Where as, the milk productivity of cow and buffaloes were 1-2 litre and 4-5 litre per day, respectively in the Bundelkhand region (Tyagi, 1997). The low milk production by the Indian cattle and buffaloes has been attributed to several reasons. However, inadequate nutrition is the single largest factor responsible for low milk production in well-defined breeds of dairy animals. Several reports that there is deficiency of green fodder, dry fodder and commercial concentrates in the country due to which animals do not get adequate feed for expression of their genetic potential for milk production are available.(NCA, 1976; Ranjhan, 1994; Anonymous, 1996, and Kundu et al., 2005). Efforts to increase feed and fodder resources during the last four decades have been offset by the bovine population increasing at an annual rate of 1.5 percent. Therefore, availability of feeds and fodder to the livestock remained unchanged during this period.

An in-depth understanding of existing feeding practices of dairy animals would help not only in getting a comprehensive account of the level of dairy farming development in the study area, but also in planning and taking up any research or development programme. Therefore, an attempt was made, to assess the existing seasonal patterns of feeding practices of dairy animals in Jhansi district of Bundelkhand region.

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METHODOLOGY

An exploratory study was conducted in Jhansi district of Bundelkhand region. Stratified random sampling technique was applied in the selection of eight villages in the district. From each selected village, 30 respondents representing large, medium, small, marginal and landless categories were included in the investigation by using proportionate random sampling technique. The respondents having at least one dairy animal at the time of investigation were interviewed. Thus Information was gleaned from 240 farmers with the help of a semi-structured interview schedule. Grazing hours, frequency of stall feeding, amount of fodder fed to an animal in terms of dry, green and concentrate in different seasons were recorded. The data were analysed for mean and standard deviation.

RESULTS AND DISCUSSION

Seasonal feeding patterns

It was observed that a combination of stall-feeding and grazing was most prevalent mode of feeding of dairy animals in Jhansi district. The data regarding type and quantity of feed for different animals in three seasons i.e. winter, summer and rainy season were analyzed and presented in Table-1.

Grazing hours

Animals were sent for grazing for a period of 5 to 7 h daily. On the basis of mean it was observed that wet cows, wet buffaloes and calves were sent for grazing 5.51, 7.02 and 7.01 h during winter, summer and rainy seasons, respectively. Where as, dry cows / buffaloes and heifers were sent separately for grazing i.e. 5.75, 7.31 and 7.36 h during winter, summer and rainy seasons, respectively. It was observed that farmers had small size of land holding and agricultural operations were performed mainly using bullocks. The bullocks were fed at home i.e. stall-feeding, and occasionally they were sent for grazing. During the survey it was also observed that stray cattle practice (Annapratha) was popular in the Bundelkhand Region. Das and Tripathi (2008) also reported that livestock feeding mainly consists of grazing complemented and supplemented with stall feeding in Sunderban delta of India.

Frequency of feeding

Stall-feeding was practiced twice a day i.e. morning and evening for all type of animals. Few farmers provided feed and fodder more than two times to bullocks, advanced pregnant animals and high milk yielders. These animals were generally kept at home and feeding was done in the morning, noon and evening.

Feeding of dry fodder

All the farmers were feeding dry fodder to their animals. Most commonly used dry fodders were wheat straw, barley straw, dried grass and stovers (dried chari). Wet cows were fed dry fodder 5.76, 6.84 and 5.60 kg /animal per day during winter, summer and rainy seasons, respectively. Whereas, wet buffaloes were fed 6.32, 6.91 and 5.63 kg dry fodder per day in respective seasons. The amount of dry fodder provided to dry cow/ buffaloes was 5.98, 6.09 and 5.11 kg/ day/ animal during winter, summer and rainy season, respectively. Whereas, heifer and calf were fed 1-2 and 3-4 kg dry fodder per day, respectively. Similarly, the bullocks were fed dry fodder up to 8.96, 9.17 and 8.42 kg per day per animal during respective seasons. During the winter and rainy season some amount of green fodder was also fed since the amount of dry fodder was comparatively less in these seasons. It appeared that feeding was done on the basis of body size of the animals and it was true that bullocks and buffaloes had more body weight than other animals in the study area. It was noticed that leftover of dry fodder was 0.5 to1 kg in a feeding manger in a day, which was later used as farm yard manure.

Feeding of green fodder

Sampled farmers were providing green fodder to their animals up to some extent except during summer season. Berseem, up rooted weeds, grasses, tree leaves and sorghum were the most common sources of green fodder in the study area. The amount of green fodder fed to wet cow was 5.71 and 4.81kg per day during winter and rainy seasons, respectively. Whereas, wet buffaloes and dry cows / buffaloes were fed 5-6 and 4-5 kg green fodder per day during the same seasons, respectively. The amount of green fodder fed to a calf and heifer was 2 kg and a bullock 4-5 kg/day per animal during winter and rainy seasons. It was observed that quantity of green fodder fed to the animals depends on the milk production of the animals. Buffaloes

Table-1. Existing seasonal patterns of feeding practices of dairy animals in Bundelkhand

S.N	Animals	Grazing (hours) per (kg / day)	Frequency of feeding per day	Dry fodder (kg/day)	Green fodder (kg/day)	Concentrate (kg/day)
			Winter season			
1	Wet cow	5.51	2.02	5.76	5.71	1.36
2	Wet buffalo	5.51	1.96	6.32	6.23	1.37
3	Dry cow/ buff.	5.75	2.03	5.98	4.88	0.38
4	Calves	5.51	2.02	1. 07	1.6	0.26
5	Heifer	5.75	1.91	3.42	2.16	0.16
6	Bullock	0.72	2.19	8.96	4.95	0.93
			Summer season			
1	Wet cow	7.02	1.83	6.84	0.05	1.27
2	Wet buffalo	7.02	1.89	6.91	0	1.61
3	Dry cow/ buff.	7.31	1.76	6.09	0	0.41
4	Calves	7.02	1.88	1.54	0.01	0.15
5	Heifer	7.31	1.72	3.14	0	0.14
6	Bullock	1.36	2.03	9.17	0	0.89
			Rainy season			
1	Wet cow	7.01	1.81	5.6	4.81	1.29
2	Wet buffalo	7.01	1.83	5.63	5.07	1.54
3	Dry cow/ buff.	7.36	1.78	5.11	4.22	0.37
4	Calves	7.01	1.82	1.5	1.42	0.21
5	Heifer	7.36	1.75	2.82	1.78	0.16
6	Bullock	1.08	2.12	8.42	4.11	0.86

being high milk yielders than cows were offered more green fodder in Jhansi district.

Feeding of concentrate

Barley/wheat flour and mustard cake were the main component of concentrate feed offered to the animals in the study area. Farmers prepared concentrate by mixing flour and oil cake in 1:1 ratio. It was observed that concentrate was fed in the form of sani (flour, cake and salt were soaked in water for 4-5 hours then mixed in dry fodder) to the animals. The data presented in Table-1 reveals that 1 to 1.50 kg/day concentrate was fed to each lactating animal and 0.34 to 0.41kg to each dry animal per day. The amount of concentrate fed to calf and heifer was negligible (0.14-0.21kg/day) where as about 1 kg concentrate was offered to each bullock in the study area during working days.

It was a common practice of feeding 20 g common salt to each adult animal along with sani. Most of the farmers fed more concentrate to lactating animals in order to get more milk.

Farmer's knowledge on recommended feeding practices of dairy animals

A cursory look on Table-2 reveals that 45.80 percent farmers have knowledge about recommended feeding practices of dairy animals. Further, the maximum (69.58 %) knowledge was observed regarding feeding of dry animal and minimum (19.14%) in case of fodder production practices. It was safe to explain that farmers of Jhansi district had less knowledge in relation to feeding practices of dairy animals. Similar findings were also reported by Sharma et al., 2007.

Table 2. Extent of knowledge on feeding practices of dairy animals

S.No.	Type of animals	Knowledge (%)
1	Calves	43.08
2	Heifer	65.00
3	Pregnant animals	54.08
4	Wet animals	61.67
5	Dry animals	69.58
6	Fodder production	19.14
7	Over all	45.80

Conclusions

In rain fed areas, feeding systems were primarily based on grazing of animals on native pastures of low productivity, which were steadily deteriorating. The major source of dry matter for dairy animals were crop residues as straw and stovers. These were supplemented with small quantity of grasses, available through scanty grazing or cut grasses. The lactating animals were offered relatively better feeding through supplementation of by-products, concentrates (flour and mustardcakes), etc. The overall knowledge on feeding practices was 45.80 per cent. Hence, there is a dire need to increase the knowledge level of the dairy farmers by various extension means.

REFERENCES

Anonymous. 1996. Estimates of nutrients requirement and availability for bovine population across major States in India. NDRI Publication No. 281.

Das, S.K. and H. Tripathi. 2008. Livestock feeds and feeding practices in rural Sundarban delta of India. Animal nutrition and feed technology. 8:137-142.

Kundu S.S., Sultan Singh, S.K. Mahanta, and G.H. Pailan. 2005. Feeding Farm Animals. Satish Serial Publisher, Delhi.

Pathak, P.S., and S.S. Kundu. 2006. Livestock Feeding Strategies for Dry Regions. International Book Distributing Co., Lucknow.

National Commission on Agriculture (1976) Part VII, Government of India.

Ranjhan, S.K. 1994. Consultant's reports on the availability and requirement of feed and fodder for Livestock and Poultry. Department of India, New Delhi.

Sharma, Purushottam., Sadhna Pandey, B.S.Meena and N.P. Singh. 2007. Knowledge and adoption of livestock feeding practices in Jhansi district of Bundelkhand. Indian J. dairy science. 60(1): 63-67

Tyagi, R.K. 1997. Grassland and fodder atlas of Bundelkhand. published by IGFRI, Jhansi (U.P.) : 97-105

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Impact of Temperature Rise on Pulmonary Dynamics, Heat Dissipation and Antioxidant Status in Karan Fries Heifers

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ABSTRACT: Five Karan Fries (Holstein Friesian X Tharparkar) heifers (18 to 24 months) were exposed to different climatic chamber temperatures viz. sham control (T_1), 32±1.5°C (T_2), 35±1.5° C (T_3) and 40±1.5° C (T_4) at a relative humidity of 50±5% and in natural climatic (T_5) conditions (dbt 36.6°C, wbt 24.6°C) for three hrs continuously to study the impact of temperature rise on pulonary dynamics, heat dissipation and antioxidant status. Physiological reactions (RR, RT and ST) were monitored at pre exposure and at hourly intervals during exposures, whereas oxygen consumption, heat loss through skin and pulmonary system, heat storage and blood antioxidant enzyme activity was recorded at pre exposure and 3 hr post exposure. Physiological reactions, oxygen consumption, heat loss through skin and pulmonary system increased significantly ($P<0.01$) due to exposure at different temperatures. Heat storage was maximum (2008.92 ± 148.11 KJ) at T_4 compared to others. Erythrocytes SOD and catalase activity increased significantly ($P<0.01$) due to heat and natural exposure and the percent increase was maximum at T_4 (THI > 90). SOD and catalase activity showed a significant ($P<0.01$) positive correlation with heat production, heat storage and heat loss through skin and pulmonary system. The study concludes that rise in temperature humidity index, negatively affect crossbred heifer's physiological functions

Key words : Oxygen consumption, Heat production, Thermal balance, Antioxidant enzyme

INTRODUCTION

The indigenous breeds of cattle are more acclimatized to wide range of weather conditions, but the crossbred cattle often show a decrease in the productive and reproductive efficiency during adverse climatic conditions (Jenkins and Ferrell 2004). With increase in the environmental temperature, the dissipation of heat by radiation, conduction, convection and evaporation increases. At higher temperature evaporative cooling through skin is the main avenue of heat dissipation. If the thermal balance is not maintained, the storage of heat starts in the animal body which causes an increased production of the free radicals and reactive oxygen species (ROS). When production of ROS is more than neutralization by antioxidant enzymes, a situation of oxidative stress is encountered, which may alters the cellular functions (Trevisan et al., 2001). The information regarding the antioxidants status and their relationship with thermal balance in domestic animals under heat stress is scanty. Keeping this in mind, trials were conducted on crossbred heifers exposed to heat stress in chamber. The change in antioxidant level was monitored and correlated with oxygen consumption, heat loss and storage, to elucidate the impact of heat stress on oxygen consumption, heat loss, storage and antioxidant enzymes in blood.

MATERIALS AND METHODS

Five healthy Karan Fries (Holstein Friesian X Tharparkar) heifers (age 18 to 24 month) were selected from the NDRI herd, and fed maize, jower, oat, silage ad libitum and concentrate mixture @1.5 kg/day under loose housing conditions. The drinking water was available round the clock for animals. These animals were subjected to five exposures viz. T_1-sham control, T_2-32±1.5°C (THI=80), T_3-35±1.5°C (THI=84.70), T_4-40±1.5°C (THI=91.80) and in natural exposure (T_5) i.e. open environment for three hours at a strach for three days consecutively and monitored for oxygen consumption, heat production, heat loss through skin and respiratory system, before exposure and

after end of three hour exposures. Blood samples were collected from jugular vein at the start and end of exposure for analysis of super oxide dismutase (SOD) and catalase enzymes. The physiological responses viz, respiration rate (RR), rectal temprature (RT) and skin temperature (ST) were recorded using standard methods at the pre exposure and at hourly interval during exposure.

Oxygen consumption was recorded using, modified method of Benedict Roth Collins Spirometer (Upadhyay and Madan, 1985) and heat production was calculated from oxygen consumption (Mclean, 1972). Heat storage was estimated from the changes in mean RT and ST over initial values at the end of 3 h exposure and specific heat of tissue (3.47KJ/ Kg/°C) and the live weight in Kg (Finch, 1985). Heat loss from skin through sweating was recorded using ventilated capsule method (McLean, 1963a) and from respiratory tract using differences in temperature and humidity in coming and outgoing air using digital hygrometer. Antioxidant enzymes i.e. catalase (EC1.11.1.6) and SOD (EC 1.15.1.1) activity in the erythrocytes were measured by using methods of Aebi (1984) and Marklund and Marklund (1974) respectively. The analysis of data was carried out using standard method (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

The mean oxygen consumption and heat production (Table-1) of heifers increased from pre exposure values of 1.79 to 2.23 l/min and 2197.40 to 2742.46KJ/h in$T_{2;}$ 1.74 to 2.31 l/min and 2133.57 to 2837.96 KJ/h in T_3 and 1.89 to 3.40 l/min and 2315.23 to 4176.29 KJ/h in $T_{4,}$ respectively. The magnitude of increase in oxygen consumption and heat production was significantly ($P<0.05$) higher due to exposures at different climatic chamber temperatures over pre exposure values. The pre exposure values of oxygen consumption were slightly lower compared to the values reported by Chikamune et al. (1986). This difference could possibly be ascribed to type of breed or body weight of animals. The increase in oxygen consumption after exposure appears to indicate increased demand of oxygen at tissue level due to a rise in THI.

Maximum heat loss (63.02%) through skin was registered during T_4 followed by T_5 (54.54%), T_3 (50.26%) and T_2 (39.2%) over initial values .The increase in heat loss through skin due to heat exposure

Table 1. Changes in oxygen consumption, heat production and heat loss applying different temperature treatments in Karan Fries heifers

Parameter			T1	T2	T3	T4	T5
Oxygen consumption	BE	l/ min	1.73 ±0.04	1.79 ±0.09	1.74 ±0.08	1.89 ±0.09	1.75 ±0.14
		cc/ w^{75}	23.65 ±0.97	24.55 ±1.78	23.70 ±0.26	25.63 ±0.74	23.81 ±1.67
	AE	l/ min	1.97 ±0.06*	2.23 ±0.15*	2.31 ±0.19*	3.40 ±0.24**	2.73 ±0.18**
		cc/ w^{75}	26.94 ±1.06	30.71 ±2.86	31.51 ±2.47	46.07 ±0.71	37.56 ±3.25
Heat production	BE	KJ/ hr	2123.75 ±54.20	2197.40 ±110.75	2133.57 ±101.41	2315.23 ±122.37	2148.30 ±176.32
		KJ/ hr/ w^{75}	29.04 ±0.19	30.14 ±2.19	29.09 ±1.20	31.45 ±0.91	29.23 ±2.06
	AE	KJ/ hr	2420.82 ±78.25*	2742.46±191.23*	2837.96±233.51*	4176.29±304.54**	1.17±224.53**
		KJ/ hr/ w^{75}	33.07 ±1.30	37.70 ±3.52	38.68 ±3.03	56.55 ±2.10	46.11 ±3.99
Heat Loss through skin	BE	KJ/ hr	5741.31 ±269.79	5961.39 ±129.41	5846.92±217.69	5923.90 ±236.51	5878.97±287.60
		KJ/ hr/ w^{75}	78.73 ±3.49	81.53 ±3.10	79.94 ±3.91	81.41 ±5.68	80.50 ±5.27
	AE	KJ/ hr	6283.57 ±339.47	8282.28±150.88**	8706.36±354.13**	9596.42±237.57**	990.80±104.97**
		KJ/ hr/ w^{75}	85.40 ±2.52	113.43 ±5.16	119.25 ±6.69	131.39 ±6.23	122.90 ±3.69
Heat loss through pulmonary system	BE	KJ/ hr	455.26 ±15.01	510.43 ±11.93	503.81 ±20.79	501.66 ±19.08	504.78 ±22.67
	AE	KJ/ hr	541.96 ±27.08	718.08 ±12.31**	771.65 ±34.01**	861.04 ±9.11**	786.62 ±9.88**

* BE - before exposure, AE- after exposure

The values are mean ± SE of five observations on five animals,

*,**indicates values differ significantly at ($P<0.05$) and ($P<0.01$) respectively.

in chamber was significantly (P<0.05) higher over pre exposed value .Heat loss through pulmonary system in KF heifers before exposure ranged from 455.26 to 504.78 KJ/hr. At T_4 a maximum (72.48%) increase in heat loss through pulmonary system was registered over pre exposure value.

The heat loss through skin at higher temperature occurs in zebu and crossbred cattle mainly through evaporation of sweat, which may depend upon the animal's ability to sweat. High ambient temperature causes an elevation of skin temperature and thus evokes a reflex sweating mechanism (Joshi et al., 1968a,b).In the present study a significant (P<0.05) positive correlation (r=0.85) between skin temperature and heat loss through skin was observed. Robertshaw (1966) also reported a positive relationship between rectal temperature and evaporation rate. Analysis of data indicated a significant (P<0.05) difference in sweating rate due to different treatments and time intervals. Upadhyay and Aggarwal (1997) also reported a major role of sweating in heat loss mechanism in crossbred cattle. During the present study the overall correlation coefficient between heat loss through skin and THI was found positively (P<0.05) correlated. Similar positive correlation among ambient temperature and heat loss through skin surface has been reported by Finch (1985).

The heat storage in heifers at different exposures (T_1-T_5) was significantly different (P<0.01). The analysis of data indicated a significant positive(r=0.87) correlation between THI and heat storage. The results of present study corroborates with the report of Finch et al. (1984) who reported a linearly higher heat storage with the increase in the intensity of solar radiation. Mc Lean (1963a, b) also reported increase in heat production at higher ambient temperature and humidity.

The mean pre exposure values of catalase and SOD (Table 2) varied from 149.01 to 155.46 µmol H_2O_2 /min/mg Hb and 3927.37 to 4319.92 U/min /g Hb respectively .The mean values of SOD activity increased with the increase in the exposure time at different chamber temperatures (Table 2). These results are in agreement with those reported by Kumar et al (2007) in cattle and buffaloes at natural acute heat exposure. Bernabucci et al. (2002) also reported a significant increase in SOD and catalase activity in transition dairy cows exposed to moderate heat stress. Correlation coefficients of SOD and catalase showed a significant (P<0.01) positive correlation with oxygen consumption, heat production, heat loss and heat storage. Analysis of variance of data showed a significant (P<0.05) difference in SOD and catalase enzyme levels among different exposures and intervals. These results are in agreement with those of Kumar et al. (2007) in cattle and buffaloes during natural exposure.

The mean values of respiration rate (RR) and rectal temperature (RT) in KF heifers have been presented in Fig 1. The highest increase in RR (171%) of KF heifers was observed in T_4 followed by T_3 (99%) and T_2 (41%) over pre exposure values. The increase in RR over the initial value after three hours of exposure was significantly (P<0.05) higher at all treatments. These results are in accordance with those reported by Spain and Spiers (1996) that RR of HF cows increased with increase of ambient temperature (21-29°C). A significant (r = 0.98) positive correlation coefficient was found among RR and THI. Similar results were also observed by Singh and Singh (2006) in KF heifers during solar exposure.

Table 2. Changes in SOD and Catalase activity in KF heifers during different treatments

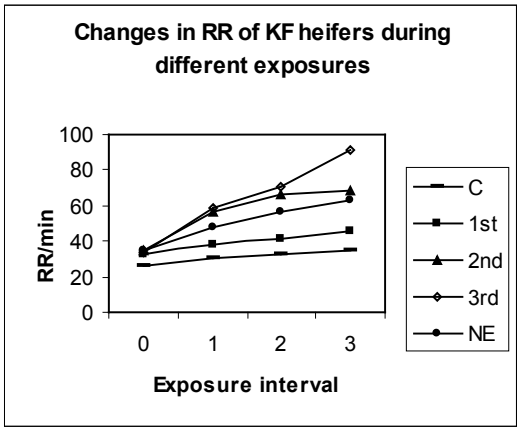
Treatments	Catalase(µmol H_2O_2/min/mgHb)		SOD(U/min/gHb)	
	BE	AE	BE	AE
T_1	155.46 ±1.94	164.17 ±1.84	4034.41 ±104.87	4185.20 ±93.41
T_2	150.34 ±2.54	167.22 ±2.76**	4030.97 ±103.29	4276.00 ±149.49
T_3	151.94 ±3.21	172.33 ±4.16**	3927.37 ±71.52	4401.97** ±114.92
T_4	149.01 ±2.92	224.95 ±5.49**	4319.92 ±165.85	5368.03 ±115.46**
T_5	152.37 ±4.81	169.56 ±3.15*	4062.72 ±92.78	4492.03 ±113.34*

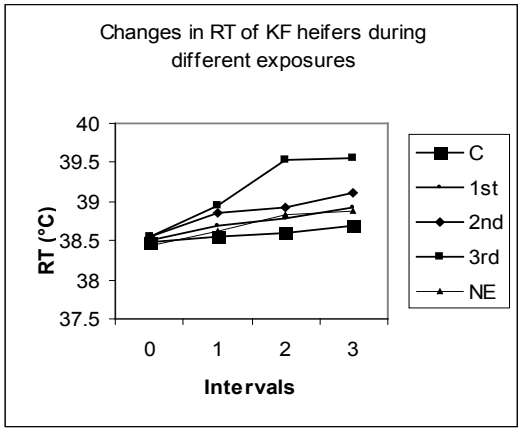
BE - before exposure, AE - after exposure

The values are mean ± SE of five observation on five animals.

***indicates values differ significantly at (P<0.05) and (P<0.01) respectively.

Fig.1. Changes in respiration rate and rectal temperature of KF cattle during different exposures





Note :- C : Indicates,T_1; Ist, T_2; 2nd, T_3; 3rd, T_4; and NE, T_5

The RT of KF heifers increased by 0.2, 0.42,0.56 and 1.02°C respectively in T_1, T_2, T_3 and T_4 after three hours exposure over the pre exposure values. Singh and Singh (2006) also reported a rise in rectal temperature in KF cattle at higher ambient temperature. The skin temperature was also increased with increase in exposure temperature from 35 to 40°C. The ST increased by 2.9, 3.5, 4.4, 5.3 and 4.5°C respectively at T_1, T_2, T_3, T_4 and T_5 after three hours exposure over the pre exposure values. These results corroborate with the findings of Ashour (1993) who reported an increase in skin temperature, which leads to heat storage in the animal's body. The study concludes that rise in temperature humidity index, negatively affect crossbred heifer's physiological functions.

REFERENCES

Aebi, H. 1984. Catalase in vitro . In: Methods in Enzymology (L. Packer, ed.). Vol. 105, Academic press, New York, pp. 121-126. .

Ashour, G. 1993. Impact of environmental temperature on heat storage in body of calf. J. Animal Prod. 64:716.

Bernabucci, U., B. Ranchi., N. Lacetara and A. Nardone. 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J. Dairy Sci., 85:2173-217

Chikamune, T., Y. Kanai., and H. Shimizu. 1986. Comparison of the effects of seasonal- climatic changes on thermo regulatory responses and plasma concentrations of thyroid hormones in swamp buffaloes and cattle. Japanese J. Zootech. Sci., 57(9): 778-784

Finch, V.A. 1985. Comparison of non evaporative heat transfer in different cattle breeds. Australian J. Agril. Res., 36: 497-508.

Finch, V.A., I.L. Bennett, and C.R. Holmes. 1984. Coat colour in cattle: effect on thermal balance, behaviour and growth, and relationship with coat type. J. Agric. Sci.(Camb), 102: 141-147.

Jenkins T. G. and C. L. Ferrell (2004) Preweaning efficiency for mature cows of breed crosses from tropically adapted *Bos indicus* and *Bos taurus* and unadapted *Bos taurus* breeds. Animal Prod. 82:1876-1881

Joshi, B.C., R.E. McDowell and D.P. Sadhu. 1968a. Body surface evaporation rates at low and high temperatures in Murrah buffaloes. J. Dairy Sci., 51: 1689-1692.

Joshi, B.C., R.E. McDowell and D.P. Sadhu. 1968b. Body surface evaporation rates at low and high temperatures for Gir and Haryana Cattle. J. Dairy Sci., 51: 1693-1697.

Kumar, A., P. Kumar and S.V. Singh. 2007. Oxidative stress marker's profile in erythrocytes of natural and heat exposed cattle and buffaloes. Indian J. Dairy Sci. 60: 114-118.

Marklund, S. and G. Marklund. 1974. Involvement of superoxide dismutase anion radical in autoxidation of pyrogallol and a convenient assay for Superoxide dismutase. European J. Biochem.47: 469-474.

McLean, J.A. 1972. On the calculation of heat production from open circuit calorimetric measurements. British J. Nutr., 27: 597-600.

McLean, J.A. 1963a. Measurement of cutaneous moisture vaporization from cattle by ventilated capsules. J. Physiol., 167: 417-426

McLean, J.A. 1963b. The partition of insensible losses of body weight and heat from cattle under various climatic conditions. J. Physiol., 167: 427-44

Robertshaw, D. 1966. Sweat gland functioning of domestic ruminants. Proc. of 4th Intern. Biometeoro.Confer. N. J: New Brunswick..

Singh, R. and S.V. Singh. 2006. Circardian changes in peripheral temperature and physiological responses under solar exposure and shed during summer in Karan Fries heifers. Indian J. Anim. Sci., 76(8): 605-608.

Snedecor, G.W. and W.G. Cochran. 1994. Statistical methods. Oxford and IBH Pub. Co., New Delhi.

Spain, J.N. and D.E. Spiers. 1996. Effect of environmental temperature variation on ear and rump of buffalo calves. J. Dairy Sci. 79: 639.

Trevisan, M. , R. Browne., M.. Ram., P. Muti., J. Freudenheim., A. N.Carosella and D. Armstrong. 2001. Correlates of markers of oxidative status in general population. Am. J. Epidemiol. 154: 348-356.

Upadhyay, R.C. and A. Aggarwal. 1997. Pulmonary and skin evaporative heat loss during exercise in hot dry conditions in crossbreds. Indian J. Anim. Sci., 67(1): 51-53.

Upadhyay, R.C. and M.L. Madan. 1985. Studies on blood and base status and muscle metabolism in working bullocks. Animal Prodn., 40: 11-16.

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Performance of Angora Rabbits Fed on Biul (*Grewia optiva*) Leaves and Kudzu (*Puereria thunbergiana*) Vine

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ABSTRACT : Forty eight adult male German angora rabbits divided in three equal groups of 16 each were fed in group T_1 a conventional hay , in T_2 group wilted biul (*Grewia optiva*) leaves and in group T_3 kudzu (*Puereria thunbergiana*) vine as a roughage source ad lib. Rabbits in all the groups were offered 150 g of concentrate in the mash form. Feed intake and body weight was monitored fortnightly for a period of 75 days. The rabbits were sheared before and after the experiment. Average initial body weights of rabbits were 2.68, 2.71 and 2.70 kg in T_1, T_2 and T_3 groups, respectively. These were 2.94, 3.19 and 3.11 kg, respectively at the end of the experiment. The wool yield was 129.0, 140.0 and 143.3 g in T_1, T_2 and T_3 groups with the significant ($P<0.05$) differences among treatment, although there were no differences between T_2 and T_3 groups. The significant ($P<0.05$) differences were recorded for daily roughage and dry matter intake between groups with the respective value of 21.2 and 148.2 , 53.3and 169.2 and 54.8 and 165.6 g in T_1, T_2 and T_3 groups. Significant ($P < 0.05$) differences were recorded for the digestibility of crude fibre, ether extract, acid detergent fibre and cellulose. It was concluded that feeding of biul (*Grewia optiva*) leaves and kudzu (*Puereria thunbergiana*) vine as potential roughage sources gave higher wool production from angora rabbits.

Key words: Wool production, Biul leaves, Kudzu vine, Angora rabbit, Digestibility, DMI

INTRODUCTION

Feed cost is one of the major factors amounting above 50-60% of total production cost and governs the success of rabbit production. Small savings made in terms of feed cost is of prime concern in rabbit farming. Rabbits can be successfully raised on diets consisting entirely of forages and cereal by products (Cheeke, 1986). The biul (*Grewia optiva*) and kudzu-vine (*Puereria thunbergiana*) are nutritious forage available during lean period i.e. September to January in sub-temperate climatic conditions when there is acute shortage of green leaves. Biul is highly valued as ruminant fodder and has been successfully incorporated in Angora rabbit diets. Kudzu-vine has been successfully utilized in broiler rabbit diet upto 25% level (Singh et al., 1994). However, the reports on the utilization of these two forages in the diet of high wool producing German Angora rabbits are scarce. Therefore, this experiment was conducted to study the comparative potential of these two forages in angora rabbit feeding as compared to conventional roughage (dry hay).

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MATERIALS AND METHODS

Forty eight adult male rabbits of German Angora breeds divided into 3 groups of sixteen rabbits in each. Group 1 (control, T_1) were offered conventional dry hay, whereas groups T_2 and T_3 were given ad lib biul (*Grewia optiva*) leaves and kudzu-vine (*Puereria thunbergiana*), respectively as a source of roughage for 75 days experimental period. Concentrate was offered @ 150g/ day with a standardized scoop in the mash form. Dry matter intake/day was monitored weekly by taking total concentrate and roughage offered and the residue left. The ingredient composition of pelleted concentrate mixture was groundnut cake 6, mustard cake 6, sunflower cake 6, soyaflakes 6, maize 20, barley 20, rice phak 25, fish meal 4, molasses 5, mineral mixture 1 and common salt 1

parts. The experiment was conducted for 75 days. The animals were weighed and sheared before and after the experiment. At the end of the feeding trial (70th day of feeding) the digestibility trial was conducted for five days on five representative rabbits in each group. During the trial period total concentrate and grass intake were recorded, faeces voided were collected and weighed daily. The wool samples were taken from the dorsal surface of each rabbit for quality evaluation. Fresh faecal samples were collected for crude protein and dry matter estimation. The feed, grass and faeces were analyzed for proximate principles (AOAC, 1990) and fiber fractions (Goering and Van Soest, 1970). Wool samples collected were studied for staple length (cm) with scale after fixing sample on the board and fibre diameter (μ), pure fiber (%) and guard hair (%) were analyzed with lanometer as per standard procedures. The dry matter required for producing 100 g wool was calculated for studying comparative economics in two regimes. The purchased cost of concentrate and the prevalent cost of roughage and fodder were used for calculating the feed cost. The data were analyzed statistically (Snedecor and Cochran, 1994).

Table 1. Chemical composition (% DM basis) of concentrate, local grass, biul leaves and kudzu-vine fed to German Angora rabbits

Nutrients	Concentrate	Local grass	Biul leaves	Kudzu-vine
Crude protein	17.76	9.97	16.27	18.12
Crude Fiber	10.21	26.8	22.91	32.9
Ether extract	1	2.3	2.16	1.69
Total ash	8.8	7.03	6.46	4.87
Nitrogen free extract	62.23	53.9	52.2	42.42
Acid detergent fibre	24.7	44.3	35.11	53.71
Cellulose	19.3	35.22	28.31	40.32
Lignin	5.4	9.08	6.8	13.39

RESULTS AND DISCUSSION

The concentrate consisted of 17.7% crude protein, 10.2 % crude fiber, 24.7 % acid detergent fiber, 19.3 % cellulose and 5.4 % lignin. The composition of diet is as per the NRC (1977) and is sufficient to take care of the nutrient requirements of rabbit. The composition (Table 1) of local grass revealed 9.97 % crude protein, 26.80 % crude fiber, 44.30 % acid detergent fiber, 35.2 % cellulose and 9.08 % lignin and is in close agreement with earlier reports (Bhatt et al., 2005) .The biul leaves and kudzu vine consisted of 16.27 and 18.12 percent crude protein, 22.91 and 32.90 percent crude fiber, 35.11 and 53.71 percent acid detergent fiber, 28.31 and 40.32 percent cellulose and 6.80 and 13.39 percent lignin, respectively. The chemical composition of biul leaves and kudzu-vine differ slightly from those reported by Singh and Negi (1986) and Singh et al., 1994. These changes in the composition may be due to the differences in harvesting stage of the forage.

The initial body weight (Table 2) of rabbits in T_1, T_2 and T_3 groups were 2.68±0.08, 2.71±0.09 and 2.70±0.1 kg, respectively and the differences between groups were statistically non- significant. The body weight of rabbits after 75 days of experimental period was highest in T_2 (3.19±0.09) followed by T_3 (3.11±0.09) and T_1 (2.94±0.1 kg) groups and were significantly ($P<0.05$) higher in T_2 and T_3 as compared to T_1 group. The average wool yield was significantly ($P<0.05$) higher in both the treatment groups (143.3±6.7 in T_3 and 140.0±5.9 in T_2) as compared to control (T_1) (129.0±4.2 g).Similar results were reported by Singh and Negi (1986) when they fed ad lib biul leaves to russian Angora rabbits though the wool production was lower in their experiment may be due to the poor wool production potential of russian Angora. In Angora rabbits there is no report on feeding kudzu-vine while in broiler rabbits Singh et al., (1994) reported encouraging results by feeding kudzu-vine leaves upto 50% level. High protein and energy yielding components in test roughages may be factors responsible for higher wool yield.

Table 2. Body weight, wool yield and wool quality of German angora rabbits

Parameters	conventional dry grass hay (T_1)	Biul (*Grewia optiva*) (T_2)	Kudzu-vine (*Puereria thunbergiana*) (T_3)	SEM
No. of rabbits	16	16	16	
Body weights				
Initial body weights (Kg)	2.68	2.71	2.7	0.09
Final body weights (Kg)	2.94[a]	3.19[b]	3.11[b]	0.09
Weight change (g)	260	480	410	
Wool yield (g) quality				
Av. Wool yield/shearing	129.0[a]	140.0[b]	143.3[b]	5.9
Wool yield/KgW$^{0.75}$	57.4	58.6	61.2	
Staple length (cm)	4.92[a]	5.38[b]	5.48[b]	0.16
Fiber diameter (m)	13.34	13.98	13.77	0.37
Pure fiber	5.56	5.03	6.73	0.67
Guard hair	3.25	4.43	3.3	0.5

Values with different superscripts in a row differ significantly (P<0.05)

Plane of nutrition (Table 3) revealed significantly (P<0.05) higher roughage intake in T_3 and T_2 groups as compared to control (T_1) indicating higher palatability of biul leaves and kudzu-vine in comparison to grass. The lower intake of concentrate in test groups (T_3 and T_2) showed higher replacement value of these fodders for concentrates. The total dry matter intake per day was significantly (P<0.05) higher in T_2 and T_3 groups as compared to T_1 group. The proportion of roughage in DMI was highest in T_3 (33.09) followed by T_2 (31.50) and lowest in T_1 (14.30%) groups indicating comparatively higher palatability of these roughages as compared to grass.

Wool quality attributes revealed improved staple length and wool/fibre diameter in test groups as compared to control, which were well correlated with improved wool yield of these respective groups as after hair density these two parameters are among the major governing the wool yield.

Digestibility coefficient data revealed significant (P<0.05) differences for crude fiber, ether extract, acid detergent fiber and cellulose digestibility whereas non-significant differences were observed for dry matter, crude protein and nitrogen free extract digestibility. Crude fibre digestibility was highest (40.63±2.93) in T_3 group followed by T_2 (32.73±7.48) and lowest in T_1 (12.20±2.83%) group. Acid detergent fiber and cellulose digestibility in T_3 group were also highest among the groups indicating that the fiber of kudzu-vine is more digestible. Singh et al., (1994) have also reported increased crude fiber digestibility by feeding kudzu-vine to broiler rabbits. The digestibility of crude protein, crude fiber, acid detergent fiber and cellulose in biul leaves fed group (T_2) was slightly lower than kudzu vine (T_3) fed group but statistical differences were non- significant. In agreement to our findings Singh and Negi (1986) also reported almost similar crude protein and crude fiber digestibility by feeding biul leaves to Angora rabbits. The ether extract digestibility was improved significantly (P<0.05) in test forages as compared to control group. The digestibility of ether extract is related to the digestibility of crude fiber in the diet and is due to the fact that the major part of the plant lipid is always associated with cell walls in non added fat diets (Xicatto, 1998). Significant (P<0.05) differences were observed for the nitrogen balance in different groups.

Dry matter intake for producing 100 g wool was 8.62 in T_1, 9.06 in T_2 and 8.67 kg in T_3 group with the respective concentrate intake value of 7.38 in T_1, 6.20 in T_2 and 5.79 kg in T_3 group indicating the T_3 group is most economical.

It is concluded that biul leaves and kudzu-vine can be used safely as forage sources in Angora rabbit feeding.

Table 3. Plane of nutrition and digestibility coefficient of nutrients in German Angora rabbits fed on biul and kutzu based diets

Parameters	conventional dry grass hay (T_1)	Biul (*Grewia optiva*) (T_2)	Kudzu-vine (*Puereria thunbergiana*) (T_3)	SEM
Plane of Nutrition				
Concentrate intake (g)	127	115.9	110.8	5.6
Roughage intake (g)	21.2[b]	53.3[a]	54.8[a]	2.1
Dry matter intake (g)	148.2[b]	169.2[a]	165.6[a]	3.4
Proportion of concentrate (%) in DMI	85.69	68.49	66.91	
Proportion of roughage (%) in DMI	14.3	31.5	33.09	
Dry matter used/100 g wool produced (kg)	8.62	9.06	8.67	
Concentrate required for producing/ 100 g wool (kg)	7.38	6.2	5.79	
Digestibility (%)				
DM	63.12	61.02	62.32	1.28
CP	74.05	71.17	75.78	0.99
CF	12.20[b]	32.73[a]	40.63[a]	3.4
EE	21.92[c]	50.14[b]	70.21[a]	4.21
NFE	73.82	69.2	68.92	1.31
ADF	27.86[b]	38.58[a]	45.56[a]	3.75
Cellulose	31.92[b]	32.49[b]	44.50[a]	3.12

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REFERENCES

AOAC. 1990. Official Methods of Analysis,(15th Ed.) Vol.2. Association of Official Analytical Chemists, Washington DC.

Bhatt, R.S., S.R. Sharma, Umesh Singh, and K.S. Risam. 2005. Effect of concentrate levels on the production performance of Chinchilla rabbits. Indian J. Anim. Sci. 75 (3): 312-315.

Cheeke, P.R. 1986. Potential of rabbit production in tropical and sub tropical agricultural systems. J. Anim. Sci. 63: 1581-86.

Goering, H.K. and P.J. Van Soest. 1970. Forage Fiber Analysis. Agricultural Handbook No. 379, ARS, USDA, Washington DC.

NRC. 1977. Nutrient Requirement of Rabbits. National Academy of Sciences, Washington, D.C

Singh, B., and S.S. Negi. 1986. Effect on wool production of supplementing black locust (*Robinia psuedocacia*) and biul (*Grewia optiva*) leaves ad lib to a concentrate diet of Angora rabbits. J. Appl. Rabbit Res. 8: 159-163.

Singh, R, R.K. Sawal, V. Bhasin and D.R. Bhatia. 1994. Nutrient digestion by rabbits on diets containing kudzu hay. Indian J. Anim. Sci. 67: 120.

Snedecor, G.W. and W.G. Cochran. 1994. Statistical Methods. 8th Ed.) Oxford and IBH Publishing Co., Calcutta.

Xicatto,G. 1998. Fat Digestion. In the Nutrition of the Rabbit. Blas, C. de and Wiseman, J. eds CAB international Wallingford Oxon OX 10 8DE UK.

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Effect of EDTA Supplementation on Phytate Phosphorus Utilization and Efficiency of Microbial Phytase in Laying Hens

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ABSTRACT : To evaluate the effects of ethylene diamine tetra acetate (EDTA) on phytate P utilization and possible synergism between EDTA and microbial phytase (MP), an experiment was conducted using 192 Hyline W36 laying hens, using a completely randomized design with a 3×2 factorial arrangement (0, 0.1 and 0.2 % EDTA and 0 and 300 FTU microbial phytase). Dietary treatments were a corn-soybean meal basal diet (0.1% available P, 3.8% Ca, and 15% CP) supplemented with different levels of EDTA and MP. Hens fed phytase supplemented diets had significantly higher hen day egg production, feed intake, egg weight and egg mass. They utilized feed more efficiently. Phytase supplementation increased egg specific gravity, haugh unit, shell thickness and dry shell weight. EDTA didn't influence any of the measured parameters. Significant interactions were observed between phytase and EDTA on egg production, egg weight, egg specific gravity, haugh unit, dry shell weight and serum P. Results suggested that EDTA was not capable of increasing phytase efficiency.

Key words : Phytate Phosphorus EDTA, Laying Hens

INTRODUCTION

A significant portion of the P in mature cereal grains and oilseeds is present as phytate P and inositol (Maga, 1982). Nonruminants have insufficient phytase to digest phytate (Nelson et al., 1971), hence inorganic P is often added to their diets. Physical methods such as soaking, drying, germination (Jongbloed et al., 1991), supplementation of diets with exogenous microbial phytase (Kornegey, 2001) and Vitamin D (Mitchel and Edwards, 1996) were found to be effective in increasing phytate phytate P availability.

Synergistic effects between microbial phytase and Vit D analogs (Biehl et al., 1995; Mitchel and Edwards.,1996) and phytase and citric acid (Boling et al .,2000) have been reported in broiler chicks, but not much work has been done to determine if organic acids, other than citric, will improve phytate P utilization in poultry. EDTA is a strong chelating agent that has been shown to improve absorption of some minerals in poultry diets. EDTA improved Zn absorption when supplemented to turkey poult (Kratzer et al., 1959) and chick (O'Dell et al., 1964) diets containing plant protein. Increased in vitro phytate P hydrolysis in canola meal was observed when it was added in conjunction with a microbial Phytase (Maenz et al., 1999). Rafacz-Livingston et al. (2005) reported in contrary that EDTA was not able to increase phytate P utilization in broiler chicks fed corn soybean meal diet. Interaction between EDTA and phytase have not been assessed in laying hens therefore the present study was aimed to investigate the effects of EDTA on phytate P utilization and the possible synergistic effect between EDTA and microbial phytase in laying hens.

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MATERIALS AND METHODS

A total of 192 Hyline-W36 laying hens were devided in a completely randomized design with a 3×2 factorial arrangement (0, 0.1 and 0.2 % EDTA and 0 and 300 FTU microbial phytase). Each of the six dietary treatments was fed to four replicate groups of 8 hens from 53 to 63 wk of age. Laying hens were placed in wire layer cages (50×50×40 cm) and provided free access to water and a balanced unmedicated corn-soybean-meal based mash layer diet that met or exceeded the NRC (1994) recommendations. Hens were exposed to a 16 h light: 8 h dark lighting schedule. Dietary treatments were 1) P-deficient negative control diet (0.1 % P) (NC), 2) NC+ 300 FTU MP 3) NC+0.1 % EDTA 4) NC+0.1 % EDTA and 300 FTU MP 5) NC+0.2 % EDTA 6) NC+0.2 % EDTA+300 FTU MP per Kg of diet (Table-1). The supplied microbial phytase[1] had 1000 IU active phytase per gram. Disodium salt of EDTA was used as EDTA source. Hen day egg production (HDP) and egg weight (EW) were recorded daily and egg mass (EM) was calculated by multiplying EW by HDP. Feed consumption and feed efficiency were recorded at 2 week intervals

Table 1. Composition and nutrient content of the experimental diets

Ingredients (%)	**Treatment**					
	1	**2**	**3**	**4**	**5**	**6**
Corn	66.44	66.38	66.34	66.28	66.24	66.18
Soybean meal	21.12	21.13	21.14	21.15	22.16	22.17
Soybean oil	1.2	1.23	1.24	1.25	1.27	1.28
Limestone	8.12	8.12	8.12	8.12	8.12	8.12
Oyster shell	2	2	2	2	2	2
Common salt	0.15	0.15	0.15	0.15	0.15	0.15
$NaHCO_3$	0.36	0.36	0.31	0.31	0.26	0.26
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Meth	0.1	0.1	0.1	0.1	0.1	0.1
EDTA	-	-	0.1	0.1	0.2	0.2
Phytase	-	0.03	-	0.03	-	0.03
Nutrient (calculated)						
ME(Kcal/kg)	2817	2817	2817	2817	2817	2817
CP (%)	15	15	15	15	15	15
Ava.P (%)	0.1	0.1	0.1	0.1	0.1	0.1
Tot.P (%)	0.32	0.32	0.32	0.32	0.32	0.32
Ca (%)	3.8	3.8	3.8	3.8	3.8	3.8
Meth+Cys (%)	0.6	0.6	0.6	0.6	0.6	0.6
Lysine (%)	0.74	0.74	0.74	0.74	0.74	0.74

1.Provided (/kg of diet): vitamin A (retinyl acetate), 8,000 IU; cholecalciferol, 2,200 ICU; vitamin E (DL-tocopheryl acetate), 8 IU; vitamin B_{12}, 0.02 mg; riboflavin, 5.5 mg; D-pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; vitamin B1 (thiamin mononitrate), 1 mg; pyridoxine, 2.2 mg; D-biotin, 0.05 mg; and vitamin K (menadione sodium bisulfate complex), 2 mg.

2.Provided (/kg of diet): manganese, 65 mg; iodine, 1 mg; iron, 55 mg; copper, 6 mg; zinc, 55 mg; and selenium, 0.3 mg.

Egg shell thickness (ET), dry shell weight, shell ash, egg specific gravity and haugh unit were measured biweekly. ET was measured with a digital micrometer after the eggs had been emptied. Shell thickness was measured as an average of 3 measurements taken at equidistant points along the equator of each egg. Haugh units were calculated using the HU formula (Eisen et al., 1962) based on the height of albumen determined by a micrometer and egg weight.

Dry shell weight was determined by breaking the egg and separating the egg shell from the liquid content. The egg shells were then dried overnight for 24 h in a oven at 100°C. Dry shell weight was expressed as a percent of whole egg weight. Dried shells were ashed in a muffle furnace at 600° C for 12h to measure shell ash. Shell ash was expressed as a percent of dried shell.

Egg specific gravity was determined using 11 gradient saline solutions varying in specific gravity from 1.050 to 1.140 at 0.004 unit increments (Holder and Bradford, 1979).

At the termination of experiment, 2 birds from each replicate were selected randomly and 5 ml of blood sample was taken from their wing vein. Serum concentration of Ca and P were measured by colorimetric method (AOAC 1995,) and the activity of alkaline phosphatase (ALP) was determined by commercial kit (Diagnostica. Cat No. 10-508, 525).

All the hens were weighed at beginning and termination of experiment. Data were statistically analyzed (SAS Institute, 1990) involving a factorial arrangement of main factor (EDTA and phytase levels) in a completely randomized design.

RESULTS AND DISCUSSIONS

The addition of phytase to diets containing 0.1% available P (aP) significantly increased hen day egg production ($P<0.0001$), feed consumption ($P<0.0001$), egg weight ($P<0.0001$), and egg mass ($P<0.0001$). Hens received diets with phytase supplementation utilized their feed more efficiently ($P<0.0001$). Different levels of EDTA didn't affect any of the production parameters (Table 2).

Table 2. Effect of phytase and EDTA supplementation on hen day egg production, feed intake, feed: gain, egg weight and egg mass.

Treatment						
EDTA (%)	**Phytase (FTU/kg)**	**Egg production (%)**	**Feed intake (g/d/hen)**	**Feed: gain (g/g)**	**Egg weight (g)**	**Egg mass (g)**
0	0(negative control)	37.34^{c}	83.50	4.75	51.38^{e}	31.03
0	300	80.51^{a}	100.37	2.22	59.77^{a}	46.10
0.1	0	37.78^{c}	81.17	4.52	52.96^{d}	29.69
0.1	300	81.27^{a}	99.35	2.09	58.05^{b}	47.57
0.2	0	42.22^{b}	80.23	4.35	54.59^{c}	31.40
0.2	300	77.77^{a}	98.87	2.22	59.29^{ab}	44.95
SEM	3.22	2.39	0.26	0.66	1.45	
				Main effect		
EDTA	0	58.92	91.94	3.48	55.57	38.56
	0.1	59.75	90.26	3.31	55.51	38.63
	0.2	60.00	89.55	3.28	56.94	38.17
Phytase	0	39.11^{b}	81.64^{b}	4.54^{a}	52.98^{b}	30.71^{b}
	300	80.00^{a}	99.53^{a}	2.18^{b}	59.04^{a}	46.21^{a}
				Probabilities		
EDTA		0.6505	0.3851	0.1898	0.0092	0.9074
Phytase		0.0001	0.0001	0.0001	0.0001	0.0001
EDTA×Phytase		0.0011	0.8740	0.2192	0.0007	0.1514

Means with no common superscript differ significantly ($P<0.05$)

Phytase supplementation to a low P diets has positive effects on egg production, egg mass and egg weight by improving P use (Van Der Klis et al., 1996; Gordon and Roland, 1997, 1998; Li et al., 1998; UM and Paik, 1999; Boling et al., 2000; Jalal and Scheideler, 2001; Roland et al., 2003; Keshavarz, 2003). This may be due to improvement in availability of some other nutrients, such as energy and amino acids. Namkung and Lesson (1999) also reported that phytase supplementation improved AME and digestibilities for some amino acids such as Val and Ile in broilers.

Egg production and egg weight significantly ($P<0.0007$) increased when EDTA level of diets without phytase increased from 0 to 0.2 % (Table 2). Addition of phytase to low P diets resulted in higher egg specific gravity ($P<0.0001$), thicker shells ($P<0.0001$), higher haugh unit ($P<0.0001$), and higher dry shell weight ($P<0.0002$) (Table 3). EDTA failed to have any significant effect on measured egg quality parameters. Gordon and Roland (1998) also reported that phytase supplementation increased egg specific gravity. Higher shell weight and shell thickness observed in phytase supplemented diets confirm the egg specific gravity results. It seems that phytase supplementation improved egg quality parameters (specific gravity, shell thickness and shell weight) by improving Ca availability. Gordon and Roland (1998) reported that phytase improved Ca utilization.

Significant interactions were observed between phytase and EDTA on specific gravity ($P<0.0055$), haugh unit ($P<0.0417$) and dry shell weight ($P<0.0002$); inclusion of EDTA had a negative effect on all mentioned parameters. It seems that in addition to a numerical depression in feed intake due to negative effects of EDTA, depression in specific gravity, haugh unit, and shell weight in hens

Table 3. Effects of Phytase and EDTA on egg specific gravity, shell thickness, dry shell weight and shell ash.

Treatment						
EDTA (%)	**Phytase (FTU/kg)**	**Egg specific gravity**	**Shell thi ckness (mm)**	**Haugh unit**	**Dry shells weight (%)**	**Shell ash (%)**
0	0(negative control)	1.060^{c}	0.264	79.03^{a}	9.62^{b}	65.65
0	300	1.070^{a}	0.284	77.40^{ab}	10.63^{a}	71.73
0.1	0	1.065^{bc}	0.269	81.19^{a}	10.27^{ab}	67.95
0.1	300	1.068^{ab}	0.277	72.99^{bc}	10.31^{ab}	65.81
0.2	0	1.064^{bc}	0.263	79.41^{a}	10.08^{b}	69.07
0.2	300	1.068^{ab}	0.273	71.35^{c}	10.20^{b}	71.38
SEM	0.001	0.004	1.68	0.23	2.27	
			Main effect			
EDTA	0	1.065	0.274	78.21	10.12	68.69
	0.1	1.067	0.273	77.09	10.29	66.88
	0.2	1.066	0.268	75.38	10.14	70.22
Phytase	0	1.063^{b}	0.265^{b}	79.88^{a}	9.99^{b}	67.56
	300	1.069^{a}	0.278^{a}	73.91^{b}	10.38^{a}	69.64
			Probabilities			
EDTA		0.351	0.1529	0.1559	0.3293	0.2475
Phytase		0.0001	0.0001	0.0001	0.0002	0.2019
EDTA×Phytase		0.0055	0.1498	0.0417	0.0002	0.1224

Means with no common superscript differ significantly ($P<0.05$)

received EDTA may be partly due to a probable decrease in the availability of calcium. The Na ion of EDTA could be replaced by available Ca in gastrointestinal tract and increase Ca excretion (Mellau and Jorgensen, 2003).

Serum ALP significantly (P<0.0432) decreased in hens received phytase supplemented diets; they also had significantly higher serum P (P<0.0001) concentration. Neither serum ALP, nor serum P concentrations were affected by different levels of EDTA. Addition of both EDTA and phytase had no significant effect on serum Ca concentration (Table 4).

Diet supplemented with phytase had significantly lower concentration of ALP. Phytase supplementation of low aP diets resulted in lower concentration of serum ALP in broiler chicks (Viveros et al., 2002; and Brenes et al., 2003). ALP is zn^{++} containing metalloenzyme that has a key roll in bone mineralization. Decreased blood aP level by any reason, will increase ALP activity. It seems that phytase increased plasma P concentration (Table 4) through liberation of phytate P and consequently resulted in decreased ALP activity. A significant interaction (P<0.0204) was observed between phytase and EDTA on serum P. Addition of both levels of EDTA significantly (P<0.0204) decreased serum P in phytase supplemented diets. Results obtained suggested that EDTA was not capable of increasing microbial phytase efficiency.

Table 4. Effects of phytase and EDTA on serum alkaline phosphatase, Ca, P and bodyweight changes

Treatment EDTA (%)	Phytase	Alkaline phosphatase (u/l)	Ca (%)	P (%)	Body weight changes
		(FTU/kg)			
0	0(negative control)	223.50[a]	15.25	1.18[c]	-20.49
0	300	135.75[c]	16.54	6.03[a]	58.75
0.1	0	233.00[a]	14.05	1.27[c]	15.50
0.1	300	156.75[bc]	16.00	3.93[b]	44.79
0.2	0	193.50[ab]	16.63	1.96[c]	-4.24
0.2	300	177.25[b]	17.58	4.55[b]	57.43
SEM	21.50	1.34	0.41	14.33	
			Main effect		
EDTA	0	179.62	15.89	3.61	19.13
	0.1	194.88	15.02	2.60	30.15
	0.2	185.38	17.10	3.25	26.60
phytase	0	216.60[a]	15.31	1.47[b]	-3.08[b]
	300	156.58[b]	16.70	4.84[a]	53.66[a]
			probabilities		
EDTA		0.1278	0.3241	0.0693	0.8364
Phytase		0.0432	0.2208	0.0001	0.0005
EDTA×Phytase		0.0703	0.9316	0.0204	0.4088

Means with no common superscript differ significantly (P<0.05)

REFERENCES

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arilngton, VA.

Biehl, R.R., D.H. Baker, H.F. Deluca.1995. 1 hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese utilization in chicks fed soy-based diets. J. Nutr. 125: 2407-2416.

Boling, S.D., D.M. Webel, I. Marromichalis, C.M. Parsons, D.H. Baker. 2000. The effect of citric acid on phytate phosphorus utilization in young chicks and pigs. J. Anim. Sci. 78: 682-689.

Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro, C. Braro. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol. 110: 201-219.

Eisen, E. J., B.B. Bohren, and H. E. McKean. 1962. The haugh unit as a measure of egg albumen quality. Poultry Sci. 41: 1461–1468.

Erdman, J. W. 1979. Oilseed phytates: Nutritional implications. J. Am. Oil Chem. Soc. 56:736–741.

Gordon, R.W., and D.A. Roland, Sr. 1997. Performance of commercial laying hens fed various phosphorus concentrations with and without supplemental phytase. Poult. Sci.76: 1172-1177.

Gordon, R. W., and D. A. Roland, Sr. 1998. Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. Poult. Sci. 77: 290-294.

Holder, D. P., and M. V. Bradford. 1979. Relationship of specific gravity of chicken eggs to number of cracked eggs and percent shell. Poult. Sci. 58: 250-251.

Jalal, M.A., and S.E. Scheideler. 2001. Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poult. Sci. 80: 1463-1471.

Jongbloed, A.W., P.A. Evert, P.A. Kemme. 1991. Phosphorus availability and requirements in pigs. In: Haresign, W., and Cole D.J.A. (eds). Recent Advances In Animal Nutrition. Butterworths, London,pp: 65-80.30:1126

Keshavarz, K. 2003. The effect of different concentrations of nonphytate phosphorus with and without phytase on the performance of four strains of laying hens. Poult. Sci. 82:71-91.

Kornegey, E.T. 2001. Digestion of phosphorus and other nutrients: The role of phytase and factors influencing their activity. In: M.R. Bedford and G.G.Partridge (eds). CABI. The United Kingdom, pp: 237-272

Kratzer, F.H., J.B. Alfred, P.N. Davis, B.J. Marshall, P. Vohra, 1959. The effect of autoclaving soybean protein and the addition of ethylene diamine tetra acetic acid on biological availability zinc for turkey poults. J. Nutr. 68: 313-316.

Li, D., X. Che, Y. Wang, C. Hong, P.A. Thacker. 1998. Effect of microbial phytase, Vitamin D_3 and citric acid on growth performance and phosphorus, nitrogen and calcium digestibility in growing swine. Anim. Feed Sci. and Technol. 73: 173-186.

Maenz, D.D., C.M. Engele-Schann, R.W. Newkirk, H.L. Classen. 1999. The effect of minerals and mineral chelators on the formation of phytate-resistant and phytase-susceptible forms of phytic acid in solution and in slurry of canola meal. Anim. Feed Sci. and Technol. 77: 177-192

Maga, J. A. 1982. Phytate: its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. J.Agric. Food Chem. 30: 1–9.

Mellau, L.S., and R.J. Jorgensen. 2003. Does EDTA-infusion affect calcium homeostasis leading to increased resistance to challenge Acta. Vet. Scand. Suppl. 97: 29-34

Mitchel, R.D., and H.M. Edwards. 1996. Additive effects of 1, 25 dihydroxycholecalciferol and phytase on phytate phosphorus utilization and related parameters in broiler chicken. Poult. Sci. 75: 111-119.

Namkung, H. and S. Lesson. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci. 78: 1317-1319.

NRC. 1994. Nutrient requirements of poultry. (9th Ed.) National Academy Press, Washington, D.C., U.S.A.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. J. Nutr. 101: 1289–1294.

O'Dell, J.M., B.L. Yohe, J. E. Savage. 1964. Zinc availability in the chick as affected by phytate, calcium and ethylene diamine tera acetate. Poult. Sci. 43: 415-419.

Rafacz-Livingston, K.A., C. M. Parsons, and R.A. Jungk. 2005. The effects of various organic acids on phytate phosphorus utilization in chicks. Poult. Sci. 84: 1356-1362.

Roland, D. A., Sr., H. A. Ahmad, S. S. Yadalam, T. Sefton. 2003. Effect of nongenetically modified phytase supplementation on commercial Leghorns. J. Appl. Poult. Res.12: 257-263.

SAS Institute. 1990. SAS/STAT User's Guide: Statistics. Release 6.04 ed. SAS Institute Inc., Cary, NC

UM, J.S., and I.K. Paik. 1999. Effects of microbial phytase supplementation on egg production, eggshell quality and mineral retention of laying hens fed different levels of phosphorus. Poult. Sci. 78: 75-79.

Van der Klis, J. D., H. A. J. Versteegh, P. C. M. Simons. 1996. Effectiveness of natuphos phytase in improving the bioavailabilities of phosphorus and other nutrients for layers. Pages 295-304 in Phytase in Animal Nutrition and Waster Management. M. Coelho and E. T. Kornegay, ed. BASF Corporation, Mt. Olive, NJ.

Viveros, A., A. Brenes, I. Arija, C. Centeno. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci. 81: 1172-1183.

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Application of a Duplex PCR Approach for the Specific and Simultaneous Detection of *Clostridium* Spp. and *Lactobacillus* Spp. in Broiler Gastrointestinal Tract

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ABSTRACT : Detection of *Clostridium* spp. and *Lactobacillus* spp. in gastrointestinal contents of broiler by conventional culture-based microbiological methods is laborious and time-consuming. A novel method for the reliable detection of *Clostridium* spp. and *Lactobacillus* spp. was designed in the form of a rapid duplex PCR method that allowed the simultaneous detection, in a single tube, of two common bacterial genera in broiler gastrointestinal tract using four primers (Clos58-f, Clos780-r, LAA-f and LAA-r) targeting 16S rDNA sequence. Results showed that there were *Clostridium* spp. and *Lactobacillus* spp. in all four gastrointestinal segments viz. duodenum, jejunum, ileum and caecum of broilers and no cross detection of other strains occurred. This duplex PCR produced two distinct bands, of 722-bp and 286-bp, for *Clostridium* spp. and *Lactobacillus* spp. respectively. The 722-bp band produced by two (Clos58-f and Clos780-r) of four primers in duplex PCR reaction, was specific only from *Clostridium* spp. and not obtained from other non-target microorganisms. On the other hand, the 286-bp band was produced by two primers, LAA-f and LAA-r, and was specific for the *Lactobacillus* spp. genus. The divergence between the size of the *Clostridium* spp. (722 bp) and *Lactobacillus* spp. (286 bp) was suitable for developing the duplex PCR assay. As *Clostridium* spp. and *Lactobacillus* spp. can be dominant genera in the chicken gut, this method could be very useful to analyze microbial populations in chicken gut or possibly other similar samples.

Keywords: Duplex PCR, Poultry, Gut, *Clostridium* spp. *Lactobacillus* spp.

INTRODUCTION

The two most commonly reported bacterial genera in poultry industry are *Clostridium* spp. and *Lactobacillus* spp. It is known that *Clostridium* spp. especially *Clostridium perfringens*, the causative agent of necrotic enteritis (NE), is a deadly pathogen. *Clostridium perfringens* is a common environmental bacterium and is readily isolated from the intestine of birds and mammals (McCourt et al., 2005; Hofshagen and Stenwig, 1992). The species is divided into five types (A, B, C, D and E) on the basis of production of major lethal exotoxins. Although *Clostridium perfringens* is often found in the intestinal tract of healthy birds it can cause outbreaks of disease, especially in broilers and turkey flocks. Presently, the incidence of NE is effectively controlled through the use of antibiotic feed additives; however their outright ban in Europe, as well as consumer concerns in North America, has led to an increased interest in alternatives for the control of intestinal pathogens. Meanwhile *Lactobacillus* spp. are the most important competent protective cultures which have been developed to reduce risk of zoonosis in chicken. Nurmi and Rantala (1973) investigated the effects of an undefined gut content matrix of adult poultry fed to chicks to protect them from *Salmonella*, a phenomenon termed competitive exclusion.

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Defined *Lactobacillus* strains in competitive exclusion preparation have been confirmed for inhibiting pathogenic growth in broiler chickens. Prevention of the intestinal epithelium from pathogenic bacterial invasion by *Lactobacillus* strains has been reported (Tsai et al., 2005; Hudault et al., 1997).

Therefore, these bacteria comprise several of the major factors affecting both the producer and consumer (Gilbert et al., 2003). Detection of these bacterial groups in a gastrointestinal sample is very imperative. There is an urgent need for rapid, sensitive, and specific techniques for detection of these bacteria (Li et al., 2005; Otero et al., 1998). Furthermore, some of these methods are known to show variable sensitivities, the reliability of which depends on factors such as specificity of the antibodies; interference components in the medium used for culture enrichment; and the relative levels of gene expression of the target antigens.

While the estimate of cultivability of gastrointestinal tract bacteria e.g. *Clostridium* spp. is relatively high (10-50%) compared to most microbial ecosystems (Zoetendal et al., 2004; Amann et al., 1995), the culturable fraction is still a minority. The reasons for this cultivation anomaly include the unknown growth requirements of the bacteria, the selectivity of the media that are used, the stress imposed by the cultivation procedures, the necessity of strictly anoxic conditions, and difficulties with stimulating the interactions of bacteria with other microbes and host cells. The circumvention of these limitations requires culture-independent methods. A dramatic increase in the application of approaches based on the sequence diversity of the 16S ribosomal RNA (rRNA) gene have been made during the past decade to explore the diversity of bacterial communities in a variety of ecosystems, including the mammalian GI tract (Vaughan et al., 2000). Meanwhile *Lactobacillus* spp. are often hard to be distinguished by classical microbiological techniques since phenotypic characteristic analysis is always time-consuming and labor-intensive (Kao et al., 2007; Stackerbrandt and Teuber, 1988; Yeung et al., 2002). Therefore, the rapid and accurate analytical techniques are important for quality control of probiotic products and gut bacteria investigation.

Rapid detection of broiler pathogens is of interest to both the poultry industry and regulatory agencies monitoring food production (Kimura et al., 2000; Lampel et al., 2000). Bacterial detection methods based on nucleic acid, such as the polymerase chain reaction (PCR), have shown tremendous potential and have been increasingly exploited. Since PCR and various PCR-based technologies provide fast results and a high degree of specificity, they constitute a valuable tool in microbiological diagnostics (Cortez et al., 2006; Woodward and Kirwan, 1996). Ordinary PCR procedures are all mono- specific and thus are either laborious or very expensive for routine use in laboratories for per bacteria detection alone.

However, multiplex PCR has been successfully applied to many areas, including detection of microorganisms (Jofre et al., 2005; Bubert et al., 1992; Soumet et al., 1999; Vantarakis et al., 2000; Houf et al., 2000; Wang et al., 2002). Mentioned limitations might be overcome by the use of the multiplex PCR that is able to detect these bacterial groups. The discrimination between these bacterial genera by PCR is particularly interesting because they show a high degree of relatedness in protein profiles (Manfreda et al., 2003; Owen et al., 1988).

With this background the present work was aimed at developing a duplex PCR-based method for the simultaneous detection of *Clostridium* spp. and *Lactobacillus* spp. in broiler gastrointestinal segments. The published primers and developed a method to detect these two bacteria groups in one rapid duplex PCR assay. The application and efficacy of this method for microbiological assessment of broiler GI tract (duodenum, jejunum, ileum and caecum) was also investigated.

MATERIALS AND METHODS

The two bacteria examined in the present study were *Clostridium* spp. and *Lactobacillus* spp. isolated from the duodenum, jejunum, ileum and caecum of 36 commercial, broiler chicks raised under conditions identical to those found in commercial broiler operations. The broilers were not exposed to competitive exclusion preparations as newly hatched chicks and were fed a diet of commercial feed (NRC, 1994). At the age 30d, eight birds were randomly selected and sacrificed by cervical dislocation. The duodenum, jejunum, ileum and caecum were removed aseptically, clamped with forceps, and placed in sterile plastic bags on ice. In the laboratory each section was inverted onto sterile glass rods and approximately one g of content was

collected into a centrifuge tube containing 9 ml of sterile phosphate-buffered saline (PBS), pH 7.4, and homogenized by vortexing with glass beads (4-mm diameter) for 3 min. Debris was removed by centrifugation at 700g for 1 min, and the supernatant was centrifuged at 13000g for 5 min. The pellet was washed twice with PBS and stored at -20°C for extraction of DNA.

DNA extraction and preparation

One ml of each gastrointestinal segment contents was added to a 1.5ml microcentrifuge tube and centrifuged at 14500g for 2 min to pellet the cells. Then supernatant was removed and cells the resuspended in 48ml of 50mM EDTA. 6ml of 10mg/ml Lysosyme was added to the resuspended cell pellet, and gently was mixed. The purpose of this pretreatment was to weaken the cell wall so that efficient cell lysis could take place. The samples were incubated at 37°C for 45 min and centrifuged for 2 min at 14500g supernatant removed the 60ml of Nuclei Lysis solution was added and the solution pipetted gently to resuspend the cells and samples incubated at 80°C for 5 min to lyse the cells. 3ml of RNase solution was added to the cell lysate after cooling toe room temperature and the tubes were mixed by inventry 2-5 times. The samples were incubated at 37°C for 30 min and cooled to room temperature after which 20ml of Protein precipitation solution was added to RNase-treated cell lysate and tubes vortexed vigorously at high speed for 20S. The samples were then incubated on ice for 5 min and centrifuged at 14500g for 3 min. The supernatant containing the DNA was transferred to clean 1.5ml microcentrifuge tubes containing 60ml of isopropanol and mixed gently by inversion until the thread-like strands of DNA formed a visible mass. The tubes were centrifuged at 14500g for 2 min and the supernatant were off and carefully the tube drined on clean absorbent paper. 60ml of 70% ethanol was added and tubes inverted gently several times to wash carefully aspirated the DNA pellets centrifuged at 14500g for 2 min and the ethanol. The tubes were drained on clean absorbent paper and pellets to air-dried for 15 min after when 10ml of DNA rehydration solution was added to the tubes and the DNA was rehydrated by incubating at 65°C for 60 min periodically mixing the solution by gently tapping of the tubes. The prepared DNA was stored at 4°C for PCR amplification.

Oligonucleotide primers

The oligonucleotide primers used in this study were synthesized by Metabion International AG. Sequences of the two PCR primer pairs for duplex PCR, their references and size of expected amplification products are given in Table 1. The GenBank program BLAST (http://www.ncbi.nlm.nih.gov/BLAST) was used to ensure that the applied primers were complementary with the target species but not with other species. Primers were compared with sequences in the Gene Bank, and none was found to have the exact sequence as that of the non-targeted sequence. Duplex PCR to confirm optimized duplex PCR reaction, and parameters and analysis of PCR products

The PCR amplification mixture (25µl) consisted of 1ml of 25 ng DNA sample, 0.08 mM of each dNTP, 1.2 mM $MgCl_2$, 1× PCR buffer, 0.28m M of each primer (Clos58-f, Clos780-r, LAA-f and LAA-r), 1 U of Taq DNA polymerase and 18.6 ml ddH_2O. Amplification was performed on a thermocycler (ABI 9700) with initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45s , annealing at 55.3°C for 45s and extension at 72°C for 80s, with a final extension at 72°C for 3 min. Negative and positive controls were used for bacterial confirmation and

Table 1. Primer sequences and size of PCR-amplified gene targets of *Clostridium sppi and Lacto bacillus spp.*

Bacteria	Primer	Primer Sequence (5-3)	Expected product size (bp)	Reference
Clostridium spp.	Clos58-f	AAAGGAAGATTAATACCGCATAA	722	Amit-Romach et al., 2004
	Clos780-r	ATCTTGCGACCGTACTCCCC		
Lactobacillus spp.	LAA-f	CATCCAGTGCAAACCTAAGAG	286	Wang et al., 1996
	LAA-r	GATCCGCTTGCCTTCGCA		

detection by PCR technique. ddH_2O was used as negative control in reaction mixtures to confirm the absence of contamination of material as well as removal of experimental errors and to prove the exclusion of non-target DNA. *Clostridium perfringens* and *Lactobacillus acidophilus* prepared using defined culture medium were used as positive controls. The positive control strains of these bacteria used in this study were prepared from the bacterial isolate archives of the Agriculture Biotechnology Research Institute of Iran and Scientific and Industrial Research Organization of Iran. Double-stranded DNA extracted from each isolate was examined along the presence of PCR-compatible DNA.

A total of 5ml of loading dye was added to each sample and negative and positive control PCR tubes and mixed thoroughly by pipetting. Then 5ml of each PCR product was analyzed by gel electrophoresis in 2% agarose gels containing ethidium bromide (Bio-Rad). DNA bands were visualized by UV illumination and photographed using the Gel Doc 1000 documentation system (Bio-Rad). A PUC Mix Marker 8 was used as molecular size marker. The expected PCR amplicons were at 722 bp and 286 bp corresponding to the genus *Clostridium* spp. and *Lactobacillus* spp. respectively.

RESULTS AND DISCUSSION

Clostridium spp. and *Lactobacillus* spp. were obtained in all four gastrointestinal segments viz. duodenum, jejunum, ileum and cecum of broilers. *Clostridium* spp. and *Lactobacillus* spp. have been reported in poultry gut (Skanseng et al., 2006; McCourt et al., 2005; Tsai et al., 2005) and support the results obtained in the present suudy.

The genus-specific duplex PCR amplified 16S rDNA of the correctly predicted size from the appropriate *Clostridium* spp. and *Lactobacillus* spp. control strains tested, but not from any other bacterial strain. Products generated from four gastrointestinal segments of chicken are shown in Fig. 1 (lanes 1-4). When the two genus-specific primer pairs were used individually, similar results were obtained as when the two reactions were run separately as a direct PCR. The main aim of this study was to develop and evaluate a method for simultaneous detection of *Clostridium* spp. and *Lactobacillus* spp. using a duplex PCR. The main step in development of the final assay was the definition of a duplex PCR assay. Although the primer pairs used in this study have previously been confirmed to have a high specificity (Wang et al., 1996; Amit-Romach et al., 2004), due to the combination of both primer sets, and the adjustment of the PCR mixture, the specificity was reconfirmed on *Clostridium perfringens* and *Lactobacillus acidophilus*. From obtained results, no cross detection of other strains occurred.

An adequate strategy to assess the validity of the PCR results is the use of positive and negative controls which are co-amplified with the target sequence by the same set of primers. Therefore, two positive controls and one negative control were PCR tested to prove the specificity of the primers and the reaction condition. This novel set of primers was found to work satisfactorily and give optimum yield of product in samples that were used as positive controls (Fig. 1, lanes 5-6). PCR products were obtained specifically only when DNA from the corresponding genus was present in the reaction either in isolation or in combination. The positive controls showed the positive band for both the controls specifically for the respective bacteria. This was confirmed from the amplified fragments of 722 and 286 bp specific for *Clostridium* spp. and *Lactobacillus* spp., respectively. In this reaction, ddH_2O was used as negative control and tested with positive samples (*Clostridium perfringens* and *Lactobacillus acidophilus*) to explain the contamination factor (Fig. 1, lane 7). The specificity of the primers was further confirmed by direct and separate PCR which showed the accuracy of this assay in detecting the respective sequences of the bacterial DNAs. Meanwhile PCR primers of both the bacterial genera were searched using NCBI BLAST search option, and showed high homology with the gene bank sequence from which the primers were applied.

A reliable mPCR method for the simultaneous detection of some genes of *Clostridium perfringens* from a heat lyzed bacterial suspension was also developed. The presence of one, two or three bands specifically indicated the *Clostridium perfringens* toxin type. *Clostridium perfringens* reference strains of different toxin types were used as controls for establishing the mPCR and the method was then introduced into bacteriological diagnostics including *Clostridium perfringens* isolates from various animals with intestinal disorders (Settanni and Corsetti, 2007).

The minimum dose of some bacteria in broiler GI tract is believed to be extremely small, while that

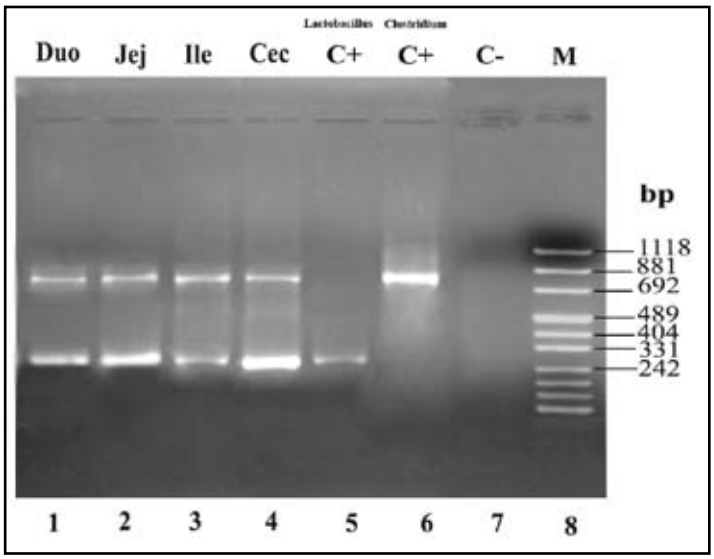

Fig. 1. Electrophoresis of duplex PCR products on 2% agarose gel stained with ethidium bromide. Intestine samples = **lanes 1-4:** Amplification products from DNA of *Clostridium* spp. and *Lactobacillus* spp., with primers Clos58-f, Clos780-r, LAA-f and LAA-r, C+ (*Lactobacillus acidophilus*) = **lane 5**: Positive control amplified DNA, C+ (*Clostridium perfringens*) = **lane 6:** Positive control amplified DNA, C- = lane 7: Negative control, M=**lane 8**: Molecular weight marker.

Duo: Duodenum; Jej: Jejunum; Ile: Ileum; Cec: Caecum

of other bacteria is extremely large (Apajalahti et al., 2004; Kothary and Babu, 2001). This emphasizes the need for high sensitivity of detection methods for bacteria. Some times enrichment of samples with defined medium can increase the number of target organisms and reduce the risk of false-positive results due to amplification from non-target cells which have similar needs for nutrients and growth conditions. Thus, it is possible to enrich these bacteria simultaneously and then detect them at one time in the same PCR system. Thus caution needs to be exercised when reporting negative results of the duplex PCR assay for samples with notably high microbial background levels (Li et al., 2005). Using a selective medium instead of a non-selective medium for enrichment might reduce competition from indigenous flora and improve detection sensitivity of the duplex PCR assay. In fact, some samples required for selective enrichment of cells may influence the effectiveness of the PCR and cause inhibitory effects (Jofre et al., 2005; Rossen et al., 1992; Al-Soud and Radstrom, 2000, 2001; Lantz et al., 2000). Thus, the implementation of PCR-based assays as routine microbiology diagnostic tools, especially in testing laboratories with quality assurance programs, requires proper controls to verify the accuracy of the results obtained.

To develop a novel method for the rapid and reliable identification of *Clostridium* spp. and *Lactobacillus* spp., a duplex PCR analysis using four primers (Clos58-f, Clos780-r, LAA-f and LAA-r) targeting 16S rDNA sequence was first designed. This duplex PCR specifically could detect both *Clostridium* spp. and *Lactobacillus* spp. among gastrointestinal contents of chickens. This duplex PCR produced two distinct bands, 722-bp and 286-bp, from both *Clostridium* spp. and *Lactobacillus* spp. The 722-bp band, which was produced by two (Clos58-f and Clos780-r) of four primers in duplex PCR reaction, is produced only from *Clostridium* spp. genus, while this band did not appear from other non-target microorganisms. On the other hand, a 286-bp band produced by two primers, LAA-f and LAA-r, was specific for the *Lactobacillus* spp. genus. The divergence between the size of the *Clostridium* spp. (722 bp) and *Lactobacillus* spp. (286 bp) was suitable for developing a duplex PCR assay. Accordingly, designed primers generated amplification products, easily distinguishable in a 2% agarose gel.

Optimization of the multiplex PCR may require the adjustment of the relative ratio of the sets of primers due to preferential amplification of certain targets over another (Jofre et al., 2005; Elnifro et al., 2000). To optimize the PCR assay, various concentrations of $MgCl_2$ at different annealing temperatures were tested with the newly designed set of primers in combination. Finally, the reaction mixture with 1.2 mM $MgCl_2$ concentration

at 55.3°C annealing temperature was found to work perfectly for detection of both the bacterium in a single step reaction. The successful amplification of the diagnostic products was also found to be dependent on the ratio of forward and reverse primers which was determined by performing several experiments with varying concentrations of different primers. The optimal ratio was 1:1:1:1 for Clos58-f (0.28 mM), Clos780-r (0.28 mM), LAA-f (0.28 mM) and LAA-r (0.28 mM).

Some single PCR protocols have been described (Skanseng et al., 2006; Gholamiandekhordi et al., 2006; Engström et al., 2003; Elegado et al., 2004; Rantsiou et al., 2006; Selim et al., 2005). Equal ratio of the two pairs of primers added to the PCR reaction may affect the ability of detecting the co-existence of *Clostridium* spp. and *Lactobacillus* spp. In the duplex PCR described here the ratio of the two pairs of primers was carefully optimized to efficiently detect mixed populations of *Clostridium* spp. and *Lactobacillus* spp. This was verified by including DNA samples from *Clostridium perfringens* and *Lactobacillus acidophilus*, individually and as mixtures for positive controls in each duplex PCR batch. Thus the duplex PCR correctly and reproducibly detected the *Clostridium* spp. and *Lactobacillus* spp. and did not cross-react with the other bacterial groups. This duplex PCR is rapid and reliable and employs an appropriate strategy. Duplex PCR, with primers for the *Clostridium* spp. and *Lactobacillus* spp are specific for only these genera without any specific binding to 16S rDNA sequences from non-target organisms. In this context, our method could rapidly detect *Clostridium* spp. and *Lactobacillus* spp. from other related organisms by duplex PCR yielding two PCR products (722 and 286 bp), compared with the production of no PCR products from other related microorganisms.

Furthermore, in an optimal reaction condition, the assay was found to be highly sensitive by successfully detecting both the bacterial groups in different gastrointestinal segments. The introduction of multiplex PCR may reduce the risk of contamination, labor time, and reagent costs through the possibility of combining assays for the detection of some genus or species into one assay (Mishra et al., 2007). PCR amplification of DNA extracted from chicken gut is known to be difficult, as this material contains uric acid and other PCR inhibitors. Initial studies in our laboratory showed that *Clostridium* spp. and *Lactobacillus* spp. PCRs conducted on DNA extracted as per our method have no inhibitors. But some samples such as food constituents, organic and phenolic compounds, glycogen, fats, and Ca^{2+}, might interfere with the amplification of DNA by PCR (Li et al., 2005; Wilson, 1997). Inhibition of PCR reactions results in poor detection sensitivity and even complete reaction failure. Since PCR can detect all of the samples that are positive, this helped to confirm its specificity in comparison with culture methods. In addition, the duplex PCR gave positive and informative results from birds from different flocks, fed on different diets, helping to confirm that the bacteria existed in broiler gut.

This duplex PCR method was applied to a collection of gastrointestinal segment of chickens that were mainly detected by biochemical, serological, microbiological and phenotypic analyses. Meanwhile additional and complementary analyses of these bacteria by DNA-DNA hybridization and 16S rDNA sequencing led us to ensure that our method was very reliable. Slot blot hybridization can confirm the specificity of the assay. In the hybridization assay, the use of radio labeled species-specific nucleotide probe facilitates the detection of bacterial genus and at the same time nullifies the chances of scoring false positive data, which is generally apprehended in PCR method due to nonspecific amplification. Although both the slot blot hybridization and sequencing are additional confirmatory experiments and could validate our results of duplex PCR, however, a simple duplex PCR is enough to detect these groups (Mishra et al., 2007).

Previously, it was shown that *Clostridium* spp. and *Lactobacillus* spp. can be dominant genera in the chicken gut (Apajalahti et al., 2004). Therefore, this method may be very useful to analyze microbial populations in chicken gut or possibly other similar samples. Our method, however, has one limitation. This method is not suitable for the classification *Clostridium* spp. and *Lactobacillus* spp., because our method depends on a few polymorphisms in 16S rRNA gene and not sequence variations in the whole genome.

Technical and economic burdens have been placed on clinical microbiology laboratories to identify gut bacteria using conventional microbiological tests, which take up several days and are prone to misdiagnosis. Hence, an effort is underway to replace conventional tests with rapid

and accurate molecular-based methods, including PCR (LaGier et al., 2004). A major advantage of the duplex PCR was that it was far more rapid than culture, potentially giving results on the same day as sample receipt rather than after 7-10 days. The availability of such rapid tests could greatly facilitate future epidemiological studies, and improve diagnostic capacity. Although the current duplex PCR was only designed to detect *Clostridium* spp. and *Lactobacillus* spp., in the future it could be expanded to include detection of other pathogenic and useful bacteria. All that would be required would be to add an additional species-specific amplification to the second step, based on portions of the 16S rRNA gene, following their amplification in the first genus-specific step.

Unlike RAPD-PCR that employs low annealing temperature, (Lee et al., 2004; Berthier and Ehrlich, 1999), the annealing temperature of our duplex PCR was relatively highly (55.3°C), because primers used were relatively long in length (23 and 20 bp for *Clostridium* spp and 21 and 18 bp for *Lactobacillus* spp.). This high and broad annealing temperature gave rise to very high reproducibility.

Our strategy for the PCR amplification was to use the sequences of two DNA fragments of the said two genera to design two forward and two reverse specific primers for both the bacteria. In our assay, the novel set of primers yielded evident differential product unique for each of the bacteria in a single step reaction. This could amplify DNA fragments of desired sizes of both the bacteria in isolation and in combination. It is known that there are always chances of competition between the samples for PCR reagents and primers particularly when there are two primer pairs and two target templates. The result shown in this manuscript not only shows the compatibility of the primer combinations but also establish the optimization of the PCR assay. The figure that includes the results of negative control as well as the positive controls not only ensure the specificity but also rule out the apprehension of contamination factor in detection (Mishra et al., 2007). Usually, the results obtained by PCR assay are equivalent or superior to those obtained by microscopy and the PCR test are also able to detect mixed samples that were missed by microscopy (Bjerrum et al., 2006). This could probably be due to dominance of one group over the other groups in a sample and general biasness of microscopic while scoring the data. Detection of mixed samples not only proves the effectiveness of this assay but also establishes the relevance of this method in diagnosis because such cases are very frequent.

Finally, today, control programs move towards the new strategy of community diagnosis. Such findings provide evidence for the possible use of this approach in monitoring microorganisms as a means for the evaluation of large scale control program. Classical technology and monoplex PCR approaches are not suitable for studies of complex flora consisting of multiple microbial groups. The current trend is towards culture-independent, PCR based methods because they are believed to overcome problems associated with selective cultivation and isolation of microorganisms from natural samples and because PCR-based methods are generally characterized by their simplicity, speed, cost-effectiveness and reliability and also to rapidly detect multiple microorganisms in a single reaction, simultaneous amplification of more than one locus is required such as multiplex PCR (Settanni and Corsetti, 2007) in which several specific primer sets are combined into a single PCR assay. Hence, multiplex PCR is undoubtedly useful to rapidly identify several genera and species.

CONCLUSION

A rapid, simple and convenient duplex PCR-based assay has been developed for the specific and simultaneous detection of *Clostridium* spp. and *Lactobacillus* spp. in chicken gut. The results show that the method may be very useful for food, and veterinary analysis laboratories dealing with many samples that contain both *Clostridium* spp. and *Lactobacillus* spp. genera.

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REFERENCES

Al-Soud, W.A., and P. Radstrom. 2000. Effects of amplification facilitators on diagnostic PCR in the presence of blood, feces, and meat. Journal Clini. Microbiol. 38: 4463-4470.

Al-Soud, W.A., and P. Radstrom. 2001. Purification and characterization of PCR-inhibitory components in blood cells. J. Clin. Microbiol. 39: 485-493.

Amann, R.I., W. Ludwig, and K.H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual cells without cultivation. Microbiol. Rev. 59: 143-169.

Amit-Romach, E., D. Sklan, and Z. Uni. 2004. Microflora Ecology of the Chicken Intestine Using 16S Ribosomal DNA Primers. Poult. Sci. 83: 1093-1098.

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult. Sci. J. 60: 223-232.

Berthier, F., and S.D. Ehrlich. 1999. Genetic diversity within *Lactobacillus sakei* and *Lactobacillus curvatus* and design of PCR primers for its detection using randomly amplified polymorphic DNA. Int. J. Syst. Bacteriol. 49: 997-1007.

Bjerrum, L., R.M. Engberg, T.D. Leser, B.B. Jensen, K. Finster, and K. Pedersen. 2006. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. Poult. Sci. 85: 1151-1164.

Bubert, A., S. Kohler, and W. Goebel. 1992. The homologous and heterologous regions within the iap gene allow genus- and specie sspecific identification of *Listeria* spp. by polymerase chain reaction. Appl. Environ. Microbiol. 58: 2625-2632.

Cortez, A.L.L., A.C.F.B. Carvalho, A.A. Ikuno, K.P. Burger, and A.M.C. Vidal-Martins. 2006. Identification of *Salmonella* spp. isolates from chicken abattoirs by multiplex-PCR. Res. Vet. Sci. 81: 340-344.

Elegado, F.B., M.A.R.V. Guerra, R.A. Macayan, H.A. Mendoza, and M.B. Lirazan. 2004. Spectrum of bacteriocin activity of *Lactobacillus plantarum* BS and fingerprinting by RAPD-PCR. Int. J. Food Microbiol. 95: 11-18.

Elnifro, E.M., A.M Ashshi, R.J. Cooper, and P.E. Klapper. 2000. Multiple PCR: optimization and application in diagnostic virology. Clin. Microbiol. Rev. 13: 559-570.

Engström, B.E., C. Fermér, A. Lindberg, E. Saarinen, V. Båverud, and A. Gunnarsson. 2003. Molecular typing of isolates of *Clostridium perfringens* from healthy and diseased poultry. Vet. Microbiol. 94: 225-235.

Gholamiandekhordi, A.R., R. Ducatelle, M. Heyndrickx, F. Haesebrouck, P. Van-Immerseel. 2006. Molecular and phenotypical characterization of *Clostridium perfringens* isolates from poultry flocks with different disease status. Vet. Microbiol. 113: 143-152.

Gilbert, C., D. Winters, A. O'Leary, and M. Slavik. 2003. Development of a triplex PCR assay for the specific detection of *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli* O157:H7. Mol. Cell. Probes. 17: 135-138.

Hofshagen, M., and H. Stenwig. 1992. Toxin production of *Clostridium perfringens* isolated from broiler chickens and capercaillies (*Tetrao urogallus*) with and without necrotizing enteritis. Avian Dis. 36: 837-843.

Houf, K., A. Tutenel, L. De Zutter, J. Van Hoof, and P. Vandamme. 2000. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiol. Lett. 193: 89-94.

Hudault, S., V. Lievin, M.F. Bernet-Camard, and A.L. Servin. 1997. Antagonistic activity exerted in vitro and in vivo by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection. Appl. Environ. Microbiol. 63: 513-518.

Jofre, A., B. Martin, M. Garrigaa, M. Hugas, M. Pla, D. Rodriguez-Lazaro, and T. Aymerich. 2005. Simultaneous detection of *Listeria monocytogenes* and *Salmonella* by multiplex PCR in cooked ham. Food Microbiol. 22: 109-115.

Kao, Y.T., Y.S. Liu, and Y.T. Shyu. 2007. Identification of *Lactobacillus* spp. in probiotic products by

real-time PCR and melting curve analysis. Food Res. Int. 40: 71-79.

Kimura, R., R.E. Mandrell, J.C. Galand, D. Hyatt, and L.W. Riley. 2000. Restriction site- specific PCR as a rapid test to detect enterohemorrhagic *Escherichia coli* O157:H7 strains in environmental samples. Appl. Environ. Microbiol. 66: 2513-2519.

Kothary, M.H., and U.S. Babu. 2001. Infectious dose of foodborne pathogens in volunteers: a review. J. Food Safety. 21(1): 49-73.

LaGier, M.J., L.A. Joseph, T.V. Passaretti, K.A. Musser, and N.M. Cirino. 2004. A real-time multiplexed PCR assay for rapid detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli*. Mol. Cell. Probes. 18: 275-282.

Lampel, K.A., P.A. Orlandi, and L. Kornegay, 2000. Improved template preparation for PCR-based assays for detection of food-borne bacterial pathogens. Appl. Environ. Microbiol. 66: 4539-4542.

Lantz, P.G., W.A. Al-Soud, R. Knutsson, Hahn-B. Hagerdal, and P. Radstrom. 2000. Biotechnical use of polymerase chain reaction for microbiological analysis of biological samples. Biotec. Annu. Rev. 5: 87-130.

Lee, J., J. Jang, B. Kim, J. Kim, G. Jeong, and H. Han. 2004. Identification of *Lactobacillus sakei* and *Lactobacillus curvatus* by multiplex PCR-based restriction enzyme analysis. J. Microbiol. Methods. 59: 1-6.

Li, Y., S. Zhuang, and A. Mustapha. 2005. Application of a multiplex PCR for the simultaneous detection of *Escherichia coli* O157:H7, *Salmonella* and *Shigella* in raw and ready-to-eat meat products. Meat Sci. 71: 402-406.

Manfreda, G., A. De-Cesare, V. Bondioli, and A. Franchini. 2003. Comparison of the BAXR System with a multiplex PCR method for simultaneous detection and identification of *Campylobacter jejuni* and *Campylobacter coli* in environmental samples. Int. J. Food Microbiol. 87: 271-278.

McCourt, M.T., D.A. Finlay, C. Laird, J.A. Smyth, C. Bell, and H.J. Ball. 2005. Sandwich ELISA detection of *Clostridium perfringens* cells and a-toxin from field cases of necrotic enteritis of poultry. Vet. Microbiol. 106: 259-264.

Mishra, K., D.K. Raj, R.K. Hazra, A.P. Dash, and P.C. Supakar. 2007. The development and evaluation of a single step multiplex PCR method for simultaneous detection of *Brugia malayi* and *Wuchereria bancrofti*. Molecular and Cellular Probes. doi:10.1016/j.mcp.2007.05.001.

National Research Council (NRC). 1994. Nutrient Requirement of Poultry. 9th ed. National Academy. Washington DC.

Nurmi, E., and M. Rantala. 1973. New aspects of *Salmonella* infection in broiler production. Nature. 241: 210-211.

Otero, A., M.L. Garcia-Lopez, and B. Moreno. 1998. Rapid microbiological methods in meat and meat products. Meat Sci. 49(Suppl. 1): S179-S189.

Owen, R.J., M. Costas, L. Sloss, and F.J. Bolton. 1988. Numerical analysis of electrophoretic protein patterns of *Campylobacter laridis* and allied thermophilic Campylobacter, from the natural environment. J. Appl. Bacteriol. 65: 69-78.

Rantsiou, K., E.H. Drosinos, M. Gialitaki, I. Metaxopoulos, G. Comi, and L. Cocolin. 2006. Use of molecular tools to characterize *Lactobacillus* spp. isolated from Greek traditional fermented sausages. Int. J. Food Microbiol. 112: 215-222.

Rossen, L., P. Noskov, K. Holmstrom, and O.F. Rasmussen. 1992. Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solution. Int. J. Food Microbiol. 17: 37-45.

Selim, A.S.M., P. Boonkumklao, T. Sone, A. Assavanig, M. Wada, and A. Yokota. 2005. Development and Assessment of a Real-Time PCR Assay for Rapid and Sensitive Detection of a Novel Thermotolerant Bacterium, *Lactobacillus thermotolerans*, in Chicken Feces. Appl. Environ. Microbiol. 71: 4214-4219

Settanni, L., and A. Corsetti. 2007. The use of multiplex PCR to detect and differentiate food- and beverage-associated microorganisms: A review. J. Microbiol. Methods. 69: 1-22.

Skanseng, B., M. Kaldhusdal, and K. Rudi. 2006. Comparison of chicken gut colonisation by the pathogens *Campylobacter jejuni* and *Clostridium perfringens* by real-time quantitative PCR. Mol. Cell. Probes. 20: 269-279.

Soumet, C., G. Ermel, V. Rose, N. Rose, P. Drouin, G. Salvat, and P. Colin. 1999. Identification by a multiplex PCR-based assay of *Salmonella typhimurium* and *Salmonella enteritidis* strains from environmental swabs of poultry houses. Lett. Appl. Microbiol. 29: 1-6.

Stackerbrandt, E., and M. Teuber. 1988. Molecular taxonomy and phylogenetic position of lactic acid bacteria. Biochemie. 70: 317-324.

Tsai, C.C., H.Y. Hsih, H.H. Chiu, Y.Y. Lai, J.H. Liu, B. Yu, and H.Y. Tsen. 2005. Antagonistic activity against *Salmonella* infection in vitro and in vivo for two *Lactobacillus* strains from swine and poultry. Int. J. Food Microbiol. 102: 185-194

Vantarakis, A., G. Komninou, D. Venieri, and M. Papapetropoulou. 2000. Development of a multiplex PCR detection of *Salmonella* spp. and *Shigella* spp. in mussels. Lett. Appl. Microbiol. 31: 105-109.

Vaughan, E.E., F. Schut, G.H.J. Heilig, E.G. Zoetendal, W.M. deVos and A.D.L. Akkermans. 2000. A molecular view of the intestinal ecosystem. Curr. Issues Intest. Microbiol. 1: 1-12.

Wang, G., C.G. Clark, T.M. Taylor, C. Pucknell, C. Barton, L. Price, D.L. Woodward, and F.G. Rodgers. 2002. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. fetus. J. Clin. Microbiol. 40: 4744-4747.

Wang, R.F., W.W. Cao, and C.E. Cerniglia. 1996. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. Appl. Environ. Microbiol. 62: 1242-1247.

Wilson, I.G., 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63(10): 3741-3751.

Woodward, M.J., and S.E.S. Kirwan. 1996. Detection of *Salmonella enteritidis* in eggs by the polymerase chain reaction. Vet. Res. 138: 411-413.

Yeung, P.S.M., M.E. Sanders, C.L. Kitts, R. Cano, and P.S. Tong. 2002. Species-specific identification of commercial probiotic strains. J. Dairy Sci. 85: 1039-1051.

Zoetendal, E.G., C.T. Collier, S. Koike, R.I. Mackie, and H.R. Gaskins. 2004. Molecular Ecological Analysis of the Gastrointestinal Microbiota: A Review. J. Nutr. 134: 465-472.

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Influence of Sugarcane Press Mud on Serum Calcium and Plasma Inorganic Phosphorous in Broilers

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ABSTRACT : Effect of sugarcane press mud (SPM) on serum calcium and plasma inorganic phosphorus in broilers was assessed in a feeding experiment on 300 birds for 60 days during starter and finisher phase. The SPM after screening for different minerals was included at four levels (1, 2, 3 and 4%) in fish based and soya based diets to prepare eight experimental diets. In addition, two BIS specified fish based and soya based control diets were formulated. Such diets were offered to three groups of ten birds each (5 males and 5 females) reared under uniform managment conditions in battery brooders. Serum calcium content(mg/dl) was more or less uniform (P>0.05) and ranged from 9.80(T5) to 11.20(T2) for starter phase, while at the end of finisher phase it was from 8.50(T9) to 11.40(T3). Similarly, plasma inorganic phosphorus (mg/dl) ranged from 8.70(T7) to 6.70(T5) for starter phase, while the range at the end of finisher phase was from 8.30(T5) to 6.90(T6). Treatment effects on serum calcium and plasma inorganic phosphorous levels at the end of starter and finisher phase, were not significantly different. It is concluded that SPM as a mineral source is effective and it appears to be a valuable non-conventional feedstuff for broilers.

Key words: Sugar cane press mud, Serum, Calcium, Plasma inorganic phosphorus.

Although the energy and protein are the most important nutrients, yet role of minerals in the overall performance of birds is a well established fact (Ranjhan, 1999). Under intensive system of feed management broiler diets are generally fortified with mineral premix or the individual mineral salts. Quite often such exercise is prohibitive due to many reasons including the cost and availability (Singh and Soloman, 1995). The SPM a byproduct from sugar cane industries, is a good source of macro as well as micro minerals. Therefore evaluated its effect as a source of minerals on serum calcium and inorganic phosphorus in broilers.

A conventional control diet containing calcite power, di-calcium phosphate and salts of pertinent trace minerals was formulated. After screening the SPM for different minerals (Table1). SPM was included at different levels (1, 2, 3 and 4%) in both fish based and soya based diets to form eight experimental diets at the expense of relevant mineral contributing salts. Each of the diets mentioned in the (Table 2 and 3) was for starter as well as finisher type and were offered to total of 300 one day old crossbred broiler chicks (Vencob) of uniform body weight divided into ten treatments of triplicate groups (5 males and 5 females) making 30 groups. Since the calcium and phosphorous are the major essential minerals, an attempt was made to maintain their levels at zero day, at the end of starter phase and at the end of finisher phase. From each group one male and one female were selected randomly and about 1 ml of blood was collected from each bird through wing vein. Blood samples from three male

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Table 1. Proximate composition and mineral profile of press mud (On DM basis)

Proximate analysis (%)		Mineral profile	
Dry matter (%)	94.45±0.57	Phosphorus (%)	01.27±0.20
Crude protein (%)	08.96±0.12	Potassium (%)	01.81±0.10
Ether extract (%)	10.24±0.21	Calcium (%)	02.40±0.18
Crude fiber (%)	16.09±0.24	Magnesium (%)	01.28±0.10
Total ash (%)	14.17±0.12	Sulphur (%)	02.62±0.19
Nitrogen free extractives (%)	44.99±0.84	Iron (ppm)	2042.0±17.70
Acid insoluble ash (%)	04.33±0.16	Manganese (ppm)	228.0±4.64
pH	06.35±0.19	Zinc (ppm)	36.50±2.56
Organic carbon (%)	40.87± 0.90	Copper (ppm)	22.60±4.41
		Cobalt (ppm)	236.7±2.82

Table 2. Ingredient composition of starter diets (kg)

Ingredients	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Maize	540	540	540	540	540	540	540	540	540	540
Rice Polish	42.25	37.85	33.52	29.15	24.81	32.78	28.4	24.1	19.7	15.34
Soybean extract	210	210	210	210	210	285	285	285	285	285
Groundnut extract	60	60	60	60	60	46	46	46	46	46
Sunflower extract	50	45	40	35	30	60	55	50	45	40
Fish	80	80	80	80	80	0	0	0	0	0
Dicalcium Phosphate	12	12	12	12	12	20.5	20.5	20.5	20.5	20.5
Calcite	5	4.4	3.75	3.1	2.44	12.22	11.6	10.9	10.3	9.66
Salt	0.75	0.75	0.75	0.75	0.75	3.5	3.5	3.5	3.5	3.5
Sugarcane press mud	0	10	20	30	40	0	10	20	30	40
Ferrous Sulphate	0.263	0.176	0.1005	0.021	0	0.273	0.1925	0.112	0.035	0
Zinc Oxide	0.05	0.0496	0.0493	0.0489	0.0486	0.056	0.0565	0.056	0.0559	0.0555
Copper Sulphate	0.004	0.0018	0	0	0	0	0	0	0	0
Cobalt Sulphate	0.0095	0	0	0	0	0.0095	0	0	0	0
Potassium iodide	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	.0034	0.0034	0.0034
Sodium Selenite	0.0002	0.0002	0.0002	0.0002	0.0002	0.0006	0.0006	0.0006	0.0006	0.0006
Magnesium Sulphate	0.203	0.1951	0.1887	0.1819	0.173	0.191	0.184	0.1773	0.1707	0.164
Total	1000.5	1000.4	1000.3	1000.3	1000.2	1000.5	1000.4	1000.4	1000.3	1000.2
Additives	+	+	+	+	+	+	+	+	+	+

Table 3. Ingredient composition of finisher diets (kg)

Ingredients	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Maize	610	610	610	610	610	610	610	610	610	610
Rice Polish	42.25	37.85	33.52	29.15	24.81	32.78	28.4	24.1	19.7	15.34
Soybean extract	170	170	170	170	170	235	235	235	235	235
Groundnut extract	30	30	30	30	30	16	16	16	16	16
Sunflower extract	60	55	50	45	40	70	65	60	55	50
Fish	70	70	70	70	70	0	0	0	0	0
Dicalcium Phosphate	12	12	12	12	12	20.5	20.5	20.5	20.5	20.5
Calcite	5	4.4	3.75	3.1	2.44	12.22	11.6	10.9	10.3	9.66
Salt	0.75	0.75	0.75	0.75	0.75	3.5	3.5	3.5	3.5	3.5
Sugarcane press mud	0	10	20	30	40	0	10	20	30	40
Ferrous Sulphate	0.263	0.176	0.1005	0.021	0	0.273	0.1925	0.1129	0.035	0
Zinc Oxide	0.05	0.0496	0.0493	0.0489	0.0486	0.056	0.0565	0.0561	0.0559	0.0555
Copper Sulphate	0.004	0.0018	0	0	0	0	0	0	0	0
Cobalt Sulphate	0.0095	0	0	0	0	0.0095	0	0	0	0
Potassium iodide	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034
Sodium Selenite	0.0002	0.0002	0.0002	0.0002	0.0002	0.0006	0.0006	0.0006	0.0006	0.0006
Magnesium Sulphate	0.203	0.1951	0.1887	0.1819	0.173	0.191	0.184	0.1773	0.1707	0.164
Total	1000.5	1000.4	1000.3	1000.3	1000.2	1000.5	1000.4	1000.4	1000.3	1000.2
Additives	+	+	+	+	+	+	+	+	+	+

Table 4. Mean serum calcium and plasma inorganic phosphorus (mg/dl) of birds during starter and finisher phase

	Calcium concentration		Plasma inorganic phosphorus concentration (mg/dl)	
Treatment	Starter phase^{NS}	Finisher phase^{NS}	Starter phase^{NS}	Finisher phase^{NS}
T1	10.00±0.40	10.25±0.35	7.35±0.10	7.40±0.60
T2	11.20±0.90	11.30±0.90	7.90±0.10	7.50±0.10
T3	10.95±0.85	11.40±0.40	7.35±0.65	7.20±0.00
T4	10.70±1.20	10.85±0.45	6.90±0.30	7.30±0.30
T5	09.80±0.30	10.50±0.70	6.70±0.20	8.30±0.90
T6	09.85±0.25	10.60±1.40	7.30±0.80	6.90±0.20
T7	10.85±0.25	09.05±0.75	8.70±0.75	7.65±0.25
T8	10.00±0.00	10.80±0.60	7.30±0.65	7.45±0.15
T9	09.95±0.75	08.50±0.10	8.30±0.70	7.25±0.05
T10	10.60±0.40	09.85±0.05	8.60±1.30	7.30±0.20

NS=Non significant

of the three groups of same treatment were pooled and similar collection was repeated for females also. The serum samples of different treatment groups were subjected to calcium estimation (Faulkner and Meites, 1982). Similarly, for plasma inorganic phosphate estimation blood was collected in a clean, sterilized and labeled vial containing Ca-EDTA as an anti coagulant. About 3 ml of blood thus collected was centrifuged and the separated plasma was subjected for plasma inorganic phosphate estimation (Tietz, 1976). Mixed factorial design (2x2x5) was employed to evaluate the data for statistical analysis and the results were interpreted accordingly (Snedecor and Cochran, 1980).

When the data (Table 4) for serum calcium were subjected to statistical analysis (CRD), it was evident that at the end of starter as well as at the end of finisher phase, the values were found to be more or less uniform and ranged from 9.80(T5) to 11.20(T2) mg/dl for starter phase, while at the end of finisher phase from 8.50(T9) to 11.40(T3) mg/dl. Similarly, plasma inorganic phosphorus ranged from 8.70(T7) to 6.70(T5) mg/dl for starter phase, while the range at the end of finisher phase was from 8.30(T5) to 6.90(T6) mg/dl. The calcium and plasma inorganic phosphorous levels of birds both during starter and finisher phase were within the normal blood serum concentration of 8-12mg/dl and 7-9mg/dl in broilers respectively. Thus, the results indicate that SPM can be used effectively as a source of calcium and phosphorous and maintain the serum calcium and plasma inorganic phosphorous concentration well within the normal range. No adverse effects were seen on supplementing the SPM at 4% both in fish and soya based diets.

REFERENCES

Faulkner, W.R., and Meites, S. 1982. American Association for Clinical Chemistry. Washington D.C. 365-373.

Ranjhan, S.K. 1999. Animal Nutrition and Feeding Practices, 6th Revised Edn. Vikas Publishing House Pvt. Ltd., New Delhi.

Singh,B.G., and S. Solomon. 1995. Sugarcane agro-industrial alternatives. Vijay Pimlani Oxford and, IBH, Pub.Co.Pvt.Ltd. New Delhi.

Snedecor,G.W., and W.G. Cochran. 1980. Statistical methods (9th Edn. The Iowa State University Press, Ames, Iowa.

Tietz, N.W. 1976. Clinical Guide to Laboratory Tests. W.B. Saunders Company, Philadelphia. p. 17.

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Studies on Different Levels of Soybean Cake on the Performance of Broiler Chicks

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ABSTRACT : The experiment was conducted on 240 day old, 60 broiler chicks divided into four groups, to study the effect of graded levels of soybean cake (Deoiled) on body weight, feed conversion efficiency and digestibility of nutrients. The chicks were fed isoproteinous and isocaloric ration having 0, 10, 20, and 30% soybean cake. In eight weeks trial, the body weights were 785.55, 833.99, 1117.98 and 978.77g, feed conversion ratio 2.47, 2.45, 2.20 and 2.48 kg/chick and performance index was 318.04, 381.22, 508.17 and 394.67 on 0, 10, 20 and 30 % soybean cake ration, respectively. The dry matter digestibility was 55.40, 56.06, 57.41 and 56.93%, protein efficiency ratio was 1.77, 1.78, 1.98 and 1.69 and efficiency of energy utilization was 7.02, 6.96, 6.30 and 7.11 Kcal/g weight gain, respectively in the four groups. The cost of ration per unit gain of body weight was minimum (Rs.16.03) on 20% soybean cake ration.

Key words: Broilers, Growth, Nutrient utilization, Soybean cake

Although animal proteins are commonly incorporated in chick rations, yet their scarcity and high cost are the factors limiting their use. So the oil cakes are being used in abundance. Among the protein feeds for poultry, soybean cake is highly nutritious. Soybean (meal) cake has good quality, 52% protein. While the whole soybean contains 42% protein and valuable amino acids, lysine (2.9 %), methionine (7.5 %), leucine (3.4 %) and glycine (2.4 %) (Aziz et al., 2001).Therefore, the present study was aimed to evaluate the graded levels of deoiled soyabean cake in Broiler chicks.

The experiment was carried out on 240 day old (IVRI strain) broiler chicks. The birds were weighed and randomly allotted to four dietary groups of 60 each. During the balance study all birds were kept in cages under similar microclimatic conditions. The four groups consisted of control group without soybean cake (T_1) with groundnut cake being only source of vegetable protein, whereas in remain group rations soybean cake was 10 (T_2), 20 (T_3) and 30% (T_4) respectively. The rations were isonitrogenous and isocaloric. Feed and water were provided ad libitium. The percentage of ingredients and composition of ration is detailed in Table 1. The proximate composition of ingredients was determined according to AOAC. (1984). The effects of different levels of soybean cake were evaluated by recording the final body weight, feed intake, feed conversion, protein efficiency, energy efficiency ratio and performance index. The data were statistically analyzed in completely randomized design. The level of significance of differences among treatment means were tested in different growth periods, following the procedure of Snedecor and Cochran (1981).

The average body weight gain data (Table 2) indicated that T_3 group consisting 20% soybean cake ration was better (1118g) than all other groups ($P<0.05$). The differences between T_1 and T_2 as well as in T_1 and T_4 were statistically significant ($P<0.05$). The finding of present study is in agreement with the findings of Haq et al. (1988) and Gupta et al. (1989), who used soybean meal in place of fish meal. Marginally better results were obtained using a slightly higher level of soybean meal (36 %) along

Table 1. Ingredients and chemical composition % of the rations.

Ingredients	Composition of ration %			
	T1	T2	T3	T4
Maize	37.5	36.5	38.5	35.0
Barley	5.0	8.0	7.0	6.0
Rice polish	13.0	14.0	15.0	20.0
Wheat bran	5.0	5.0	6.0	6.0
Groundnut cake	36.5	23.5	10.5	-
Soybean cake	-	10.0	20.0	30.0
Mineral mixture (a)	2.87	2.87	2.87	2.87
Vets (b)	0.02	0.02	0.02	0.02
TM 50 (C)	0.01	0.01	0.01	0.01
Neftin-200 (d)	0.05	0.05	0.05	0.05
Amprol plus (e)	0.05	0.05	0.05	0.05
Vit. D_2, D_3.(Supplement)	Liquid form	Liquid form	Liquid form	Liquid form
Chemical composition				
Dry matter	92.69	92.54	92.38	92.34
Crude protein	22.97	22.98	22.97	23.86
Ether extract	6.01	5.84	5.65	5.05
Crude fiber	6.32	6.38	6.26	6.42
Nitrogen free extract	58.37	57.68	58.32	56.78
Total ash	6.33	7.12	6.80	7.89
Metabolic energy	2848	2835	2859	2864
C: P ratio	126:1	125:1	126:1	122:1

with maize (60 %) and non-phytin phosphorus (0.35 %) supplemented with lysine and methionine in broiler diet (Panda et al., 2002). Similarly, in contrast to present results higher body weight gain have been reported by Aziz et al., (2001) using soybean cake at different levels along with soybean oil levels at 0.5 to 1 % level to meet out energy and methionine requirements. Reddy and Eshwariah (1989) showed that the fish meal can be successfully replaced with soybean meal and sesame cake up to a level of 75 % without supplementing lysine and methionine.

The average feed intake per chick during eight weeks of experimental period in different treatment groups ranged between 1936 to 2461 g. The feed conversion ratio was 2.47, 2.45, 2.20 and 2.48 in T_1, T_2, T_3 and T_4 groups, respectively. The highest feed intake and feed conversion ratio was in T_3 and the lowest in control (T_1). Higher feed requirements, were reported by Thyangarajan et al. (1988) and Reddy et al. (1998). The present findings are in agreement with earlier workers (Zatari and Jerry, 1990; Aziz et. al., 2001 and Medhi et. al., 2002) who also used soybean cake in the ration of broilers. It is evident from these results that the feed conversion efficiency increased with low fiber content in diet, enzyme supplementation and supplementation of deficient amino acids and the minimum level of trypsin inhibitors. The performance index of group (T_1) was found to be lowest (318.04) and it was highest in 20 % (T_3) soybean cake group (508.17). The index was higher than reported by Kumar, (1997), using Ambadi cake in broiler ration but comparable to 10 and 30% soybean cake diets.

The nutrients digestibility (Table 3) ranged between 55 to 57 % and the metabolizable energy values between 61 to 63 % in different treatment groups. Chicks in group T3 digested more dry matter (57.41 %) and showed best performance

Table 2. Feed consumption (g/chick), body weight gain (g/chick), feed conversion ratio and performance index during 0-8 week's period in different rations.

Treatment	Body weight gain (g)	Feed consumption (g)	Feed conversion ratio (FCR)	Performance index (PI)
T1	785.55	1936.36	2.47	318.04
T2	933.99	2291.49	2.45	381.22
T3	1117.98	2461.82	2.20	508.17
T4	978.77	2428.18	2.48	394.67

Table 3. Effect of dietary treatments on dry matter digestibility, protein efficiency of ration and efficiency of energy utilization in chicks

Treatment	Apparent dry matter digestibility (%)	Protein efficiency utilization	Efficiency of energy (Kcal/g gain)	Gross Energy metabolized gain (%)	Cost of feed per unit (Rs.)
T1	55.4	1.77	7.02	62.2	17.38
T2	56.06	1.78	6.96	61.66	17.58
T3	57.41	1.98	6.3	61.31	16.03
T4	56.93	1.69	7.11	63.28	18.51

(6.3 calories/g gain) than other treatment groups. During the period of growth the protein efficiency ratio ranged between 1.69 and 1.98. The protein efficiency was superior on 30 % soybean diet. The present findings are agreement with earlier worker (Kumar, 1997), where Ambadi cake (20%) was used as the source of protein along with fish meal, similar results were also observed by Medhi et al. (2002).

In the present investigation the cost per unit gain was also apparently low in 20 percent soybean cake ration (Rs. 16.03) and almost similar i.e. in rest two groups. By increasing the level of soybean cake upto 30 percent the cost of ration increased upto Rs. 18.51 per kg weight gain. Thus, 20% level of soyabean appeared economical.

REFERENCES

A.O.A.C. 1984. Official Methods of Analysis. (14th Ed). Association of official analytical chemists. Arlington, Virginia, USA.

Aziz, M.A., Z. H. Khandaker, and M.M. Islam. 2001. Effect of replacing protein from fish meal with soybean on the performance of broiler chickens. Indian J. of Anim. Nutri. 18: 23-28.

Gupta J.J., B.P.S. Yadav, and A. Verma. 1989. Utilization of damaged soybean grain in broiler ration. Indian J. of Poultry Sci. 24: 166-178.

Haq, Inam-UI, M.Z. Alam, A. R. Barque, T. H. Shah and A.U. Rehman. 1988. Biological evolution of soybean oil meal as a substitute for fish meal in broiler rations. Pakistan Vet. J. 8: 10-14.

Kumar P. 1997. Incorporation of Ambadi cake in broiler ration. M. Sc. Thesis, Rajasthan Agriculture University, Bikaner.

Medhi, D. H.F. Ahmed., and B. K. Kanwar. 2002. Influence of enzyme supplementation on the performance of broiler feed diets containing different levels of Ajar seed meal. Indian J. of Poultry Sci. 37: 99-101.

Panda, A.K., S.V. Ramarao, and G. Shyamsunder. 2002. Performance of commercial broiler, feed diet with different levels of non-phytin phosphorus. Indian J. Poultry Sci. 37: 45-48.

Reddy, C.P., S.P. Reddy, S.P.V. Reddy and D.S. Rao. 1998. Influence of cotton seed cake on the performance of broilers. Indian J. of Anim. Nutri. 15: 188-193.

Reddy, R.V. and H. Eswaria. 1989. Effect of graded replacement of fish meal with vegetable proteins in broiler starter rations. Indian J. Anim. Nutri. 6:166-168.

Snedecor, G.W. and W.G. Cochran. 1981. Statistical methods. 7th ed. the Iowa state university press Ames, Iowa, USA.

Thyangarajan, D., J. Kalatharan, M. Babu, M.K. Abdul and V. Sundarasu. 1988. Indian J. Poultry Rev. 9:25-26.

Zatari, I.M., and L.S. Jerry. 1990. Sunflower meal as a component of fat supplemented diet for broiler chickens. J. of Poultry Science. 69: 1503-1507.

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Factory Tea Waste with or without Enzyme Supplementation as a Feed Ingredient for Broiler Chicks

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ABSTRACT : An experiment was conducted to assess the feeding value of Factory Tea Waste (FTW) with or without enzyme cocktail supplementation on the performance of broilers. Three hundred sixty day old Vencob broiler chicks (BW of 47.62g) were divided into 8 groups of 45 chicks each. Eight concentrate mixtures for broiler starters(0-4 weeks) and finisher (4-6 weeks) were formulated by replacement of wheat bran in the control ration at 0, 5, 10, 15% level (w/w) with FTW with or without enzyme supplementation. Each diet was fed to triplicate groups of 15 chicks. Lower body weight gain ($P<0.05$) of chicks at 6 weeks was noticed when FTW was included above 10% in the diet. However no significant difference was observed between 5 and 10% level on enzyme supplementation. Feed efficiency, protein efficiency and dressing percentage were not affected due to inclusion of FTW at different levels. The cost per kg gain was lowest at 10% level with enzyme supplementation. Therefore, the study reveals that the FTW can be used up to 10% level with enzyme supplementation in the ration of broilers.

Key words: Factory Tea Waste, Multienzyme, Broilers chicks

Due to the high cost of cereal grains and milling byproducts, it is necessary to explore alternative and inexpensive ingredients that are locally available, and are not used for human food. Tea (*Camellia assamica*) waste, a tea industry by product, is available to the tune of 9,06,876 tonnes (GTAC, 2003) annually from 2472 tea gardens (Deka, 2001) in Assam, and it contains 21.8 per cent protein (Das et al. 2005).The tea waste protein, contains quite high level of all the essential amino acids compared to other by products like salseed meal, babul pods, mahua seed cake, mango seed kernel, leucaenea leaf meal, cotton seed cake. (Barman and Rai, 2003). Enzymes are primarily used in poultry diets containing high amount of cellulose and hemicellulose, which are reported to be helpful in enhancing the nutritive value of feedstuff . The present experiment was conducted to study the growth of broiler chicks fed diets containing varying levels of Factory Tea Waste (FTW) alone or in combination with an enzyme cocktail.

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Three hundred sixty day old broiler chicks (Vencob) of the same hatch were randomly distributed into 8 equal groups of 45 chicks in each. Each group was further divided into three replicates of 15 chicks in each. Broiler starter (0-4 weeks) and finisher ration (4-6 weeks) were prepared as per BIS (1992) for control ration. The experimental rations viz T_1,T_2, T_3 were prepared from the respective control(C) ration by incorporating, factory tea waste each at 5, 10, 15 percent levels replacing matching quantity of wheat bran (w/w) (Table 1). The control and experimental rations without or with enzyme supplementation were designated as control - C_1E_1,C_1E_2(0%FTW,0%FTW+enzyme); experimental rations T_1E_1,T_1E_2 (5% FTW,5% FTW +enzyme); T_2E_1,T_2E_2 (10% FTW, 10% FTW + enzyme); T_3E_1,T_3E_2 (15% FTW,15% FTW + enzyme) and randomly allotted to respective groups housed in thermostatically controlled battery brooders. The experiment was conducted for a period of 6 weeks in 2 phases; starter (0-4 weeks), finisher (4-6 weeks).Weekly body weight, feed consumption and mortality were recorded. At the end of experiment,

Table 1. Composition (%) of broiler rations varying in factory tea waste (FTW)

Ingredients	Starter ration (0-4 weeks)				Finisher ration (4-6 weeks)			
	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Maize	45.50	47.00	48.46	49.03	58.87	57.33	59.64	59.64
Wheat bran	15.00	10.00	05.00	00.00	15.00	10.00	05.00	00.00
Factory Tea Waste	00	05.00	10.00	15.00	00	05.00	10.00	15.00
Fish meal	06.00	06.00	05.00	06.00	07.00	04.00	03.50	04.50
Rice polish	02.50	02.00	02.00	01.00	-	-	-	-
Til oil cake	13.50	16.00	15.54	14.97	02.00	10.00	09.00	10.00
Ground Nut Cake	16.50	13.00	13.00	13.00	16.53	13.07	12.26	10.26
Mineral Mixture	0.60	0.60	0.60	0.60	0.40	0.40	0.40	0.40
Salt	0.40	0.40	0.40	0.40	0.20	0.20	0.20	0.20

Mineral mixture contains Ca 25%, P 5%, NaCl 23%, I 10 ppm, Cu 100 ppm, Mg 2000 ppm, cobalt 10 ppm.

Each 500g of Multienzyme (Alvizyme Plus) contains : Alvizyme plus contains, *Lactobacillus acidophilus* 5,00,000 million CFU, *Saccharomyces cervisiae* 5,00,000 million CFU, Amylase 2,50,000Units,Protease 3,50,000 Units, Lipase 20,000Units, Cellulase 30,000 Units, Phytase 45,000 Units, Galactosidase 45,000 Units, Glucanase 70,000 Units, Pectinase 1,20,000 Units, Xylanase 35,000 Units.

Table 2. Performance of broilers fed diets containing different levels of factory tea waste (FTW)

Parameters SE	C_1E_1	C_1E_2	T_1E_1	T_1E_2	T_2E_1	T_2E_2	T_3E_1	T_3E_2	Pooled
Av. wt. gain at 6 weeks (g)	1388.67^{cd}	1418.72^{d}	1382.26^{bcd}	1400.47^{cd}	1340.47^{b}	1340.33^{bc}	1372.60^{a}	1090.40^{a}	12.25
Feed consumed per birds (g)	3234.49^{b}	3272.85^{b}	3226.47^{b}	3224.61^{b}	3161.90^{b}	3188.09^{b}	2659.87^{a}	2692.46^{a}	72.10
Feed/gain ^{NS}	2.41	2.39	2.42	2.38	2.45	2.41	2.55	2.50	0.054
PER ^{NS}	1.98	1.99	1.97	1.99	1.94	1.97	1.86	1.90	0.12
N-balance (g)	57.03^{c}	58.29^{c}	55.57^{bc}	58.05^{bc}	54.64^{b}	56.49^{b}	53.06^{a}	53.35^{a}	0.64
Dressing Percentage ^{NS}	71.32	72.52	71.47	71.35	71.13	71.33	70.89	70.98	0.38
Cost of feed / kg gain in wt.(Rs)	24.53	24.64	23.23	23.13	22.71	22.62	23.92	23.78	-
No. of chicks died	1	1	2	1	1	2	3	2	-

* Figures with different superscripts in a row differs significantly ($P<0.05$)

3 birds from each group were selected for nitrogen balance trial for a period of 3 days. After the entire feeding and balance trial 3 birds from each group were slaughtered to study carcass quality. Feed samples were analysed for nitrogen and proximate principles (AOAC 1990) and analysis of data was carried out statistically in a 2x4 factorial design as per Snedecor and Cochran (1980).

The data on average body weight gain, feed consumption, feed efficiency, nitrogen balance dressing percentage, mortality and feed cost per kg live weight gain are presented in table 2. The chemical composition of FTW on DM basis was CP 24.57, EE 3.5, CF 18.00, NFE 48.43, total ash 5.50, Ca 3.80, P 0.52 and tannic acid 6.02 per cent respectively. The percentage of CP, EE was found to be higher than those reported by Konwar et al. (1985). The average body weight gain in 6 weeks significantly ($P< 0.05$) decreased with increased level of factory tea waste in the diet with or without enzyme supplementation. Total gain in body weight

at 6 weeks was significantly different ($P < 0.05$) between control C (C_1E_1, C_1E_2), T_2 (T_2E_1) and T_3 (T_3E_1, T_3E_2). However, no significant difference was observed between C (C_1E_1, C_1E_2) and T_1E_1, T_1E_2 (5% FTW), T_2E_2 (10% FTW) with enzyme supplementation. Results of the present study are similar to that of Daimary et al. (1988) who reported linear decrease in total gain in body weight with increased inclusion of decaffeinated tea waste from 5-15% in the ration although the gain in body weight at 15% inclusion of tea waste was comparable to control. In another study (AICRP, 1982), body weight gain was progressively depressed when level of incorporation of FTW was more than 5% in broiler starter ration. The least body weight gain observed in T_2E_1 (10 % FTW, without enzyme), and T_3E_1, T_3E_2 (15% FTW, without or with enzyme) groups might be due to higher inclusion of FTW which contain 6.02 % tannic acid. No significant difference was observed between control and T_2E_2 (10% FTW, with enzyme), which could be due to positive effect of enzyme supplementation as it might help in improving the digestibility of organic nutrients. Mean digestibility of DM, CP and NFE was significantly lower in T_3E_1, T_3E_2 groups (15 % FTW, 15% FTW + enzyme). Digestibility of ether extract and CF was also lower in T_3E_1,T_3E_2(15% FTW,15% FTW + enzyme). However, no significant difference was observed in feed consumption among C, T_1 and T_2. Feed intake was significantly depressed due to inclusion of 15 percent FTW. Daimary et al. (1988) reported linear decrease in feed intake when higher level of decaffeinated tea waste was incorporated in broiler diet. Vohra et al. (1966) reported reduced feed intake when tannic acid content of ration was 0.5 per cent. The feed efficiency and protein efficiency ratio was decreased with increased level of FTW incorporation in the diet, although non significantly. AICRP (1982) reported lower feed conversion efficiency at 10 and 15 per cent level of FTW incorporation in the diet. The percentage nitrogen retention decreased with increasing level of FTW in the diet which might be due to lower utilization of protein by the birds. The percentage nitrogen retention was lowest in T_3 group (15% FTW). However, improvement in nitrogen retention in the enzyme supplemented groups is probably due to multienzyme supplementation. Dressing percentage was not affected by the level of FTW in the diet. The cost of feed/ kg live weight gain was lowest with inclusion of 10 percent FTW with enzyme supplementation. The percentage survivability of chicks fed different levels of FTW and control diets were uniformly good and few deaths that were noticed could not be attributed to dietary treatments. From the results it is concluded that FTW can safely be included upto 10 per cent level with enzyme cocktail supplementation in broiler rations without any detrimental effect on their growth rate, feed efficiency and performance.

REFERENCES

AOAC. 1990. Official Methods of Analysis. (15th Ed.,) association of analytical chemists Washingtin, D.C.

AICRP.1982. Annual report of All India Coordinated Research Project on Agril. By Products. Department of Animal Nutrition. Assam Agricultural University, Khanapara, Guwahati-781022.

Barman, K., and S.N. Rai . 2003. Tannins profile in some agro-industrial by-products. Proc.4th Asian Buffalo Cong. "Buffalo for food security and rural employment." Held at New Delhi, Vol. 11 Pp: 171-178.

BIS. 1992. Indian Standard of Poultry feeds (IS:1374:1992)

Daimary, P., A. Saikia ,K.K.Baruah, P.C. Das, and B.N. Saikia. 1988. Possibility of including decaffeinated tea waste as a source of protein in the diet of broiler chicks. Indian J. Anim. Prod. Magmt.,4-5:51-52.

Das, J., B.N Saikia, K.K. Baruah, D.C Milli. 2005. Effect of Tea waste on the performance of kids. Indian J.Anim.Nutr.,22:244-246.

Deka, A. 2001. Tea industry in Assam Agriculture. Directorate of Extension Education, Assam Agril. University., Jorhat-13, Assam, India, pp132-152.

GTAC. 2003. Guwahati Tea Auction Center. Annual Report Ganeshguri, Dispur, Guwahati-6, Assam, India.

Konwar, B.K., A.K. Medhi, H.F. Ahmed, A. Saikia and P.C Das. 1985 Effect of feeding factory tea waste in starter chicks. Indian J.of Poult. Sci., 20:122-123.

Snedecor, G.W. and W.G. Cochran. 1980. Statistical Methods. (6th Ed.) Oxford and IBH Publishing Co. Pvt., New Delhi

Vohra,P., F.H Kratzer, and N.A. Joalyn. 1966. The growth depressing and toxic effect of tannins to chicks. Poult. Sci., 45;135-142.

NEWS, AWARDS/HONOURS

Prof. M.J. MODAYIL, Ph.D., D.Sc.
MEMBER, A.S.R.B.

Professor Mohan Joseph Modayil who is currently Member, Agricultural Scientists Recruitment Board, New Delhi is an accomplished academician-researcher-manager with 38 years of work experience in various spheres of fisheries in India and abroad. He has a first class Masters in Marine Biology and Fisheries from Kerala University, Ph.D. in Bio Science from Mysore University and D.Sc. in Aquatic Biology and Fisheries from Kerala University. He had his postdoctoral research at the School of Marine Sciences, University of North Wales, United Kingdom. He is also an Elected Fellow of the Zoological Society of London. The areas of his specialization include Marine Biology, Marine Fisheries, Fisheries Management, Coastal Mariculture, Fisheries Governance and Policy, Research Management, Development and Administration, Human Resource Development & Management, Postgraduate Research Guidance.

He started his research work on marine cryptofauna along the south east coast of India which was a pioneering study to understand the interrelationships of cryptofauna to marine algae. He started his academic career in the area of manpower development at the College of Fisheries of the University of Agricultural Sciences where he served in various capacities for 30 years and while leaving held the position of Professor and University Head of Department of Fisheries Resources and Management. He was selected to the position of Director, Central Marine Fisheries Research Institute, Cochin in 2000 which he held till December 2007 when he moved over to the Agricultural Scientists Recruitment Board as Member (Animal Sciences) after being selected by a National Search Committee.

During the past 38 years of his service to the country, he has been instrumental in developing need based course curriculum for the professional fisheries education in the country. He has been an excellent teacher in the field of taxonomy, population dynamics, estuarine biology, , molluscan biology and ecophysiology. His research on the biology and population dynamics of marine bivalves has been noteworthy. In the field of reproductive biology, his scheme of classification of maturity of bivalves has been adopted as a standard tool by other workers. In the early seventies, he was able to demonstrate the feasibility of growing mussels and edible oysters in the backwaters of the south west coast. He, along with the large number of students who worked with him, developed a school of thought on the reproductive strategies of marine bivalves in the Indian waters. His work on developmental research took him on assignments in the Philippines, Thailand, Malaysia, Islamic Republic of Iran and West Indies. He also visited USA, Canada, Australia, UAE, China, Singapore, Thailand for various short assignments. He had coordinated and IDRC and DFID Projects in India with extremely useful developmental outputs.

He has edited over a dozen books and has over 100 research publications, 50 articles, numerous reports, bulletins, Special Publications and a dozen invited book chapters. He served as Managing Editor, Indian Journal of Fisheries (2000 to 2007), Editor in Chief, Journal of Marine Biological Association of India (2000 to 2007), President, Marine Biological Association of India (2000 to 2007), Council Member, Asian Fisheries Society, Philippines (2005 Onwards), Member, Academic Council, Kerala University, Member, Academic Council, Kerala Agricultural University, Member, Senate, Cochin University of Science & Technology, Consultant/ Researcher to IDRC in Philippines, Thailand, Malaysia, West Indies, Consultant for IFRTO, Islamic Republic of Iran, Faculty Attachment at the University of North Wales, U.K., Programme Coordinator, DFID Project of the British Government, India, Chairman, National Committee on Monsoon Trawl Ban, Member, National Coastal Zone Authority, National Exotic Species Committee, National Biodiversity Committee, Member, Editorial Board of Indian Journal of Marine Science, Indian Hydrobiology, President, Marine Biological Association of India, Vice President Asian Fisheries Society Indian branch.

His service as Director, Central Marine Fisheries Research Institute for the seven years from 2000 to 2007 resulted in great advancements for the CMFRI which has been turned in to a world class organization through his administrative, research and financial management initiatives, proving his abilities as an able administrator. For the first time in the country, he pioneered Open Sea Cage Mariculture of Finfishes, flagging a historic leap in fish farming initiatives in India. The recently concluded 8th International Asian Fisheries Forum is a standing testimony for his organizational abilities.

ANNOUNCEMENT

VIth Regional Conference

Nutritional Interventions to Improve Animal Production

On

15th October, 2008

Venue

Animal Nutrition Department, College of Veterinary Science, Tirupati.

VIth Regional conference of Animal Nutrition society of India, South zone is being organized in collaboration with Sri Venkateswara Veterinary University, Tirupati with the theme "Nutritional interventions to improve animal production" on 15th October, 2008 (Wednesday) at Animal Nutrition Department, College of Veterinary Science, Tirupati. The conference will be focusing on different areas of technological advances in various aspects of Animal Nutrition and allied subjects during the technical deliberations. It includes presentation of lead papers on the feed processing technologies developed on feed resources by eminent Scientist / Policy makers. There will be a Farmer-Industry and Scientist interaction on current and emerging Animal Nutrition issues for improving livestock production.

Further information can be obtained form Professor J. Rama Prasad, organizing secretary, ANSI, South University Professor & Head, Department of Animal Nutrition, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati – 517 502.

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Dr. Chander Datt honoured with Young Scientist Award

Dr. Chander Datt working as Scientist, SS (Animal Nutrition) at ICAR Research Complex for NEH Region, Tripura Centre, Lembucherra was conferred with Dr. K. Pradhan Young Scientist Award (2005-07) on October 4, 2007 during the inaugural session of International Tropical Nutrition Conference-2007 (Tropnutricon-2007) which was held from Oct. 4 to 7, 2007 at National Dairy Research Institute, Karnal (Haryana) auditorium. Dr. Dayanand Dongaonkar, Secretary General of Association of Indian Universities was the Chief Guest of the function. The Conference was held under the aegis of Animal Nutrition Society of India (ANSI). Other dignitaries on the dais included Dr. Sushil Kumar, Director, National Dairy Research Institute, Karnal, Haryana, Dr. E.R. Orskov, an eminent animal nutritionist from U.K. Dr. K.K. Singhal, Organising Secretary of the conference, Dr. T.K. Walli, President, ANSI and Dr. S.N. Rai, Secretary, ANSI. Dr. K. Pradhan, an internationally acclaimed animal nutritionist, presented the award to Dr. Datt. About 500 reputed animal scientists, educationists and people from feed industry from India and abroad (USA, UK, France, Italy, Germany, Iran, Sri Lanka, Gambia etc.) attended this conference. The award has been conferred on Dr. Chander Datt in recognition of his research work carried out in the field of Animal Nutrition. He worked on nutritional evaluation of local feed resources of Tripura and also on the introduced cultivated forages. He also studied the essential mineral status of soils, feed/fodders and cattle in Tripura so that corrective measures could be taken in case of imbalance/deficiency.

Earlier, he was presented with ASPEE Gold Medal for graduation (B.Sc. (Hons.) Agriculture). He was honoured with Jawahar Lal Nehru Award-1999 (National Award) for his Ph. D. work for outstanding contribution in the field of animal nutrition. He joined as Scientist at ICAR Research Complex for NEH Region, Tripura Centre in January, 1999. He also received Best Paper Award-2006 presented by Indian Dairy Association, New Delhi. His biography was published in "Marquis Who's Who-2007" (USA) and International Biographical Centre (UK) for his excellent academic record. He has 15 research aricles, 40 research abstract papers, 15 popular articles, 4 book chapters, 3 book/bulletins and 4 booklets/scientific folders to his credit.

Announcement

The CEC of ANSI in it's meeting held on 19/07/2008, decided to confer one biennial ANSI **award of Rs. 5000/-** to be shared equally by authors in addition to the existing Dr. S. P. Arora best paper award so as to recognize the quality research contribution in the form of published research articles in the Indian Journal of Animal Nutrition. All the research workers in India and abroad are requested to send their quality manuscripts, to chief editor, IJAN, so as to compete for the above two awards.

(Dr. S. S. Thakur)
Secretary,
ANSI, NDRI Karnal

Guidelines for author(s) Revised as on March 08
Submission of manuscripts

Manuscripts should be written, so that these are intelligible to the professional reader. The Editor or the Publisher reserves the right to modify typescripts to eliminate ambiguity and repetition and improve communication between author and reader. If extensive alterations are required, the manuscript will be returned to the author for revision. Refer web page www.nutrisocietyindia.com

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The original manuscript, two copies and a CD should be submitted to:

Dr. S. S. Kundu
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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. Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

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Manuscripts should be presented in the following order: (i) TITLE PAGE, (ii) ABSTRACT and Keywords, (iii) INTRODUCTION, (iv) MATERIALS AND METHODS, (v) RESULTS AND DISCUSSION, (vi) REFERENCES, (vii) 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.

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Articles must have a structured abstract that states in 500 words or fewer, the purpose, basic procedures, main findings and principal conclusions of the study. The abstract should not contain abbreviations or references. Five or fewer key words (for the purpose of indexing) should be supplied below the abstract.

Acknowledgments

The source of financial grants and other funding should be acknowledged, including a frank declaration of the author's industrial links and affiliations. The contribution of colleagues or institutions should also be acknowledged.

References

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Books and Articles within Edited Books

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arligton, VA.

NRC. 1989. Pages 90-110 in Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Dutta, T.K., and S.S. Kundu**.** 2005. Probiotics in small ruminant's nutrition. Page 193-214 in Animal Feed Technology.. S.S. Kundu, S.K. Mahanta, Sultan Singh and P.S. Pathak. Eds Satish Serials Publishing House, Delhi.

Handbooks, Technical Bulletins, Theses, and Dissertations

Goering, H.K., and P.J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA , Washington, DC.

Sachan, C.B. 2004 Analysis of production performance and management practices of Bhadawari buffaloes in its home tract and at organized farm. PhD Diss. Bundelkhand University, Jhansi (U.P.) India.

Journal Articles and Abstracts

Singh,S., G.Suresh, S.S. Kundu, and P.S. Pathak. 2008. Effect of organic and inorganic fertilizers on nutritive value of sorghum fodder in cattle ration. Indian J. Anim. Sci.78:406-409.

Singh,S., S.S. Kundu,and L.K. Karnani. 2007. Effect of supplanting the *Dicanthium annulatum* hay diet with tree leaves on microbial activity in the rumen of goats. J. Anim. and Feed Sci.16, Suppl 2,124-129

Conference Proceedings

Kundu, S.S., M.M. Das, K.K. Singh, and A.K. Samanta. 2002. Evaluation of some locally available feeds for carbohydrate and protein fractions. Page No. 140 – 141in Proc. of Natl Symp. on Grassland and Fodder Research in the new millennium. Indian Grassland and Fodder Research Institute, Jhansi (U.P.) India.

NMC. 1995. Summary of peer-reviewed publications on efficacy of pre milking and post milking teat disinfections published since 1980. Pages 82-92 in Natl. Mastitis Counc. Reg. Mtg. Proc., Harrisburg, PA. Natl. Mastitis Counc., Arlington, VA.

Talmant, A., X. Fernandez, P. Sellier and G. Monin. 1989. Glycolytic potential in longissimus dorsi muscle of Large White pigs as measured after in vivo sampling. Page 1129 in Proc. 35th Int. Congr. Meat Sci. Technol., Copenhagen, Denmark.

Van der Werf, J. H. 1990. A note on the use of conditional models to estimate additive genetic variance in selected populations. Proc. 4th World Congr. Appl. Livest. Prod. Edinburgh, Scotland XIII: 476-479

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.PDFAccessed Dec. 14, 2001.

Huntington, G.B., D.L. Harmon, N.B. Kristensen, K.C. Hanson, and J.W. Spears. 2006. Effects of a slow-release urea source on absorption of ammonia and endogenous production of urea by cattle. Anim. Feed Sci. Technol. Doi: 10.1016/j.anifeedsci.2006.01.012

Le Neindre, P., C. Terlouw, X. Boissy, and J. Lensink. 2001. Behavioral research and its application to livestock transport and policy: A European perspective. J. Anim. Sci. 79 (E-Suppl.)

http://www.asas.org/jas/jas0905.pdf Accessed Oct. 7, 2001.

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