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CONTENTS

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REVIEW

Grain Sprouts as Green Feed with Hydroponic Technique: Review of Merits and Limitations

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ABSTRACT

Hydroponic is an alternative technology to grow green feed without soil. This technology requires less land, water and is resilient to weather conditions, guaranteeing green feed production round the year. This method of producing green feed has both merits and limitations. Cereal grains and legume seeds can be utilized for sprouting to produce green feed. The most commonly used cereal grains are barley and oat in temperate countries, and maize in India due to easy availability and lower cost. During the sprouting process, beneficial changes occur in grain in terms of increased enzyme activity as well as protein content and more bioavailable amino acids and vitamins. The facility for production of grain sprouts can be modern hi-tech type of climate-controlled chambers or low-cost device as per the financial status of the farmer, availability of building material and local conditions. Hi-tech chambers are fully automatic, maintain optimal conditions and yield more biomass but relatively expensive. In tropical countries, low cost devices are more accepted to grow grain sprouts by small dairy farmers. The merit of hydroponic grain sprouts is ease of producing a green feed with better protein and micronutrient profile in a short period for supplementation along with available crop residues like straw and stover, especially in places where there are constraints of land and water. The limitations of this technique include loss of dry matter, reduction in energy content and chances of fungal contamination. Perusal of published reports suggest that the green biomass produced using hydroponic technique is a new category of feed resource and calling it as green fodder may not be appropriate, as it can't replace the conventional forage. Hence, in view of the nutrient profile, merits and limitations of production, grain sprouts can be termed as green feed for strategic supplementation with more utility during contingency situations. The need to review some of the pertinent work done on this aspect has been realized and an attempt is made to provide holistic view from the perspective of livestock feeding.

Key words: Contingency supplement, Grain sprouts, Green feed, Hydroponic, Livestock, Nutritive value

INTRODUCTION

Livestock rearing is an important income generating activity in rural India. It backs about 4% to National GDP and is a chief source of employment and ultimate livelihood for 70% of inhabitants in rural areas (IGFRI Vision, 2011). Milk production has been growing annually at an average rate of 6.65% (GOI, 2019). But average milk yield per animal is low because of less nutrition and genetic potential. Presently, land available for fodder production is just 4-5% of cultivated area and it has been predicted that the shortage by 2025 will be about 40% for green and around 20% for dry fodder. With an objective to augment green fodder availability, research effort on hydroponics was renewed in India in the last 10 years and good amount of data has been generated from the studies conducted in many Institutions. The technology of growing fodder without soil and by sprinkling water in short time is called Hydroponics (Bakshi *et al.,* 2017). Hydroponics is the method of growing plants without soil, but with water or nutrient solution under regulated temperature and humidity (Dung *et al.,* 2010a). Grains contains enough nutrients to sustain growth for a period of 7-10 days without any additional nutrient supplementation (Naik and Singh, 2014; Gunasekaran *et al.,* 2019). Sprouting grains is an ancient method practiced by Indians and included in many of the dishes. Some farmers practice feeding of sprouted grains to working and milking animals. By extending the sprouting period from 3 to 10 days, grains yield a fairly good quantity of green

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biomass. Study has showed that up fodder may be a sensible supply of nutrients and that they include a grass juice issue that offers increased performance to farm animal (Nutrigrass, 2007). Sprouting process leads to increased enzyme activity, bioconversion of nutrients from one form to another leading to increased protein, vitamins and fibre compared to the seed (Chavan and Kadam, 1989). In times of acute shortage of green fodder during natural calamities like drought, hydroponic fodder can be the solution to some extent as it is not influenced by season (Bakshi *et al.,* 2018). Thus, due to these beneficial changes in nutritive value, sprouted grains could have a good potential in improvement of livestock performance, especially when there is scarcity of land and water. On perusal of nutritive value reported by researchers on this aspect, use of the term "hydroponic fodder" may not be very appropriate due to low fiber content and does not truly qualify to be called as a fodder. Hence, the researchers at ICAR-NIANP, Bengaluru have termed the green biomass obtained 6 to 7 days after germination with hydroponic technique as "grain sprouts". However, the term hydroponic fodder is already reported in the published articles and the same is being retained in this review paper to avoid confusion.

GRAIN SPROUTS BY HYDROPONIC TECHNIQUE

In normal circumstances, soil acts as a mineral nutrient reservoir but the soil itself is not crucial to plant growth. When the mineral nutrients in the soil are dissolved in water, plant roots are able to absorb them (Bakshi *et al.,* 2017). When the required nutrients are supplied through water, soil is no longer required for the plant to thrive. The green growth plus root mass, seeds and any ungerminated seeds together referred to as sprout fodder / hydroponic fodder / hydroponic feed or hydroponic grass (Naik *et al.,* 2015). The word hydroponics has been derived from the Greek word which means 'water working'. The term Hydro depicts 'water' and ponic means 'working' and it is a technology of growing plants without soil, but in water or nutrient rich solution for a short duration in an environmentally controlled house or machine. The term hydroponics was coined by W.F. Greicke in 1936 who developed first commercial hydroponic fodder production unit in USA (Runjun and Yora, 2018). Dr. Allen Cooper introduced the concept of hydroponic fodder during 1930's. A range of cereals and legume seeds were utilized for fodder production, including barley, oats, wheat, maize, horse gram, cowpea, sun hemp *etc.* The most commonly used cereal grain is barley in temperate countries. Different types of grains successfully used to grow sprout fodder hydroponically are maize (Naik and Singh, 2014; Gunasekaran *et al.,* 2019; Chethan *et al.,* 2021); oat and wheat (Snow *et al.,* 2008);cowpea, sorghum and alfalfa (AI-Karaki and AI-Hashimi, 2012); horse gram and sun hemp (Jemimah *et al.,* 2017) and barley (Reddy *et al.,* 1988). Giridhar and Gowda (2018) suggested that sprouts of jowar (sorghum) should never be used for feeding of livestock as they contain hydrocyanic acid. However, the selection of the hydroponics fodder to be made depends on the agro-climate, price and availability of seeds. In Indian context, maize grain is most suitable for production of hydroponics fodder because of its simple accessibility, lower cost, good biomass yield and quick growth (Naik *et al.,* 2011; Naik, 2012).

METHOD OF GRAIN SPROUTS PRODUCTION

Method of grain sprouts production has been reported by many workers (Naik *et al.,* 2016a; Bakshi *et al.,* 2017; Gunasekaran *et al.,* 2019; Jemimah *et al.,* 2020; Chethan *et al.,* 2021). The resultant green shoots and root mat are harvested and are fed to livestock. The grain responds to the supply of moisture and nutrients by germinating, sprouting and then producing a slender vegetative green shoot with interwoven roots within 5 to 8 days. The facility for production of sprouted grains can be of many types as per the financial status of the farmer, availability of building material and local conditions. Advanced modern hi-tech greenhouse is highly advanced, closed, insulated, illuminated, thermostatically controlled, fully automatic and is relatively expensive. The requirement for water, light, temperature and humidity is maintained by water fogger or sprinkling and artificial lights, controlled automatically through sensors. Such devices are electrically powered,

require skilled manpower and suitable for organized farming (Nailk, 2015). In this type of green houses, the biomass yield will be more with less chances of fungal growth.

LOW COST GREEN HOUSE

In an attempt to reduce the cost of production, low cost devices have been tried using locally available materials with reasonable success (Naik and Singh, 2013; Giridhar and Gowda, 2018). The shade net structure can be constructed with bamboo or wood or MS / GI / PVC pipes or brick masonry. The existing wall of a house can also be used to construct lean-to-shade net green house, which reduces the cost of fabrication. The hydroponics fodder can be irrigated by micro-sprinklers (manual or automatic) or a knapsack sprayer at regular intervals. In shade net structure, the internal environment of the greenhouse is more effected by the outside climatic condition.

PROCEDURE FOR GRAIN SPROUTS PRODUCTION (HYDROPONIC FODDER)

The method adopted by many workers are compiled and reported (Sneath and Mclntosh, 2003; Naik, *et al.,* 2015; Gunasekaran *et al.,* 2017a; Chethan *et al.,* 2021). Procurement of quality seeds with good germination and devoid of fungal infestation is the key. The seeds are soaked in a plastic bucket /drum with potable water for 12 to 24 hours depending on the grain type and hardness of the seed coat. This is followed by decanting the water, wrapping the seeds in a porous cloth material like a clean gunny bag for 48 hours for germination with intermittent spray of water to maintain moisture. Germinated seeds are spread as a thin layer in plastic trays / crates with small holes for drainage of excess water. With the passage of time, the sprouts turn greenish with slender and slimy roots emerging from the seed. In a period of 6-7 days post germination, the total height of sprout would be about 20 cm depending on the quality of grain, water quality, climate and moisture management. The whole process requires moderate light and medium temperature. The fodder / grain sprout can be best grown in tier system to take the advantage of space with shade net (sides) and metal sheet (roof) at a room temperature of 22-28ºC and relative humidity of 65 -70%. Watering can be done manually for 5 to 7 times in a day with sprayer, when the straw bedding is used. In automated hydroponic units, sprinklers/foggers with timers to spray normally at an interval of 60 to 90 minutes. The sprouts will reach a height of about 20 cm in 6-7 days period excluding the time spent for soaking and germination. The green biomass looks like a mat of interwoven root, seed and shoot and ready for feeding. The harvested green biomass can be wilted for few hours to remove excess moisture before feeding to livestock. Maintenance of hygiene is most essential and between two cycles, the trays must be cleaned, often with chlorine-based cleaning solutions (0.1% sodium hypochlorite (Devendar *et al.,* 2020) to minimize the risk of mould growth.

Seed rate: It is the quantity of seeds loaded per unit surface area of the hydroponic tray. It varies with the type of seed used. If the density is too high, chance of mould growth is more and if it is spread sparsely on the tray, the benefit of maximum growth in small space cannot be achieved. Hence, seed rate of 7.6 kg/m^2 (1.5 kg maize seed per tray of 78 cm × 24 cm) for maize is recommended (Naik *et al.,* 2016a) for growing hydroponics maize fodder for higher biomass output. However, most of the other business units advocate seed rate of 6-8 kg/m^2 (Morgan *et al.,* 1992). The advised seeding rate for cultivation of hydroponic barley or wheat is 4-6 kg/m^2 (Al-Karaki and Al-Momani, 2011; Starova Jeton, 2016). Kide *et al.* (2015) reported that African tall maize selection was spread on the hydroponic tray at a rate of five hundred grams per two sq. feet tray. Gunasekaran *et al.* (2019) considered the impact of seed rate (100, 150, 200, 250 and 300 g/sq.ft of maize seed) on biomass yield of hydroponic fodder maize on 9th day of growth and found significantly ($P<0.05$) lower biomass yield (3.60 ± 0.05 kg/kg of seed) at a seed rate of 100 g/sqft and significantly ($P<0.05$) higher biomass yield (4.50 ± 0.57kg/ kg of seed) was observed at a seed rate of 250 g/sqft equivalent to 2.77 kg/m^2. When seed rate was increased beyond 300 g/sqft, a significant ($P<0.05$) decline in biomass yield was observed. However, Chethan *et al.* (2021) observed an optimal seed rate

density of 4 kg/m^2 which yielded average of 4.5 kg biomass per kg of maize grain.

Water requirement/efficiency: The water used for sprouting of grains should be clean and free from contamination. According to Calder (2002) hydroponic system needs a fraction of the water usage of standard farming whereas still supplying top quality feed. It needs one to two litres of water to provide one kg of fodder as compared with 80-90 litres of water to grow a kg of standard green grass. High water use efficiency is, however, a major advantage of this technique which saves about 95-97% of used water in comparison to conventional agriculture with minimum usage of land space. The hydroponics forage production requires only about 3-5 % of water needed to produce same amount of forage produced under conventional system (Al- Karaki and Al-Momani, 2011; Al- Karaki and Al-Hashmi, 2012; Naik *et al.,* 2014). However, the total water required for the complete cycle of sprouts production (soaking, germination, cleaning of trays and the green house) is about 4.2 liter per kg of biomass yield (Chethan *et al.,* 2021).

Optimum temperature and humidity: Temperature of the water used for soaking also affects the seed germination rate. The optimum temperature for soaking the seeds is 23ºC (Sneath and Mcintosh, 2003). The trays are placed in hydroponic racks, and seeds are sprayed sporadically with fresh water or nutrient enriched solution. The trays ought to never be exposed to direct daylight, robust wind and significant rain. During the germination period, the seeds are kept moist by keeping them wrapped in a wet cloth or gunny bag. The environmental factors for optimum growth include temperature between 19 to 22ºC, humidity between 40-80% (optimum being 60%) and aeriation for 3 minutes after every 2 h (El-Deeba *et al.,* 2009; Starova Jeton, 2016).

Light: Photosynthesis is not important for the metabolism of the seedlings until day 5 when the chloroplasts are activated (Sneath and Mclntosh, 2003). Therefore, light isn't needed for germination of cereal grains. However, moderate light within the last half of the germination period encourages photosynthesis and greening of the sprouts. Bakshi *et al.* (2018) reported optimum light of 2000 lux intensity for 12-16 h in a day.

Nutrient solution: The nutrient solution for cultivating hydroponic forage isn't necessary because it can even be created by normal potable water. There are reports of non-significant improvement in the nutrient content of the sprouts with nutrient solutions, that don't justify the additional expense of using the nutrient solution comparatively than H_2O (Dung *et al.,* 2010a). The comparative analysis of husbandry barley created by using tap H_2O or nutrient disclosed that sprouts grown with nutrient had higher crude protein and ash contents than those grown with tap H_2O. The calcium, potassium, phosphorous, magnesium, sodium, iron, copper and zinc concentrations were higher in barley fodder created using nutrient solution (Peer and Leeson, 1985; Dung *et al.,* 2010a; Fazaeli *et al.,* 2012). However, there was no significant distinction in dry matter loss and *in sacco* degradability of nutrients. Moreover, previous reports indicated that the nutrient needs of the seedlings are satisfied from the nutrients reserves in the seeds (Bewley, 1997; Dung *et al.,* 2010b). Gunasekaran *et al.* (2017b) studied the effect of various nutrient solutions *viz.*, 2.5% biogas slurry, 10% diluted cow urine, 0.1% urea and 10% vermiwash and found that the highest biomass yield was in group supplemented with water as nutrient solution. Use of nutrient solution also will increase value of fodder / sprout production. Thus, it was determined by several workers that there was no extra good thing about nutrient solution for producing hydroponic fodder / sprouts (Dung *et al.,* 2010a; Fazaeli *et al.,* 2012).

Duration of growth and harvesting time: The starting of sprouting and visibility of roots varies with the kind of seeds. In case of maize and cowpea seeds, sprouting starts after 1 or 2 days and the roots were clearly visible after 2 or 3 days, respectively. The grains are usually allowed to sprout for about 7 days inside the greenhouse and on 8^{th} day they are harvested as a fodder / sprouts for feeding livestock. Normally, the farmers producing hydroponics fodder / sprouts by means of low cost devices in field conditions allow the sprouted grains to grow for 7-10 days; however, it may

enhance the chances of mould growth due to increasing moisture beneath the root system (Chethan *et al.,* 2021).

Biomass yield: Naik *et al.* (2011) stated that 1 tray containing 1500 grams maize seeds produces 7-9 kg green fodder / sprout with fodder height of 20-25 cm. There is upsurge in fresh weight and decrease in the DM content during sprouting of seeds. Harvests of 5-6 folds on fresh basis (1000 grams seed yield 5-6 kg fodder/sprout) and DM content of 11-14% are common for hydroponics maize fodder; however, occasionally DM content up to 18% has also been noted (Naik *et al.,* 2017). One kg of maize seed produced 4.5 kg green sprout in 10 days in studies of Chethan *et al.* (2021). The variation in biomass yield is due to type and quality of seed material and managemental conditions. Height of the plant differs with the type of the seed and quality. In maize hydroponic fodder, it is stated that the usual height will be 22-24 cm (Chethan *et al.,* 2021) grown in plain water and it can differ from 11to 30 cm or more (Dung *et al.,* 2010b; Naik and Singh, 2013).

Nutrient composition: There is increase in fresh weight and decrease in the DM content during sprouting of seeds, which is attributed to the imbibition of water and enzymatic activities.The crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and Ca content were increased, but organic matter (OM) and non-fibrous carbohydrates (NFC) content decreased (P<0.05) in the hydroponic green forage compared with the original seed on a DM basis (Fazaeli *et al.,* 2012; Kide *et al.,* 2015; Mehta and Sharma, 2016; Chethan *et al.,*2019). Hydroponic fodder/sprout is a good source of vitamins A, E, C, thiamin, riboflavin, niacin, biotin, free folic acid, anti-oxidants like β-carotene (Finney, 1982; Cuddeford, 1989; Naik *et al.,* 2015) and minerals (Chung *et al.,* 1989; Fazaeli *et al.,* 2012). Shipard (2005) and Naik *et al.* (2014) found that hydroponic fodder is also a rich source of bioactive enzymes, with the highest activities in sprouts being generally between germination and 7 days of age (Chavan and Kadam, 1989). Nutrient and mineral profile of grain sprouts has been compared with maize grain and maize green fodder (Tables 1 and 3; Chethan *et al.,* 2019; 2021). The fatty acid concentration showed a significant (P<0.05) positive relationship with the growth period. The concentrations of linoleic, linolenic and stearic acids increased (P<0.05) linearly with sprouting time (Peer and Leeson, 1985). Besides helping in the elimination of the anti-nutritional

Table 1. Chemical composition and nutritive value of Maize grain, maize grain sprout and conventional maize green fodder on DM basis (Chethan *et al.,* 2021)

Attributes	Maize grain	Maize grain sprout	Conventional maize green fodder
Organic matter (%)	98.6	97.1	94.5
Crude protein (%)	8.50	13.0	7.81
Ether extract (%)	2.44	4.40	1.93
Crude fiber (%)	2.43	10.0	27.6
Total ash (%)	1.42	2.89	5.49
Total carbohydrates (%)	87.6	79.5	84.7
Neutral detergent fiber (%)	13.26	32.9	52.8
Acid detergent fiber (%)	3.42	15.5	25.7
Acid insoluble ash (%)	0.17	0.46	1.20
Acid detergent lignin (%)	0.44	0.98	2.09
Total digestible nutrients (%)	76.6	65.2	61.2
IVDMD (%)	90.1	67.6	60.9
IVOMD (%)	90.6	68.0	61.2
Metabolizable energy (MJ/kg)	11.5	9.78	9.18

Table 2. Chemical composition of hydroponics maize fodder at different days of sprouting (% DM basis) Limba *et al.* (2016)

Attributes	Maize Seed (0 day)	Days of sprouting under hydroponics system						
		1	2	3	4	5	6	7
Organic matter	99.2	98.9	98.7	98.8	98.6	98.2	97.6	97.2
Crude protein	9.97	11.37	12.7	13.4	14.8	15.1	16.6	18.4
Crude fibre	2.63	3.14	3.80	4.63	5.12	6.20	6.38	7.30
Ether extract	2.54	2.51	2.59	2.96	3.14	3.21	3.29	3.50
Nitrogen free extract	84.1	81.9	79.7	77.7	75.5	73.7	71.3	68.0
Total ash	0.80	1.10	1.26	1.20	1.42	1.74	2.40	2.80

factors such as phytate in the grains, hydroponic fodders/sprout are good sources of chlorophyll and contain a grass juice factor that improves the performance of livestock (Naik *et al.,* 2015). The resultant crop is free from pesticides or herbicides (Naik and Singh, 2014) and almost organic in nature.

NUTRIENT CHANGES DURING GROWTH OF HYDROPONIC SPROUTS

Naik *et al.* (2016b) examined the growth rate of hydroponics cowpea at different stages and reported that fresh yield (kg/kg seed) of hydroponically sprouted cowpea increased while that of DM yield decreased ($P<0.05$) with the advancement of growing period and respective values remained highest and lowest on 6th day of growing period. The CP content of the hydroponics cowpea fodder was the lowest ($P<0.05$) on 1st day (21.04), then increased and was the highest on 6th day (27.2%) day of sprouting period. The EE content increased and was the highest on 6th day (1.77%) of sprouting. During growth period of the hydroponics cowpea sprouts, the CF content increased and NFE level decreased. Similarly, Al-Karaki and Al-Hashimi (2012) reported 5.5 times increase in fresh weight of cowpea with 15% DM after sprouting. Fresh yield of 3.5-6 folds in 7-8 days with DM content of 10.3 and 18.5% has been reported for hydroponic barley and maize fodder, respectively (Naik *et al.,* 2015). Limba *et al.* (2016) reported increase in CP, EE, CF and ash content with reduction in NFE content with the progress of sprouting of maize grain (Table 2).

EFFECT OF HYDROPONIC FODDER IN LIVESTOCK

Voluntary intake: Early report of Pandey and Pathak (1991) indicated a voluntary intake of 50.38 kg fresh hydroponics sprouts/day that supplied 7.13 kg DM (1.93 kg/100 kg BW) to the animal. However, they reported that the DM intake was a limiting factor on sole feeding of hydroponic green fodder. Sometimes, animals take the leafy parts of the hydroponics fodder and the roots portions are not consumed which can be avoided by mixing the former mixture with the other roughage components of the ration (Reddy *et al.,* 1988, Naik *et al.,* 2014). However, there are reports of decrease in the DM intake of the animals when hydroponics fodder/sprout is fed (Fazaeli *et al.,* 2011; Naik *et al.,* 2014; Abhishek *et al.,* 2019), which might be due to

Table 3. Mineral profile of maize grain, maize grain sprouts and maize green fodder (Chethan *et al.,* 2021)

Item	DM	Ash	AIA	Ca	P	Mg	Cu	Zn	Fe	Mn	Se	B
	(% DM)						ppm DM					
Maize grain	91.1	1.44	0.20	0.04	0.25	0.09	5.65	39.5	31.1	7.45	0.94	8.11
Maize grain sprouts	90.2	3.29	0.46	0.20	0.39	0.16	8.86	58.6	65.0	9.95	0.85	9.21
Maize green fodder	90.5	8.94	1.20	0.38	0.16	0.28	13.7	43.7	161	36.5	0.99	23.5

higher water content of the sprouts leading to limited DM intake. Contrarily, Verma *et al.* (2015) observed higher DM intake in hydroponic fodder/sprout supplemented group compared to control in Hariana breed of dairy calves. However, study at ICAR-NIANP in sheep showed lower DM intake due to inclusion of fresh maize grain sprouts replacing conventional green fodder (Unpublished data).

Digestibility of nutrients: Naik *et al.* (2014) reported that hydroponic maize fodder had higher CP (13.3 vs 11.1 %), EE (3.27 vs 2.20 %), NFE (75.3 vs 53.5%) and lower CF (6.37 vs 22.2 %) as compared to conventional green fodder. Digestibility of CP (72.4 vs 68.8%) and CF (59.2 vs 53.2%) were higher in comparison with conventionally grown green fodder of Napier grass. Naik *et al.* (2016c) conducted feeding trial in heifers by feeding hydroponic maize fodder (HMF) and recorded DM of HMF as 15.4% and CP, EE, CF, NFE and TA as 13.5, 2.4, 8.96, 73.4 and 1.56 %, respectively on DM basis and also reported digestibility of 65.6, 68.1, 80.9, 59.7 and 69.9%, respectively for DM, OM, CP, EE, CF and NFE. Similarly, there was increase in digestibility of DM, OM, CP, CF, EE and NFE in milch cattle fed on hydroponic fodder/sprout (Reddy *et al.,* 1988). Verma *et al.* (2015) reported that feeding of male calves of Hariana breed on hydroponically grown barley feed for 90 days resulted in increase in DM intake by 3.38%, digestibility of nutrients by about 9% for OM and about 7% for CP. Dung *et al.* (2010b) reported 21.9% loss in DM from the original seed after sprouting. A loss of 2% gross energy was observed in the sprouts as compared to the original grain. The ash, crude protein and all other minerals but potassium was higher in concentration on a Dry matter basis in the barley grain sprouts than in the grain. Similarly, there was no remarkable difference (P>0.05) in *in sacco* degradation when grain was compared with hydroponic barley sprouts. Feeding study conducted in sheep at ICAR-NIANP has showed that the digestibility (%) of nutrients with respect to DM, OM, CP and EE was significantly higher due to inclusion of fresh maize grain sprouts replacing conventional green fodder and there was no significant difference on digestibility of NDF and ADF (Unpublished data). The digestibility of nutrients of the hydroponic fodder was alike with the digestible legumes fodder like berseem (Pandey and Pathak, 1991). Adebiyi *et al.* (2018) stated that addition of hydroponic maize fodder/sprouts enhanced the performance and nutrient digestibility of diets in weaned pigs. Abhishek *et al.* (2019) also stated substantial increase in the digestibility of crude protein and crude fiber and opined

Photos: Low cost hydroponics for grain sprouts production with or without straw bedding

it could replace part of protein in concentrate mixture of lactating cows' diet.

Effect on growth: Kide *et al.* (2015) found that feeding of hydroponically grownup maize and barley fodder/sprouts for growing Konkan Kanyal goats inflated the overall DM intake, higher feed conversion quantitative relation and weight gain and additionally economically helpful. Muthuramalingam *et al.* (2015) conducted trial on Tellicherry kids for 5 months by feeding hydroponic maize fodder/sprouts and reported the average body weight at 16, 30, 45, 60, 75, 90, 120 and 150 days as 3.1±0.1, 3.64±0.19, 5.83± 0.31, 9.0±0.18, 13.6±0,46, 14.8±0.93, 17.8±0.36, and 22.1± 0.58 kg, respectively. The average daily weight gain of kids was 140.9 ± 0.31 g. Grigorev *et al.* (1986) and Tudor *et al*. (2003) stated an enhancement in performance due to the feeding of hydroponic fodder when compared to the original unsprouted grain in dairy cattle. However, Myers *et al.* (1974) presented that there was no advantage with regards to animal performance when hydroponic grain sprouts was fed. Similarly, Fazaeli *et al.* (2011) found no effect of the sprout fodder on average daily gain (ADG), but feed cost was increased by 24%. Jemimah *et al.* (2015) reported no adverse effects on average daily gain and FCR in rabbit kittens and goat kids fed hydroponic horse gram or sun hemp fodder replacing 50% of concentrate mixture. Feeding hydroponic barley fodder improved ($P<0.05$) feed intake, ADG and FCR significantly compared to those fed a ration containing barley grains (Mysaa Ata, 2016). Similar results of improved total body weight gain, ADG and lower cost per kg body weight gain upon feeding hydroponic maize fodder was observed by Rajkumar *et al.* (2018). Studies in sheep by Chethan *et al.* at ICAR-NIANP fed maize grain sprout as partial replacement of conventional green fodder and concentrate mixture showed lower($P<0.05$) body weight gain (Unpublished data). The variation in reports of feeding trials with hydroponic forage is mostly attributed to difference in experimental planning and feeding schedule. In view of this, it is difficult to precisely conclude the effects of feeding the sprouts on growth.

Meat quality: Feeding of hydroponic fodder to beef cattle resulted in leaner meat comprising more omega-3-fatty acids and vitamins (Maxwell Salinger, 2013). However, Devendar *et al.* (2020) stated there was no significant change in carcass characteristics and meat quality of Deccani lambs fed hydroponic barley fodder-based rations with replacement of concentrate mixture at 50 and 75 per cent levels.

MILK YIELD AND COST ECONOMICS

Naik *et al.* (2014) reported increase in milk yield by 13.7% and net profit of ₹12.67/cow/day on feeding hydroponic maize fodder against conventionally grown green fodder of Napier grass as control. Reddy *et al.* (1998) reported increase in milk yield in crossbred milch cows fed artificially grown fodder compared to conventional NB-21 fodder and concluded that artificially grown fodder was superior to conventional NB-21 fodder. Naik *et al.* (2017) conducted study in lactating cows replacing maize of concentrate mixture with hydroponic maize fodder (HMF) and observed that feeding of HMF resulted in non-significant increase in milk yield (7.97 vs 8.59 kg/day) and fat (%) (4.20 vs 4.50) in HMF fed group than the control group.

Verma *et al.* (2015) reported 33% reduction in feeding cost per kg weight gain per calf per day where 50 % of CP and energy requirement was met by hydroponic barley fodder. On the other hand, Fazaeli *et al.* (2012) stated that hydroponic sprout had no benefit over barley grain in feeding of calves, relatively it increased the cost of feeding. Ayurvet Research Foundation Ltd. (India) has reported that feeding hydroponic fodder/sprouts to bulls reduced the cost of feeding by ₹ 21 per bull per day.

EFFECT ON REPRODUCTIVE PERFORMANCE

The Surat District Milk Producers Union Limited (SUMAL) carried out experiments in heifers by feeding on hydroponic maize green to 20 heifers while another 20 heifers were given conventional fodder. The group fed with hydroponic fodder had 30% higher reproductive efficiency. Ayurvet Research Foundation Ltd. (India) reported increased concentration of sperm from 1185 to 1199 million/ml and increased number of

straw produced from 1310 to 1433 per ejaculate by feeding hydroponic fodder to bulls (Khanna, 2014). Improvement in reproductive traits like higher level of sex hormones and higher body weight at puberty in rams fed barley grain sprouts has been reported (Al-Saadi, 2017). These reports suggest the role of micronutrients and anti-oxidant compounds present in hydroponic fodder/sprouts.

ECONOMICS OF HYDROPONIC SPROUT PRODUCTION

Traditional fodder production requires a major investment for land, in addition to investment in agricultural machinery, equipment, infrastructure required for pre- and post-harvesting, including handling, transportation and conservation of fodder. It additionally needs labour, fuel, lubricants, fertilizers, pesticides, and weedicides. On the other hand, hydrophonic sprout production needs only seed and water as production inputs with modest labour inputs. Hydroponics minimizes post-harvest losses, with no fuel needed for harvesting and post harvesting processes. Additionally, in hydroponic systems it takes merely 7 to 8 days to grow from seed to sprout while it takes 45 to 60 days under conventional systems. But, the initial investment required for fixing subtle, sophisticated, machine-driven industrial hydroponic fodder production systems, with environmental control, and operational prices are higher than soil-based fodder production farming. Such husbandry systems need far more specialized instrumentation and technical information than is needed in traditional farming. Mold growth in hydroponics is likely and thus prevention or treatment could further involve investment. So, with encouragement of feeding schedule with hydroponic fodder, the profits are typically outweighed by the input costs (Tranel, 2013; Reddy, 2014). Bakshi *et al.* (2017) related the cost of fodder production in hi-tech devices Vs low cost device and observed cost of production was 4-4.50/kg and 2-3/kg (if maize seeds are home grown), respectively. The feed cost per kg milk was expensive when animals were fed maize fodder produced from a hi-tech hydroponic system, mainly due to higher cost of hydroponic fodder production than green fodder produced by traditional farming (Reddy *et al.,* 1988; Naik *et al.,* 2014). However, farmers of the Satara district of Maharashtra stated that the cost of milk production was reduced with use of hydroponic sprout fodder in a low cost shade net system with home-grown or locally purchased seeds (Naik *et al.,* 2013).Consequently, when fodder sprout was produced in low cost hydroponic system, the feed cost per kg milk was reduced exceptionally (25 to 30%) and net profitability was improved notably (Naik *et al.,* 2014; Jemimah *et al.,* 2018).Thus, with proper planning and use of low cost devices to produce hydroponic fodder may reduce the cost of fodder/sprout production.

Effort to produce green fodder by utilizing fibrous materials like rice straw as base material for sprouting of barley grain has been made with good success in improving nutrient content and utilization in sheep (Fayed, 2011). Similarly, in an attempt to improvise and economize such a practice for small holder system, researchers at ICAR-NIANP, Bengaluru have devised grain sprouting method over fibrous material bedding using locally available cereal straw at a ratio of 7 parts of grain and 1 part of chaffed straw (Giridhar and Gowda, 2018). This method involves pre-treatment (soaking) of seeds with 4% vinegar solution prior to germination for preventing mold growth and straw bedding ensures longer availability of moisture between two water sprays, thus economizing the usage of water. The nutritive value of the final product in terms of CF (>20%) and protein (>8%) is similar to conventional green fodder. When the grain sprouts are fed to livestock along with straw bedding, the problem of low fiber in grain sprouts is also solved. In the feeding trials in dairy cattle under field condition with this technique of grain sprouting, supplementation of 4 kg of maize sprouts per day improved daily milk output by at least 1.2 liters besides marginal improvement in fat and SNF contents.

MERITS OF HYDROPONIC FODDER |PRODUCTION

Several merits of hydroponic system have been reported (Sneath and Mclntosh, 2003; Naik *et al.,* 2015; Bakshi *et al.,* 2017) and are summarized in this section.

The green fodder/ grain sprout from hydroponics is fairly more nutritious and of better quality as compared to conventionally grown fodders (CGF). In contrast to conventional green fodders, hydroponic green fodder (HGF) contains more CP with increased levels of amino acids, enzymes, vitamins, and fatty acids. As biochemical changes occur during the process of sprouting leading to more bioavailable form and higher digestibility of nutrients. Grain sprout is soft and very much liked by the animals. Hydroponic technology takes the pressure off the land as it requires only 10 m X 5 m to grow 600-650 kg of fodder/day while to produce the same quantity, one hectare of land would be needed under CGF system. This amount of fodder is sufficient to rear 20-25 milch bovine. This eliminates the dependency on land, encouraging even landless persons to undertake dairy farming as an occupation using HGF technology. The HGF system needs solely 2-3 litres of water to supply one kg of green fodder as compared to fifty five to seventy five litres of water needed for the normal CGF. Besides, there's no wastage of water because the offered water is recycled. The green biomass is succulent and attractive and animals relish it. The feeding of HGF leads to improvement in health and productivity of farm animals. The CGF system needs laborer's to start land preparation, sowing, irrigation, cutting, conveying fodder from field to bovine shed, cutting the chaff and at last feeding the bovine, while in the HGF system, only 1 laborer will complete the complete method in 2-3 hours per day.

HGF system permits fodder to be fully grown at intervals within a temperature range of 15-33^{o}C and relative humidity (RH) range of 70- 80 % without any mold growth. The technology is environmental friendly. The hydroponics system can simply be scaled down or up to cater to the requirements of farmers. The HGF can be fed to cows once the plants are at simply 7-8 days**.** In HGF method, the biomass conversion ratio is as more as 6-7 times that of the CGF fully grown for sixty-five to eighty days. HGF can be made around the year because it is grown under semi-protected conditions. There's no wastage in HGF system, because the entire succulent tender plant (at 7-8 days), comprising of roots, leaves, grain and stem is fed to the animals, whereas the CGF consists of solely leaves and stem. Soil-borne pests and diseases are eliminated in HGF husbandry system. There aren't any herbicides or fungicides employed in the method of manufacturing grain sprouts and hence, the usage of chemicals will be completely avoided.

LIMITATIONS OF HYDROPONIC FODDER/ SPROUT PRODUCTION

On working with hydroponic system of fodder/ sprout production, few workers have noticed limitations also (Naik *et al.,* 2015; Gowda *et al.,* 2019). Under Hi tech system, hydroponic units require continuous and reliable electricity supply to maintain constant temperature and relative humidity, where as in low cost devices it is difficult to maintain the optimum conditions. Constant attention and daily labour for about minimum of two to three hours is required for grain sprouts production. Use of quality and germination grade seeds is of paramount importance for getting maximum biomass yield. Mould growth and mycotoxins production is common, if moisture is not managed properly. Loss of 15-20% dry matter during sprouting is inevitable when compared grain. Rodents and predators are the frequent threats under low cost production system. As grain sprout is highly succulent (contains about 85% moisture and less fiber), in small ruminants like sheep and goat dry matter intake may be reduced and may lead to soft faeces when fed as sole fodder. Hence feeding the green sprouts mixed with dry fodder is a most strategic approach.

FUTURE RESEARCH NEEDS

Scientific research in developing countries needs to focus on developing area specific low-cost devices for production of hydroponics grain sprouts considering agro-climatic conditions and locally available materials. Commonly available cereals and legume seeds should be evaluated with regards to their suitability for green biomass production and nutrient profile. The package of practice for each grain that can ne sprouted hydroponically need to be developed and popularized among farmers. Long term feeding trials with different

types of hydroponics green feed on different categories of livestock with regard to their productive and reproductive performance should be assessed. Grain sprouts using combinations of cereal and legumes appear to be promising feed for equines, that need some research. Solar powered green house should be developed to overcome the problem of electricity in rural areas. As mould growth is one of the major drawbacks in low cost devices an alternative organic mould control measures should be developed to make sprout fodder mould free.

CONCLUSIONS

Hydroponic grain sprouts can be grown in short time with less water and space requirement. Grain sprouts has a better nutritive value in terms of protein and micronutrients as compared to grain. But it does not provide adequate fiber as compared to conventional green fodder. Hydroponically grown grain sprouts as green feed has the potential in improving performance of livestock especially during scarcity of land and limited water availability. This technology is of use to farmers in places where land cost is high or land is unfit for green fodder production. The challenges of sprout production by hydroponic technology are many as it has certain advantages and also some limitations. Thus, low cost hydroponic technology needs to be perfected with suitable modification for location specific livestock based farming system. Hydroponically grown grain sprout is considered to be a new feed resource with entirely different nutritive value than either the grain or green fodder. It is a green feed that can be fed along with dry fodder as a strategic supplement to provide some limiting nutrients for livestock during scarce availability of green fodder.

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Green Synthesis, Characterization and Antimicrobial Evaluation of Selenium Nano Particles on Mastitis Causing Bacteria

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ABSTRACT

Nanotechnology is now creating a growing sense of excitement in the field of life science. Nano particles can be synthesized by chemical, biological and green methods, but green synthesis method is the most emerging approach of preparation due to eco friendly and less time consuming procedure. This study aimed at green synthesis, characterization and evaluation of antibacterial effect of nano selenium on mastitis causing bacteria. Aqueous extract of *Coriandrum sativum* leaf extract were used for the production of selenium nano particles. Characterization of synthesized nano particles was done by zeta sizer analysis and transmission electron microscope. The result of zeta sizer analysis reveals that the diameters of nano particles are within the range of nano scale *i.e.* less then 100 nm. Transmission electron microscopy analysis of selenium nano particles was done to confirm the shape, size and morphology. Antibacterial activity of nano minerals was investigated on mastitis causing bacteria such as *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa* by well diffusion method. The antibiosis test result indicated that selenium nano particles were able to inhibit *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa.* So, it may be concluded that the advances in nanotechnology has enabled us to utilize particles of nano size of selenium as a therapeutic application against mastitis causing bacteria in the coming years.

Key words: Antibiosis, Green synthesis, Nanoparticles, Selenium

INTRODUCTION

A nano material is defined as elements having at least one dimension in length, ranges between 1 nm and 100 nm (Khan, 2017). Nanoparticles (NPs) have wide range of applications in areas such as health care, cosmetics, food, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics and space industries (Zhang *et al.,* 2012). It creates and uses structures that have novel properties because of their small size. It has been referred to as the next industrial revolution. NPs can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time, but chemicals used for NPs synthesis and stabilization are toxic and lead to non-eco friendly by products (Elumalai and Velmurugan, 2015). Thus, there is an increasing demand for green nanotechnology. Green synthesis of nano particles makes use of environment friendly and non-toxic. In other words, Green nanotechnology is the utilization of various plant resources for the biosynthesis of metallic nano particles. Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, tollens and biological method, which have advantages over conventional methods involving chemical agents associated with environmental toxicity (Solanki *et al.,* 2008). Among plants, *Coriandrum sativum* are easily available and has been used in synthesis of various nano particles including selenium (Zhang *et al.,* 2012). Selenium nano particles (SeNPs) have been widely used as an effective antimicrobial agent against bacteria, fungi, and viruses (Zhang *et al.,*2004). Their effect was recognized already in ancient times. Although the SeNPs mechanism of action is still not clear, small diameter SeNPs have a superior antimicrobial effect to those of a larger diameter. Considering the advantages of green synthesis, the present investigation was aimed at production of SeNPs by green synthesis method, its characterization and evaluation of its anti microbial effect on mastitis causing microorganisms such as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

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MATERIALS AND METHODS

Coriandrum sativum leaves were washed thoroughly with the tap water followed by double distilled water to remove debris and other contaminants. The leaves were heated with distilled water at 60 ºC for 15min. Filtration was done by using Whatman filter paper (0.2µm). 5ml prepared leaf extract were added to 100ml of 1mM sodium selenite in an Erlenmeyer flask. The flask was placed on the magnetic stirrer for 24 hours for constant stirring. Reduction of selenite into elemental selenium was confirmed by appearance of red colour of the solution. In order to determine the particle size and potential, Zeta sizer (Malvern Zetasizer Nano ZS90) was used under room temperature and the peak was observed. Transmission electron microscopy (TEM) analysis was performed to determine the shape and size of synthesized nanoparticles. NPs samples were diluted in eppendorf tubes using double distilled water and placed in an ultrasonic bath for 15 minutes. After that one drop of NPs samples were placed over a copper grid and leave for overnight drying and finally observed under TEM (JEOL-2010, Japan).

Mastitis causing microorganisms likes *staphylococcus aureus* (*S. aureus)* (MTCC-1144), *Escherichia coli (E. coli) (MTCC-443), Pseudomonas aeruginosa (P. aeruginosa)* (MTCC-1019) were obtained from Institute of Microbial Technology (IMTECH) Chandigarh, India. Antibacterial effect was observed on mastitis causing microorganisms like S. *aureus, P. aeruginosa, E. coli* by well diffusion method. Muller Hinton agar medium (Hi-media) was prepared for lawn culture of the supplied microorganisms. Then by using a puncher well diffusion method 50µl of synthesized NPs was added. Incubation was done at 37ºC for 24 hours. Antibacterial activity was measured on the basis of the inhibition zone around the well.

RESULTS AND DISCUSSION

The first indicator of synthesis of SeNPs using *C. sativum* leaf extracts is the formation of dark red coloured pigment. The change in colour from pale yellow to red confirmed a quick reduction of Se ions to nano selenium. This difference in colour could be considered as a primary evidence of reducing potential of *C. sativum* leaf extract (Fig. 1). The particle size of the synthesized SeNPS were analysed by using Zeta potential, particle size analyzer which measure the average particle size. For this study the SeNPs solution was placed in a particle size analyser (Malvern Zetasizer Nano ZS90) at 25 ºC shows the average particle size of nanoparticle below 100 nm. Whereas the high intensity peak observed at 79.8 nm denotes that SeNPs synthesized by coriander leaf extract have high poly-disparity index and standard in size (Fig. 3). The TEM analysis result of SeNPs demonstrated that the particles are predominantly spherical in shape. These are well dispersed and homogenous. The average diameter of SeNPs was around 40-60nm (Fig. 2). The red coloured formation confirmed a quick reduction of Se ions to nano selenium. The transmission electron

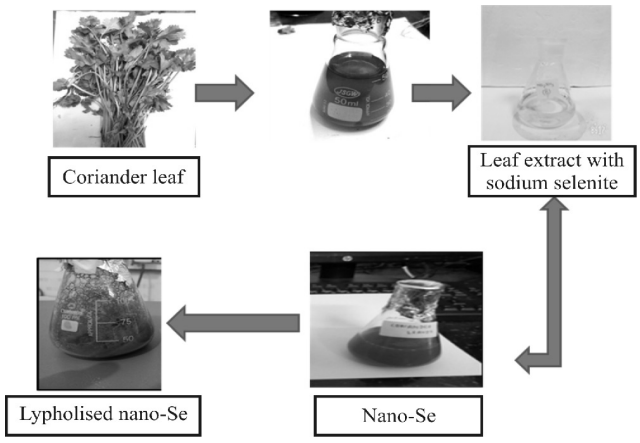


Fig 1. Green synthesis of nano se by using Coriandrum sativum

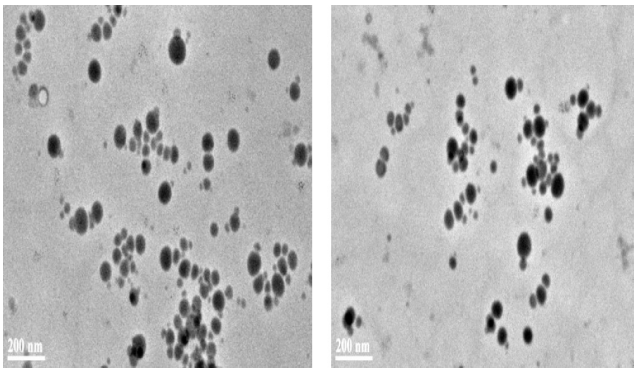


Fig 2. Transmission electron microscopy of synthesized nano-Se

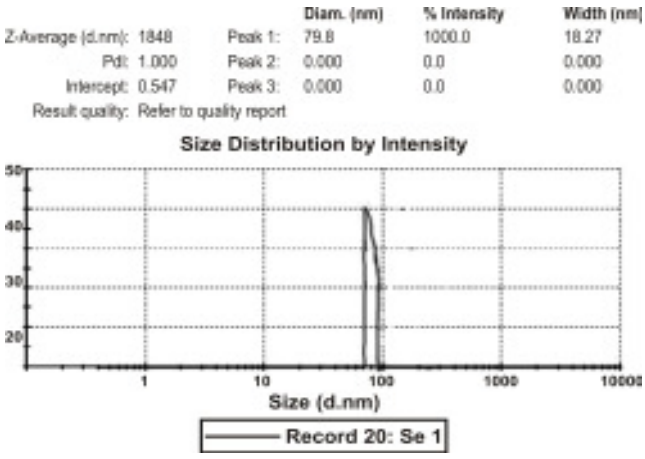
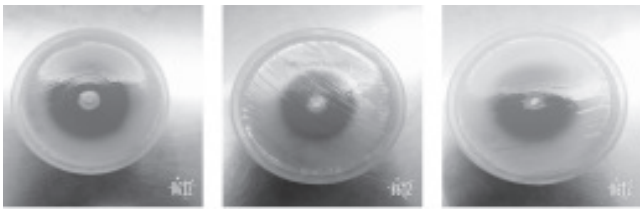


Fig 3. Zeta sizer analysis of synthesized nano-Se

microscope (TEM) analysis demonstrated that the particles are predominantly spherical in shape. The average diameter of SeNPs was around 40-60nm. Ingole *et al.* (2010) also reported that by using glucose as a reducing agent, the size of SeNPs synthesized, as determined by TEM, was in the range of 20-80 nm. Dwivedi *et al.* (2011) also reported that by using an organic acid as a reducing agent, the size of the Se nanoparticles synthesized as determined by TEM was in the range of 40-100 nm.

Plants leaves contain a substantial number of organic constituents, like phenolic compounds and various types of glycosides that help in synthesis of metal nano particles. Therefore, research work of Balamurugan and Sami Nathan (2014), Ahmed *et al.* (2015) and Santhoshkumar *et al.* (2014) taken as a reference to synthesize Se nano particles using various leaves extract. However, for the first time *Coriandrum sativum* was used for the synthesis of Se nano particles.

Zeta potential analysis of synthesized nano particles was done which indicate the average size of the particles and determine the surface charge of nano particles in solution. This study revealed that *Coriandrum sativum* leaf extract is a good candidate for synthesizing metal nanoparticles high stabilization properties. TEM analysis of NPs determines the size and shape of individual particles. Result indicate *Coriandrum sativum* synthesized NPs having average size range bellow 100 nm with spherical in shape. That denote the quality of the NPs synthesized by green method is as good as synthesized by chemical or biological process (Zhang *et al.,* 2011).



Staphylococcus aureus | Pseudomonas aeruginosa | Escherichia coli

Fig 4. Antibiosis of Se- nano particles on mastitis causing bacteria

The antimicrobial activity of newly synthesized SeNPs was assessed against mastitis causing bacterial pathogens showed well observed inhibition zone in case of *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Fig. 4). Nano- Se at a concentration of 1mM prevent the growth of mastitis causing bacteria namely *Pseudomonas aeruginosa, Staphylococcus aureus* and *E. coli* which were commonly isolated from milk samples produced by mastitis infected cows. The minimum inhibitory concentrations (MICs) of NPs against *P. aeruginosa* and *S. aureus* were found to be 1 and 2ìg/mL, respectively. Our findings suggest that NPs exert antibacterial effects in a dose and time dependent manner. Results from the present study demonstrate that the antibacterial activity of NPs may be due to the generation of reactive oxygen species (ROS), malondialdehyde (MDA), and leakage of proteins and sugars in bacterial cells (Zhang *et al.,*2004). Se NPs at a concentration of 1mM prevent the growth of mastitis causing bacteria. When selenium nanoparticles were mixed with bacterial solution, the growth of bacteria was inhibited after 3 hours (compared with the control). The inhibitory effects continued after 4 and 5 hours. Similarly, Erskine *et al.* (1989) reviewed Selenium is a cofactor for enzyme glutathione peroxidase. Inside the cell, cytoplasm contains (70 %) and mitochondrial matrix contains (30 %) glutathione peroxidase activity. Feeding cows 2mg of supplemental selenium/day starting 3 months before calving and throughout lactation reduced the severity and duration of mastitis

caused by experimentally challenging cows with *Escherichia coli.* Malbe (2006) suggested dietary Se supplementation to diets deficient in Se reduced the rate of new intra-mammary gland infections in dairy cows.

CONCLUSION

Coriandrum sativum can be used as a suitable medium for green synthesis of nano Se and the synthesized nano Se have antibacterial affects against mastitis causing bacteria.

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Quality of Lablab (*Lablab purpureus*) Forage Preserved as Hay or Silage

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ABSTRACT

Shortage of livestock feeds during the dry season is a major constraint to livestock production in Kenya. This scenario is due to dependence on rain fed forage production resulting in shortages during the dry season and excess during the wet season. This situation can be ameliorated through conservation, but losses occur when forages are conserved. The main objective of this study was to assess the effects of on-farm conservation methods on quality of lablab fodder. Fodder from eight varieties of lablab;DL1002, Ngwara Nyeupe, Echo-Cream, Black Rongai, Eldo-Kt-Cream, Eldo-Kt-Black1, Brown Rongai and Eldo-Kt-Black2 were conserved on-farm either as hay or silage. The conserved and fresh fodder were analyzed for dry matter content, crude protein, ash content, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and *in vitro* dry matter digestibility *(*IVDMD). Lablab silage was analyzed for pH and total ammonia nitrogen. Crude protein content declined significantly; by 4.2 g/100 g when fodder was conserved as hay and by 6.0 g/100 g in silage. The NDF content increased significantly by 7.6 g/100g in lablab hay but declined by 4.2 g/100 g in silage while ADF increased by 6.1 g/100 g in hay and declined by 5.0 g/100 g in silage. A significant decline of 3.2 g/100 g of lignin was observed in silage with no difference in the hay. The IVDMD declined significantly by2.8 g/100 g in lablab hay and increased by 4.5 g/100 g in silage. The pH of lablab silage ranged from 4.37 to 4.89 while total ammonia nitrogen ranged from 27 to 41 g/100 g for different lablab varieties. Conservation of lablab as silage was found to be a superior on-farm method compared to hay making.

Key words:Forage quality, Hay, *Lablab purpureus*, Silage, Varieties

INTRODUCTION

Fodder growth of tropical and subtropical perennial forages often surpasses the feed requirements of dairy herds during the wet season(Amuda and Tanko, 2019). Feed conservation through ensiling or hay making has been recommended as the favorite choice during the wet season when feeds are excess for the sake of the dry period (Amodu *et al.,* 2005;Shishi and Agbo, 2018;Ishiaku *et al.,* 2020).*Lablab purpureus* has been used as animal fodder and can be fed either fresh or conserved as hay or silage. The whole plant is one of the most palatable legumes for animals, the leaves being more palatable compared to stems (Abdallah *et al.,* 2015). It is among the favored fodder crops with high potential for incorporation into livestock production systems (Amodu *et al.,* 2005) and is highly persistent to a wide range of agronomic conditions with dry matter yields of 2.7–7.7 t/ha in localities varying from the semi-arid to the sub-humid regions (Amodu *et al.,* 2005).The whole lablab plant has a protein content of 13-24.5% on dry matter basis (Heuze *et al.,* 2014).

Hay from the whole lablab foliage is an important feed resource for livestock and can substitute costly supplements that are expensive and not readily available to small-scale dairy farmers (Mthembu *et al.,* 2018; Ishiaku *et al.,* 2020).With the improvement of small-scale intensive systems and increasing costs of concentrate feeds, conservation of forage crops should be an essential part of livestock production. Conserving high quality fodder reduces the necessity of purchasing protein concentrates for use in ruminant rations (Ngongon *et al.,* 2008).

Legume fodder can be conserved as hay or silage, however, when conserved alone they do not ensile well due to their high moisture content and high buffering ability, causing high nutrient losses and

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unstable silage of unacceptable pH. Under the circumstances, proper conditions can only be achieved when the right silage additives and proper procedure are followed (Ngongon *et al.,* 2008 and Seppälä *et al.,* 2019). However, during conservation, the preserved materials change in quality depending on method used, especially loss of leaves during curing for legume hay and decline of protein in final products of legume silage due to proteolysis (Husein and Diriba, 2018).This study therefore aimed at assessing quality of lablab fodder preserved on-farm either as hay or silage.

MATERIALS AND METHODS

Three sites with different climatic conditions and soil fertility were selected within Nandi South Sub-County, Nandi county, Kenya: Koibem with temperature of 18°C, high fertile soils with nitrogen content of 0.38% and carbon content of 3.91%, Kiptaruswo with temperature of 20°C, medium soil fertility with nitrogen content of 0.26 % and carbon content of 1.87%, and Kapkarer with temperature of 22°C, low soil fertility of nitrogen content of 0.16% and carbon content of 1.44 % (Omondi *et al.,* 2011 and Landon, 2014).

Eight lablab varieties namely: DL1002, Ngwara Nyeupe, Echo-Cream, Black Rongai, Eldo-Kt-Cream, Eldo-Kt-Black1, Brown Rongai and Eldo-Kt-Black2 were established within the three sites. The treatments were made up of composited fresh lablab, hay and silage from eight lablab varieties in three sites of Nandi South Sub-County. Complete randomized design was used with three treatments of lablab (fresh, hay and silage) replicated three times after compositing of the eight lablab varieties from each of the three sites.

Lablab fodder was harvested at 50% flowering after two months of emergence and wilted for two days to reduce moisture content. The material was chopped into pieces of approximately 2.5 cm length using forage chopper. A two kg sample was then mixed thoroughly with 5% w/w of maize flour to increase dry matter content of the resultant silage due to high moisture content during harvest. Molasses (diluted with 1:3 parts water) was included at 3% v/w of lablab forage. The material was placed into heavy gauge plastic bags in duplicate in case of any damage by one for each variety per site making a total of 48 bags. After compacting by hand to expel all air, the bags were sealed with cello tape to ensure that they remained airtight. They were stored in a shade and raised floor for 60 days prior to opening for nutrient analysis. After 60 days of storage, sub-sample of the silage was collected from top, middle and bottom part of the bag to make a 220 g sample from each silage sample for chemical analysis. One hundred and twenty grams was oven dried at 60°C for one week and ground through a Wiley mill standard model No.3 with sieve of 0.5 mm and stored for further nutrient analysis while the other 100 g was used for pH and ammonia nitrogen determination.

Lablab fodder was air dried for four days after harvesting at 50% flowering. The material was turned regularly to allow even drying before baling to avoid mold formation. Each bale of hay weighed approximately 20 kg and was prepared manually for each variety namely: DL1002, Ngwara Nyeupe, Echo-Cream, Black-Rongai, Eldo-Kt-Cream, Eldo-Kt-Black1, Brown Rongai and Eldo-Kt-Black2 in duplicate per site using a baling box of 85 cm length 55 cm width and 45 cm height open on both ends. After baling, the bales were stored under waterproof shade on raised wooden base for 60 days.

The procedures of Nadeau *et al.* (2000) and Fellner *et al.* (2000) were followed. A mixture of 100 g silage and 1 litre of distilled water were blended for 60 seconds using a kitchen blender. The blended material was then covered with aluminum foil and left to settle for two hours. The pH of the silage extract was measured using a glass electrode pH meter that was standardized with buffers of pH 4 and pH 7. The blended material was filtered through two layers of cheese cloth and centrifuged at 2500 rpm for 30 minutes. The aliquot measuring 100 ml was treated with 15 ml 20% sulphuric acid and frozen for ammonia nitrogen determination. The total ammonia nitrogen was estimated by Kjeldahl method (AOAC, 2016).The dry matter and crude protein of fresh lablab, silage and hay were analyzed following standard procedures (AOAC, 2016).Neutral

detergent fibre (NDF), acid detergent fibre (NDF) and acid detergent lignin (ADL) were determined using the method of Van Soest *et al.* (1991).The two-stage *in vitro* dry matter digestibility (IVDMD) was determined following the procedure of Tilley and Terry (1963).

The data obtained was subjected to analysis of variance (ANOVA) using Genstat Inc. 15th edition 9 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) to determine whether there was difference in lablab quality preserved using the two methods of conservation compared with the fresh lablab. The means were separated using Tukey's statistical test at a significant level of 5%.

RESULTS AND DISCUSSION

The nutrient composition of fresh and conserved lablab forage is shown in Table 1. Nutrient composition of lablab fodder varied significantly (P<0.05) between fresh and conserved except for the ash content. The CP content was highest for fresh fodder compared to hay and silage which was the lowest. Lablab hay had the highest neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) compared to silage which had lowest fibre fractions. IVDMD was highest for lablab silage while lowest was for hay. The dry matter content of lablab hay and silage was 834.8 and 427.4 g/kg. The dry matter content of lablab hay in this study was below 854 g/kg reported by Kabirizi *et al.* (2000), 918 g/kg by Mpangwa *et al.* (2000) and 904 g/kg by Hassan *et al.* (2016). The low dry matter content could be due to the drying conditions prior to baling and time of harvesting as reported by Husein and Diriba, (2018).Moisture content prior to hay baling should be reduced to 15.0-20.0 g/100 g in order to restrict plant actions and microbial enzymes that could lead to development of molds in hay.

Studies that were done by Ngongoni *et al.* (2008) by Morris and Levitt, (2010) and by Heinritz *et al.* (2012) reported dry matter content in lablab silages to be ranged from 171–208 g/kg, 185-370 g/kg and 313 g/kg, lower than 427.4% observed in this study. The difference could be due to harvesting age of the forage, ensiling conditions and variation in length of time of wilting prior to ensiling, as a long wilting time is associated with high DM in silages as opposed to short time (Husein and Diriba, 2018). However, the DM content in the silage satisfied the recommended level (McDonald *et al.* 1991) of above 300 g/kg in order to restrict the growth of clostridia bacteria that usually develop in silages with low DM content (below 300 g/kg). The *Clostridium botulinum* and *Listeria monocytogenes* bacteria in low DM silage was reported to be responsible for the poor quality silage as they cause botulism and meningoencephalitis in animals that consume such silage (McDonald *et al.,* 1991). They also noted that too much dry matter in ensiled material can also restrict fermentation process resulting to both high pH and soluble carbohydrates in the end product.

Crude protein is one of the quality parameters that is used to measure the quality of a forage plant (Ishiaku *et al.,* 2020). In this study, crude protein for fresh lablab, hay and silage was 22.0, 17.8 and 16.0 g/100g. The hay CP content was within an earlier range

Table 1. Nutrient content (DM) of fresh and conserved lablab fodder

Treatments	DM g/Kg	CP g/ 100g	Ash g/ 100g	NDF g/ 100g	ADF g/ 100g	ADL g/ 100g	IVDMD g/ 100g
Fresh	168.7^{c}	22.0^{a}	8.7^{a}	46.6^{b}	33.6^{b}	10.5^{a}	70.9^{b}
Hay	834.8^{a}	17.8^{b}	8.8^{a}	54.2^{a}	39.7^{a}	10.4^{a}	68.1^{b}
Silage	427.4^{b}	16.0^{c}	8.9^{a}	42.4^{c}	28.6^{c}	7.3^{b}	75.4^{a}
Means	477.0	18.6	8.8	47.7	33.9	9.4	71.5
S. E.	8.29	0.42	0.28	0.83	0.5	0.32	0.94
P-value	<.001	<.001	0.803	<.001	<.001	<.001	<.001

DM, dry matter; CP, crude protein, NDF; neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin, IVDMD, in vitro dry matter digestibility; Column means with different superscripts are significantly different (P≤0.05)

(12-19 g/100g) reported by various authors (Aganga and Autlwetse, 2000; Kabirizi *et al.,* 2000; Mpangwa *et al.,* 2000, Mupangwa *et al.,* 2000;Hassan *et al.,* 2016). The decline in CP content of hay compared with fresh lablab was due to loss of lablab leaves during hay baling and drying. There was also considerable decline of crude protein during the ensiling process which could be related to protein degradation that has been reported during fermentation of many legumes (Uchide and Kitemine, 1987). Amodu *et al.* (2005) reported that the reduction in CP in Columbus grass silage was attributed to proteolysis before full fermentation during the ensiling process. The CP content of the preserved lablab in this study met the CP requirements for the beef cattle (7.0 g/100 g), sheep and goats (10-12 g/100 g).

The fibre fractions content in lablab hay in this study (54.2 NDF, 39.7ADF, and 10.4 ADL g/100 g) were within range reported elsewhere (Kabirizi *et al.,* 2000 Mupangwa *et al.,* 2000) The previously cited studies reported a range of 47.3-58.3% for NDF, 29.4-41.5 for ADF and 6.4-11.3 % for ADL. The increase in fibre fractions fresh fodder was attributed to the high rate of leaf shattering and loss increasing the stem content that was experienced during drying process unlike silage that was ensiled at low moisture content (Castro Montoya and Dickhoefer, 2018).

The NDF content of lablab silage (42.4 g/100 g) in this study was slightly higher than 39.5 g/100 g reported by Contreras-Govea *et al.* (2009). These authors also reported an ADF content of 21.2 g/100g in lablab silage. Rooke and Hatfield, (2003) explained the low NDF and ADF content in silages as due to low pH conditions that is favorable for cell wall hydrolysis, that declined the NDF and to some extent ADF content.

Lablab hay baling is a challenge as leaves dry faster than stems resulting in leaves detaching from the undried stems before and during baling (Manyawu *et al.,* 2016). The complete drying of stems was necessary to discourage molds formation within hay after baling (Manyawu *et al.,* 2016). In an attempt to even drying, most of leaves were lost through shattering hence increasing the percentage of lignin in the resulting hay as opposed to silage (Husein and Diriba, 2018; Castro Montoya and Dickhoefer, 2018). The ash content in both lablab hay and silage in this study concurred with those reported by Hassan *et al.* (2016) as 8.78 g/100 g.

The *in vitro* dry matter digestibility of lablab hay was 68.1 g/100 g and 75.4 g/100 g for silage compared to 70.9 g/100 g for fresh fodder. Diribsa *et al.* (2014) reported IVDMD of lablab hay as 62.03 g/100 g, lower than in this study. Digestibility of lablab silage from this

Table 2. Ammonia nitrogen content and pH of lablab silage

	Silage quality	
Lablab variety	**pH**	**NH_3N g/100 g**
Black Rongai	4.42^{b}	30.3^{a}
Brown Rongai	4.37^{b}	27.0^{a}
DL1002	4.63^{ab}	33.6^{a}
Echo Cream	4.52^{ab}	30.3^{a}
Eldo-Kt-Black1	4.43^{b}	31.5^{a}
Eldo-Kt-Black2	4.38^{b}	34.8^{a}
Eldo-Kt-Cream	4.49^{ab}	41.0^{a}
Ngwara Nyeupe	4.81^{a}	29.1^{a}
Mean	4.508	32.2
S.E.	0.068	3.67
P Value	0.006	0.282

NH_3-N, ammonia N; Eldo, Eldoret; Kt, Kitale; DL, dry land variety; Column means with different superscripts are significantly different ($P<0.05$).

study was in agreement with Contreras-Govea *et al.* (2009) who reported the *in vitro* dry matter digestibility (IVDMD) of 77.0 g/100g. The difference in IVDMD of hay and silage was due to variation in leaves to stem ratio and fibre fractions as reported by Rooke and Hatfield (2003). The authors reported that, decline in fibre fractions in silage was associated with low pH that enabled cell wall hydrolysis. This might have caused similar effects of lablab silage having high IVDMD compared with hay that had more fibre fractions due to loss of leaves during baling and drying in this study (Castro Montoya and Dickhoefer, 2018).The ammonia N and pH of lablab silage is shown in Table 2. The pH of lablab silage varied significantly ($P<0.05$) between the varieties as opposed to ammonia nitrogen (NH_3-N) content. High pH was observed in silage from Ngwara Nyeupevariety while low pH was recorded with Brown Rongai variety.

All silages in this study had pH ranging from 4.37 to 4.89. Previous studies have reported pH range of 4.0 to 4.9 and 4.0-4.5 in lablab silage (Morris and Levitt, 2010; Heinritz *et al.,* 2012; Abdulrahman *et al.,* 2018). In another study, Abu-Bakr *et al.* (2015) reported the pH range of 4.3 to 5.6 in silages made with a mixture of legumes and other fodder materials. Higher pH values in legume silages (5.3–5.7) have been reported (Muhammad *et al.,* 2008, Muhammad *et al.,* 2009). Tjandraatmadja *et al.* (1993) recommended a pH of 4.2 for well-preserved tropical silages. As such, the pH of silages in this study ranged from good to average based on the classification by Kung and Shaver, (2001); pH <4.0 (excellent), between 4.1 to 4.3 (good), pH between 4.4 to 5.0 (average) and > 5.0 (bad). The pH of an ensiled material is an indication of its acidity, and is influenced by the buffering capacity of the crop (Abdulrahman *et al.,* 2018). Buffering capacity measures to what extent a forage sample will tolerate a change in pH, with all forages having different buffering abilities (Abdulrahman *et al.,* 2018). Fresh forage with a high buffering capacity and low DM will need more acid to reduce its pH than forage with a low buffering capacity (Kung and Shaver, 2001; Amuda and Tanko 2019). Legumes are associated with low DM content at the time of harvest for silage making, high buffering capacity and low soluble carbohydrates that makes it difficult in making quality silage from them (Phiri *et al.,* 2007; Kuppusamy, 2020). Silage from legumes can be made successfully through pre-wilting (up to 50% moisture) and use of silage additives (like molasses), mixing with other cereals and microbial inoculants. This results into improved rate of fermentation and increases soluble carbohydrates thus resulting into a better silage (Abu-Bakr *et al.,* 2015).Ammonia-N content in silage is pointer of fermentation quality and a gauge of the degree of protein degradation during conservation (Driehuis *et al.,* 2001). Well-conserved silage contains less than 10 g/100 g of NH_3-N/ (10%) of total Nitrogen (McDonald *et al.,* 1991). All silages in this study appeared acceptable based on pH but the NH_3-N content was unsatisfactory as it ranged from 27 to 41 g/ 100 g, above the recommended in silage (McDonald *et al.,* 1991). Buxton and O'Kiely (2003) reported an increase in ammonia-N as the quantity of lablab increased in the mixture of corn silage. They reported the results as expected because legumes have high CP content that is favorable to greater proteolysis than corn silage. In addition, they reported legumes to have superior buffering capacity than grasses hence higher NH_3-N content (Buxton and O'Kiely, 2003). Therefore, higher ammonia-N formation was expected in the mixture as the proportion of legumes increased (McDonald *et al.,* 1991).

CONCLUSION

Results from this study show that conservation of lablab fodder as silage results in a better retention of the nutritive value.

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Impact of Green Fodder Replacement with Corn Silage on Performance and Metabolizability in Growing Hariana Cattle

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ABSTRACT

This study was conducted to determine the effect of green fodder replacement with corn silage on feed intake, growth performance and feed utilization efficiency in growing Hariana cattle. Corn silage was prepared in bunker silo and after ensiling for 60 days, silage was evaluated for nutrients content and physical and chemical characteristics. Low pH value, ammonia-nitrogen (NH_3-N) and butyric acid content and high lactic acid, individual volatile fatty acids (VFAs), total VFAs content and buffering capacity (BC) denoted that prepared corn silage was well preserved and of very good quality. To study the effect of green fodder replacement with corn silage on animal performance, 18 growing Hariana heifers were randomly allocated into three groups (n=6) on body weight (130±3.0 kg) and age basis (14±1.5 months). Experimental heifers either received a basal total mixed ration (TMR) devoid of corn silage ($S_{0\%}$) or were fed on TMR of which 50% ($S_{50\%}$) and 100% ($S_{100\%}$) green fodder were replaced (DM basis) with corn silage. As the level of inclusion of corn silage increased, dry matter intake (DMI) also increased significantly ($P<0.05$) while average daily gain (ADG) was similar among all groups. Residual metabolizable feed consumption (RMFC) measured as the difference between metabolizable energy (ME) intake (MEI) and ME required (MER) showed a linear increase with silage levels. However, residual intake and body weight gain (RIG) showed an inverse trend than RMFC. Other studied feed efficiency measures showed a non-significant effect of treatment. The apparent digestibility of crude protein (CP) was higher in the $S_{0\%}$ group while digestibility of the other nutrients was similar among the three groups. In conclusion, the replacement of green fodder with corn silage reduced metabolizability and increased feed intake without altering growth performance in growing Hariana cattle.

Key words: Blood metabolites, Cattle, Corn silage, Efficiency, Green fodder, Performance

INTRODUCTION

The feed itself accounts for 60 to 65% of the total cost of production in dairy cattle and is the main determinant of production system profitability. Shrinkage of irrigated lands for fodder production, higher labor cost, and small landholdings further increases the cost of rearing dairy animals. Out of the available dry matter, most of it is available in the form of agricultural by-products and dried grass which is of inferior quality. This indicates that as most of the livestock are underfed, they are not able to perform optimally. It is imperative to arrange sufficient good quality feed and fodder for efficient utilization of the genetic potential of the various livestock species and sustainable improvement in productivity. The only way to meet the increasing fodder needs of livestock is to look for alternative options of fodder. Among these, silage is one of them. Availability of nutritious fodder throughout the year can be maintained by converting green fodder into silage.

Silage or grass pickle is the green succulent roughage preserved under controlled anaerobic fermentation in the absence of oxygen by compacting green chops in air and watertight receptacles. Silage is green succulent roughage preserved more or less in its original condition, with a minimum deterioration and minimum loss in respect of various nutritive constituents of fodders. Well-fermented silage is readily consumed by animals and may improve their health and production characteristics (Varadyova *et al.,* 2010). Recent findings on silage production indicate that it could replace conventional fodder without any

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ill-effect on intake, efficiency, digestibility, and performance of dairy animals (Chaudhary *et al.,* 2014). In a well-managed system, where losses are low, the silage dry matter content, digestibility and ME content will be similar or slightly lower and crude protein content might be similar to parent fodder (Kaiser and Piltz, 2004). Preference for cereal green fodder including maize, sorghum, pearl millet, *etc.* is due to more sugar content than protein, as sugar is utilized in the fermentation process to make lactic acid by microorganisms (Nazli *et al.,* 2019).

The biological basis that contributes to the variation in metabolizability of diet includes, feed intake, composition of ration, digestion of feed and associated energy use, activity and metabolism (Nguyen *et al.,* 2005). Approximately one-third of the variation can be explained by known processes and the remaining 67% of the variation is believed to be caused by processes in the body including but not limited to protein turnover, ion pumping and protein leakage (Richardson and Herd, 2004). Feed efficiency tended to be correlated with apparent digestibility of nutrients especially DM and protein, indicating that animals with higher efficiency had higher nutrients digestibility compared to less efficient animals. Therefore, evaluation of the association between the efficiency of feed utilization, and blood variables permits the constant identification of efficient and productive animals (Nkrumah *et al.,* 2007).

A major factor that affects the efficiency of animals is dietary composition. Therefore, a corollary to this concept exists in the utilization of diets, suggesting that when different diets are fed to cattle, the analyses of efficiency measures should be able to differentiate between the efficiencies of the utilization of diet. Considering the significance of feed efficiency measures and limitation of green fodder availability, the present study was designed to investigate the effect of replacement of green fodder with corn silage on feed intake, growth performance and feed utilization efficiency.

MATERIALS AND METHODS

To prepare the best quality silage, maize fodder was used in this study. Fodder harvest was performed at a height of 5 cm above the ground when the moisture content of maize fodder was in the range of 70-75%. Silage was prepared in a bunker silo having a dimension of 20 m×10 m. The whole fodder was chopped into 2-3 cm pieces by using tractor operated forage chopper (Ensiladeria JF40Max, NB Maquinas Ltd., Brazil). Chopped material was filled in the silo and compressed with a tractor fitted with a labeler. For adequate compaction and perfect anaerobic condition, the chopped fodder was covered with two layers of polythene sheets (0.2 mm thickness) followed by a layer of sand and tires. After 60 d of ensiling, the silo was opened from one end and the silage sample was collected in a zip lock polythene pack from the core area of the opened portion of the filled bunker silo. Collected silage samples were used for the evaluation of nutrients content, and physical and chemical characteristics.

The representative samples of silage were analyzed for moisture content by using the toluene distillation method (Method 925.04; AOAC, 1990). Crude protein (CP ; Method 4.2.08), ether extract (EE; Method 920.85) and acid insoluble ash (AIA; Method 923.03) contents were analyzed by following protocols of AOAC (2005). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined as per the method of Van Soest *et al.* (1991). Ca and P content were determined by titration method (Talpatra *et al.,* 1940) and spectrophotometric method (AOAC, 2005), respectively. The total carbohydrate and non-fiber carbohydrate (NFC) content in silage samples was determined by using the equation of Sniffen *et al.* (1992) and Detmann and Valadares Filho (2010), respectively. Physical characteristics like the smell, colour, structure, *etc.* of prepared corn silage were evaluated by following the guidelines of Horiguchi and Takahashi (2007). The temperatures of the core, lateral and apical parts of the working face of silage were measured by mercury in a glass clinical thermometer (Qingdao Dacon Trading Co. Ltd., Shandong, China). The pH in the silage extract

before stabilizing with 5% meta-phosphoric acid was measured by using a pH meter (Systronics pH System-361, India). The estimation of lactic acid in silage samples was done as per the method of Barker and Summerson (1941) and modified by Barnett (1951). Acetic, propionic, butyric, valeric and isovaleric acid was determined with gas chromatography-mass spectroscopy (5975C VL MSD with Triple-Axis Detector, Agilent Technologies India Pvt. Ltd, with 30 m×320 ìm×0.25 ìm capillary column, condition: column temperature 130ºC, injection temperature 220ºC) with flame ionization detector (FID). NH_3-N and total-N were measured in the extract by phenol-hypochlorite assay (Weatherburn 1967) and Method 4.2.08 of AOAC (2005), respectively. BC was determined by the hydrochloric acid-sodium hydroxide method of Playne and McDonald (1966). The concentration of WSC in silage samples was determined by method of McDonald and Henderson (1964). To assess the quality of the silage, the V-Score (Takahashi *et al.,* 2005) and Flieg index (Kilic, 1986) were also calculated.

Animal care procedures were approved (approval number, 121/IAEC/18) and conducted under the established standard of the Institutional Animal Ethics

Table 1. Ingredients and nutrients composition of TMR fed during feeding trial (g/kg DM basis or as mentioned)

Attribute	Group[α]		
	S0%	S50%	S100%
Ingredients composition			
Berseem fodder	400	200	0
Corn silage	0	200	400
Wheat straw	200	200	200
Mustard oil cake, solvent extracted	128	128	128
Ground barley grain	104	104	104
Gram chuni	80	80	80
Wheat bran	80	80	80
Micronutrient mixture[β]	8	8	8
Nutrients composition			
DM	598.9	622.7	646.5
CP	167.4	147.0	126.6
EE	31.7	30.4	29.2
Ash	88.7	78.8	68.9
NDF	467.6	475.9	484.2
ADF	252.6	259.4	266.2
ADL	44.2	44.0	43.7
Total CHO	712.2	743.8	775.3
NFC	244.6	267.9	291.1
Ca	11.1	11.3	11.5
P	4.4	7.4	10.3
ME, Mcal/kg DM	2.24	2.25	2.26

[α]S0%, TMR without corn silage; S50%, TMR of which 50% green fodder was replaced with corn silage and S100%, TMR of which 100% green fodder was replaced with corn silage; [β]Micronutrient mixture consisted (kg-1) of 700,000 IU of vitamin A, 70,000 IU of vitamin D3, 250 mg of vitamin E, 190 g of Ca, 90 g of P, 50 g of Na, 19 g of Mg, 1.2 g of Cu, 9.6 g of Zn, 1.5 g of Fe, 6.0 g of Mn, 325 mg of I, 150 mg of Co, 10 mg of Se.

Committee (IAEC), constituted as per article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India. A total of 24 growing Sahiwal heifers were selected from the cattle herd maintained at LFC, DUVASU, Mathura. All heifers were housed in a well-ventilated shed having the proper arrangement for individual feeding and watering without having access to the other animal's diet. Deworming of all the experimental animals was done before the start of the experiment by oral administration of Fentas bolus (Intas Pharmaceuticals Pvt. Ltd., India) at the dose level of 10 mg/kg body weight. Animals were let loose every fortnightly for exercise. Selected heifers were randomly assigned into three groups (n=6) on body weight (130±3.0 kg) and age basis (14±1.5 months). Heifers were either fed on basal TMR consisted of compounded concentrate: green fodder: wheat straw in the proportion of 40: 40: 20 ($S_{0\%}$) or TMR of which 50% green fodder (DM basis) was replaced with corn silage ($S_{50\%}$) and TMR of which 100% green fodder (DM basis) was replaced with corn silage ($S_{100\%}$). Ingredients and nutrients composition of TMR fed in different groups are presented in Table 1. TMR was prepared daily by hand mixing and offered at 0900 h. The calves were fed the TMR in such an amount that at least 5% refusals were left daily per animal. Fresh drinking water was offered *ad libitum* twice daily at 0800 h and 1700 h. A digestion trial with 4 days adaptation and 6 days collection period was conducted at the end of the study. TMR offered and refusal left were sampled daily for chemical analysis. Feces excreted during 24 h were collected and measured daily for 6 days.

The experimental calves were monitored daily for DMI and fortnightly for growth performance and feed efficiency measures. To increase the accuracy of estimation of ADG, calves were weighed at fortnightly intervals in the early morning (0600 h) before offering feed and water. RMFC was used to determine metabolizability by using the following methodology and guidelines of Fan *et al.* (1995) and NRC (1996).

$$RMFC = MEI - MER$$

MEI and MER were calculated as per equations derived by Sharma *et al.* (2016).

$$MEI\ observed\ (Mcal/d) = DMI \times ME\ of\ feed$$

$$MER\ (Mcal/d) = MERm + MERg$$

$$MERm\ (Mcal/d) = 122 \times BW^{0.75}/1000$$

$$MERg\ (Mcal/d) = (4.1 + 0.0332 \times W\text{-}0.000009 \times W^2) / (1\text{-}0.1475 \times WG)$$

Where, W is the live weight (kg) and WG is the live weight gain (kg/d).

Gross feed efficiency (GFE), net feed efficiency (NFE), energy balance (EB), energy conversion efficiency (ECE), residual body weight gain (RWG), residual intake and body weight gain (RIG), FCR and FCE were used as feed efficiency measures. GFE and NFE were calculated as per guidelines of Okine *et al.* (2001) however; ECE and EB were calculated by following guidelines of NRC (2001).

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

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

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

Where; *Yijk* is the dependent variable, *ì* is the overall mean of the population, *Ti* is the mean effect of the treatment, *Dj* is the mean effect of day of sampling (j=0, 30, 60 and 90 days of dietary treatment), *(T×D)ij* is the effect of the interaction between treatment and period and *eijk* is the unexplained residual element assumed to be independent and normally distributed. Individual animals were used as the experimental unit

for all data. The pair-wise comparison of means was carried out using "Tukey's honest significant difference (HSD) test". Significance was determined at $P<0.05$ and the values are presented in the tables. Error bars in figures depict standard error. The data were also analyzed for correlation coefficient (r) and coefficient of variation (CV).

RESULTS AND DISCUSSION

In the present study, corn silage was well preserved as indicated by their high lactic acid content and low pH value, NH_3-N content and butyric acid content (Table 2). Ensilaged maize fodder retained its physical characteristics and silage smelled slightly acidic and fruity, appeared brownish-yellow with loose and soft and non-viscous texture. The core silage temperature in the bunker was 32ºC which was slightly higher than the ambient temperature. Flieg points calculated by means of the pH values and DM content also denoted a very good quality of prepared corn silage. The prepared corn silage was well preserved and of very good quality. The DM content and values of other nutritive constituents were within ranges reported previously by NRC (2001) for typical corn silage. The corn silage prepared in the present study had a DM concentration of 270 g/kg fresh silage which was near the optimum stage of maturity at harvest as reported by Phipps *et al.* (2000), Keady *et al.* (2002b) and Keady *et al.* (2007). Fisher and Lessard (1987) reported that the DM, CP and ADF contents were 32.5, 8.0 and 27.0% for corn silage which was in accordance with the findings of the present study. The CP recorded from the silage is well above the threshold of 60 g/kg required by rumen microbes to build their body protein. Below this threshold, intake of fodder by ruminants and rumen microbial activity would be adversely affected (Van Soest, 1994). The pH of silage is one of the simplest and quickest ways of evaluating its quality, as silage properly fermented will have a much lower pH. Kung and Stoke (2001) also reported pH values in the range from 3.7- 4.2 for maize silage. Similar results were obtained by Church (1991), Etman *et al.* (1994), McDonald *et al.* (1995), Sheperd and Kung (1996), and they reported pH values for maize silage ranging around

Table 2. Nutrient composition, physical and chemical characteristics of corn silage (g/kg DM or as mentioned)

Attribute	Amount/characteristics
Nutrient composition	
DM	270
OM	958
CP	83
EE	31
Ash	42
NDF	461
ADF	285
ADL	27
Total CHO	844
NFC	383
Ca	1.50
P	1.90
ME, Mcal/kg DM	2.33
Physical characteristics	
Aroma	Slightly acidic and fruity smell
Colour	Brownish-yellow
Structure/texture	Loose and soft, non-viscous/firm
Temperature, ºC	32
Moldiness	Absent
Chemical characteristics	
pH value	4.37
Lactic acid, g/100g DM	67.35
Acetic acid, g/kg DM	24.18
Propionic acid, g/kg DM	3.69
Butyric acid, g/kg DM	0.29
Valeric acid, g/kg DM	0.18
Isovaleric acid, g/kg DM	0.25
Total acids, g/kg DM	95.94
Lactic: acetic acid ratio	2.79
Total-N g/100g DM	1.52
NH_3-N g/100 g DM	0.13
BC, ml of alkali required	8.94
WSC, g/100 g DM	6.50
V- score	94.57
Flieg index	84.20
Quality classification	Very good

4.2-4.5. Lactic acid is the most abundant acid and imparts around 75% of the total acids contained in silage. In high-quality corn silage, it ranged between 4-7% (Seglar, 2003). The lactic acid content of the present study was within the range and resulted in an optimum pH. Langston *et al.,* (1958) stated that high-quality silage is characterized by low NH_3-N concentration. The NH_3-N content of the corn silage in the present study fell within the range between 0.04 and 0.15% of DM (Sheperd and Kung, 1996). V-score and Flieg index are methods for evaluating the quality of silages. Silage is considered to be very good when it's Flieg index ranges between 81 and 100 (Moselhy *et al.,* 2015). Mafakher *et al.* (2010) studied the chemical composition and quality characteristics of corn, sunflower, and corn-sunflower mixture silages and found the highest Flieg index (103.01) and the best pH value (3.66) for corn silage compared to other silages.

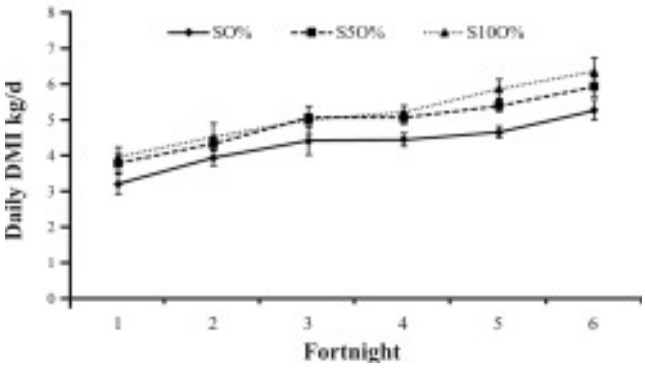

Fig. 1. Effect of green fodder replacement with corn silage on DMI

Effect of green fodder replacement with corn silage on DMI, ADG, feed efficiency measures and

Table 3. Performance, feed efficiency and nutrient digestibility of animals fed TMR containing different levels of corn silage

Attribute	Group			SEM	P value	r	CV$^{\beta}$
	S0%	S50%	S100%				
Animal performance and feed efficiency							
DMI, kg/d	4.33^{a}	4.93^{ab}	5.16^{b}	0.16	0.032	0.217	0.298
ADG, g/d	618.52	620.74	633.33	19.50	0.950	0.048	0.344
MEI, Mcal/d	9.95^{a}	10.96^{b}	11.66^{b}	1.25	0.032	0.243	0.284
MER, Mcal/d	9.82	9.86	9.92	0.80	0.933	0.024	0.206
RMFC, kg/d	0.13^{a}	1.10^{b}	1.74^{c}	0.53	0.047	0.480	0.386
GFE, Mcal/kg BW	0.054	0.052	0.052	0.004	0.440	-0.070	0.285
NFE, Mcal/kg BW	0.153	0.280	0.218	0.061	0.057	0.146	0.218
RWG, kg/d	-0.09	-0.08	-0.09	0.03	-0.020	-0.811	0.346
RIG index	-0.22^{c}	-1.17^{b}	-1.84^{a}	1.29	-0.838	-0.459	0.395
FCR	7.02	7.99	8.19	0.58	0.510	0.144	0.369
FCE	0.144	0.126	0.124	0.011	0.161	-0.144	0.370
EB, Mcal	279.78	307.76	326.41	21.46	0.088	0.356	0.112
ECE	0.035	0.036	0.036	0.003	0.951	0.034	0.223
Apparent nutrient digestibility, %							
DM	64.53	65.79	64.50	1.04	0.276	-0.004	0.033
OM	68.69	69.95	69.11	0.91	0.291	0.080	0.028
CP	74.10^{b}	71.04^{b}	65.63^{a}	0.90	0.048	-0.884	0.021
EE	77.62	78.62	78.12	0.65	0.299	0.133	0.017
NDF	58.43	60.10	58.80	1.21	0.285	0.053	0.043
ADF	54.89	53.10	54.78	1.37	0.217	0.056	0.049

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

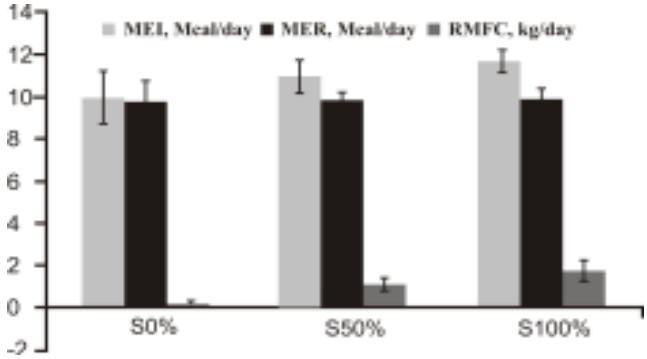

Fig. 2. Effect of green fodder replacement with corn silage on metabolizability

nutrients digestibility are depicted in Table 3. Statistical analysis of data revealed that the replacement of green fodder with corn silage affected daily DMI significantly (P<0.05) and intake was reported to be highest in the $S_{100\%}$ group (Figure 1). However, the replacement of green fodder with different levels of corn silage did not exert any effect on ADG. Although the DMI increased with an increased level of inclusion of corn silage but ADG was similar among all three groups. It is perhaps predictable that the substitution of green fodder by maize silage would cause a positive effect on voluntary forage intake without altering cattle performance due to their high palatability. There are several examples in the literature where maize silage inclusion has stimulated higher voluntary forage intake (Hameleers, 1998), although that was generally accompanied by little or no effect on the performance of dairy animals. Keady *et al.* (2007) offered grass silage either as the sole forage or in addition to maize silage in continental cross beef steers and found that the inclusion of corn silage in the diet increased DM intakes. The corn silage intake was higher than cattle fed with fresh Napier grass (Siddque *et al.,* 2015) and Napier grass silage (Bureenok *et al.,* 2012). Juniper *et al.* (2005) replaced different levels of grass silage with corn silage in the basal diet of Simmental × Holstein-Friesian steers and found that the forage DM intake increased linearly with the increased level of inclusion of maize silage. The linear increase in intake response to forage substitution observed in the current experiment corroborate well with earlier reports in dairy cows (O'Mara *et al.,* 1998; Phipps *et al.,* 2000). El-Ayouty *et al.,* (2000) also found that up to 100 % of maize silage or berseem silage can be fed to rabbits without affecting the growth performance. Fazaeli *et al.,* (2006) also observed the non-significant effect of the inclusion of different levels of corn and sorghum silage on body weight gain of yearling male calves. Thus, the results suggest that corn silage has the potential to replace green fodder in diets fed to growing cattle.

MEI was increased with an increased level of corn silage while MER was similar among all three groups. RMFC measured as the difference between ME intake and ME required showed significant (P<0.05) effect of replacement of green fodder with corn silage. As the level of replacement of green fodder with corn silage increased RMFC increased. Heifers in group $S_{50\%}$ and $S_{100\%}$ consumed 0.97 and 1.61 kg more DM/day than the $S_{0\%}$ group yet gaining at a similar rate (Figure 2). Replacement of green fodder with corn silage did not affect RWG, GFE, NFE, FCR, and FCE. However, feed efficiency measure like RIG index showed significantly (P<0.05) higher value in the $S_{0\%}$ group followed by $S_{50\%}$ and $S_{100\%}$ groups. Lower RMFC and higher RIG index in $S_{0\%}$ group are favorable and showed better metabolizability in green fodder fed animals. As inclusion levels of corn silage increased, EB also increased due to increased DMI but showed a non-significant effect. ECE also showed a non-significant effect of treatment.

Although the mean digestibility of CP was significantly (P<0.05) higher in $S_{0\%}$ and $S_{50\%}$ compared to $S_{100\%}$ groups but mean apparent digestibility of other nutrients was similar among all groups. RMFC, NFE, EB, ECE and FCR showed positive whereas; GFE, RWG, RIG, and FCE showed a negative correlation with treatment. Replacement of green fodder with corn silage showed a positive correlation with OM, EE, NDF, and ADF digestibility coefficient whereas; negative correlation with DM and CP digestibility coefficient.

Several feed efficiency measures calculated as a function of individual intake and body weight gain have been proposed over the years in an attempt to quantify the capacity of animals to convert the ingested feed into a product. Heifers fed on green fodder based TMR showed better metabolizability of TMR than corn silage

fed groups. Ideally, ME intake should equal ME required and RMFC should be nearly zero on a population basis (Fan *et al.,* 1996). Okine *et al.,* (2001) observed similar ME requirement between steers fed silage and silage-grain based diets. However, they reported an 8% higher ME intake for steers fed alfalfa compared to fenugreek silage. Accordingly, other studies also reported that animals with less metabolizability consumed more feed and had a less efficiency of feed utilization than animals with high metabolizability (Smith *et al.,* 2010; Kelly *et al.,* 2011a; Sobrinho *et al.,* 2011). The relative differences in the heifers with lower RMFC when fed berseem fodder compared to the higher RMFC when fed silage based diet could also indicate that green fodder may cause heifers to be more efficient in converting feed to gain. It appears from the analysis of RMFC that heifers were more efficient in converting feed to gain and maybe less wasteful when fed green fodder compared to corn silage based diet.

The digestibility varies between individuals and is affected by physiological state, physical and chemical characteristics of the diet, intake, and feed availability (Titgemeyer, 1997). Compared to fresh forage, the digestibility of silages is lower (Neto *et al.* 2009). The mean digestibility of CP was significantly ($P<0.05$) higher in $S_{0\%}$ and $S_{50\%}$ compared to $S_{100\%}$ groups, however; mean apparent digestibility of other nutrients was similar among three different groups. High CP content in TMR of S0% group than $S_{50\%}$ and $S_{100\%}$ groups might be the reason behind higher CP digestibility. Mahmoud and Ebeid (2014) offered one of the three rations with different kinds of forage berseem, berseem plus corn silage or corn silage to multiparous Egyptian buffaloes. They found significantly higher digestibility coefficients of DM, CP, CF, and cellulose in a berseem fed group compare to other tested rations. However, no significant differences were noticed among tested rations for OM, EE, NFE, NDF, ADF, and hemi-celluloses. These results are in agreement with those obtained by El-Aidy (2003) and Khalafalla *et al.* (2007) who found higher digestibility of nutrients for cows or buffaloes fed rations containing berseem fodder. In general, the higher digestibility values obtained for CP in the S_0 group may be attributed to the effect of feeding such high quality forage which provided stimulatory factors to cellulolytic and other rumen bacteria (Das and Singh, 1999).

CONCLUSIONS

In the present study, nutritional, physical and chemical attributes denoted that prepared corn silage was of very good quality and well preserved. Green fodder replacement with 50 and 100% of corn silage increased feed intake and reduced metabolizability while growth performance was similar. The metabolizability of diet was reduced with an increased level of corn silage yet the performance of growing cattle was similar. So, corn silage can be used as an alternative to high quality green fodder.

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Influence of Different Sources of Supplementary Chromium on Growth, Immunity and Liver Function of Buffalo Calves

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ABSTRACT

Different sources of chromium were used to assess the effect on the growth and immunity, and liver function of buffalo calves. A total of 21 Murrah buffalo calves with body weight (BW) of 82.66±1.73 kg were used in a 90 day trial. Calves were randomly allocated into 3 treatments. The treatments were as follows: 1) Control comprised of total mixed ration (TMR) without any supplementation; 2) CrC, TMR+1mg/kg DMI/day/calf chromium in the form of chromium chloride hexahydrate; 3) CrP, TMR+1mg/kg DMI/day/calf chromium in the form of chromium picolinate. Bodyweight, weight gain, and average daily gain showed significant (P<0.05) improvement with supplementation of chromium either as chromium chloride or chromium picolinate in the diet of calves. The feed: gain ratio was lower (P>0.05) in the both CrC and CrP groups. Dietary supplementation of chromium from different sources did not affect the total leukocyte, urea, glucose and alkaline phosphatase concentration in blood. An improvement (P<0.05) was detected in the circulating concentration of total immunoglobulin and lymphocyte% as result of supplementation of both chromium picolinate and chromium chloride. Plasma concentration of total cholesterol, HDL cholesterol, alanine aminotransferase and aspartate aminotransferase were decreased with supplementation of chromium from both the sources. Dietary supplementation of chromium from both the sources exerted beneficial effects on the growth performance, immune status,and liver function, but the chromium picolinate supplementation was observed to be more beneficial.

Key words: Murrah buffalo calves, Chromium chloride, Chromium picolinate,Growth performance

INTRODUCTION

The strategies that maximize the use of available nutrient sources are required for better livestock production. Chromium (Cr) is known as an essential trace element for animals and humans (European Food Safety Authority Panel on Dietetic Products, Nutrition, and Allergies, 2014). Trivalent Cr is a component of oligopeptide low molecular weight Cr-binding substance, chromodulin, contributing as a part of the insulin signalling auto-amplification mechanism, thereby influencing the metabolism of carbohydrates, protein, and lipids (Pechova and Pavlata, 2007). It is needed for the normal metabolism of fat and protein (Xu *et al.,* 2017).Feeding supplemental dietary Cr to dairy cows increases immune response and resistance to diseases (Spears *et al.,* 2012).The Cr deficiency comprises impaired nutrient metabolism, compromised immunity, and decreased body weight gain (BWG) and health status, specifically under stressful conditions (Mousavi *et al.,* 2019b). Urinary excretion of Cr was increased in animals exposed to stress or fed diets rich in carbohydrates (Pechova and Pavlata, 2007), suggesting the need for supplementation of Cr in the diet from exogenous sources (Kargarand Kanani, 2019).The immunity was improved by trivalent Cr and its effects appear to be more evident during the stress (Lien *et al.,* 2014).Chromium improves immune responses by promoting immunoglobulin production, and reducing serum cortisol concentration (Weiss and Spears, 2005).Kargarand Kanani (2019) recorded higher body weight (BW) and BWG in calves fed diets with supplemental Cr.In the view of these facts, present experiment was conducted to study the possible effects of different sources of Cr supplementation on growth,

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immunity and liver function of buffalo calves.

MATERIALS AND METHODS

All the procedures followed in this study were sanctioned by the Institutional Animal Ethics committee, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut.

Twenty-one clinically healthy Murrah buffalo calves were taken from LRC, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, and randomly assigned into three groups (n=7) after blocking by BW (82.66±1.73 kg) and age (5.02±0.15 month). The diet was provided to the experimental calves in the form of a total mixed ration (TMR). The TMR (Table 1) was prepared as per recommendations of NRC (2001) and offered daily in the morning (07:30 h), noon (13:30 h), and evening (18:45 h). The calves were fed a TMR without Cr (control) or with Cr supplementation at 1 mg/kg DMI/day/calf (CrC) in form of Cr chloride hexahydrate ($CrCl_3.6H_2O$, molecular weight 266.45, minimum assay 97%; HiMedia Laboratory Pvt. Ltd., Mumbai, India) or 1 mg/kg DMI/day/calf (CrP) in the form of chromium picolinate $(CrC_6H_4NO_2)_3$, molecular weight 418.3, minimum assay 96%; HiMedia Laboratory Pvt. Ltd., Mumbai, India) for 90 days of the study period. Chromium chloride and Cr picolinate were fed to each calf of concerned group individually by mixing with 150 g of concentrate once daily before morning feeding.The rest amount of compounded concentrate was added to TMR. All calves were provided fresh water *ad libitum*.

The offered and refused feeds were recorded daily for the calculation of feed intake. Body weight (BW) was determined at fortnightly intervals using the electronic weighing balance in the morning before feeding and watering. BW gain (BWG) was calculated by subtraction of BW of the previous fortnight from the BW of the current fortnight.The ratio of feed consumption and BWG was used in the calculation of feed conversion ratio (FCR).

5 ml of blood sample was collected from the jugular vein in heparinized vacutainer tubes (BD Franklin, USA) at 0, 15, 30, 45, 60, 75, and 90 days of Cr supplementation in the morning (07.00 h) before feeding. Day "0" represented the start of the experiment. Chilled ice boxes were used in the transportation of blood-filled vacutainer tube to the laboratory.White blood cells and lymphocyte count were performed in fresh whole blood. The rest of the blood samples were then centrifuged at 1600 g for 20 min at 4°C and plasma was harvested with the help of a

Table 1. Ingredient composition (g/kg DM) of TMR fed during study period

Attributes	Control	CrC	CrP
Berseem fodder	180	180	180
Oat fodder	100	100	100
Wheat straw	130	130	130
Grounded yellow maize	320	319	319
Wheat bran	50	50	50
Mustard cake	115	115	115
Soybean cake	93	93	93
Common salt	3	3	3
Mineral and vitamin premix*	9	9	9
Chromium chloride	-	1	-
Chromium picolinate	-	-	1

*Premix composition (per 2.5 kg): Vitamin A 50 lac IU, Vitamin D_3 10 lac IU, Vitamin E 750 IU, Vitamin B_{12} 6 mg, Vitamin K 1 g, Nicotinamide 10 g, Vitamin B_2 2 g, calcium pantothenate 2.5 g, choline chloride 150 g, calcium 750 g, phosphorus 150 g, zinc 15 g, manganese 27.5 g, copper 2 g, iodine 1 g, cobalt 0.45 g, selenium 100 mg; CrC, group supplemented with chromium chloride ($CrCl_3.6H_2O$, HiMedia Laboratory Pvt. Ltd., Mumbai, India) @ 1 mg/kg DMI/day/calf; CrP, group supplemented with chromium picolinate {$Cr(C_6H_4NO_2)_3$, HiMedia Laboratory Pvt. Ltd., Mumbai, India} @ 1 mg/kg DMI/day/calf

dropper without disturbing the packed blood cells. Plasma was kept in Eppendorf tubes and stored at -20°C until ready for analysis for immunological profile and liver function enzymes.

Total immunoglobulin (TIG) was measured in the plasma by the zinc turbidity method (McEvan and Fisher, 1970).White blood cells (WBC) were counted according to the procedure described by Feldman *et al.* (2000). Lymphocyte was recorded in Giemsa stained blood film following the technique given by Jain (1986).Plasma concentration of glucose, total cholesterol, HDL cholesterol, urea, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were measured by commercial kits (Span Diagnostics Ltd Surat, India).

Statistical analysis

Analysis of variance techniques was used to analyze the data using the GLM procedure of SPSS (V20: SPSS Inc.,Chicago, IL, USA). Duncan's Multiple Range Test was conducted to compare the means.

RESULTS AND DISCUSSION

Statistical analyses revealed no significant (P>0.05) effects of dietary Cr supplementation from inorganic and organic sources on initial body weight and average daily feed intake over a period of 90 days (Table 2). However, final BW, BWG, and average daily gain (ADG) were higher (P<0.05) in treatment groups either supplemented with Cr chloride or Cr picolinate (Table 2). The feed:gain ratio was lower (P<0.05) in treatment groups (Table 2) during the 90 days of the study period.

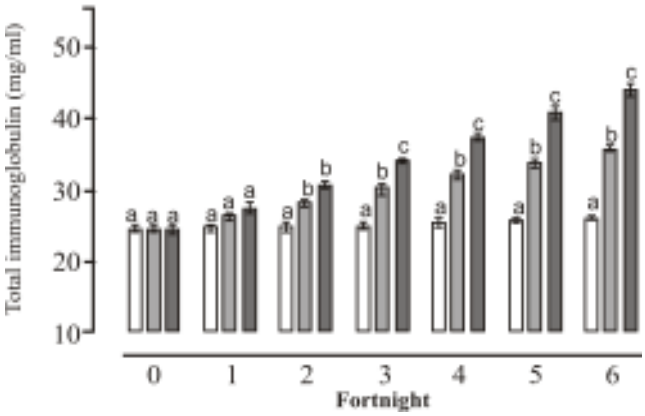

Fig. 1. Changes in total immunoglobulin of buffalo calves in control (□), CrC group supplemented with chromium chloride @ 1mg/kg DMI/day/calf (▭), and CrP group supplemented with chromium picolinate @ 1mg/kg DMI/day/calf (▬) groups between fortnight 0 and 6; Bars bearing different small letter (a, b, and c) differ significantly at P<0.05

Irrespective of sources Cr inclusion in the diet improved BW and ADG in this experiment. Presnt finding is similar to that of Mousavi *et al.* (2019b). The positive response of Cr on BW and BWG might be due to increased efficiency of tissue accretion in calves (Ghorbani*et al.,* 2012). However, Kumar *et al.* (2016) reported no effect of dietary Cr (0.5 mg/kg DM) supplementation on BW and ADG of Sahiwal calves. The dietary addition of Cr in both forms of Cr chloride and CR picolinate did not affect the daily feed intake

Table 2. Growth performance of Murrah buffalo calves supplemented with different sources of chromium

Variable	Control	CrC	CrP	SEM	P-value
Initial body weight (kg)	82.16	82.86	82.97	1.73	NS
Final body weight (kg)	109.76[a]	113.31[b]	114.61[b]	1.79	*
Weight gain (kg)	27.6[a]	30.45[b]	31.64[b]	0.25	*
Average daily gain (kg/d)	0.31[a]	0.34[b]	0.35[b]	0.05	**
Average daily feed intake (kg/d)	2.66	2.53	2.55	0.08	NS
Feed conversion ratio (feed: gain ratio)	8.68[a]	7.49[b]	7.24[b]	0.12	**

CrC, group supplemented with chromium chloride @ 1mg/kg DMI/day/calf; CrP, group supplemented with chromium picolinate @ 1mg/kg DMI/day/calf; SEM, standard error mean; Means bearing different superscript (a, b, and c) in a row differ significantly (P<0.05)

that corroborates well with the findings 0f Kumar *et al.* (2013) in buffalo calves and Mousavi *et al.* (2019b) in Holstein calves. In this experiment, Cr supplementation showed positive effect on feed conversion efficiency, which is in agreement twith he findings of Haldar *et al.* (2009). However, Mousavi *et al.* (2019a) observed that Cr supplementation did not affect the feed conversion efficiency.

The plasma concentration of the TIG was highest (P<0.05) in Cr picolinate supplemented group followed by Cr chloride and control groups (Table 3). Similar trends were also observed at 3, 4, 5 and 6th fortnights of the study period (Figure 1). Mean total leukocyte did not show a significant difference among the groups (Table 3). Mean lymphocyte% was higher in treatments supplemented either with Cr chloride or Cr picolinate than control (Table 3). Similar patterns of lymphocyte % were also reported at 3, 4, 5 and 6th fortnight (Figure 2).The increased circulating TIG in both Cr chloride and Cr picolinate fed groups is accordace with the results of Kumar *et al.* (2017). In the present study, Cr supplementation had a positive response on the plasma concentration of TIG might be dietary fed Cr increased the blood concentration of lymphocyte and production of immunoglobulin from lymphocytes (Terramoccia *et al.,* 2005).

Dietary Cr supplementation did not affect the

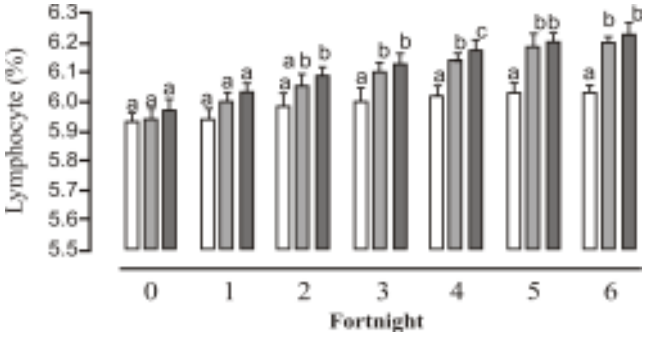

Fig. 2. Changes in leukocyte concentration of buffalo calves in control (□), CrC group supplemented with chromium chloride @ 1mg/kg DMI/day/calf (▭), and CrP group supplemented with chromium picolinate @ 1mg/kg DMI/day/calf (■) groups between fortnight 0 and 6; Bars bearing different small letter (a, b, and c) differ significantly at P<0.05

blood concentration of WBC count is in the line of the research reported by Kegley *et al.* (1996). In line with the present finding, Kumar *et al.* (2016) observed that the dietary supplemental Cr (0.5 mg/kg DM) in the form of Cr picolinate did not influence total blood leukocyte count in Sahiwal calves. Increased blood lymphocyte count in Cr supplemented groups is in agreement with the findings of Zhumabaeva *et al.* (2014) who also reported that Cr supplementation improved peripheral blood lymphocytes at the expense of higher counts of B

Table 3. Immuno-biochemical profile of Murrah buffalo calves supplemented with different sources of chromium

Variable	Control	CrC	CrP	SEM	P-value
Total immunoglobulin (mg/ml)	25.09[a]	30.04[b]	34.05[c]	0.56	**
Total leukocyte count (×10³/µl)	7.15	7.30	7.42	0.33	NS
Lymphocyte (%)	59.90[a]	60.90[b]	61.16[b]	0.55	**
Urea (mg/dl)	52.01	52.05	50.32	0.74	NS
Glucose (mg/dl)	54.18	54.98	56.13	0.50	NS
Total cholesterol (mg/dl)	157.58[a]	149.70[b]	142.74[c]	1.11	**
HDL cholesterol (mg/dl)	98.68[a]	89.73[b]	82.17[c]	0.76	**
Alkaline phosphatase (KA Unit)	10.58	11.03	10.11	0.42	NS
Alanine aminotransferase (IU/L)	45.30[a]	43.47[b]	40.54[c]	0.46	**
Aspertate aminotransferase (IU/L)	131.85[a]	128.09[b]	127.72[b]	0.84	**

CrC, group supplemented with chromium chloride @ 1mg/kg DMI/day/calf; CrP, group supplemented with chromium picolinate @ 1mg/kg DMI/day/calf; SEM, standard error mean; Means having different superscript (a, b, and c) in a row differ significantly (P<0.05)

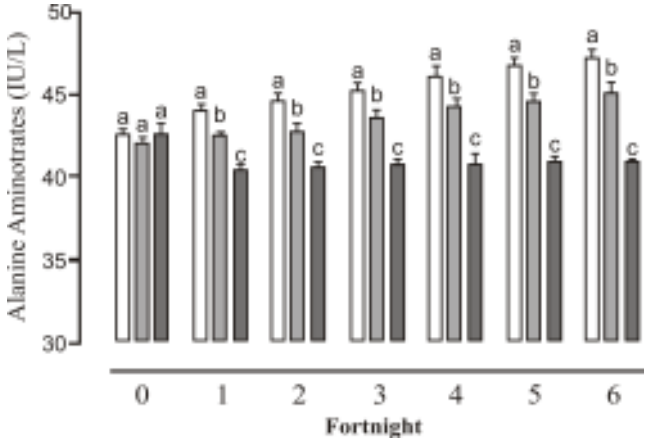

Fig. 3. Changes in alanine aminotransferase concentration of buffalo calves in control (▭), CrC group supplemented with chromium chloride @ 1mg/kg DMI/day/calf (▭), and CrP group supplemented with chromium picolinate @ 1mg/kg DMI/day/ calf (▭) groups between fortnight 0 and 6; Bars bearing different small letter (a and b) differ significantly at P<0.05

and T lymphocytes. Deka *et al.* (2014) reported higher B and T-cell proliferation in peri-parturient Murrah buffaloes supplemented with inorganic Cr (1.5 mg/kg DM).

Mean plasma urea and glucose concentration did not vary statistically among the groups (Table 3).The Cr supplementation either in the form of Cr chloride or Cr picolinate did not affect plasma urea concentration. In agreement with the present findings Pechova *et al.* (2002) also observed that urea levels were unaffected with supplementation of Cr in the form of Cr enriched yeast in peripartum dairy cows. The non-significant effect of Cr on blood urea concentration is in line with the report of Mousav *et al.* (2019a).

Plasma concentration of the glucose was not affected by supplementation of any source of Cr. Similarly, Ghorbanifar *et al.* (2012) reported that plasma glucose concentration was unaffected by dietary supplementation of Cr. In contrast to the present findings, Chang *et al.* (1994) have reported a reduction in plasma glucose concentration with supplementation of Cr. Total cholesterol and HDL cholesterol concentrations were lower in Cr picolinate supplemented group as compared to Cr chloride supplemented and control groups (Table 3).A decline was observed in circulating concentration of cholesterol and HDL cholesterol in buffalo calves fed diet with Cr. Similar to the present finding Ghorbani *et al.* (2012) reported a decline in the level of cholesterol. This might be regulatory effect of Cr in enhancement of glucose tolerance that caused reduction in circulating level of cholesterol. Mousavi *et al.* (2019a) reported that Cr supplementation did not affect blood level of total cholesterol.

Supplementation of Cr chloride and Cr picolinate did not affect the mean activity of ALP (Table 3). Mean ALT (Table 3) and ALT activity on 1, 2, 3, 4, 5, and 6th fortnights (Figure 3) were found lower (P<0.05) in Cr picolinate supplemented group followed by Cr chloride supplemented and control groups.AST activity was lower (P<0.05) in both the groups fed Cr chloride andCr picolinate groups in comparison to control (Table 3). But there was no significant difference observed between CrC and CrP (Table 3). A similar pattern of AST activity was also observed on 2, 3, 4, 5, and 6 fortnights of the study period (Figure 4).Observation that

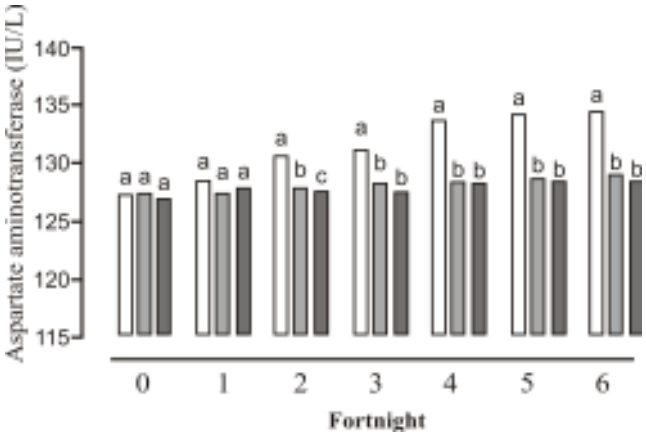

Fig. 4. Changes in aspartate aminotransferase concentration of buffalo calves in control (▭), CrC group supplemented with chromium chloride @ 1mg/kg DMI/day/calf (▭), and CrP group supplemented with chromium picolinate @ 1mg/kg DMI/day/ calf (▭) groups between fortnight 0 and 6; Bars bearing different small letter (a, b, and c) differ significantly at P<0.05

chromium supplementation did not affect the level of ALP activity is corroborating with the finding of Keshri *et al.* (2019). However, Yazaki *et al.* (2009) reported positive influence of chromium on ALP level. The Cr supplemented calves had shown a decline in the concentration of AST and ALT over 90 d experimental periods, indicating that Cr supplementation improved the function of the liver in calves. However, Keshri *et al.*(2019) did not observe any positive response of Cr on liver function. Pechova *et al.* (2002) also reported that AST level was not affected with addition of Cr in the diet of prepartum dairy cows.Yazaki *et al.* (2009) reported an increase in AST and ALT levels by Cr supplementation.

CONCLUSIONS

Cr supplementation increased the body weight and weight gain, total immunoglobulin, lymphocyte and lowered the total cholesterol, HDL cholesterol, ALT, and AST, thereby improved the growth performance, immunity and liver function of growing Murrah buffalo calves. But the Cr supplementation in the form of Cr picolinate is more beneficial in the term of improvement of immunity and liver function.

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Effect of Bypass Fat Supplementation on Growth, Onset of Puberty, Biochemical Metabolites and Reproductive Parameters in Jersey Crossbred Prepubertal Heifers

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ABSTRACT

Bypass fat can increase the energy density of ration without having any adverse effects on feed intake and digestion. The experiment was conducted to investigate the effects of supplementation of bypass fat on the onset of puberty in dairy heifers. The trial was conducted with 12 prepubertal divided into control (C) and Treatment (T) groups. The ration for the experimental animals in control group were not supplemented with bypass fat, whereas heifers in treatment group received bypass fat at 50g/day/h for a period of 150 days. Bypass fat supplementation significantly (P<0.05) improved the mean live body weight, body condition score (BCS) and body fat thickness (BFT) in supplemented heifers. Bypass fat supplementation had no significant (P>0.05) effect on the proportion of heifers attaining puberty (50.00 *vs.* 33.33%) as well as the age at onset of puberty (605.67±22.67 *vs.* 626.50±28.50 days). There was no significant (P>0.05) difference observed between the control and treatment groups in the mean size of dominant follicle, CL and ovary. The overall mean uterine horn diameter during the trial period was significantly (P<0.05) higher in bypass supplemented group than in control group. The number of small, medium and large follicles did not vary between groups (P>0.05) in heifers during the study period. No significant (P>0.05) effect on plasma NEFA level was observed, however plasma glucose level was significantly (P<0.05) higher in bypass fat supplemented heifers. Hence, it can be concluded that bypass fat supplementation at 50g/day/h improved the body growth of supplemented heifers but did not had any beneficial effect on onset of puberty in heifers.

Key words: Bypass fat, Energy, Heifers, Jersey, Onset of Puberty.

INTRODUCTION

The dairy industry is dependent on dairy cow's reproductive success; since the lactation cycle is reliant on the cow's ability to become pregnant and produce milk after delivery. Nutrition is one of the many significant factors responsible for reproductive activity. Various reproductive problems, such as delayed puberty, anoestrous, repeat breeding, retained foetal membranes, abortion, weak calf syndrome, *etc.,* could eventuate due to poor nutrition in cows, thus reducing reproductive efficiency.

Malnutrition can delay the onset of puberty in dairy cattle during the heifer stage. Early maturing cows will yield more milk during their entire productive time, but delayed puberty is a big concern for dairy cows. The nutritional plan is the most significant aspect affecting the age of maturity (Poy and Panday, 1971). In the tropical zone, puberty occurs in the *Bos indicus* breeds between 16 and 40 months (McDowell *et al.,* 1976) but is associated with the body weight instead of the age of the animal. The body weight of heifers at puberty was observed to be between 55% and 60% of adult body weight (Perry, 2016). Malnourished females lack ovarian activity due to inhibition of pulsatile luteinizing hormone (LH) (Rasby and Funston, 2016). The LH pulse generation system in the hypothalamus and its

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pre-pubertal spike is affected by the nutritional plane (Schillo *et al.,* 1992). Nutrition influences the production and release of GnRH (gonadotrophin releasing hormone), FSH (follicle stimulating hormone), LH, and GH (Growth hormone) owing to its function on the hypothalamus and the anterior pituitary. Ovarian follicular development and steroid production are also affected by diet. Thus, greater growth rates will reduce the negative feedback of estradiol on the secretion of LH and thus promote follicular growth.

Balanced nutrition and effective management will help to promote growth and early sexual maturity (Heinrichs *et al.,* 2005). Supplementation of high energy concentrates or fats have been used as a technique to increase the energy density of the ration. However, various negative impacts of such supplementation *viz.* decrease in intake and digestion of fibre, acidosis, and chelation of minerals may be observed in such animals. To reduce the detrimental effects of high energy concentrate and fat supplementation, a specialised rumen-inert lipid, popularly known as bypass fat was developed. Bypass fat can resist lipolysis and biohydrogenation in rumen but is digested in abomasum and can help overcome negative energy balance (NEB) without negatively affecting the ruminal digestion and fermentation process. Initially bypass fat was only considered as a source of energy during transition period in dairy cows to reduce NEB and maintain milk production, however later on, it was demonstrated that the fatty acids present in bypass fat may have a positive effect on reproduction (Staples *et al.,* 1998; Rahbar *et al.,* 2014). Thus, bypass fat supplementation may provide the energy required for achieving optimum growth in growing heifers to attain puberty without any adverse effects. Hence, the main objective of this study was to determine the effects of bypass fat supplementation in crossbred Jersey heifers on the onset of puberty and reproductive parameters.

MATERIALS AND METHODS

This study was conducted in the Dairy farm complex of ICAR-National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal, India. Kalyani is located at the lower Gangetic basin in the Nadia district of West Bengal at an altitude of 11m above sea level. The coordinates of Kalyani are: 22° 58'30" N latitude and 88° 26'4" E longitude. The climatic condition is tropical hot and humid. The study was conducted between the months of October, 2020 to March, 2021. The average climatic conditions during the trial period were *viz.* temperature: 28.3° C (31.8-23° C), rainfall: 16.6 mm (0.00-94.1 mm), and humidity: 46 % (34-73 %).

Twelve (*n*=12) Jersey x Zebu crossbred heifers above 16 months of age with no history of observed sign of estrus and ovaries without any functional structures (corpus luteum or matured follicles), were selected and assigned to one of the two dietary treatment groups for the study. Heifers were similar in age (16.84± 0.36 months), body weight (184.54± 7.46 kg), and BCS (2.75± 0.08), and distributed equally to one of the two groups with six cows in each group: Control (non-supplemented) or treatment (supplemented) group. The trial was conducted for a period of 150 days (5 months). The BW and BCS were measured at fortnightly interval for both the groups. Body weight was recorded using a digital weight recording platform (Spider – 300, Future weighing & engineering). BCS were determined by the same individual for the entire experiment according to a 1 (thin) to 5 (obese) scale (Edmonson *et al.,* 1989). The experimental cows were dewormed and vaccinated regularly according to farm schedule. Subcutaneous back fat thickness was measured at the end of trial period by real time ultrasound (Linear ultrasonograph DIGI 1100 CD-E VET, SSMED Ltd. probe- 6.5 MHz).

All the experimental animals were housed in the experimental sheds, which was well-ventilated having cemented floor with individual feeding and watering arrangement throughout the experimental period. Proper cleanliness and healthy surroundings were ensured throughout the experimental period. All cows were fed with concentrate mixture, 20 to 30 kg of seasonally available green fodders and paddy straw according to the farm protocol. The treatment group was supplemented with bypass fat at the rate of 50 g/

animal/day for a period 05 150 days. Bypass fat was procured from ICAR- Central Coastal Agricultural Research Institute, Old Goa, Goa.

Blood samples were collected at early morning at fortnightly interval from both the groups by means of jugular vein puncture using sterile needles. The blood samples were collected into centrifuge tubes containing EDTA (10%), put in an ice bucket and carried to the laboratory within 20 minutes of collection. Blood samples were centrifuged at 3000 rpm for 30 min and plasma was separated and kept in the labelled storage vials of 5 ml capacity and stored at -20°C till analysis. Samples were later analysed for blood glucose and plasma non-esterified fatty acids (NEFA) levels. Blood glucose was analysed by spectrophotometry using AUTOSPAN® Glucose (ARKRAY, Inc.). Plasma NEFA was analysed by the copper soap extraction method modified by Shipe *et al.* (1980).

The onset of puberty in heifers was monitored by observing the behavioural signs of estrum, presence of cervico-vaginal mucus discharge, rectal palpation of genital organs and ultra-sonographic examination of ovaries. Heifers were observed for the visual signs of estrus twice in a day and the ultra-sonographic evaluation of reproductive tract was carried out at 7 days interval. Reproductive tract score (RTS) values are gauged through rectal palpation and ultrasound examination of the uterine horns and ovaries as per the method of Andersen *et al.* (1991). Puberty and sexual maturity of heifers was gauged based on ovarian development and palpable size of the reproductive tract.

Intensity of estrus in all the experimental animals was scored, based on the score card (maximum score: 100) designed by Layek *et al.* (2011). Each observation of sign of the estrus was assigned a score as per the scale and the sum of the scores was recorded. Duration of behavioural estrus in all the animals was estimated in hours from the time of first appearance of estrous to the time of detection of last estrous sign.

Ultrasound examination (Transrectal ultrasonograph DIGI 1100 CD-E VET, SSMED Ltd. probe- 9 MHz) was carried out at weekly intervals in experimental animals. Monitoring of follicular growth (small<4 mm; medium 4-8 mm and large>8 mm) was carried out. On each examination and for each animal in control and treatment groups, the diameter of uterine horn (cm) was assessed. The diameter and area of ovaries, presence of corpora lutea and dominant follicles and their sizes were also measured.

Statistical analysis

The data were captured in excel and analysed using suitable statistical package. The model contained the diet and interval as fixed parameters, two-way ANOVA was used to compare the effect of bypass fat supplementation on the mean differences between the control and treatment group. Data were presented as least square means ± standard error of the mean (SEM). The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The live body weight increased along with increasing age in both the groups throughout the trial period. The overall mean body weight during the 150 days trial period was significantly higher ($P<0.05$) in treatment (220.47±5.85 kg) group than in control (209.34±4.95 kg) group. There was no significant ($P>0.05$) difference in final body weight at the end of trial period between control (231.83±17.18 kg) and treatment (251.5±11.94 kg) group. The average daily weight gain during the 150 day trial period was non-significantly ($P>0.05$) higher in treatment group (417.78±18.65g/day) than in control group

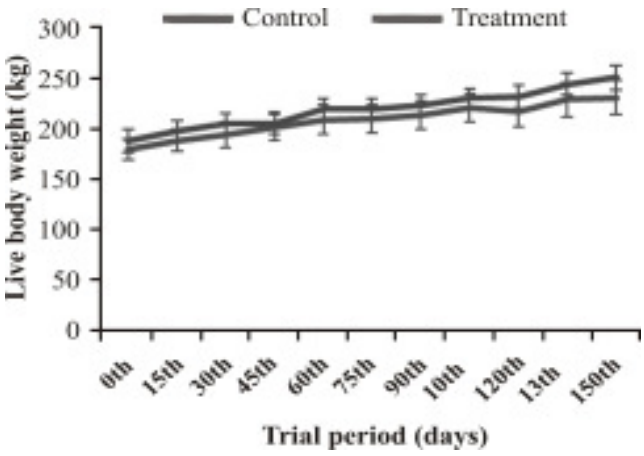

Fig 1. Live body weight (kg) of heifers during trial period (Mean ±SEM)

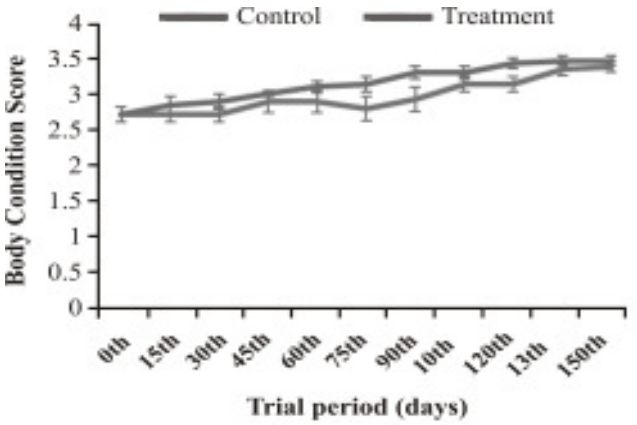

Fig 2. Body Condition Score (BCS) during trial period in experimental heifers (Mean ±SEM)

(343.89±47.94g/day). Vahora *et al.* (2012) observed significantly higher average daily gain in bypass fat supplemented growing buffalo heifers. Gajera (2013) observed significant improvement in total weight gain and average daily body weight gain in bypass fat supplemented Jaffarabadi heifers. Rosadiuk *et al.* (2021) observed that the overall, average daily gain and body weight were greater for heifers offered increased plane of nutrition during both the pre- and post-weaning phases. The ADG can be improved by increasing levels of nutrition, and in general around 750 g/day ADG is optimal for dairy heifers (Wathes *et al.,* 2014) with lower rates delaying puberty and age at first calving (AFC). In the present study, we observed the ADG to be lower than 750 g/day in both control (343.89±47.94g/day) and treatment (417.78±18.65g/day) group, which may be the reason of delayed puberty in both the groups during our study.

The overall mean BCS for 150 days period was significantly (P<0.01) higher in treatment group (3.18±0.08) than the control group (3.00±0.07). The average gain in BCS during the trial period was non-significantly higher in treatment group (0.75±0.09) than in control group (0.67±0.11). Campanile *et al.* (2010) found significant improvement in BCS of Murrah buffalo heifers fed high energy diet in contrast to those fed a low energy diet. Carvalho *et al.* (2013) found higher energy intake to increase the BCS of zebu heifers significantly without altering the BW at puberty. The BFT (mm) was significantly higher (P<0.05) in treatment group (22.94±0.85 mm) than in control group (21.17±0.61 mm) at the end of 150th day trial period. Similar to our findings, Ortega *et al.* (2020) observed significant improvement in fat thickness in the rump region in heifers fed with high energetic-protein supplement in post-weaning period. Chelikani *et al.* (2003) observed back fat thickness to be lower in dairy heifers fed with low protein and energy containing diet in comparison to that of high protein and energy containing diet.

The overall mean RTS during the trial period of 150 days was similar (P>0.05) in both treatment (3.42±0.08) and control (3.32±0.11) group. RTS was developed as an indirect technique of predicting pubertal age and an indication of heifer nutritional requirements before the breeding season (Anderson *et al.* 1991). Heifers were assigned a score ranging from 1 to 5 for their reproductive tract. A RTS of one suggested an immature, non-cycling reproductive system, whereas an RTS of five suggested a cycling heifer with a functional corpus luteum. On the final trail day (150th day) of the experiment, two heifers in the control group and three heifers in the treatment group had an RTS of 5, suggesting cyclicity with a functional corpus luteum. Parallel to our findings, Speer *et al.* (2020) observed that increased energy diet supplementation had no effect on heifer RTS and that all heifers showed similar reproductive maturity near

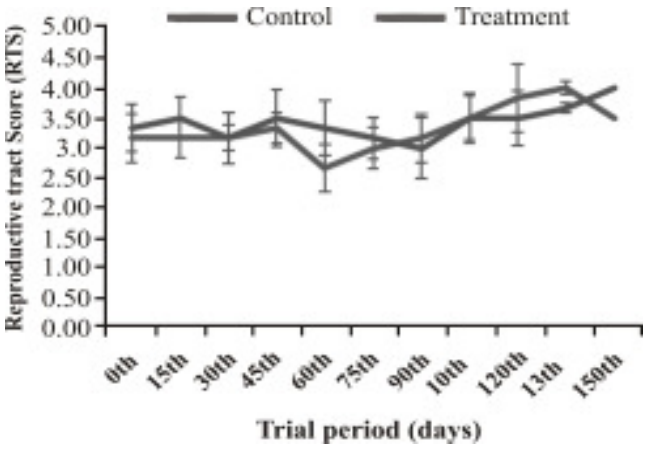

Fig 3. Reproductive tract score (RTS) in experimental heifer groups (Mean ±SEM)

the breeding season. Rosasco *et al.* (2017) found that increasing nutritional supplementation had no effect on RTS in beef heifers.

The average numbers of specific type of follicles based on size (small<4 mm; medium 4-8 mm and large>8 mm) at a given interval throughout the trial period was observed using ultrasound examination. The overall mean number of small follicles was 6.05±0.46 and 5.67±0.36 in control and treatment groups, respectively. The overall mean number of medium follicles was 4.83±0.24 and 4.98±0.37 in control and treatment groups, respectively. The average number of large follicles was 0.91±0.07 and 0.92±0.08 in control and treatment groups, respectively. There was no significant (P>0.05) difference in number of small, medium as well as large follicles between the groups. Similar to our findings, Rosasco *et al.* (2017) found no effect of increased nutritional supplementation on antral follicle count (AFC) in beef heifers, which is consistent with our findings. Stokes *et al.* (2018) observed that trace mineral supplementation had little influence on the AFC of Angus breed heifers. Speer *et al.* (2020) found that supplementing a higher calorie diet had no effect on total follicle count in heifers across treatment groups. The similarity in follicular growth between groups can be attributed to the absence of an energy shortage in the research animals (Mattos *et al.* 2000).

The mean maximum diameter of the follicles (mm) during the 150 day trial was almost similar (P>0.05) between treatment (12.67±0.99 mm) group and control group (12.00±0.97 mm). The overall mean area of the largest follicle (mm^2) during the 150 days trial was slightly higher in treatment (434.89±77.06) group than in control group (391.98±58.28), however the difference was non-significant (P>0.05). In a study on postpartum buffaloes, Ganie (2011) observed no significant effect of bypass fat supplementation on the largest follicle and preovulatory follicle diameter; nevertheless, it was improved in contrast to the control. Similarly, Petit *et al.* (2002) and Katiyar *et al.* (2017) observed that supplementing with bypass fat had no effect on the size of dominant follicles in postpartum buffalo cows.

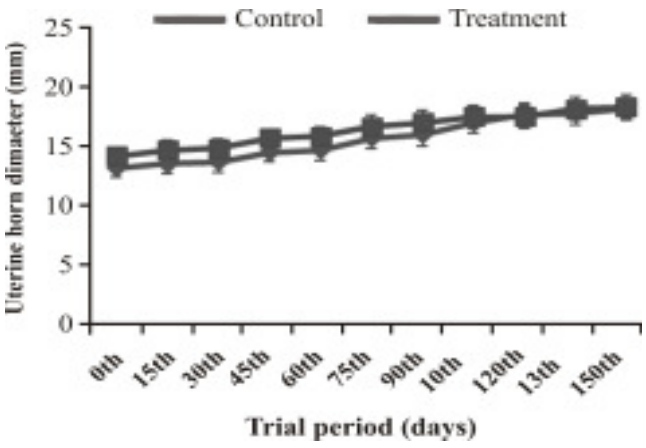

Fig 4. Fortnightly uterine horn diameter (mm) in experimental heifers (Mean ±SEM)

The overall mean ovarian diameter (mm) during the 150 day trial was insignificantly higher (P>0.05) in treatment (20.70±0.39) group than in control group (20.12±0.39). Similarly, the mean area of the ovary (mm^2) during the 150 day trial was higher in treatment (974.54±33.94) group than in control group (939.72±36.17), however the difference was non-significant (P>0.05). In support of the present findings, Freetly *et al.* (2014) found that diet had no influence on ovarian weight and size in peripubertal heifers. Speer *et al.* (2020) observed that increased energy diet supplementation had no effect on ovarian length and height in heifers across treatment groups.

The overall mean uterine horn diameter during the 150 day trial period was significantly (P<0.05) higher in treatment group (16.39±0.40 mm) than in control group (15.62±0.56 mm). The uterine horns of the prepubertal heifer are often flaccid and undeveloped. However, as puberty approaches and in response to increasing stimulation by ovarian estrogens, the uterus and cervix become bigger and display more smooth muscle tone when palpated manually (De Carvalho, 2014). Akhtar (2002) observed a substantial improvement in uterine horn diameter in Nili-Ravi buffalo heifers fed a greater amount of dietary energy. Summers *et al.* (2012) recorded increased uterine horn diameter in heifers administered a corn gluten feed-based supplement throughout development period.

The heifers were monitored for onset of puberty by observing the estrus signs and development of

corpus luteum (CL) in ovaries during 150 days trial period. Out of 6 heifers, 2 in control group and 3 in treatment group attained puberty during the trial period. The difference in percentage of heifers that attained puberty was insignificant (P>0.05) between treatment (50.00%) and control group (33.33%). The age at onset of puberty (days) was insignificantly (P>0.05) lower in treatment group heifers (605.67±22.67 days) than in control heifers (626.50±28.50 days).

Using the concept of "targeted body weight," nutritional management involving continuous, high rates of growth is one approach for promoting the timely onset of sexual maturity. As thumb rule, this strategy sets a target of 60 to 65 percent of mature body weight (BW) for individual heifers to have achieved puberty. Feeding heifers a high-concentrate diet during crucial developmental windows causes alterations in metabolic endocrine state, as seen by increased circulatory concentrations of leptin, insulin, and IGF-1, which can trigger the onset of puberty months later (De Carvalho *et al.,* 2014; Speer *et al.,* 2020). The results of our current investigation show that bypass fat supplementation has no influence on the age at pubertal onset in prepubertal heifers. Similar to the current observation, Lammoglia *et al.* (2000) fed dietary fat to beef heifers and found no effect on pubertal age, but did found an effect on the proportion of heifers reaching puberty. Chelikani *et al.* (2003) provided prepubertal Holstein heifers with diets differing in dietary

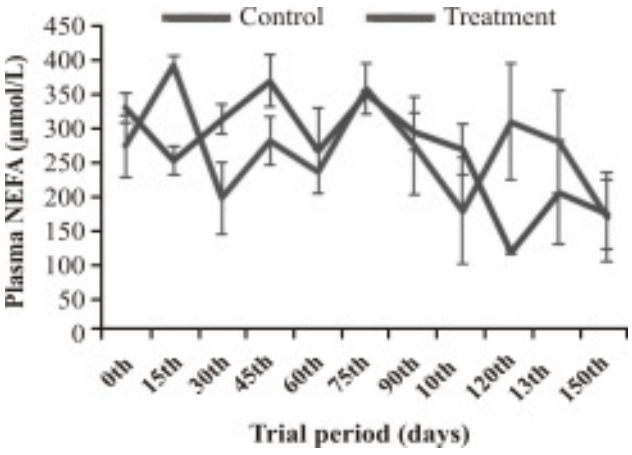

Fig 5. Plasma NEFA (µmol/l) during trial period in experimental heifers (Mean ±SEM)

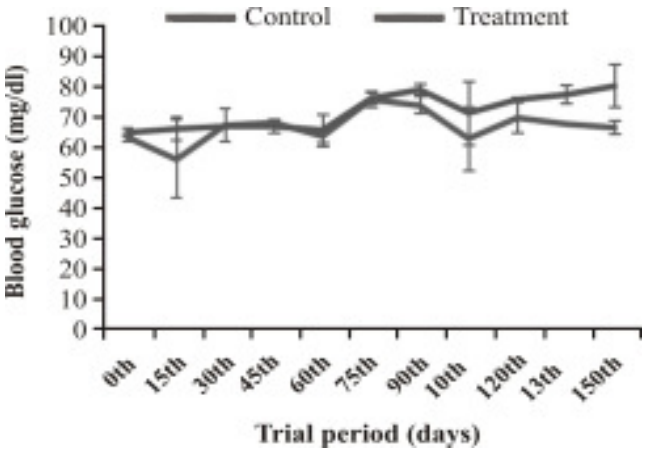

Fig 6. Blood glucose (mg/dl) during trial period in experimental heifers (Mean ±SEM)

energy and protein density, but observed that dairy heifers achieved puberty with consistent body weight and body composition independent of dietary modification. Shike *et al.* (2013) investigated the effect of supplementary fat on beef heifers but found no change in the proportion of pubertal heifers. Manthey *et al.* (2017) fed dairy heifers with distillers' dry grain of high fat content but found no effect of diet on heifer age and body weight at puberty. Oliveira *et al.* (2008) opined that the influence of a greater protein and energy diet on the age at puberty was unclear; however exposing heifers to a male significantly lowered the age at puberty in heifers.

The estrus intensity score (P>0.05) in heifers attaining puberty was similar in both treatment (56.00±1.15) and control group (55.50±1.50). Layek *et al.* (2011) reported that the estrus intensity score in *Bos indicus* breeds is generally around 50%, which was similar to the findings of the present study. Mucus discharge was observed as the most commonly expressed estrus behaviour in heifers attaining puberty. The duration of estrus (hours) in treatment group (21.33±4.81) and control group (18±6.00), did not differ statistically (P>0.05).

The mean blood NEFA level during 150 day trial period was similar (P>0.05) in both treatment group (267.92±23.26 µmol/l) and control group (268.76±21.23 µmol/l) and did not differ significantly. There have been conflicting studies of plasma NEFA levels in dairy cows

fed with bypass fat. Manriquez *et al.* (2019) found that bypass fat supplementation had no effect on NEFA levels in supplemented cows. Postpartum cows given bypass fat had significantly lower plasma NEFA levels, according to Singh *et al.* (2016) and Nirwan *et al.* (2019). However, Dhami *et al.* (2017) and Ranaweera *et al.* (2019) observed an elevated level of plasma NEFA in bypass fat supplemented postpartum cows. Jorritsma *et al.* (2003) observed the plasma NEFA levels to be increased in heifers subjected to acute fasting indicating NEFA level rises in case of negative energy balance.

The mean blood glucose level during 150 day trial period was significantly ($P<0.01$) higher in treatment group (72.04±1.81 mg/dl) than in control group (66.98±1.65 mg/dl). Similar to our findings, Yelich *et al.* (1996) observed plasma glucose levels to be higher in heifers fed to high-gain in comparison to those fed to low-gain diet. Campanile *et al.* (2010) found significantly greater concentration of glucose in Murrah buffalo heifers fed high energy diet in contrast to those fed a low energy diet. Cappellozza *et al.* (2014) observed that beef heifers supplemented with cracked corn, soybean meal and urea had higher glucose levels in contrast to heifers without any supplementation. Ortega *et al.* (2020) observed significant increase in the mean glucose level in heifers supplemented with high levels of energy and protein supplement in the post-weaning period. Rosadiuk *et al.* (2021) observed overall glucose level to be higher in heifers fed with high plane of nutrition in both preweaning as well as postweaning period. The increase in glucose level in dietary fat supplemented animals might be due to glucose sparing effect of dietary fatty acids (Voigt *et al.,* 2005). Additionally, fat feeding is known to induce insulin resistance therefore leading to increase in glucose level of animals fed with dietary fats (Palmquist and Moser, 1981).

CONCLUSIONS

Based on the present study it was concluded that, bypass fat supplementation at 50 g/day over a period of 150 days improved the body growth and condition in supplemented heifers. However, supplementation of bypass fat at the above mentioned level did not have any significant effect on reproductive tract development and onset of puberty in prepubertal heifers. Further studies on bypass fat supplementation on prepubertal heifers at higher inclusion level, larger sample size, different age group and trial interval can be done to further validate the effect of bypass supplementation on onset of puberty in prepubertal heifers.

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Seasonal Variation in Nutrient Utilization and Growth Performance of Small Ruminants Under Grazing on Silvipasture System

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ABSTRACT

Seasonal variation in chemical composition as well as nutrient utilization and growth performance of *Jalauni l*ambs and *Bundelkhandi*kids were studied under grazing on two tier silvipasture consisting (stocking rate of 2ACU/ha) of *Hardiwickia binata* tree (one tier) and grass species namely *Cenchrus ciliaris, Crysopogon fulvus, Panicum maximum and Stylosanthes hamata* (another tier) during growing (August-October) as well as post-growing (November-January) seasons in Bundelkhand region along with supplementation (1% of body weight) of concentrate mixture. Average dry matter (DM) content of pasture forages increased with advancement of maturity from 31.56% during growing season to 49.44% in post growing season, with concomitant increase in neutral detergent fiber (NDF) content from 70.23% to 73.11%, and reduction in crude protein(CP) content from 8.29% to 5.45%. DM intake was comparable in both the species, however, significantly ($P<0.05$) higher in growing than post growing season. Similarly, digestibility of nutrients namely DM, OM, NDF and CP were higher in growing season than post growing season in both the species. Intake (g/$W^{0.75}$) of digestible crude protein (DCP) in both lambs and kids was higher (5.76 vs 5.64) in September as compared to December (2.98 vs 3.14). Metabolizable energy (ME) intake (kj/$W^{0.75}$) also followed the same trend. Daily live weight gain (g/d) in both lambs and kids was comparable, however, significantly ($P<0.05$) higher in growing season than post growing season. It was concluded that nutrient intake, nutrient utilization and growth performance were significantly ($P<0.05$) affected during post growing season due to deterioration of nutritive value of available pasture biomass, however, comparable results were observed in both the species.

Key words: *Bundelkhandi* Kids, *Jalauni* lambs, Chemical composition, Growth performance, Nutrient utilization, Seasonal variation, Silvipasture

INTRODUCTION

Small ruminants play an important role in the food and nutritional security of millions of rural people especially the landless, marginal and small farmers in arid and semiarid rain-fed regions of India. The socio-economic value of small ruminant rearing as compared to other livestock species, for poor farmers is immense (Salem and Smith, 2008).Goats and sheep are multipurpose animals which provide hair, wool, meat, milk and skin. The production of meat from goats and sheep play a vital role in the supply of animal protein for the people of our country. Agricultural farming in semiarid and arid regions is practiced on a limited scale due to scanty and uncertain rains and shortage of irrigation water leaving most of these regions to be used as rangeland grazing. Sheep and goats raised under these conditions are generally grazing on degraded rangelands and or offered low quality fibrous feedstuffs like cereal straws and stubbles. Small ruminant production in village systems in tropical countries is often characterized by poor growth rates and high mortality (Devendra and Burns, 1983). The productivity of small ruminants can be improved by improving the nutrition either through concentrate feeding or provision of additional forage (Pathasarathy *et al.,* 1984; Das and Singh, 1999;Das *et al*, 2007; Das *et al.,* 2011a). Silvipasture system is an efficient and integrated land use management system of tree species, fodder and or livestock specially suited for rearing small ruminants. However, nutrient composition of pasture varies according to season (Das *et al.,* 2011b). The present experiment was conducted to study the seasonal variation in nutrient utilization and growth performance of sheep and goat under grazing on silvipasture system.

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MATERIALS AND METHODS

The experiment was conducted on 10-12 months aged growing lambs and kids. Twenty five each of Jalauni lambs (average body weight 26.20±0.91 kg) and Bundelkhandi kids (average body weight 25.15±0.78k g) were allowed to graze stocking rate of 2ACU/ha) on two tier silvipasture consisting of *Hardiwickia binata*tree (one tier) and grass species namely *Cenchrus ciliaris, Crysopogon fulvus, Panicum maximum ands tylosanthes hamata* (another tier) during growing (August-October) as well as post growing (November-January) seasons at Central Research Farm, ICAR-Indian Grassland and Fodder Research Institute, Jhansi. The total annual forage production potential of the silvi-pasture system from grass and tree component were estimated as per the procedure described by Prajapati(1980).The animals were allowed to graze on *H. binanata* based silvi-pasture for 7 hours from 9 am to 4 pm daily. All the animals were also supplemented with concentrate mixture (consisted of mustard cake, maize, wheat bran, mineral mixture and common salt at 35: 30: 33: 1: 1) at1.0% of their body weight at stall after returning back from grazing. Live weight changes of animals were recorded fortnightly. After 45 days of experimental grazing, digestion trial of 6 days duration was conducted in the month of September and December on 6 animals each from lambs and kids using lignin as internal marker (Costa *et al.,* 2019). Total faeces voided during 24 hrs were collected using faeces collection bags. A direct observation and simulation method was used to determine the botanical composition of the diet consumed by the animals. Forage samples of the ingested species that were consumed by the individual animals were hand clipped for three consecutive days for the entire grazing period from 9 am to 4 pm. The representative samples of forage as well as concentrate and faeces collected during digestion trial were analyzed for dry matter (DM), ash and ether extract according to AOAC (1995). Samples were also analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Goering and Van Soest, 1970).Total nitrogen was determined by micro-kjeldhal method (AOAC 1995). Data were subjected to analysis of variance. Mean data were compared for species and also compared over different seasons for statistical differences using Student's t-test (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Rain fall (Fig 1) during growing season (August to October) which is considered as the main season of the vegetative growth was 341.8 mm and during non-growing season (November to January) there was very little rainfall (24.4 mm). The mean biomass yield from pasture was 5.89 ton DM/ha and pruned foliage yield as top feed was 0.90 ton DM/ha. Thus, the total yield was 6.79 ton DM/ha, was similar (6.8 ton/ha) to *Albizia procera* based silvipastoral system (Palsaniya *et al.,* 2012).Ram *et al.* (2016) also recorded a similar biomass yield in *H. binata* based silvipastures of different grasses and legumes under semiarid climatic conditions of Bundelkhand region.

Average chemical composition of pasture indicated that DM content varied from 31.56% during growing season to 49.44% in post growing season (Table 1). The total ash contents in all the feeds were similar during both the seasons. CP content varied from 10.10% in *S. hamata* to 6.26% in *C. ciliaris* during growing season, and 7.67% in *H. binata* to 4.02% in *C. fulvus* during post growing season. Keba *et al.* (2013) also reported similar CP content in *C. ciliaris* and *P. maximum*. However, Singh and Singh (2017) reported lower CP content in *C. ciliaris* (5.35%), *C. fuvus* (3.60 %)and in *H. binata* (7.80%) than the present findings which might be due to different stage of harvesting of plant samples. NDF contents of legume component varied from 62.21% in *H. binata* to 65.36% in *S. hamata,* whereas in grasses the NDF content ranged

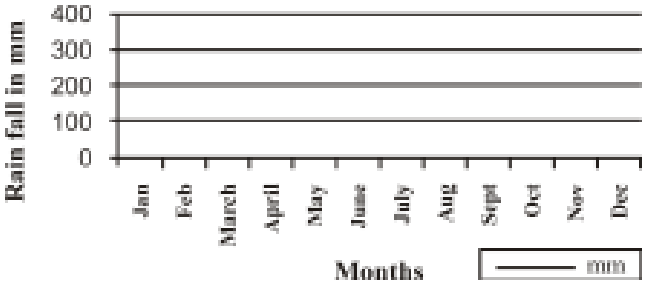

Fig. 1. Annual rain fall

from 73.86% in *P. maximum* to 80.35% in *C.fulvus*. Higher lignin content in *H. binata* leaves as compared to other component of pasture was observed in both the seasons and corroborated with the earlier study of Chitra (2018).The average crude protein content of pasture biomass varied from 8.29% in September to 5.45% in December whereas, on the other hand, NDF content increased from 70.23% during growing season to 73.11% in post growing season. Similarly, lignin content ranged from 7.92% in September to 9.36% in December. The differences in fiber components between season suggested that less amount of rainfall (Fig. 1) caused faster maturation during post growing season and this resulted in higher cell wall contents and lower cell contents than those of growing season. Shinde *et al.* (2001) earlier observed that crude protein (CP) content of ground vegetation under semi-arid condition declined from 9.9% in monsoon to 7.2% during winter. Evitayani *et al.* (2004) also observed that the crude protein (CP) content of pasture grasses declined from rainy season to dry season. Salim *et.al.* (2003) however, reported higher CP content during post growing season as compared to monsoon season which might be due to fertilizer application in the pasture 15 days before harvesting. Mero and Udén (1998) have reported that the levels of protein at young stages of plant growth during monsoon are usually high; at the end of vegetative stage achieve the maximum values and declining as plants matured. Subhalakshmi *et al.* (2011) reported that the chemical composition of pasture is influenced by season, type of soil, stocking density, type grazing pasture and climate. The nutritional content of any forage is dependent on its nutrient content such as protein, which is essential for the growth, development and production status of ruminant animals. During post growing season in the present experiment the crude protein content of herbaceous biomass was far below the critical level as recorded earlier by Mahala *et al.* (2009).

The plant species that were consumed by small ruminants under the trial primarily included *C. ciliaris, C. fulvus, P. maximum, S. hamata and H. binata.* DM intake data indicated that DM intake by lambs and kids was comparable in both the seasons (Table 2) and corroborated with the findings of Kabir *et al.* (2004) where no difference in DM intake was observed in sheep and goat grazed with protein supplementation. Salim *et al.* (2003),however, recorded significantly ($P<0.05$) higher herbage DM consumption by sheep than goat. Intake level of 40-90 g DM/kg$W^{0.75}$ has been reported as normal for grazing ruminants (Cordova *et al.,* 1978). The DM intake values in goats and sheep in the present

Table 1. Seasonal variation in chemical composition of pasture vegetation (%DM basis)

Forage species	Season					
	Growing season					
	DM	CP	NDF	ADF	ADL	Ash
C. fulvus	31.35	8.17	75.08	46.93	4.97	10.70
P. maximum	21.22	7.51	73.86	49.82	6.47	10.79
S. hamata	29.32	10.10	62.41	43.82	8.56	5.88
C. ciliaris	37.63	6.26	77.60	48.20	9.25	10.79
H. binata	38.29	9.40	62.21	44.20	10.38	9.12
Post growing						
C. fulvus	52.56	4.02	80.35	48.33	6.60	10.22
P. maximum	37.71	4.89	75.95	48.55	7.93	10.02
S. hamata	58.74	6.30	65.36	51.43	10.75	4.88
C. ciliaris	51.77	4.35	78.29	55.82	8.59	7.99
H. binata	46.43	7.67	65.61	50.37	12.94	8.09

study also fall within this range and were in agreement with earlier report of Sharma *et al.* (1998) and Chaturvedi and Sahoo(2013). In the present experiment, total DM intake or DM intake as percentage of body weight was comparable in both kids and lambs and intake of pasture dry matter decreased by 19.93% in kids and21.48% in lambs from growing to post-growing season. Similarly, Sankhyan *et al.* (1999) also observed that the DMI of sheep decreased from monsoon (2.1%BW) to winter(1.8% BW). Pasture intake depends upon digestibility of pasture, rumen fill, metabolic factors, chemical and physical properties of concentrate and stage of growth (Das and Singh, 1999).Sharma *et al.* (1998),however, found no seasonal difference in feed consumption by goats grazing in semi-arid pasture. In the present study, more biomass intake by both sheep and goats during growing season than post growing season might be due to differences in palatability and lower nutritive value of pasture biomass. The digestibility of DM, OM and NDF were comparable in

Table 2. Nutrient utilization and growth performance of *Jalauni* lambs and *Bundelkhandi* kids grazed on *H. binata* based silvopasture system

Particulars	Season/Species				
	Growing		Post growing		
	Kids	Lambs	Kids	Lambs	SE
DMI%, BW	3.84^{b}	4.05^{b}	3.39^{a}	3.50^{a}	0.08
Pasture intake,% BW	2.90^{b}	3.15^{b}	2.34^{a}	2.45^{a}	0.09
Pasture intake,% DM	75.63	77.76	68.85	69.88	1.07
DMI (g/$W^{0.75}$ kg)	85.96^{b}	91.54^{b}	77.77^{a}	80.39^{a}	1.70
DCP intake (g/d)	64.56^{b}	65.35^{b}	35.80^{a}	38.10^{a}	3.12
DCPI (g/$W^{0.75}$ kg)	5.76^{b}	5.64^{b}	2.98^{a}	3.14^{a}	0.29
DOMI (kg/d)	0.63^{b}	0.68^{b}	0.53^{a}	0.54^{a}	19.93
DOMI (g)/kg $W^{0.75}$	56.64^{b}	59.01^{b}	43.39^{a}	44.05^{a}	1.67
N intake(g/kg DOMI)	27.92	27.48	31.09	30.50	0.50
ME (MJ/d)	9.97^{b}	10.31^{b}	8.31^{a}	8.55^{a}	0.17
ME (kJ/$W^{0.75}$)	890^{b}	893^{b}	687^{a}	696^{a}	23.92
Apparent digestibility (%)					
DM	68.55^{b}	66.44^{b}	58.67^{a}	56.84^{a}	1.18
OM	71.67^{b}	69.85^{b}	62.05^{a}	61.10^{a}	1.09
CP	58.30^{b}	55.83^{b}	35.49^{a}	37.84^{a}	2.31
NDF	69.15^{b}	67.00^{b}	58.75^{a}	55.60^{a}	1.30
ADF	63.43^{b}	61.78^{b}	47.36^{a}	46.87^{a}	1.75
EE	73.94	72.20	71.24	69.55	0.78
NFE	76.03^{bq}	71.91^{bp}	67.42^{a}	64.24^{a}	1.12
Nutritive value (%)					
DCP	6.70^{b}	6.16^{b}	3.87^{a}	3.96^{a}	0.29
TDN	68.31^{b}	64.48^{b}	58.44^{a}	57.19^{a}	1.13
ME(MJ/kg)	10.36^{b}	9.78^{b}	8.86^{a}	8.67^{a}	0.17
Daily gain (g/d)	55.74^{b}	52.38^{b}	20.56^{a}	21.67^{a}	2.98

^{a,b}Means bearing different superscripts in each species class indicate difference (P<0.05)due to season; ^{p,q}Means bearing different superscripts in each species class indicate difference (P<0.05) due to species

both the species as reported earlier by Salim *et al.* (2003) and Ranilla *et al.* (2005). However, significant($P<0.05$) seasonal variation in digestibility was observed in both the species which might be related to changes in the chemical composition particularly in fiber, lignin and silica contents and corroborated with the findings of Sun *et al.* (2014) where it was reported that the nutrient digestibility of forages in goats under grazing were higher during rainy season and decreased significantly during the winter ($P<0.05$).Digestibility of crude protein of ingested forage was higher ($P<0.05$) in both the species during growing season than non-growing season and corroborated well with earlier findings of Shinde *et al.* (1998) and Sun *et al.* (2014). DCP intake (g/kg$W^{0.75}$) by both lamb and kids was significantly ($P<0.05$) higher (5.76 vs 5.64) in September as compared to December (2.98 vs 3.14), which might be due to decreased in protein content of available pasture biomass during post growing season. Chaturvedi and Sahoo (2013) however, observed much higher DCP intake in sheep from similar type of ration which might be due to superior quality of supplemented concentrate and roughage fed to the experimental animals. Efficient microbial growth in the rumen requires a balanced supply of nitrogen (amino acids and ammonia) and energy (Das and Singh, 1999). Present study indicated that the N intake per kg DOM intake in kids and lambs during September and December was sufficient for efficient rumen fermentation and did not show any significant difference but availability of energy in terms of ME (KJ/ $W^{0.75}$) decreased significantly ($P<0.05$) during post growing season. Nutritive value in terms of DCP and TDN were comparable in both the species and significantly ($P<0.05$) lower during post growing season as compared to growing season. Similar DCP and ME contents were recorded by Salim *et al.* (2003) in small ruminants under grazing with concentrate supplementation. The quality and quantity of tropical herbage is known to decline markedly after rainy season during onset of winter and causes major constraint for small ruminant production as it was observed in the present study.Daily live weight gain was comparable for both the species, however, both the species showed significant difference in daily gain between growing and non-growing season. Salim *et al.* (2003) also recorded no differences between sheep and goats for live weight gain under grazing with concentrate supplementation. Daily gain (g/d) in both the species in present experiment corroborated with the earlier findings of Dutta *et al.* (2003). Similarly, Mekuriaw and Asmare(2018) also observed a daily gain of 53-5 g/d in Washera lambs fed natural pasture hay supplemented with concentrate and tree leaves. However, Chaudhary *et al.* (2015) reported much higher body weight gain in Sirohi kids which might be due to better quality of pasture and higher rate of supplemental concentrate mixture.

CONCLUSION

It was concluded that nutrient utilization and growth performance were comparable between lambs and kids, however, nutrient intake and utilization in both the species reduced significantly during non-growing season due to deterioration of pasture quality which is reflected from the lowered body weight gain in both the species. Present findings also indicated that *H. binata* based silvipastures under semiarid situation may be utilized for rearing small ruminants for sustainable production.

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Effect of Different Additives on Bale Silage Quality of Maize Fodder

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ABSTRACT

The present study was conductedto evaluate the effectof different additives on the bale silage quality of maize fodder. An experiment was conducted with four different treatments of maize fodder before bailing them. These treatments consisted of; control without additive (MF_0), *Lactobacillus casei* at 10^6 CFU/g (NCDC-17) of fresh fodder (MF_{LAB}), Silotan, a commercial product at 15ml/100 kg of fresh maize fodder (MF_{ST}) and formaldehyde at 0.5% of fresh maize fodder (MF_{FA}) as silage additives. The bale silage of four such treatments MF_0, MF_{LAB}, MF_{ST} and MF_{FA},in replications (3*9 treatments) was prepared by using a high power 100 kg bailing machine. Each treatment was evaluated after 60 days of ensiling in terms of quality parameters like chemical composition, fermentation characteristics, microbial (LAB) counts and *in vitro* DM degradability. The evaluation of bale silage revealed a significant increase (P<0.001) in pH and decrease (P<0.001) in ammonia nitrogen in MF_{FA}. There was no significant effect on DM, CP, EE and ADF contents of any treatment, however a significant increase (P<0.001) in WSC was observed in MF_{FA}, with a decrease (P<0.001) in WSC of MF_{LAB}. The TVFAs were significantly increased (P<0.001) in MF_{LAB} and MF_{ST}, with a decrease (P<0.001) in MF_{FA}. There was a significant decrease (P<0.001) in acetate content of MF_{FA} while a significant reduction (P<0.001) in butyrate was observed in MF_{ST} and MF_{FA}. There was a significant decrease (P<0.001) in Lactic acid content of MF_{FA}. The *in vitro* DM degradability was significantly increased (P<0.05) in MF_{LAB}. There was no significant effect of treatments on methane (ml/g DM) production in any of the treatments. The microbial counts were found significantly increased (P<0.05) in MF_{LAB}, whilst a decrease (P<0.05) of microbial count was noted in MF_{FA}, Yeast counts were significantly decreased (P<0.05) in MF_{ST} with increase in MF_{FA}. It was concluded that Silotan or MF_{LAB} can be used to improve the quality of bale silage.

Key words: Bale Silage, Formaldehyde, *Lactobacillus casei,* Silotan

INTRODUCTION

The principle of silage fermentation is to achieve a sufficient quantity of lactic acid to inhibit the growth of undesirable epiphytic microorganisms and the activity of endogenous plant catabolic enzymes, thus maximizing nutrient preservation. In previous times, pit/silo methods of making silage were preferred as the basic conditions required for the effective preservation of silage (*i.e.* anaerobic storage, high density and low pH) were difficult to achieve in big bale silage, because the herbage was not properly chopped due to which it was difficult to achieve proper density in the bales. However with the advancement in bailing machines, high power bailing machines are available now to chaff the fodder and consequently densify/ensile it compactly to make good quality silages. Silage additives may be chemical or biological, and can be categorized as stimulants, inhibitors, nutrients or absorbents (McDonald *et al.,* 1991). Information regarding the effects of the additives on the quality of these bales is limited. However, it is known that silage quality can be improved by adding LAB, molasses, formic acid and other organic acids, or mixtures of organic acids and salt to the bales. Limited information is available about use of "Silotan" as a silage additive. Our objective was to study the effect of three different additives viz *Lactobacillus casei*, formaldehyde and Silotan (a commercial product containing Chest nut extract) on the quality of the bale silage.

MATERIALS AND METHODS

Treatments consisted of different additives as MF_0 as control group (without the use of any additive), MF_{LAB} with addition of *Lactobacillus casei* at 10^6 CFU/g of fresh fodder, MF_{ST} with the use of Silotan, a commercial product made from chest nut extract at 15 ml/100 kg of fresh maize fodder and MF_{FA} with the

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addition of formaldehyde at 0.5% of fresh maize fodder. The bale silage was prepared by using a 100 kg bailing machine. Duration of ensiling was 60 d. The silage was then evaluated for silage quality, namely chemical composition, and microbial quality and *in vitro* digestibility and methane production.

The silage sample was analyzed for colour, texture, and smell. The texture was observed by pressing the silage between two fingers. Colour was observed visually. Mildly ammonical, pleasantly acidic and natural yogurt smell was preferred (Breirem and Ulvesli, 1960). Silage samples were oven dried at 60°C for 72 h. The dried samples were ground to pass through a 1 mm screen for subsequent analyses of neutral detergent fibre (NDF), acid detergent fibre (ADF), water soluble carbohydrates (WSC), crude protein (CP), and *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility IVOMD. NDF and ADF were determined according to the method of Van Soest *et al.* (1991). WSC was determined by the method of Yemm and Willis (1954). CP was determined according to the method of Association of Official Analytical Chemists (AOAC, 2005). Ash was estimated by combustion of the samples in a muffle furnace at 550°C for 5 h. The IVDMD, IVOMD and *in vitro* gas production was measured following methods of Menke and Steingass (1988). Approximately 200 mg of sample was weighed, placed into a graduated glass syringe provided with a piston, and ûlled with 30 ml of buffered rumen ûuid. Each of the incubation was run in triplicate. The glass syringes containing samples, rumen ûuid and buffer mixtures were incubated in a water bath at 39°C and the gas production was subsequently measured before incubation (0 h) and at 8, 24, and 48 h after incubation. Total gas values were corrected for blank. After the incubation of feeds, suitable aliquot of gas was withdrawn from the tip of the syringe using Hamilton gas tight syringe and was analyzed methane, using Gas chromatograph (Nucon 5700, India). For the measurements of pH, ammonia nitrogen, total volatile fatty acid (TVFA), water extract of silage was prepared by adding 90 ml of distilled water to 10 g of fresh samples in a beaker and homogenized by mechanical homogenizer. A few drops of 0.1% mercuric chloride were added in the sample, stirred thoroughly and kept in refrigerator at 4ºC. After 48 hours, the material was filtered through four layers of cheese cloth and stored in refrigerator at 4ºC. The pH was measured by using a Eutech pH meter (Oakton Instruments, IL USA) and the buffering capacity of the sample was measured as per the method of Playne and McDonald (1996). For determining individual VFA, 5 ml of water extract was taken in a beaker and 1 ml of 25 % metaphosphoric acid (prepared in 5N H_2SO_4) was added. Samples were kept overnight and centrifuged at 3500-4000 rpm for 15-20 minutes. The supernatant was injected in gas chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless steel column packed with Chromosorb-101 to serve as a stationary phase. Analytical conditions for fractionation of VFA were as follows: Injection port temperature, 250ºC; column temperature, 190ºC and detector temperature, 260ºC. The flow rate of carrier gas (nitrogen) was 40 ml/min; hydrogen 30 ml/min; air 300 ml/min. Injection volume was 3 µl. The injection was performed by means of 10 µl Hamilton syringe (Hamilton, Nevada, USA). Different VFA's of the samples were identified on the basis of their retention time and their concentration (mM) was calculated by comparing the retention time as well as the peak area of standards after deducting the corresponding blank values. The nitrogen (N) content was measured using the Kjeldahl method (AOAC, 1990). The CP was calculated as N*6.25. Lactic acid (LA) concentration was determined following Barker and Summerson (1941) method. Estimation of lactic acid bacteria and yeast and moulds was done by plate count method after using Rogosa agar medium (selective medium for *Lactobacillus*) and potato dextrose agar (selective medium for yeast and molds).

The effects of the treatment on the quality characteristics of silage were evaluated by analyses of

variance (ANOVA). If the ANOVA was significant, the means of variables were further compared using the procedure of Tukey-Kramer comparison. All these analyses were performed by SAS program (SAS Inst. Inc. Cary, NC, 2001).

RESULTS AND DISCUSSION

The chemical composition and pH, buffering capacity, TSC, and microbial counts of maize fodder have been presented in Table 1. After opening of the silage a preliminary sensory evaluation was performed by an expert. The silage was evaluated for colour, smell, visible fungal growth and acidic tastes. The results are presented in Table 2. The fermented aroma and acidic taste of the treatments were related to the amount of fermented acids generated. None of the treatments had visible fungal growth and/or sliminess. Absence of fungal growth and sliminess in all the treatment were because of preservative effect of fermented acids produced during ensiling or very low pH of the ensiled products, which prevented the growth of fungi and moulds.

Table 3 is showing the DM content, moisture, weight loss and ammonia nitrogen of bale silage. There was no difference among different group in comparison to control group for all these parameters *i.e.* DM, moisture and weight loss. The DM (%) content ranged from 28.16 to 28.89 % (MF_{FA} Vs MF_0). A significant weight loss per 100 kg bale was observed ($P<0.05$) with the treatment of *Lactobacillus* culture. It seems that silage made by bales wrapping in plastic densely compact the fodder leaving no air pocket behind for any DM losses. Nishino *et al.* (2007) observed that the DM of the maize silage was 28.4 in comparison to the control group *i.e.* 28.1, when treated with *Lactobacillus casei*. The pH in treatment groups was quite good at 3.85 in MF_{LAB}, 3.87 in MF_{ST} and as compared to 3.91 in control MF_0. It indicates that supplementation of both *Lactobacillus casei* and silotan are quite effective to bring down the pH of bale silage. Herremans *et al.* (2019) observed the addition of oak and chestnut tannin extract at different levels did not influence pH values of silages but reduced the ammonia nitrogen levels. However, a significant increase ($P<0.001$) in pH was observed with in MF_{FA}. The ammonia- N was within the range of permissible limits

Table 1. Chemical composition of maize fodder before ensiling

Parameter	Values
DM (%)	30.82±1.07
Ash (%DM)	7.33±0.10
CP (%DM)	9.09±0.002
EE (%DM)	3.62±0.001
ADF (%DM)	33.95±0.28
NDF (%DM)	56.99±0.006
TSC g/100g DM	13.9±0.6
BC meq. /100g FM	27.00±0.01
LAB (log_{10}cfu/g)	5.66±0.04
Yeast and mold (log_{10}cfu/g)	5.02±0.08
pH	6.65±0.029

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; TSC, total soluble carbohydrates; BC, buffering capacity; FM, fresh matter

Table 2. Sensory evaluation of the silage

Treatment	Treatments[#]			
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}
Colour	Dark Brown	Dark Brown	Dark Brown	Dark Brown
Smell	Ammonical	Ammonical	Ammonical	Mild Ammonical
Acidic	Highly Acidic	Highly Acidic	Mild Acidic	Mild Acidic
Visible	No moulds	No moulds	No moulds	No moulds
Sliminess	Little slimy	Little slimy	Slimy	Little slimy

[#]Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively.

Table 3. Effect of Different Additives on DM, weight Loss, pH, ammonia nitrogen and buffering capacity of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
Dry matter (%)	28.89[a]±1.46	28.36[a]±0.79	28.73[a]±1.11	28.16[a]±1.04	0.965
Weight loss (kg/bale)	3.24[ab]±0.69	4.87[b]±0.66	2.33[a]±0.08	1.70[a]±0.53	< 0.05
pH	3.91[a]±0.05	3.85[a]±0.01	3.87[a]±0.03	4.44[b]±0.05	<0.001
NH_3-N (g/100 g DM)	0.22[b]±0.02	0.26[b]±0.02	0.24[b]±0.02	0.17[a]±0.01	<0.05
Total (g/100 g DM)	1.42[a]±0.01	1.43[a]±0.02	1.39[a]±0.01	1.40[a]±0.01	0.221
BC (mEq NaOH/100 g)	81.26[b]±3.88	75.77[b]±2.62	72.22[b]±3.73	40.89[a]±2.50	<0.001

BC, buffering capacity; ≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; [a, b] Values bearing different alphabet in a row differ significantly (P<0.05)

at 0.26 in MF_0, 0.27 in MF_{LAB} and 0.24 in MF_{ST}, however a significant decrease (P<0.05) in ammonia nitrogen levels was observed in MF_{FA} (0.17). Kaiser *et al.*(1981) observed that the addition of formaldehyde increased the pH and decreased the ammonia nitrogen levels of the maize silage. Supplementation of lactobacilli culture and Silotan were not able to alter buffering capacity of the bale silage. However, supplementation of formaldehyde significantly reduced (P<0.001) the buffering capacity of bale silage.

Data pertaining to chemical composition of bale silage is presented in Table 4.The crude protein content of MF_0 (8.08), MF_{LAB} (8.94), MF_{ST} (8.80) and MF_{FA} (8.94) were not statistically significantly different. The treatment of *Lactobacillus casei* significantly (P<0.001) decreased the EE and WSC, with no effect on CP, NDF and ADF content of silage. The treatment of Silotan significantly (P<0.05) reduced NDF content, with no significant effect on the protein, organic matter, EE, ADF and TSC content of the silage. The treatment of formaldehyde significantly (P<0.001) increased the ash, NDF, WSC, hemicellulose, with no prominent effect on CP, EE, and ADF content of silage.

Data pertaining to VFA content of bale silage are presented on Table 5. Treatment of *Lactobacillus casei* significantly (P<0.001) increased TVFAs, with no significant effect on acetic acid, lactic and butyric acid content of silage. Nishino *et al.* (2005) found similar

Table 4. Effect of different additives on chemical composition of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
Dry matter	28.89[a]±1.46	28.36[a]±0.79	28.73[a]±1.11	28.16[a]±1.04	0.965
On % dry matter basis					
Crude protein	8.08[a]±0.08	8.94[a]±0.05	8.80[a]±0.73	8.94[a]±0.96	0.217
Ether extract	3.19[a]±0.22	2.52[a]±0.22	2.51[a]±0.42	3.04[a]±0.29	0.261
Ash	5.92[a]±0.07	5.92[a]±0.15	6.02[a]±0.21	6.66[a]±0.09	0.012
Organic matter	93.34[a]±0.21	94.08[a]±0.15	93.68[a]±0.07	93.94[a]±0.09	0.033
NDF %	24.97[a]±0.96	25.15[a]±0.63	22.33[a]±0.62	26.43[a]±0.36	0.433
ADF %	52.67[b]±0.30	53.14[b]±0.74	47.06[a]±1.30	53.76[b]±1.66	<0.05
TSC	4.75[b]±0.17	3.30[a]±0.11	4.64[b]±0.11	9.46[c]±0.40	<0.001

≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; NDF, neutral detergent fibre; ADF, acid detergent fibre; TSC, total soluble carbohydrates; [a, b] Values bearing different alphabet in a row differ significantly (P<0.05)

Table 5. Effect of different additives on volatile fatty and lactate content of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
Total volatile fatty acids	30.98^{b}±3.09	38.07^{c}±2.20	34.51^{c}±1.83	14.90^{a}±0.89	<0.001
Acetate	23.83^{b}±2.38	28.31^{b}±1.57	24.86^{b}±1.46	12.07^{a}±0.67	<0.001
Propionate	8.68^{c}±0.78	6.17^{b}±0.65	6.02^{b}±0.61	1.28^{a}±0.17	<0.05
Butyric acid	0.49^{a}±0.06	0.56^{a}±0.04	0.26^{b}±0.20	0.49^{a}±0.60	<0.001
Lactate	6.48^{b}±0.02	6.59^{b}±0.06	6.76^{b}±0.02	3.69^{a}±0.15	<0.001

≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; ^{a,b,c} Values bearing different alphabet in a row differ significantly (P<0.05)

results with *Lactobacillus casei* on both direct cut and wilted grass silages. Treatment of Silotan significantly (P<0.001) increased TVFAs, with no significant effect on acetic acid and lactic acid content of silage. Treatment of formaldehyde significantly (P<0.001) decreased TVFAs, particularly acetic acid and propionic acid, with a significant (P<0.001) reduction in lactic acid content of silage.

Table 6 is showing the data on *in vitro* digestibility of bale silage. The data revealed that the *in vitro* DMD ranged from 67.45 to 69.68 with no significant difference in the treatment of Silotan and formaldehyde, while a significant (P<0.05) increase was observed in *in vitro* DMD with the treatment of *Lactobacillus casei* (MF_{LAB}). There was no significant difference in their methane production per

Table 6. Effect of different additives on *in-vitro* digestibility and gas production of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
IVDMD (%)	68.96^{a}±0.87	69.85^{b}±0.95	67.89^{a}±0.80	67.45^{a}±1.20	<0.05
IVOMD (%)	72.39^{b}±0.57	71.23^{ab}±1.96	68.70^{ab}±1.52	67.50^{a}±0.52	<0.05
ME (MJ/kg DM)	6.46^{b}±0.09	5.89^{ab}±0.05	5.67^{a}±0.24	6.38^{b}±0.16	<0.05
CH_4(% total gas)	36.11^{b}±1.80	30.36^{a}±0.58	27.92^{a}±0.12	27.30^{a}±1.01	<0.05

IVDMD, *in vitro* dry matter digestibility; IVOMD, *in vitro* organic matter digestibility; ME, metabolizable energy; ≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; $^{a, b}$ Values bearing different alphabet in a row differ significantly (P<0.05)

Table 7. Effect of different additiveson microbial counts of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
On the day of opening					
Microbial Counts	8.41^{b}±0.01	8.48^{c}±0.02	8.45^{bc}±0.01	7.91^{a}±0.02	<0.05
Yeast and Moulds	4.04^{b}±0.09	4.07^{bc}±0.02	4.02^{a}±0.04	4.15^{c}±0.03	<0.05
pH	3.91^{a}±0.05	3.85^{a}±0.01	3.87^{a}±0.03	4.44^{b}±0.05	<0.001
After three days of exposure					
Yeast moulds	5.52^{a}±0.05	5.31^{a}±0.14	6.32^{b}±0.01	6.40^{b}±0.06	<0.001
pH	4.66^{a}±0.17	5.53^{b}±0.15	4.55^{a}±0.06	5.71^{b}±0.18	<0.001

≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; $^{a, b, c}$ Values bearing different alphabet in a row differ significantly (P<0.05)

gram DM and ME values. The percent methane gas of total gas production was reduced significantly (P<0.05) in MF_{LAB}, MF_{ST} and MF_{FA}.

Table 7 is showing the data on microbial quality of the silage at the time of opening silage and three days after exposure to the air. There was a significant (P<0.001) reduction in the microbial counts in MF_{ST} (7.91) in comparison to control group MF_0 (8.41). Supplementation of *Lactobacillus casei* and Silotan significantly (P<0.001) increased the microbial counts to 8.48 in MF_{LAB} and 8.45 in MF_{ST}. Further, a significant (P<0.001) increase in the yeast and mould counts in MF_{FA} (4.15) was observed,while no effect was observed in MF_{LAB} and a significant (P<0.001) decrease in the yeast and mould counts was observed in MF_{ST} (4.02). There was a significant (P<0.001) increase in yeast and moulds counts after 3 days exposure to the air in MF_{ST} (6.32) and MF_{FA} (6.40) treatments in comparison to MF_0 (5.52) group, however no significant difference was observed in MF_{LAB} (5.31) treatment. A significant (P<0.001) increase in pH after 3 days exposure to the air was observed in MF_{FA} (5.71) and MF_{LAB} (5.53) with no significant difference in MF_{ST} (4.55) treatment.

CONCLUSION

Silotan or MF_{LAB} supplementation improved the bale silage quality of maize fodder in terms of acetic acid, lactic acid and microbial quality, and further reduced the production of butyric acid. Further it also reduced the growth of yeast in the silage.

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Effect of Micro-nutrients Supplementation and Seasonal Variability on Production Performance, Antioxidants and Immune Status in Crossbred Heifers

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ABSTRACT

Twenty four Karan Fries crossbred heifers were allocated into 4 groups of 6 animals each to evaluate the effects of micronutrient supplementation on animal performance during summer and winter seasons.The animals were fed concentrate mixture, maize fodder and wheat straw to meet nutrient requirements (ICAR, 2013). In group G_1, the mineral mixture added in concentrate mixture was devoid of iodine while in groups G_2 and G_3, iodine was supplemented at 0.25 and 0.5 ppm of diet. In group G_4, a micro-nutrient mixture was supplemented that contained chromium, niacin, vitamin E and Zn at 1.5, 600, 40 and 40 ppm of dietary DM, respectively. Daily feed intake, body weight gain, feed conversion ratio were similar in different groups irrespective of seasons.Activities of glutathione peroxidase, super oxide dismutase and catalase did not vary due to treatments and seasons. However, overall ferric reducing ability of plasma values was reduced ($P<0.05$) in group G_4 in both seasons and the values were lower ($P<0.05$) in winter than summer. The thiobarbuturic reactive substances values were also comparatively higher ($P<0.01$) in summer. Plasma total immunoglobulins concentration was higher ($P<0.05$) in winter irrespective of groups. Hence, micronutrient supplementation could help to mitigate oxidative stress in crossbred heifers during summer.

Key words: Antioxidant status, Crossbred heifers,Immunity, Performance, Seasonal variation

INTRODUCTION

In tropical countries, environmental temperature varies greatly during winter and summer seasons. Increased ambient temperature, humidity and radiation may lead to increased heat gain in comparison to heat loss from the body and cause thermal stress in animals (Cooke *et al.,* 2020). Heat stress results in reduced gut motility, ruminal contractions, saliva secretion and depressed appetite (Zha *et al.,* 2009) and also induces oxidative stress which results in altered physiological, antioxidant enzyme activity and immunity in cattle (Pradhan *et al.,* 2015; Das *et al.,* 2016). Therefore, maintaining better performance during period of seasonal stress induced immunosuppression is critically important. Nutritional management is one of the key factors in reducing the effects of thermal stress wherein certain micro-nutrients play crucial roles. Supplementation of micro-nutrients improved growth, physiological responses and immune functions in animals under heat stress (Keshri *et al.,* 2019). Oxidative stress can be ameliorated by antioxidants (Higdon and Frei, 2003). Anti- oxidative functions could be either enzymatic like catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) or non- enzymatic (vitamins C, A, E and minerals like Cu, Zn, chromium *etc.*). Changes in metabolic activity of animals during seasonal variation in ambient temperature and air humidity are related to thyroid activity. Supplementation of iodine during high environmental temperature reduced feed intake and decreased the synthesis of thyroid hormones in cattle, sheep and goats (ARC, 1980). Pattanaik *et al.* (2001) reported that iodine supplementation at 0.05 and 0.075 mg/animal/d had no significant impact on utilization of

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nutrients, and seasonal variations affected thyroid gland activity which is associated with air humidity and ambient temperature. Provision of high levels of iodine (0, 2.5, 5, 7.5 mg/kg) in the diet of Holstein cows decreased DM intake but no significant differences were observed between the groups (Norouzian *et al.,* 2009). The information on the effects of supplementation of combination of micronutrients on antioxidant status in different seasons is scanty. Therefore, the present investigation was undertaken to study the effect of micronutrient mixture (Zn, Cr, vitamin E and niacin) and iodine supplementation on growth performance, antioxidant enzyme activity and immunity status in crossbred heifers during summer and winter seasons.

MATERIALS AND METHODS

The study was conducted at Livestock Research Centre (LRC) at ICAR-NDRI, Karnal, Haryana (India) which is located at an altitude of 250 m above msl. The maximum and minimum ambient temperature in this area goes up to 48°C and 4°C, respectively with a diurnal variation to the order of 15-20°C. The average annual rainfall is 700 mm, most of which is received from early July to mid September. This study was conducted under the recognized standards of the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the Committee for Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) rules (Government of India).

Twenty four female KF calves were selected from LRC of ICAR-NDRI, Karnal. Based on their body weight and age they were distributed into 4 groups of 6 animals in each group as per randomised block design (RBD). All calves were fed experiment diet in form of total mixed ration (TMR) comprising of concentrate mixture, maize (green fodder) and wheat straw mixed in the ratio of 40:40:20 (on DM basis) to meet the requirements (ICAR, 2013). The concentrate mixture (maize 35, ground nut cake 15, soybean meal 15, wheat bran 32, mineral mixture 2 and common salt 1%) of group G_1 consisted of mineral mixture which was devoid of iodine. The animals in groups G_2 and G_3 were supplemented with iodine at a level of 0.25 and 0.5 ppm of dietary DM while in group G_4, a micronutrient mixture was provided supplying Zn, Cr, vitamin E and niacin at 40, 1.5, 40 and 600 ppm of dietary DM, respectively.

The dry bulb and wet bulb temperatures were recorded daily at 07:30 h and 14:30 h using Zeal dry bulb wet bulb thermometer and THI was calculated using formula (NRC, 1971) as given below:

$$THI = 0.72 (Tdb+Twb) + 40.6$$

Tdb= Dry bulb temperature

Twb= Wet bulb temperature

During 150 days of feeding trial, DM intake (DMI) and body weight of the animals were recorded at fortnightly intervals. Feed offered, residue leftover and DMI of the animal were recorded daily. Recording of body weights was done in morning hours before feed and water intake. Blood samples were collected at monthly intervals from the animals by jugular vein puncture in heparinised vacutainer and tubes were rotated between the palms to ensure proper mixing of blood and anticoagulants and were brought to the laboratory after placing over ice. Thereafter in order to separate plasma, samples were centrifuged for 20 min. at 3000 rpm. The plasma samples were stored at -20°C for estimation of catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD), ferric reducing ability of plasma (FRAP), thiobarbituric acid reducing substances (TBARS) and total immunoglobulins.

Dry matter, organic matter (OM), crude protein (CP), ether extract (EE) and total ash were determined as per AOAC, 2005 and neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as per Van Soest *et al.* (1991). Urine samples were analyzed for nitrogen content (AOAC, 2005). Concentration of zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and chromium (Cr) in feed samples were determined in an air-acetylene flame on an atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) after digestion in tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4"3:2:1 ratio). Sulphur was estimated in feed sample by turbidimetric method

(Massoumi and Cornfield, 1963).

For haemolysate preparation, 2 ml of whole blood was collected in vials containing acid citrate dextrose (300 µl/2 ml blood) as anticoagulant and centrifuged for 15 min at 4°C at 2000 rpm to separate buffy coat and plasma. The erythrocyte pellet (packed RBC) was washed with phosphate buffer saline (PBS) solution (NaCl, 137 mM; KCl, 2.7 mM; Na_2HPO_4, 10 mM and KH_2PO_4, 1.8 mM), pH was adjusted to 7.4. The packed RBC was mixed with an equal volume of PBS to form RBC suspension. Haemolysate (1:20 dilution) was prepared by mixing 0.5 ml RBC suspension with 4.5 ml of stabilizing solution (EDTA, 2.7 mM and 0.7 mM, 2-mercaptoethanol).The levels of SOD, catalase, GPx, FRAP and TBARS in blood plasma was estimatedusing the procedures of Madesh and Balasubramanian (1998), Aebi (1984), Pagliaand andValentine (1967), Benzie and Strain (1999) and Mc Ewan and Fisher (1970), respectively.

The data were analyzed by two-way ANOVA and difference between the means were compared by Tuckey's-B multiple range test as per Snedecor and Cochran (1994) using software package SPSS version 20.0 (2012).

RESULTS AND DISCUSSION

The chemical composition of feed ingredients (proximate and cell wall constituents) *viz.* concentrate mixture, maize fodder and wheat straw are presented in Table 1. There were significant (P<0.05) seasonal variations in terms of THI. The THI ranged from 61.42±0.6 to 85.60±0.48 and (Fig. 1). The THI was lower in winter as compared to summer (March to May). The THI values were higher during 6thto 10th fortnight. When THI exceeded 76, the animals showed heat stress (Srikandakumar *et al.,* 2003).

Supplementing iodine (G_2 0.25, G_3 0.5 mg kg/DM) and micronutrient mixture (G_4, Zn, Cr, vitamin E and niacin at 40, 1.5, 40 and 600 ppm, respectively) did not affect feed intake, daily gain and feed conversion ratio irrespective of seasonal variation in THI (Table 2). However, an improvement was observed in live weight gain of goats on iodine supplementation at 0.05 and 0.075 mg/animal/d (Pattanaik *et al.,* 2001). Supplementation of inorganic source of Zn at 20 (Wright and Spears, 2004), 100 and 200 ppm (Malcolm-Callis *et al.,* 2000) or Zn as propionate at 35 ppm (Mandal *et al.,* 2008) did not affect body weight gain in calves. Jadhav *et al.* (2008) supplemented Zn at 35 and 70 ppm to the basal diet containing about 35 ppm Zn in male Murrah buffalo calves and found that ADG was higher (P<0.05) in 70 ppm Zn fed group (601.4 g) as compared control (547.4 g) and 35 ppm Zn (566.7 g) supplemented group. Zinc supplementation at 60 ppm in either organic or inorganic form to the basal diet containing 40 ppm Zn to Holstein Friesian calves for 90 d after birth did not cause any significant difference in their growth performance (Array *et al.,* 2002). Supplementary Zn at 35 and 70 ppm through $ZnSO_4$ to the basal diet containing 32.5 ppm Zn did not affect nutrient intake and body weight gain in crossbred calves (Mandal *et al.,* 2008). Wright and Spears (2004) showed that weight gain, feed intake and feed efficiency of calves were not markedly affected by addition of $ZnSO_4$ in Holstein male calves.

Feed intake improved in lactating buffaloes supplemented with inorganic Cr at 1.5 ppm in the ration (Deka *et al.,* 2015). Halder *et al.* (2009) reported that intake of DM, OM and CP was not affected by the level of supplemental Cr but digestibility of these nutrients tended to increase linearly with the level of Cr in the diet. N retention increased proportionately with the level of supplemental Cr. When rumen protected niacin was supplemented at 12 g/d to lactating dairy

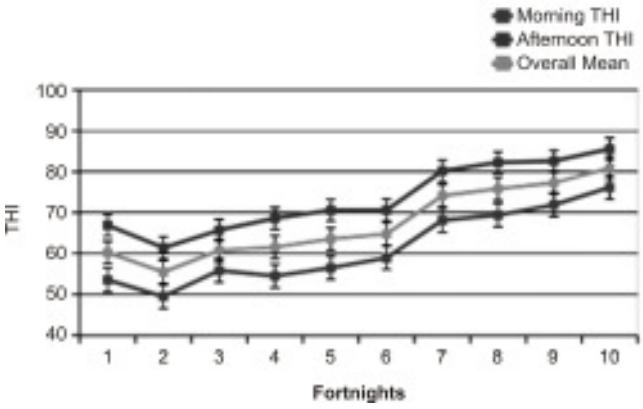

Fig. 1. THI values during different fortnights

Table 1. Chemical composition (% DM basis) of feed ingredients

Parameter	Concentrate mixture	Maize fodder	Wheat straw
Dry matter	90.99	13.98	92.26
Organic matter	92.74	89.38	88.44
Crude protein	20.34	10.75	2.27
Total ash	8.26	10.62	11.56
Ether extract	3.99	2.66	0.71
Neutral detergent fibre	33.96	47.55	72.97
Acid detergent fibre	13.81	36.21	54.62
Total digestible nutrients*	68.38	62.66	45.34
Mineral composition			
Zn (ppm)	49.56	30.20	6.08
Cu (ppm)	26.12	16.66	2.16
Mn (ppm)	56.42	32.85	2.38
Cr (ppm)	0.84	1.76	0.37
Fe (ppm)	402.5	380.83	208.41
Iodine (ppm)	0.32	0.25	0.19
S (%)	0.29	0.21	0.15

(* = calculated as per NRC, 2001)

cows during heat stress there was no significant effect on DM intake (Zimbelman *et al.,* 2013). There was no any significant effect of supplementing 185 IU of vitamin E/kg of DM in comparison to diet supplemented with 15 IU/kg DM on calves' DMI, average daily weight gain and feed conversion efficiency (Cusack *et al.,* 2005).Samanta *et al.* (2006) also did not observe any significant effect on weight. gain in calves supplemented with 125 IU of vitamin E/day when compared with control group. Likewise, supplementation of vitamin E at 40 and 20 mg/kg DM had no positive effect on growth (Dass *et al.,* 2009).

The activity of SOD, GPx and catalase was found to be similar among groups and also there were no significant differences between seasons (Table 3). It was reported that SOD activity increased ($P<0.05$) under heat stress conditions (Manish *et al.,* 2011). Under the heat stress conditions, cellular oxidative damage and lipid peroxidation increase due to reactive oxygen species generation. Supplementation of Zinc may have many nutritional benefits for livestock but has an inhibitory effect on the spontaneous lipid peroxidation. Zinc is present as cofactor for SOD and reduces superoxide radicals to H_2O_2 through cascading enzyme

Table 2. Effect of micronutrients supplementation on dry matter intake and feed conversion ratio in Karan Fries heifers

Variable	Group			
	G_1	G_2	G_3	G_4
Initial body weight (kg)	116.18±16.35	117.65±11.81	115.15±13.60	116.61±14.04
Final body weight (kg)	197.87±19.09	201.06±14.68	197.86±16.39	199.66±13.12
Feed intake (kg/d)	4.49±0.23	4.56±0.23	4.70±0.27	4.59±0.24
Average daily gain (g)	543.55±9.98	542.99±10.81	551.37±10.47	549.72±24.79
Feed conversion ratio (kg feed consumed/kg gain)	8.23±0.35	8.21±0.37	8.50±0.44	8.28±0.38

systems (Chan *et al.,* 1998). Supplementation of vitamin E (Lallawmkimi *et al.,* 2013) and niacin (Khan *et al.,* 2013) in heat stressed cows reduced plasma SOD activity compared to the un-supplemented group. Aliarabi and Chabbra (2006) reported that 40 ppm of chelated Zn supplementation in crossbred calves increased (P<0.05) SOD activity. The SOD and catalase activities were lower in vitamin E supplemented (100 IU and 150 IU/kg) groups of Murrah buffalo calves (Dass *et al.,* 2009). Serum SOD activity was not affected but serum GPx activity was significantly decreased by iodine supplementation (Qin *et al.,* 2011).

The GPx and catalase activity was not affected due to micronutrient supplementation or seasons. Similarly, Khan *et al.* (2013) reported that the plasma catalase activity showed no significant difference between the periods on niacin supplementation to lactating cows. However, supplementation of Zn at higher level increased GPx and catalase activity in calves fed basal diet with 140 ppm supplemental Zn compared to those fed control diet (Parashuramulu *et al.,* 2015). In present study, supplementation of iodine and micronutrient mixture had no marked effect on antioxidant status *viz.*, SOD, catalase and GPx in heat stressed crossbred heifers.

The TBARS levels during winter and summer in groups G_1, G_2, G_3 and G_4 and monthly variation in TBARS activity in different treatment groups under winter and seasons are presented in (Table 3). Plasma TBARS values were found to be lower (P<0.05) during winter than summer season. The blood lipid peroxidation activity was lower (P<0.05) in group G_4 supplemented

Table 3. Effect of micronutrients supplementation on antioxidant status in different groups of Karan Fries heifers

Season	Group					P value		
	G_1	G_2	G_3	G_4	Period mean	T	S	T×S
Superoxide dismutase activity (Unit/g Hb/min)								
W	2012±142	2005±157	2151±141	2054±148	2055±72	0.85	0.64	0.74
S	2155±170	2229±134	2297±176	2178±231	2227±89			
Overall mean	2083±110	2117±103	2224±112	2116±136	2135±58			
Blood glutathione peroxidase activity (µmole NADPH oxidized/g Hb/min)								
Winter	21.80±1.25	21.31±1.25	23.57±1.71	21.00±1.36	22.23±0.70	0.10	0.10	0.94
Summer	23.39±1.34	24.29±1.40	24.85±1.29	22.32±2.11	24.17±0.78			
Overall Mean	22.59±0.91	22.80±0.96	24.21±1.06	21.66±1.24	22.82±0.53			
Blood catalase activity (µmol of H_2O_2 consumed/min/mg Hb)								
Winter	109.54±5.29	111.41±5.52	112.02±6.29	111.80±2.61	111.19±2.61	0.91	0.70	0.97
Summer	110.49±8.91	113.44±7.79	117.12±5.26	110.79±3.53	112.96±3.53			
Overall Mean	110.02±5.11	112.43±4.71	114.57±4.07	111.08±2.19	112.08±2.19			
Plasma TBARS values (nmol/mg protein)								
Winter	5.35±0.29	4.94±0.20	5.03±0.27	4.50±0.33	4.95^{A}±0.12	<0.01	<0.01	0.15
Summer	6.63±0.18	6.02±0.25	6.23±0.30	4.75±0.17	6.29^{B}±0.16			
Overall Mean	5.99^{b}±1.00	5.48^{b}±0.91	5.63^{b}±0.94	4.62^{a}±0.77	5.43±0.45			
Ferric reducing ability of plasma (µmol/l)								
Winter	1327±60	1309±34	1428±53	1221±41	1321^{A}±25	0.003	0.01	0.87
Summer	1375±50	1397±70	1505±52	1348±41	1406^{B}±27			
Overall Mean	1351^{ab}±38	1353^{ab}±39	1467^{b}±37	1284^{a}±30	1364±19			

Values bearing different superscripts in a row (a, b) and column (A, B) differ significantly (P<0.05)

Table 4. Plasma total imunoglobulins levels (mg/mL) in different groups as affected by micronutrients supplementation in Karan Fries heifers

Season	Group					Significance		
	G_1	G_2	G_3	G_4	Overall mean	T	S	T×S
	Superoxide dismutase activity (Unit/g Hb/min)							
Winter	27.03±1.04	28.73±1.51	27.11±0.32	28.47±1.28	27.83^{B}±0.44	0.40	0.03	0.34
Summer	22.77±0.73	22.87±0.91	22.59±0.68	22.09±0.62	22.58^{A}±0.17			
Overall Mean	24.77±0.73	25.80±1.53	24.85±1.06	25.28±1.56	25.20±0.14			

^{A,B} Values bearing different superscripts in a column differ significantly (P<0.05)

with micronutrient mixture, however, no significant interaction was observed between the treatment effect and seasonal variation. Overall mean of blood plasma FRAP activity (µmol/l) during winter and summer seasons differed significantly (P<0.05) (Table 3) and group G_4 showed the lowest activity and significantly higher value was found in group G_1. Zade *et al.* (2014) observed that Cr supplementation increased peripheral blood glucose concentration along with decreased level of NEFA and TBARS. It was suggested that addition of $CrCl_3$ might have positive effects on antioxidant status and reduced the susceptibility of blood to free radical oxidative damage. The FRAP activity was higher during summer. In the present study, FRAP activity was reduced in response to micronutrient supplementation which is an indicative of oxidative stress mitigation. Similarly, the FRAP activity was found to be lower on vitamin E supplementation in Murrah buffalo calves (Dass *et al.,* 2009).

The supplementation of micronutrients did not have any significant effect on plasma total Ig, however, total Ig levels were found to be higher (P<0.05) in winter than in summer (Table 4). Higher levels of total Ig levels during winter than in summer season indicated that a prolonged exposure to severe heat stress was responsible for a decline of immune cell's reactivity (Cook *et al.,* 2002). They suggested that vitamin E enhanced the ability of immune cells of the mammary gland to produce more immunoglobulin. Dietary supplementation of Zn at 500 ppm level in grower pigs improved gamma globulin concentration (Borah *et al.,* 2014). Serum concentration of IgM and IgG increased by 13.2 and 20.6% due to Cr supplementation 200 µg/kgDM as nano-Cr (Wang *et al.,* 2007). Deka *et al.* (2014) also reported improved plasma total Ig and IgG in peri-parturient Murrah buffaloes due to supplementation of inorganic Cr at 1.5 ppm of dietary DM.

CONCLUSIONS

The feed intake and growth performance was not affected by seasons in Karan-Fries heifers in winter and summer irrespective of micronutrients inclusion in the diet. Micronutrient supplementation could mitigate oxidative stress and reduced FRAP and TBARS activity in cross bred heifers under summer stress conditions.

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Effect of Dietary Coriander Seed Powder Supplementation on the Serological and Immunological Parameters of Broilers

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ABSTRACT

An investigation on the effect of supplementing coriander seed powder (CSP) in broilers' diet on serum profile and immunological parameters was done. One hundred and twenty day-old commercial broiler chicks were divided into five treatment groups of 24 birds, and each group had three replicates of 8 birds. T_1 served as control and was offered basal diet as per BIS (2007) supplemented with antibiotics but no CSP. Treatment groups T_2, T_3, T_4 and T_5 were offered control diet plus 0.5, 1.0, 1.5 and 2.0% of CSP but no antibiotics as growth promotors. All the diets were isocaloric and iso-nitrogenous in nature. Plasma glucose and serum total cholesterol was decreased significantly ($P<0.05$) in the CSP supplemented groups in comparison to control group. Also, dietary supplementation of CSP resulted in increased ($P<0.05$) HDL and decreased ($P<0.05$) LDL-cholesterol. Serum triglyceride decreased significantly ($P<0.05$) upon CSP supplementation at 1.0% and above. Activity SGOT and SGPT decreased significantly ($P<0.05$) upon CSP supplementation in the diets of broiler. Relative mRNA expression of TLR2 and TLR7 of broilers was found to be enhanced ($P<0.05$) while that of TLR4 was down regulated in all the CSP supplemented groups in comparison to control group. Based on the findings of current study, it was inferred that coriander seed powder supplementation in the broilers diet has beneficial effects on various blood biochemical and immunological indices and can be preferred as feed additives over antibiotics.

Key words: Coriander seed powder, Broiler, Biochemical and Immunological parameters

INTRODUCTION

Poultry including chickens, turkeys, geese and ducks are major groups of food animals which had been domesticated for the production of meat or eggs. The chicken (*Gallus gallus domesticus*) is a type of domesticated fowl that is a subspecies of the red jungle fowl. It is one of the most common and widespread domestic birds. Humans keep chicken primarily for food, consuming both their meat and eggs. In India, the growth in poultry sector has been phenomenal during the last few decades. This has been primarily achieved due to the exploitation of various modern growth-promoting strategies and appropriate measures of disease prevention (Angelakis *et al.,* 2013). But the main constraint for an economic poultry rearing especially in the un-organized poultry sector is threat posed by the pathogens that are present in and around the poultry farms. This has led to reduced profitability of small-scale poultry farming in the country due to high cost of treatment using antibiotics. Moreover, injudicious use of antibiotic may cause antimicrobial resistance along with antibiotic residues in food chain. Considering the ever increase public awareness of ill effects of antibiotics, many countries have banned use of antibiotics as growth promoter in livestock feed. Thus, use of aromatic plants has been an important part of achieving modern intensive poultry production (Ghazanfari *et al.,* 2015).

Coriander (*Coriandrum sativum* L.) is a member of *Apiaceae* family, native to eastern Mediterranean region and southern Europe. It reputedly has health advantages and regarded as both herb and spice. Linalool, an essential oil present in coriander seeds has anthelmintic, antifungal, antioxidant and antimicrobial properties (Silva *et al.,* 2011; Saleh *et al.,* 2014). Additionally, coriander has been advocated as an anti-diabetic remedy (Lewis and Elvin-Lewis, 1977). Anti-diabetic agents can exert beneficial effects in the diabetic patient by improving and or mimicking insulin action and/or by enhancing insulin secretion (Gray

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and Flatt, 1997). Keeping in view the health benefits of coriander, the present study was conducted to investigate the effect of supplementation of coriander seed powder on the serological and immunological parameters in the broiler chickens.

MATERIALS AND METHODS

The present study was conducted at poultry shed of the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar for a period of six weeks. One hundred and twenty day-old broiler chicks were purchased from a local commercial hatchery. The chicks were wing banded, individually weighed and randomly distributed into five treatment groups *viz.* T_1, T_2, T_3, T_4 and T_5. Every treatment group had three replicates of eight birds each. The group T_1 served as control and was fed a basal ration formulated as per BIS (2007) specifications with the supplementation of antibiotics as growth promoting feed additives. In the diets of treatment group T_2, T_3, T_4 and T_5 coriander seed powder (CSP) was supplemented in place of antibiotics at the rate of 0.5, 1.0, 1.5 and 2.0%, respectively. Birds were vaccinated against F_1 strain of Newcastle disease (NCD) on 3rd day and Infectious Bursal Disease (IBD) on 14th day through intraocular/intranasal route. The birds were kept in deep litter system using chopped dry wheat straw. The shed was fumigated with formaldehyde gas (35 ml of commercial formalin and 17.5 g potassium permanganate per hundred cubic feet area) and a thin layer of calcium carbonate was spread on floor prior to placing the bedding material. *Ad-libitum* feeding programme consisted of a pre-starter diet till 7 days, a starter diet fed for succeeding 21 days, and a finisher diet fed at the end until 42 days of age. Fresh drinking water supplemented with hepatoprotective liver tonic was made available round the clock.

Blood samples were collected from the slaughtered birds in non-heparinised tubes.The samples were centrifuged at 3000 rpm for 15 minute and serum obtained was stored at -20°C until analysed. Serum parameters like total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glucose, SGOT and SGPT were determined using Ebra EM-200 Biochemistry Analyzer (Sr. No. B110318, Ebra Mannheim, Transasia Bio-medicals ltd).

Total RNA was isolated from blood samples by using TRIZOL® kit (Ambion by life technologies) as per the manufacturer's instruction. One ml of TRIZOL® reagent, 200 µl of chloroform was added to 600 µl of blood followed by centrifugation for phase separation and precipitation with isopropanol. Total RNA extracted was dissolved in 20 µl NFW (Nucleus free water) and quantified using Qubit® 2.0 fluorometer (Invitrogen). Reverse transcription was carried out with total reaction volume of 20 µl using Onescript® cDNA synthesis kit (fire script RT-cDNA synthesis kit). The polymerase chain reaction (RT-PCR) cyclic conditions were as initial incubation at 25°C for 10 min, reverse transcription at 42°C for 50 min and deactivation at 85°C for 5 min in thermal cycler (Applied Biosystem, virti). The cDNA was stored at -20°C till further use.

For the analysis of temporal expression profile of different genes, real-time PCR was carried out using Step I plus real-time PCR system. For the real-time PCR reaction, SYBR green dye based universal PCR master mix (Luna) was used. The reaction for various TLRs gene, (TLR 2, TLR 4 and TLR 7) and the endogenous control, β-actin gene was carried out in triplicate along with non-template control as a negative control for each sample. The reaction mixture used to carry out the real-time PCR reaction for TLRs 2, 4 and 7; and β-actin gene contains 1X SYBR green PCR mastermix (Luna, 5 µl), Forward primer (0.3µl), Reverse primer (0.3µl), NFW (1.4µl) and template (3 µl). Amplification was done with initial denaturation for 60 seconds at 95°C, followed by 40 cycles of denaturation for 15 s at 95°C and extension for 30 s at 60°C, and a final melting curve analysis at 60ºC.The average C_T (Threshold cycle) value obtained for the TLRs 2, 4 and 7 gene were normalized to β-actin (endogenous control). The data obtained were subjected to comparative C_T method for the analysis of the

expression levels of targeted TLR gene and an endogenous control. Amplicons were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) on an automatic ABI 3130 xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). The sequence obtained shows 100% nucleotide identity with the TLR sequence of chicken available in the global database.

Statistical analysis

Data was analysed statistically as described by Snedecor and Cochran (1994). Analysis of variance was performed and differences among treatment means were compared by using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1956).

RESULTS AND DISCUSSION

Biochemical parameters of broilers fed different diets are presented in table 1. All the CSP supplemented groups had significantly (P<0.05) lower plasma glucose concentration than the control group. Further, plasma glucose concentration was found to decrease significantly (P<0.05) as the level of CSP was increased from 0.5% in T_2 to 2% in T_5. Decrease in plasma glucose in the CSP supplemented groups might be due to the anti-diabetic property of coriander seeds. In a similar study, Al- Mashhadani *et al.* (2011) reported that inclusion of coriander seed oil at 0.5% and 1% of diet resulted in significant (P<0.05) decrease in serum glucose in broilers. Similarly, serum total cholesterol was also decreased significantly (P<0.05) with the increasing level of CSP supplementation. Control group supplemented with antibiotics had highest while treatment group supplemented with CSP at 2.0% level had lowest serum cholesterol concentration. CSP supplementation at 1.0% or above resulted in significant (P<0.05) increase in serum HDL-cholesterol. On the other hand, serum LDL-cholesterol was decreased significantly (P<0.05) in the CSP supplemented groups as compared to control with the lowest LDL concentration (43.56 mg/dl) being observed in T_5 group supplemented with 2.0% CSP. Hypocholestermic effect of coriander seeds could be due inhibition in the enzyme 3-hydroxy 3-methyl glutaryl CoA reductase (HMG-CoA reductase) that catalyzes the conversion of HMG-CoA to mevalonate. Mevalonate is required for cholesterol biosynthesis (Dhanapakiam *et al.,* 2008). Coriander oil extract interferes with production of mevalonate by acting as a reversible competitive inhibitor of enzyme HMG-CoA reductase (Ciftci *etal.,* 2010), thus results in hypocholesterol-aemia. Chithra and leelamma (1997) have also reported that coriander enhance bile acid synthesis with increased degradation of cholesterol to fecal bile acid and natural sterols which resulted in lowering serum cholesterol level. Additionally, Dhanapakiam *et al.* (2008) also reported decreased LDL and increased HDL cholesterol (P<0.05) in coriander seed-based diet fed animals. A significant (P<0.05) reduction in serum triglyceride was also reported on CSP supplementation at 1.0% or or higher level of inclusion. Similarly, Al-Jaff (2011) illustrated that the inclusion of coriander seed as diet ingredient at levels of 2% and 3% resulted in a significant decreased in serum concentrations of cholesterol and triglyceride. CSP supplemented groups had significantly (P<0.05) lower Serum glutamic

Table 1. Mean values of biochemical parameters of broilers under different dietary treatments

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)	SGOT (IU/l)	SGPT (IU/l)
T_1	$225.26^{e}\pm1.60$	$142.52^{d}\pm1.26$	$74.44^{a}\pm2.35$	$60.06^{d}\pm1.05$	$86.33^{b}\pm0.88$	$275.10^{e}\pm0.92$	$7.10^{d}\pm0.15$
T_2	$214.46^{d}\pm0.37$	$139.33^{c}\pm1.20$	$78.08^{a\pm}1.27$	$55.01^{c}\pm0.65$	$84.67^{b}\pm1.76$	$260.72^{d}\pm1.19$	$6.53^{c}\pm0.08$
T_3	$208.08^{c}\pm0.98$	$136.07^{b}\pm0.89$	$82.44^{b}\pm0.52$	$52.42^{c}\pm1.09$	$80.00^{a}\pm1.15$	$251.50^{c}\pm1.87$	$6.20^{c}\pm0.17$
T_4	$192.20^{b}\pm1.01$	$134.28^{ab}\pm0.68$	$85.13^{bc}\pm1.10$	$48.51^{b}\pm1.02$	$77.67^{a}\pm1.20$	$243.05^{b}\pm2.28$	$4.93^{b}\pm0.12$
T_5	$183.72^{a}\pm1.68$	$132.55^{a}\pm0.86$	$88.43^{c}\pm0.43$	$43.56^{a}\pm1.24$	$75.33^{a}\pm1.85$	$231.16^{a}\pm0.63$	$4.20^{a}\pm0.06$

Mean values bearing different superscripts in a column differ significantly (P<0.05)

oxaloacetic transaminase (SGOT) concentration as compared to control group. Also, increase of every 0.5% supplementation of CSP from group T_2 to T_5 lowered the serum SGOT concentration significantly (P<0.05). Serum glutamic pyruvic transaminase (SGPT) concentration was also reduced by CSP supplementation as compared to the control group, however, groups supplemented with 0.5 and 1.0% level of CSP had statistically (P>0.05) comparable SGPT concentration. Our findings are in agreement with Al-Mashhadani (2011) and Al-Jaff (2011) suggesting that coriander seeds might have hepato-protective role in birds, and hence it could replace antibiotics as feed additives in their diets.

Relative mRNA expression of TLR2 and TLR7 of broilers was found to be enhanced (P<0.05) in all the CSP supplemented groups, and a down regulation (P<0.05) trend was seen in CSP supplemented groups for TLR4 expression (Tables 2 and 3).Treatment group T_4 (1.5% CSP) had highest relative quantification (RQ) value showing maximum expression of TLR2 while treatment group T_3 (1.0% CSP) had highest RQ value showing maximum expression of TLR7. In case of TLR4 mRNA expression study, a significant (P<0.05) down regulation of RQ value was obtained, being lowest for treatment T_5 (2% CSP) followed by T_4 (1.5% CSP), T_3 (1.0% CSP) and T_2 (0.5% CSP) as compared to control group T_1. TLR2 denotes presence of Gram positive (+) bacteria like *Lactobacillus* that are beneficial for the health of the gut. Up regulation of the TLR2 RQ values for birds fed CSP in comparison to control group suggests an enhanced population of these bacteria in the gut. TLR4 depicts the presence of the Gram negative (-) bacteria like *E. coli* in the gut microflora, therefore, down regulation of TLR4 RQ values for birds fed CSP in comparison to the control group indicates an elevated anti-bacterial activity against these harmful bacteria.TLR7 represents the anti-viral activity of immune system and thus, up regulation of

Table 2. Relative expression of TLRs of the broiler birds under different dietary treatments

Target Gene	TR	CT MEAN	CT SE	$^{\Delta}$CT Mean	$^{\Delta\Delta}$CT Mean	R.Q.
	T_1	21.65	0.07	4.11	0	1.00
	T_2	21.32	0.04	3.97	-0.14	1.36
	T_3	20.78	0.02	3.84	-0.27	1.21
	T_4	20.24	0.10	3.42	-0.69	1.71
TLR2	T_5	20.51	0.11	3.24	-0.48	1.39
	T_1	24.34	0.02	6.8	0	1.00
	T_2	24.20	0.04	6.85	0.05	0.97
	T_3	24.34	0.02	7.4	0.6	0.66
	T_4	24.86	0.00	8.04	1.24	0.52
TLR4	T_5	28.24	0.84	10.7	0	0.46
	T_1	24.45	0.62	9.3	-2.20	1.00
	T_2	27.67	0.68	10.32	-0.38	1.30
	T_3	27.19	0.18	10.25	-0.45	1.37
	T_4	27.02	0.38	10.2	-0.5	1.33
TLR7	T_5	17.54	0.04	9.06	-2.20	1.25
	T_1	20.31	0.65			
	T_2	19.98	0.22			
	T_3	18.84	0.81			
	T_4	17.74	0.20			
β-Actin	T_5	17.85	0.02			

Table 3. Oligonucleotide sequences of sense and antisense primers for real-time PCR products determined

Gene[1]	Primer	Primer sequence	AccessionNo.	ProductSize
β-Actin	Sense	52 -GAGAAATTGTGCGTGACATCA-32	L08165	152
	Antisense	52 -CCTGAACCTCTCATTGCCA-32		
TLR 2	Sense	52 -CATTCACCATGAGGCAGGGATAG-32	AB046533	157
	Antisense	52 -GGTGCAGATCAAGGACACTAGGA-32		
TLR 4	Sense	52 -TTCAGAACGGACTCTTGAGTGG-32	AY064697	131
	Antisense	52 -CAACCGAATAGTGGTGACGTTG-32		
TLR 7	Sense	52 -TTGCTGCTGTTGTCTTGAGTGAG-32	AJ627563	182
	Antisense	52 -AACAACAGTGCATTTGACGTCCT-32		

[1]TLR 2 = Toll-like receptor 2; TLR 4 = Toll-like receptor 4; TLR 7 = Toll-like receptor 7

TLR7 RQ values upon CSP supplementation signifies a boosted immune system response. Modulatory functions of medicinal herbs on immune system have also been reported by earlier study. In a study on medicinal herbs, Jyotsana (2018) had reported that TLR2 mRNA expression was increased ($P<0.05$) and TLR4 mRNA expression was down regulated in broilers fed Ashwagandha root powder supplemented diets. Our results are in harmony with Hosseinzadeh *et al.* (2014) who found that coriander improved performance indices, ileum microflora and immune response in broiler chicks.

CONCLUSION

From the findings of this study, it was concluded that supplementation of coriander seed powder to broiler diets had favourable effects on serum biochemical profile in term of decreased SGOT and SGPT indicating hepato-protective effects and immunological indices of broiler chicken in terms of stimulatory effect on relative mRNA expression of TLR2 and TLR7 and down regulation pattern of TLR4 indicating improved immunity. Therefore, coriander seed powder can be a preferable feed additive in place of antibiotics.

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Effects of Graded Levels of Boiled Rubber (*Hevea brasiliensis*) Seeds Meal on Growth and Carcass Characteristics of Rabbits

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ABSTRACT

The present study was conducted at the Teaching and Research Farm of the University of Dschang-Cameroon, in order to evaluate the effects of inclusion level of rubber seed meal (RSM) on growth performances and carcass characteristics in rabbits. Sixty rabbits (30 males and 30 females) aged 2 months and weighing 900± 80 g were randomly assigned to the 5 experimental rations, that is 12 rabbits (6@& and 6B&) for each ration, in a completely randomized design. The 1st group received ration free from RSM (T0); the 2nd group received ration containing 20% of raw RSM (R20); and the 3rd, 4th and 5th groups received rations containing respectively 20, 25 and 30% of boiled RSM (B20, B25 and B30). The feed intake (FI) and live weight (LW) were recorded weekly for 3 months. At the end of the trial, 8 rabbits (4 males and 4 females) per treatment were randomly selected and slaughtered for carcass characteristics evaluation.Results showed that the FI and the feed conversion ratio (FCR) decreased insignificantly (P>0.05) in rabbits fed with rations containing boiled RSM (B30 for the FI and B25 for the FCR) compared to those receiving T0 ration. Live weight and weight gain increased non-significantly (P>0.05) in rabbits fed with the B25 ration compared to those receiving T0 and R20 rations. The carcass yield, the weights of liver, kidneys, heart, abdominal fat, caecum, big intestine, ileum, and the lengths of ileum and large intestine were comparable (P>0.05) among the groups. Cost of a kg of feed was 184.17 and 180.25 CFAF, respectively for B25 and B30 rations, that is a reduction of 17.51 and 19.26% compared to T0 (223.25 CFAF). The production cost of a kg of live weight was 1676.61, 1084.76 and 1130.17 CFAF, respectively for T0, B25 and B30 rations. Therefore, the inclusion of boiled RSM in feed increased the growth performances and reduced the cost of production. Hence, boiled rubber seed meal can be used in rabbit ration upto 25% without any adverse impact on growth performance.

Key words: Boiling, Growth performances, Production cost, Rabbit, Rubber seed meal

INTRODUCTION

The prices of various feed ingredients in recent years have risen to the extent that the cost of feed is now very high, making animal production more expensive and its products inaccessible, leading to animal protein deficiency. According to Ekenyem and Oneagoro (2006), the deficiency of animal protein affects more than 70% of the total population of African countries. Feed accounts for 70 to 80% of production costs in intensive farming (Defang *et al.,* 2014). This high cost of feed coupled with non-availability of cheaper feed ingredients is among important factors that hinder commercial animal production. In this regard, use of non-conventional feed such as rubber seeds needs to be explored to be used in the ration of fast-growing animals like rabbit.

Hevea (*Hevea brasiliensis*) or rubber tree is known and used for its latex, but its seeds can be used in animal feeding. It is reported that raw rubber seed is rich in crude protein which range from 20-32.98%, fat (50.2%), crude fiber (6.5%) and a reasonable amounts of minerals including trace elements (Ahaotu*et al.,* 2018; Udo *et al.,* 2018). According to Aguihe *et al.* (2017) and Amanidja *et al.* (2019) rubber seeds showed potentials for animal feeding. Despite the nutritional potential of these seeds, its utilization as animal feed ingredient is limited, because the raw seed contains anti-nutritional factors such as tannins, oxalate, saponins,

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phytinand hydrocyanic acid (Syahruddin *et al.,* 2014; Ahaotu *et al.,* 2018). A variety of processing procedures such as boiling is commonly used to reduce the negative effect of anti-nutritional factors present in tropical legumes. It was hypothesized that boiling of rubber seed meal (RSM) would reduce the toxic principle and thus it would be possible to incorporate RSM in broiler ration. Specific objective was to study the effect of inclusion of different levels of RSM in rabbit diet on growth performances, carcass characteristics and the cost of production.

MATERIALS AND METHODS

The study was carried out at the rabbitry of the Teaching and Research Farm of the University of Dschang, under ethical regulation of Dschang University for animal welfare. Sixty (30 males and 30 females) local breed rabbits, aged 2 months and weighing 900±80 g were used. The rabbits were lodged in separate cages made from wire with dimensions of 100cm x 45cm x 25cm. Cages were raised above the ground level for ease of cleaning. Water and feed were provided *ad libitum* throughout the period of experiment.The rabbits were treated against parasitic infection with ivermectin® (0.2 ml/kg body weight) injected subcutaneously and multivitamins added to their water.

The rubber seeds were harvested in the rubber plantations of SOCAPALM® (Cameroon Company of Palm Groves) in the Moungo Division, Littoral Region. They were decorticated, separated into 2 batches and

Table 1. Ingredients and chemical composition of experimental rations

Ingredients (%)	Experimental rations				
	T0	**R20**	**B20**	**B25**	**B30**
Maize	32	15	21	16.5	14
RSM	0	20	20	25	30
Wheat brand	20	38	35	40	40
Soybean meal	7	2	3	1	0
Palm kernel cake	10	4	5	4.5	6
Cotton seed cake	5	3	2	2.5	2
Trypsacumlaxum	20	15	11	7.5	5
Sea shell	2	2	2	2	2
Palm oil	3	0	0	0	0
Salt	0.5	0.5	0.5	0.5	0.5
*Premix 0.5	0.5	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**	**100**
Chemical compositions of rations					
Dry matter (%)	94.92	95.21	96.33	95.57	96.50
		on % DM basis			
OM	85.99	89.11	90.39	90.30	90.28
Ash	8.64	8.11	6.41	5.85	6.59
CP	16.012	16.076	16.042	16.072	16.11
ME (kcal/kg DM)	2427.78	2463.77	2432.1	2435.85	2480.48
CF	13.14	13.29	13.24	13.21	13.32

*Composition of the 0.5 premix: vit A: 3.000.000 IU, vit D: 50.0000 IU, vit E: 6.000 mg, vit K: 600 mg, vit B_1: 600 mg, vit B_2: 800 mg, vit B_3: 1800 mg, vit B_6: 400 mg, Vit_{12}: 6 mg, folic acid: 250 mg, niacin: 600 mg, Cl: 86.500 mg, Fe: 12.000 mg, Cu: 1200 mg, manganese: 12.000 mg, Zn: 10.000 mg, I: 100 mg, Se: 40 mg, magnesium: 3397 mg, Na: 283 mg, CA: 215.166 mg, Methionine: 130.000 mg, lysine: 50.000 mg. RSM: rubber seed meal. DM= Dry Matter, OM= Organic Matter, CP,Crude Protein; CF, crude fiber; T0, ration containing no rubber seeds meal; R20, ration with 20% untreated rubber seed meal; B20, ration with 20% of boiled rubber seed meal ; B25, ration with 25% of boiled rubber seed meal and B30, ration with 30% of boiled rubber seed meal.

processed differently. First batch was sun dried and the second batch was cooked for 45 minutes in a cooking pot containing water sufficient to cover the seeds. After cooking, the seeds were decanted and sun-dried. This was to ensure complete reduction of moisture to ease milling. The processed rubber seeds were separately hammer-milled prior to experimental diet formulation, to produce the respective meals as raw rubber seeds meal (RRSM), and boiled RSM (BRSM).

Five experimental diets were formulated to meet the nutritional requirements of the rabbits. T0 (control) diet was free of rubber seed meal, R20 (control) contained 20% of raw RSM, while B20, B25 and B30 contained boiled rubber seeds meal at 20, 25 and 30%, respectively, (Table 1).Sixty (30 males and 30 females) rabbits were weighed and randomly assigned to one of the five (5) groups, each containing twelve (12) animals (6 males and 6 females), corresponding to five different dietary treatments. Group 1 rabbits (control group) received T0 ration, free from rubber seeds meal (RSM); group 2 rabbits received R20 ration, containing 20% of raw RSM; while animals of groups 3, 4 and 5 were fed with rations B20, B25 and B30, containing respectively 20, 25 and 30% of boiled RSM. Amount of feed offered left overs were collected and weighed every morning before serving new feed. Weekly feed intake (FI) was calculated by subtracting the left-over from the quantity of feed offered to each rabbit in each treatment, according to the following formula:

FI (g) = Quantity of feed served – Left over feed

Each rabbit was weighed at the start of the trial and thereafter at weekly interval, using an electronic scale of 5000 g capacity and 1g precision. Therefore, the weekly weight gain (WG) was calculated using the following formula:

$$WG\ (g) = Wn\text{-}Wn\text{-}1$$

Where WG: is the weight gain, Wn: weight at the end of the current week and Wn-1: weight at the end of the previous week.

The weekly feed conversion ratio was calculated using the formula bellow:

$$FCR = \frac{\text{Total FI (g)}}{\text{Total WG (g)}}$$

At the end of the experiment, 8 (4 males and 4 female) rabbits per treatment were randomly selected, starved for 24 hours, stunned and slaughtered for carcass characteristics evaluation according to Jourdain (1980).

Carcass yield was calculated as the ratio of the dressed carcass weight to the live weight (after fasting) and multiplied by hundred.

$$\text{Carcass yield } (\%) = \frac{\text{Weight of carcass}}{\text{Live weight}} \times 100$$

The relative weight of organs and body parts (liver, kidneys, head, skin, legsand heart) were calculated as the ratio of the weight of organ or body part to the live body weight.

$$\text{Relative organs or body part weight } (\%) = \frac{\text{Weight of organs}}{\text{Live weight}} \times 100$$

The cost of per kilogram feed was evaluated from the price of ingredients in the local market. The cost of production per kilogram of live weight of the rabbit was calculated from the cost of the feed multiplied by the amount of feed consumed.

Data collected were submitted to two ways Analysis of Variance (ANOVA), to test the effects of rations and sex on the studied parameters. Means were separated for significant differences (Pp<0.05), using Duncan's multiple range test (Steel and Torrie 1980). The analyses were performed using SPSS 20.0 software.

RESULTSAND DISCUSSION

In general, feed intake increased from the start to the end of the trial regardless the incorporation rate of the rubber seeds meal. The increase in feed intake was more accentuated in rabbits fed T0 ration and less so in rabbits receiving the ration containing 30% of boiled rubber seeds (B30). At the end of the trial, the total feed intake (Table 2) was significantly (P<0.05) higher in rabbits receiving the T0 ration compared to those fed with rations containing rubber seeds meals, regardless

Table 2. Effects of feeding different levels of rubber seed mean on feed intake in rabbits

	Sex	Rations					P
		T0	R20	B20	B25	B30	
FI (g)	♂	8371.67±773.34[a]	6994.50±218.31[b]	7334.67±440.84[b]	7212.83±466.95[b]	6681.00±486.32[b]	0.000
	♀	8060.11±581.83[a]	7001.78±196.03[b]	7295.89±532.21[b]	7197.44±508.39[b]	6385.38±441.28[c]	0.000
	♂♀	8184.73±657.26[a]	6998.87±197.47[b]	7311.40±481.30[b]	7203.60±475.00[b]	6512.07±467.83[c]	0.000
P		0.423	0.949	0.881	0.953	0.268	

[a,b,c]values with the same letter, on the same row are not significantly different (P>0.05); T0, ration containing no rubber seeds meal; R20, ration with 20% untreated rubber seed meal; B20, ration with 20% of boiled rubber seed meal ; B25, ration with 25% of boiled rubber seed meal and B30, ration with 30% of boiled rubber seed meal.

of the sex. Feed consumption was comparable (P>0.05) between females and males. Irrespective of sex, feed intake was lower (P<0.05) in rabbits receiving the B30 ration as compared to those fed raw rubber seeds meals (R20). Overall feed intake decreased with the inclusion of rubber seeds meal in rations. This result is in agreement with that of Amanidja *et al.* (2019), who reported similar response in ducks fed RSM. Udo *etal*. (2018) reported that rubber seed is not quite palatable and appetizing to West African Dwarf Goats However, this result remains in contradiction with that of Loba (2017), which showed a high feed consumption in ducks having received feed containing the treated rubber seed powder.

Irrespective of the inclusion of rubber seed meal live weight increased from the start until the end of the trial. The increase in live weight was more rapid in rabbits fed with ration containing 25% of boiled rubber seed meal as compared to other rations. At the end of the experiment, the final body weight was significantly (P<0.05) higher in the rabbits fed with the ration containing 25% of boiled rubber seed meal compared to those receiving T0 or R20 ration (Table 3). However, no significant difference (P>0.05) was recorded between females and males. The gradual increase in body weight from the start to the end of the trial could be attributed to the fact that the animals used in this work were growing rabbits. According to Laffoley (1985), the live weight of rabbits increases considerably between 1 and 21 weeks of age. According to Smith (1997), this growth could be due to three phenomena: the increase in the number of cells (hyperplasia), the increase in the size of cells (hypertrophy) and/or the increase in intercellular substances (accretion). These phenomena are the consequence of the accumulation of nutrients from the food consumed. Kouakou *et al.*

Table 3. Effects of feeding different levels of rubber seed mean on live weight in rabbits

	Sex	Rations					P
		T0	R20	B20	B25	B30	
Initial live weight (g)	♂	1154.17±79.06	1197.00±85.60	1190.17±53.94	1203.67±60.82	1263.00±69.90	0.151
	♀	1212.22±74.77	1205.56±85.98	1242.67±54.21	1253.00±74.49	1247.13±58.00	0.514
	♂♀	1189.00±79.34	1202.13±82.83	1221.67±58.54	1233.27±71.54	1253.93±61.29	0.128
P		0.184	0.853	0.093	0.185	0.661	
Final live weight (g)	♂	2217.33±138.47[b]	2271.63±147.42[b]	2397.98±117.18[a]	2420.20±146.15[a]	2334.71±149.59[ab]	0.048
	♀	2245.43±99.82[b]	2292.59±131.63[b]	2316.59±147.98[ab]	2435.20±131.14[a]	2317.64±159.72[ab]	0.044
	♂♀	2234.19±112.90[c]	2284.21±133.33[bc]	2349.15±138.28[ab]	2429.20±132.34[a]	2324.96±149.73[bc]	0.004
P		0.679	0.784	0.258	0.843	0.841	

[a,b,c]values with the same letter, on the same row are not significantly different (P>0.05); T0, ration containing no rubber seeds meal; R20, ration with 20% untreated rubber seed meal; B20, ration with 20% of boiled rubber seed meal ; B25, ration with 25% of boiled rubber seed meal and B30, ration with 30% of boiled rubber seed meal.

Table 4. Effects of feeding different levels of rubber seed mean on weight gain in rabbits

	Sex	Rations					P
		T0	R20	B20	B25	B30	
Weight gain (g)	♂	1029.09±140.44^{c}	1074.64±125.17^{bc}	1340.73±133.72^{aA}	1320.36±275.11^{ab}	1158.64±262.48^{ab}	0.037
	♀	1186.36±136.70	1087.03±141.49	1166.36±154.04^{B}	1257.97±296.99	1102.64±243.95	0.436
	♂♀	1123.45±176.77^{ab}	1082.07±130.67^{b}	1218.11±175.17^{ab}	1282.93±280.06^{a}	1126.64±243.66^{ab}	0.036
p		0.079	0.862	0.018	0.685	0.692	

^{a,b,c}values with the same letter, on the same row are not significantly different (P>0.05); T0, ration containing no rubber seeds meal; R20, ration with 20% untreated rubber seed meal; B20, ration with 20% of boiled rubber seed meal ; B25, ration with 25% of boiled rubber seed meal and B30, ration with 30% of boiled rubber seed meal.

(2018) showed that the inclusion of rubber seed cake at a rate of 15% in the pig ration for fattening gave better results after weaning, with exponential growth. Suprayudi *et al.* (2015) reported an improved response in terms of the growth of juvenile carp fed with treated rubber seeds meal with an inclusion level of 50%.

The total weight gain (Table 4) was significantly (P<0.05) higher in rabbits receiving the ration containing 25% of boiled rubber seed meal as compared to those of R20 ration. In males, the increase in total weight gain was significant (P<0.05) higher in animals fed with B20 ration, males gained significantly (P<0.05) weight as compared to females. This result is in agreement with those of Amanidja *et al.* (2019), who reported increased weight gain in local ducks fed with rubber seeds flour. All the rabbits receiving boiled rubber seeds meal in the diets recorded betterlive weight and weight gain than R20. This indicates a possible elimination or reduction of anti-nutritional factors such as hydrocyanic acid, in boiled rubber seeds meal, due to the cooking process as reported by Udedibie (1991) and Umar *et al.* (2007). In addition, the nutrients present in the feed have been well utilized by these animals even though the feed intakes were lower in these treatments. In fact, the elimination or reduction of anti-nutritional factors in the feed through the boiling process could have favored a better feed digestibility, nutrients absorption, metabolism and accumulation (Matho *et al.,* 2021). The nutrients accumulation induces cells hypertrophy, hyperplasia and/or accretion, resulting in the increase of body weight and weight gain, as observed in the current work.

At the end of the trial, the feed conversion ratio (Table 5) was significantly (P<0.05) lower in the animals fed with rations containing rubber seeds meal as compared to those of the T0 ration. When B20 ration was fed, the feed conversion ratio was significantly (P<0.05) lower in males as compared to females.Overall feed conversion ratio decreased slightly in rabbits fed with feed containing boiled rubber seeds meal. This result is in agreement with those of Loba (2017), in ducks (*Cairinamoschata*) fed with rubber seed cake.

Table 5. Effects of feeding different levels of rubber seed mean on feed conversion ratio in rabbits

Growth performance	Sex	Rations					P
	T0	R20	B20	B25	B30		
FCR	♂	8.41±1.53^{a}	6.58±0.78^{b}	5.51±0.44^{bB}	5.64±1.06^{b}	6.50±1.34^{b}	0.001
	♀	6.91±0.87	6.55±0.95	6.52±0.91^{A}	6.06±1.14	6.10±1.41	0.440
	♂♀	7.51±1.36^{a}	6.56±0.86^{b}	6.11±0.90^{b}	5.89±1.09^{b}	6.27±1.34^{b}	0.000
p		0.064	0.939	0.015	0.483	0.601	

^{a,b,c}values with the same letter, on the same row are not significantly different (P>0.05); T0, ration containing no rubber seeds meal; R20, ration with 20% untreated rubber seed meal; B20, ration with 20% of boiled rubber seed meal ; B25, ration with 25% of boiled rubber seed meal and B30, ration with 30% of boiled rubber seed meal.

Data pertaining to the effect of the inclusion of boiled rubber seeds meal on the carcass yield and characteristics are summarised in Table 6. The carcass yield in male rabbits fed ration B30 was higher (P<0.05) than those in other groups. At B20 ration, the skin was significantly (P<0.05) heavier in males compared to females.The poor carcass yield in rabbits fed with ration containing raw rubber seeds meal could be attributed to impaired nutrients utilization due to the presence of anti-nutritional factors. Schrews (2000) and Mathew *et al.* (2010) reported that nutrition exerts several influences on the development of carcass characteristics, organs and muscle growth in an animal.

Data pertaining to the effects of the inclusion level of boiled RSM on the relative weights of the heart, liver, kidneys and abdominal fat in rabbits are presented in Table 7. All these attributes were comparable among the groups. It is a common practice in feeding trials to use weights of some internal organs like liver and kidneys as indicators of toxicity. Studies have shown that if there is any major effect of anti- nutritive factors in diet, organs such as kidney, liver would be affected by increasingtheir weight, since they are the major detoxifying organs (Carew *et al.,* 2003). The observations in this study suggest that the test diets did not contain any appreciable amount of toxin to cause organ damage during the period of experimentation.

Data pertaining to feed cost are presented in Table 8. Feed cost was higher for the T0 ration and lower at B30 ration, witha reduction of 19.26% compared to the T0 ration.The production cost per kg of live weight was higher with the T0 ration and lower with the B25 ration. A decrease of 35.50% compared to the T0 ration was registered.The highest feed cost and production cost

Table 6. Effects of feeding different levels of rubber seed mean on carcass yield and body parts weight in rabbits

Growth performance	Sex	Rations					P
	T0	R20	B20	B25	B30		
Carcassyield	♂	49.12±0.73^{ab}	48.12±0.91^{b}	49.29±0.95^{ab}	49.73±0.62^{ab}	50.17±1.83^{a}	0.048
(%)	♀	49.89±1.75	47.63±1.78	49.01±1.84	48.60±0.71	47.63±1.01	0.230
	♂♀	49.51±1.30	47.87±1.34	49.17±1.26	49.17±0.86	48.97±1.88	0.142
p		0.463	0.647	0.825	0.055	0.073	
Weight of the	♂	9.49±1.05	9.06±0.71	8.95±0.89	9.09±0.90	9.18±0.64	0.913
Head(%)	♀	8.72±0.58	8.81±0.47	8.78±0.90	8.82±0.60	8.74±0.48	0.999
	♂♀	9.11±0.89	8.93±0.57	8.87±0.82	8.95±0.72	8.96±0.57	0.977
P		0.259	0.582	0.815	0.633	0.326	
Weight of	♂	10.53±1.61	11.15±1.72	11.87±1.13^{A}	10.94±1.64	10.51±1.00	0.621
the Skin(%)	♀	10.50±0.76	11.20±1.05	9.99±0.35^{B}	10.35±1.18	10.05±1.22	0.492
	♂♀	10.52±1.17	11.18±1.32	11.07±1.03	10.64±1.36	10.27±1.06	0.613
P		0.973	0.963	0.007	0.583	0.577	
Weight of	♂	2.65±0.65	2.59±0.32	2.51±0.40	2.61±0.37	2.66±0.40	0.991
the legs(%)	♀	2.62±0.37	2.87±0.33	2.57±0.55	2.32±0.40	2.71±0.44	0.468
	♂♀	2.64±0.49	2.73±0.34	2.54±0.43	2.46±0.39	2.68±0.39	0.742
P		0.951	0.274	0.881	0.334	0.852	

^{a,b}values with the same letter, on the same row are not significantly different (P>0.05); T0, ration containing no rubber seeds meal; R20, ration with 20% untreated rubber seed meal; B20, ration with 20% of boiled rubber seed meal ; B25, ration with 25% of boiled rubber seed meal and B30, ration with 30% of boiled rubber seed meal.

Table 7. Effects of feeding different levels of rubber seed mean on the relative weights of internal organs and abdominal fatin rabbits

Organs	Sex	Rations					P
weight	T0	R20	B20	B25	B30		
Heard (%)	♂	0.23±0.03	0.25±0.15	0.23±0.04	0.26±0.04	0.24±0.24	0.645
	♀	0.24±0.04	0.24±0.04	0.23±0.08	0.22±0.05	0.30±0.05	0.201
	♂♀	0.23±0.03	0.25±0.41	0.23±0.05	0.24±0.05	0.27±0.05	0.367
p		0.778	0.724	0.998	0.222	0.084	
Liver (%)	♂	2.24±0.17	2.36±0.24	2.47±0.48	2.31±0.27	2.36±0.54	0.932
	♀	2.36±0.17	2.36±0.23	2.28±0.08	2.35±0.32	2.24±0.15	0.899
	♂♀	2.30±0.17	2.36±0.22	2.37±0.36	2.33±0.28	2.30±0.38	0.985
P		0.356	0.993	0.490	0.858	0.689	
Kidneys (%)	♂	0.51±0.09	0.54±0.14	0.60±0.12	0.58±0.02	0.51±0.04	0.537
	♀	0.64±0.09	0.57±0.05	0.56±0.9	0.52±0.05	0.55±0.11	0.396
	♂♀	0.57±0.11	0.56±0.10	0.59±0.90	0.55±0.05	0.53±0.08	0.806
P		0.092	0.742	0.623	0.088	0.571	
Abdominals	♂	1.07±0.42	1.75±0.56	2.17±0.49	1.94±0.84	1.81±0.84	0.228
fat (%)	♀	1.74±0.59	1.33±0.33	1.90±0.73	1.30±0.57	1.96±0.49	0.331
	♂♀	1.41±0.59	1.54±0.48	2.06±0.57	1.62±0.74	1.88±0.64	0.272
P		0.116	0.250	0.613	0.261	0.773	

T0, ration containing no rubber seeds meal; R20, ration with 20% untreated rubber seed meal; B20, ration with 20% of boiled rubber seed meal ; B25, ration with 25% of boiled rubber seed meal and B30, ration with 30% of boiled rubber seed meal.

were recorded in T0 (control) and the least was registered in B30 having 30% level of boiled rubber seed meal. The result obtained in this study was desirable in rabbit diet because the inclusion of rubber seed meal decreased the feed and production costs, giving better returns. This observation agreed with the report of Apata and Ojo (2000), that the high cost of feed was largely due to the exorbitant price and scarcity of conventional feed ingredients. Similarly, Smith *et al.* (1981) reported that feeding of non-conventional plant protein sources drastically reduced feed cost and these gave better feed cost per kg of weight gain.

CONCLUSION

It was concluded that boiled rubber seed meal can be incorporated into the ration of rabbit to enhance productivity and to reduce production cost.

Table 8. Effects of feeding different levels of rubber seed mean on feed cost

Parameters	Rations				
	T0	R20	B20	B25	B30
Price of the kg of feed (CFAF)	223.25	185.25	190.83	184.17	180.25
Percentage of variation/T0		-17.21	-14.52	-17.51	-19.26
FCR	7.51	6.56	6.11	5.89	6.27
Price of the kg of LW (CFAF)	1676.61	1215.24	1165.97	1084.76	1130.17
Percentage of variation/T0		-27.52	-30.46	-35.30	-32.59

LW: live weight; FCR: feed conversion ration; T0, ration containing no rubber seeds meal; R20, ration with 20% untreated rubber seed meal; B20, ration with 20% of boiled rubber seed meal ; B25, ration with 25% of boiled rubber seed meal and B30, ration with 30% of boiled rubber seed meal.

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Performance and Carcass Characteristics of Japanese Quail fed Dietary Cumin *(Cuminum cyminum)* Seed Powder as Natural Feed Additive

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ABSTRACT

An experiment was conducted to evaluate the effect of dietary inclusion of cumin seed (CS) powder on growth, carcass characteristics and cost economics in Japanese quails. One hundred and fifty, day-old quail chicks were distributed randomly to five dietary groups each with three replicates of ten chicks and were offered experimental diets to meet the nutrient requirements (NRC, 1994). During the experiment, cumin seed powder was included at 0.0% (T_1: Control), 0.50% (T_2), 1.0% (T_3), 1.5% (T_4) and 2.0% (T_5) level. Chemical analysis indicated that cumin seed powder contained 18.26% CP, 14.02% EE, 30.02% CF, 27.96% NFE, 7.79% TA and 0.42% AIA. Results indicated that body weight gain, performance index ($P<0.05$) and PER ($P<0.01$) increased significantly, feed consumed / kg gain ($P<0.01$) decreased significantly while there was no effect ($P>0.05$) on feed intake with inclusion of CS powder in the diet. Further, carcass yield (g), dressing percentage and ready to cook yield (g) increased ($P<0.01$) significantly while there was no effect ($P>0.05$) on weight of heart, liver, gizzard and giblet. The feed cost/ kg gain decreased by ₹ 4.08 in T_2 and ₹ 7.69 in T_3 while it is increased by ₹ 2.70 in T_4, and ₹ 8.70 in T_5 groups of quails as compared to the control (T_1). Thus, it was concluded that cumin seed powder can be included up to 1.0% level in the diet for improved performance of Japanese quail.

Key words: Carcass Characters, Cost economics, Cumin, Growth, Japanese Quail

INTRODUCTION

Several phyto-biotic additives have been examined as alternatives to antibiotic growth promoters (AGP) in broiler production, while many researchers believe that herbs, medicinal plants and spices can be considered as suitable alternatives for promoting animal health. Cumin, cardamom, anise, ginger, black pepper, and clove are a group of aromatic plants that are used widely due to their positive effects on growth and health of poultry, probably as a result of their immune stimulatory properties (Chowdhury *et al.,* 2018).

Cuminum Cyminum Linn. (cumin) is a popular aromatic spice in the world, and its vernacular name in India is Zeera. It is native to Egypt and extensively grown in India. Cumin seed is a well-known spice in human nutrition and feed industries (Hajlaoui *et al.,* 2010). Cumin seeds in whole or ground form and its essential oil have long been used in traditional medicine for the treatment of various diseases, particularly digestive disorders (Muthamma *et al.,* 2008). Cumin seeds contain several phytochemicals which possess carminative and anti-flatulent properties. In addition, antioxidant, antibacterial, antifungal and anti-inflammatory properties of cumin have also been reported earlier (Gachkar *et al.,* 2007; Einafshar *et al.,* 2012). Cuminaldehyde, cymene, terpenoids, polyphenols and flavoids are the major bioactive components of cumin (Bettaieb *et al.,* 2011). Consumption of cumin seed would increase appetite, taste perception and digestive activities of the intestine (Mnif and Aifa, 2015).Positive effects of cumin on growth, feed conversion ratio (Al-Kassi *et al.,* 2010; Jang *et al.,* 2011; Ali-Mohammadi *et al.,* 2013; Rafiee *et al.,* 2014; Ali *et al.,* 2018; Shafiee *et al.,* 2020), and carcass characteristics (Al-Kassi *et al.,* 2010; Jang *et al.,* 2011) were reported earlier in broilers, whereas, studies in Japanese quails (Cetinkaya and Filik, 2020) are very limited. Hence, the present study was conducted to evaluate the effect of inclusion of cumin seed powder at varying levels in the diet on growth and carcass

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characteristics of Japanese quail.

MATERIALS AND METHODS

Cumin seeds, de-oiled rice bran, maize, soybean meal, fish meal, mineral mixture and salt were procured from the local market. All the ingredients were ground and mixed to prepare a basal diet to meet the nutrient specifications as per NRC (1994). During the trial, cumin seed (CS) was ground and was included at 0.0% (T_1: Control), 0.50% (T_2), 1.0% (T_3), 1.5% (T_4) and 2.0% (T_5) level in iso-nitrogenous and iso-caloric broiler quail diets.

In a 5 weeks duration trial, one hundred and fifty, day-old quail chicks were purchased locally and used for conduct of the trial. All the chicks were weighed individually (av. body wt. 8.09 ± 0.03 g), wing-banded and were divided into five equal treatments, each treatment contained three replicates with ten birds per replicate allotted at random. All the chicks were housed in battery brooders throughout the experiment. Irrespective of the treatments, all the chicks were fed *ad libitum* 2 times a day with respective broiler quail diet from 0-5 weeks of age. Fresh and clean drinking water was made available at all the time. B-complex vitamins were offered in water for 3 days during first week. Except for feeding experimental diets, other managemental practices were uniform throughout the experimental period.

The chemical composition of cumin seed powder and other feed ingredients used in the experiment and the experimental diets were analyzed as per AOAC (2007). The individual body weight of quails was recorded at weekly interval up to 5 weeks of age. The feed offered and feed leftover was weighed daily, to quantify the feed utilized. Daily feed consumption was recorded replicate wise and feed efficiency was calculated weekly. Performance index (PI) was calculated for each treatment by dividing the average weight gain by the feed conversion ratio, while protein efficiency ratio (PER) was calculated by dividing the weight gain by protein consumed.

At the end of study period (5th week), two birds per replicate and thus a total 6 birds per treatment were randomly selected, weighed and slaughtered. The data on dressing percentage, carcass yield, ready-to-cook yield and percent weight of heart, liver, gizzard and giblet were recorded. Economics were worked out basing on the prevailing market price of ingredients and feed efficiency of birds. The data was analyzed statistically (Snedecor and Cochran, 1994) and tested for significance using Duncan's multiple range test (Duncan, 1955) using SPSS 24.0 version.

RESULTS AND DISCUSSION

The chemical composition of cumin seed (CS) powder used in the present study was 90.34, 92.21, 18.26, 14.02, 30.02, 27.96, 7.79, 0.42, 0.94 and 0.50 per cent, for DM, OM, CP, EE, CF, NFE, TA, AIA, Ca and P, respectively (on DM basis except for DM). The chemical composition of quail diets containing CS powder at varying levels is presented in Table 1.

The results on body weight gain, feed intake, FCR, PI and PER of different dietary treatments are presented in Table 2. The body weight gain was higher ($P<0.05$) in quails fed diets containing 1.0% CS powder as compared to the control (Table 2). In line with the present findings, Al-Kassi (2010) reported that supplementation of cumin seed powder at 1.0% level resulted in improved body weight gain in broiler chicks. Similarly, Shafiee *et al.* (2020) reported that supplementation of CS powder at 0.25 and 0.75% in the diet had resulted in increased ($P<0.01$) body weight in broiler chicken. The increased body weight gains observed in quails upon feeding CS powder in the diet might be attributed to the biological functions of cumin that are essential for growth. Further, it can be attributed to its stimulant, carminative, digestive and antimicrobial properties. On the other hand, the slight depression in growth rates observed at higher levels (2.0%) of inclusion could be attributed to the damage caused to the intestine, liver and kidneys as explained by the mechanisms by which the plant constituents may cause a damage to body tissues (Ibrahim *et al.,* 2007). Similarly, increased body weight gains upon feeding diets containing CS powder were reported by Al-Anbari *et al.* (2013), Elagib *et al.* (2013) and Rafiee *et al.* (2014) in broiler chicken and Ali *et al.* (2018) in laying Japanese quail. In contradiction to the present findings,

Ali *et al.* (2011) reported decreased (P<0.05) body weight gain in broilers fed CS at 0.2% level in the diet as compared to the control. However, no effect (P>0.05) on body weight gain upon feeding CS powder in the diet were also reported earlier in broilers (Sharifi *et al.,* 2013; Alimohamadi *et al.,* 2013; Berrama *et al.,* 2017; Amiri *et al.,* 2020; Cetinkaya and Filik, 2020) and laying hens (Saleh *et al.,* 2020).

Inclusion of CS powder up to 2.0% level in the diet had no effect (P>0.05) on feed intake of quails as compared to those fed control diet (Table 2).These results agree with the findings of Shafiee *et al.* (2020) who reported that supplementation of cumin seed powder at 0.25 and 0.75% levels in the diet had no effect (P>0.05) on feed intake in broiler chickens. Similarly, no effect (P>0.05) on feed intakes upon feeding diets containing cumin seed powder were also reported earlier in broiler chicken (Sharifi *et al.,* 2013; Elagib *et al.,* 2013; Alimohamadi *et al.,* 2014; Berrama *et al.,* 2017; Amiri *et al.,* 2020; Cetinkaya and Filik, 2020) and in laying Japanese quail (Ali *et al.,* 2018). In contrast, Rafiee *etal*. (2014) reported significantly increased (P<0.05) feed intake in broiler chicks fed cumin powder at 0.2% in the diet as compared to those receiving control diet while Saleh *et al.* (2020) reported decreased (P<0.05) feed intake in laying hens when cumin seed meal replaced wheat bran at 0, 50 and 100% wheat bran (4% in total ration) in the diet.

The FCR improved (P<0.01) significantly in quails with inclusion of CS powder up to 2.0% level in the diet as compared to the control (Table 2).The feed consumed/kg gain was lower (P<0.01) in quails fed diets containing CS powder at 1.0% level as compared to the other treatments (Table 2). In corroboration, Shafiee *et al.* (2020) reported significantly lower (P<0.05) feed consumed/kg gain in broiler chicken fed cumin seed powder up to 0.75% in the diet. The improved feed utilization upon feeding CS powder might be attributed to the improved gut function and balance of intestinal micro-flora resulting in enhanced efficiency and utilization of feed (Johri, 2011). Thus, better FCR in cumin fed birds may be attributed to its nutrient sparing effect. Further, the positive effect of cumin seed powder on body weight gain and the feed conversion ratio could be related to the increased efficiency of feed utilization. Similarly, several authors reported improved FCR upon feeding diets containing cumin seed powder (Mansoori *et al.,* 2006;Elagib *et al.,* 2013; Almohamadi

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

Constituent	T_1	T_2	T_3	T_4	T_5
Maize	47.8	47.4	47.1	46.75	46.5
De-oiled rice bran	5.80	5.85	5.80	5.80	5.65
Soybean meal	39.05	38.90	38.75	38.60	38.5
Fish meal	5.00	5.00	5.00	5.00	5.00
Cumin powder	0.00	0.50	1.00	1.50	2.00
Di-calcium phosphate	0.20	0.20	0.20	0.20	0.20
Shell grit	1.20	1.20	1.20	1.20	1.20
Salt	0.25	0.25	0.25	0.25	0.25
Trace mineral mixture	0.15	0.15	0.15	0.15	0.15
Feed additives	0.55	0.55	0.55	0.55	0.55
Total	100	100	100	100	100
Crude protein (%)	26.79	26.73	26.67	26.65	26.73
ME kcal/kg (calculated)	2900.91	2900.03	2900.31	2900.00	2901.10
Proten: Energy ratio	120.87	120.84	120.85	120.83	120.88
Feed cost/100 kg (₹)	2992.5	3089.8	3187.6	3285.1	3384.6

*Calculated value

Table 2. Effect of inclusion of cumin seed powder at varying levels in the diet on growth performance of quails

Treatment	Body weight gain (g)	Feed intake (g)	Feed conversion ratio	Performance index (g)	Protein efficiency ratio	Feed cost per kg gain (₹)
T_1	175.37^{a}±8.11	627.67±26.39	3.58^{c}±0.03	49.00^{a}±2.51	1.03^{a}±0.01	107.42^{bc}±0.89
T_2	185.63^{ab}±6.01	620.37±12.76	3.34^{b}±0.04	55.56^{ab}±2.46	1.10^{b}±0.01	103.34^{ab}±1.22
T_3	196.25^{b}±5.67	613.10±10.07	3.13^{a}±0.04	62.84^{b}±2.62	1.19^{c}±0.02	99.73^{a}±1.34
T_4	180.07^{ab}±4.22	603.33±9.13	3.35^{b}±0.04	53.75^{a}±1.80	1.09^{b}±0.01	110.12^{c}±1.22
T_5	173.75^{a}±4.63	595.57±8.52	3.43^{b}±0.06	50.72^{a}±2.14	1.06^{ab}±0.02	116.12^{d}±1.88
SEM	3.11	6.43	0.04	1.56	0.02	1.59
SS	*	NS	**	*	**	**

^{a,b,c}Values in column bearing different super scripts differ significantly; **(P<0.01); *(P<0.05); NS – Non significant

et al., 2014; Rafiee *et al.,* 2014; Berrama *et al.,* 2017; Saleh *et al.,* 2020) in broiler chicken. However, Sharifi *et al.* (2013) and Cetinkaya and Filik (2020) reported that feeding cumin seed powder in the diet had no effect (P>0.05) on FCR in broiler chicken.

The performance index (P<0.05) and protein efficiency ratio (P<0.01)) were higher in quails fed diets containing 1.0% cumin seed powder as compared to the control (Table 2). The increased PIa nd PER observed in quails with inclusion of CS powder at 1.0% level in the diet may be attributed to the increased body weight gains, reduced feed intakes and improved feed consumed/kg gain in quails at 1.0% inclusion level as observed in the present study.

Inclusion of CS powder at 1.0% level in the diet resulted in increased (P<0.01) carcass yield (g), dressing percent and ready to cook yield (g) of quails as compared to the control (Table 3). The increased carcass yield (g), dressing % and ready to cook yield observed in quails upon feeding cumin seed powder in the diet might be attributed to the fact that aromatic plants in poultry have stimulatory effects on the digestive system by increasing the production of digestive enzymes and improving utilization of digestive products *via* enhanced liver function (Hernandez *et al.,* 2004). However, several authors reported that feeding cumin seed in the diet had no effect (P>0.05) on carcass yield (Sharifi *et al.,* 2013; Habibi *et al.,* 2016; Rafeeq *et al.,* 2016; Berrama *et al.,* 2017; Cetinkaya and Filik, 2020) and dressing percentage (Elagib *et al.,* 2013) in broiler chicken. Inclusion of cumin powder up to 2.0% level in the diet had no effect (P>0.05) on weight

Table 3. Effect of inclusion of cumin seed powder at varying levels in the diet on carcass characteristics of quails

Treatment	Carcass yield (g)	Dressing %	Ready to cook yield (g)	Heart(g)	Liver(g)	Gizzard (g)	Giblet (g)
T_1	111.86^{ab}±3.81	62.42^{a}±0.43	122.97^{a}±4.34	2.18±0.10	4.35±0.27	4.59±0.19	11.12±0.55
T_2	118.32^{b}±2.91	63.25^{ab}±0.29	129.72^{a}±3.39	2.27±0.07	4.43±0.21	4.70±0.22	11.40±0.50
T_3	127.54^{c}±2.55	64.31^{b}±0.50	139.53^{b}±2.82	2.34±0.07	4.77±0.10	4.89±0.11	11.99±0.27
T_4	115.66^{ab}±3.19	63.21^{ab}±0.32	126.85^{a}±3.54	2.20±0.04	4.38±0.13	4.62±0.18	11.19±0.35
T_5	109.01^{a}±1.59	62.08^{a}±0.32	119.93^{a}±1.78	2.16±0.02	4.29±0.08	4.47±0.10	10.91±0.19
SEM	1.69	0.21	1.88	0.03	0.08	0.07	0.18
SS	**	**	**	NS	NS	NS	NS

^{a,b,c}Values in column bearing different super scripts differ significantly; **(P<0.01); *(P<0.05), NS – Non significant

of heart, liver, gizzard and giblet (g) in Japanese quails (Table 3). These results agree with Rafeeq *et al.* (2016) who reported that supplementation of cumin at 0.5 and 1.0% level in the diet had no effect (P>0.05) on weight of heart, liver and gizzard in broiler chicken. Similar findings were also reported earlier (Al-Kassie *et al.,* 2011; Sharifi *et al.,* 2013; Habibi *et al.,* 2016; Berrama *et al.,* 2017).

Inclusion of CS powder at 1.0% level in the diet had resulted in significant (P<0.01) decrease in the feed cost/kg gain in quails as compared to those fed diets containing CS powder at 0, 1.5 and 2.0% levels. The study indicated that the feed cost/kg gain decreased by ₹ 4.08 in T_2 and 7.69 in T_3 while it is increased by 2.70 in T_4 and 8.70 in T_5 groups of quails fed diets containing varying levels of CS as compared to the control (T_1).

CONCLUSION

It was concluded that cumin seed powder can be included at 1.0% level in the diet of quails for improved growth and decreased cost of production.

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Growth and Non-specific Immunity Parameters of Rohu Fed with a Floating Feed Prepared from Locally Available Ingredients (CIFA-Carp Grower)

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ABSTRACT

A Grower feed carp (CIFA-Carp Grower) was formulated and prepared using locally available ingredients, minerals (with nano-selenium and nano-zinc) and vitamins mixture through extrusion technology and it's performance was compared with an existing feed used by farmers of eastern region. The developed feed contains 28 percent crude protein (CP) and 4 percent crude fat. The feed has been commercialized, IP protected and reached to aquaculture farmers covering about 10-12 states of India. As per the scheduled methodology of demonstration, rohu fingerlings (average 105 g) were stocked randomly into triplicate ponds for both control and treatment following a completely randomised design and stocking density of 7500/hectre was maintained for each pond. The fish of control ponds were fed with a mixture of groundnut oil cake (GNOC) and rice bran (50:50) with minerals and vitamins mixture where as fish of treatment ponds were fed with a grow out feed developed by ICAR-CIFA(CIFA-Carp Grower). During the experimental period of 10 months, growth, survivability, feed conversion ratio (FCR) and non-specific immunity parameters of rohu fed with CIFA-Carp Grower were recorded and compared with rohu fed with an existing feed used by farmers of this region. The average size of fish after feeding with CIFA- Carp Grower were 1088 gm at 10 months of feeding with average feed conversion ratio (FCR) of 1.290 and it was better than existing/traditional method of feeding. The results of the demonstration showed that weight gain, survivability, feed convetrsion ratio (FCR) and nonspecific immunity parameters of grow out rohu fed with CIFA-Carp Grower was better as compared to existing feed practiced by many farmers in this region. So, CIFA- Carp Grower may be popularised among farmers, entrepreneurs and researchers for aquaculture development of the whole country.

Key words: CIFA-Carp Grower, Immunity, Floating feed, Growth performance, Rohu

INTRODUCTION

The extrusion process improves nutrient digestibility, palatability, pellet durability, water stability and pellet storage life (Barrows and Hardy, 2000). It also increases *in vivo* digestibility of dry matter and energy of feed in rainbow trout *Oncorhynchus mykiss* (Cheng and Hardy, 2003). Good quality floating feed on locally available ingredients could be produced through extrusion technology by maintaining extrusion temperature of 130°C and moisture of 20 percentages (Das *et al.,* 2018). It has been experimented that, the quality of til oil cake based floating feed for *Labeo rohita* are superior as that of soybean meal based floating feed and that is also economical for the farmers (Das *et al.,* 2016).

ICAR-CIFA, Bhubaneswar has developed a grower feed for carp (CIFA-Carp Grower) out of locally available ingredients through extrusion technology. This is a floating feed for carp which ensures better growth, improved FCR, higher palatability to fish, disease resistance and production of tasty fish. This is suitable for carp grower to enhance their production and profitability. It contains 28 percent crude protein, 4% fat, and nano zinc and nano selenium. This feed is suitable for carp growers to enhance their production and profitability. This feed has been IP protected and commercialized for increasing aquaculture production and productivity in the country and have been reached to aquaculture farmers covering about 10-12 states of India. However, farmers still use traditional method of practices and a survey revealed that 65.4 percent of Indian major carp farmers in Andhra Pradesh used mash feed as their sole feed source(Ramakrishna *et al.,* 2013). In this experiment,

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pelleted floating feed was produced through extrusion technology and performance evaluation was done in terms of growth, feed efficiency and immunological parameters by comparing with an existing mash feed used by farmers of this region.

MATERIALS AND METHODS

Fish feed was formulated using locally available feed ingredients *i.e.* maize, soyabean meal, til oil cake (TOC), groundnut oil cake (GOC), mahua oil cake, de-oiled rice bran (DORB) and minerals (with nano selenium and nano-zinc) and vitamin mixture as per requirement. The extruded floating feeds were produced in the feed mill facilities of ICAR-CIFA, Bhubaneswar. The ingredients were pulverized, mixed, extruded and then dried (Fig 1). Experimental feed were then analyzed for physical and chemical characteristics to ascertain the quality of the feed (Tables 1 and 2).

Fig 1. CIFA-Carp Grower developed by ICAR-CIFA

CIFA-Carp Grower after production was transported and demonstration was done in grow out ponds of KVK, Ranital, Bhadrak. Odisha. The demonstration was carried out as per scheduled methodology. Rohu fingerlings (average 105 g) were stocked randomly into triplicate ponds for both control and treatment following a completely randomised design and stocking density of 7500/hectre was maintained for each pond. The fertilizer was applied as per normal schedule. The fish of control ponds were fed with mash feed used by farmers (ground nut oil cake and rice bran mixture, 50:50 supplemented with minerals and vitamin mixture) whereas fish of treatment ponds were fed with CIFA-Carp Grower developed by ICAR-CIFA. The experiment was continued for 10 months period (Fig 2).

Table 1. Physical characteristics of CIFA-Carp Grower

Parameters	%
Floating percentage	100
Sinking percentage	0
Apparent Density (gram/ cubic cm)	0.96

Table 2. Chemical characteristics of CIFA-Carp Grower

Parameters	% % on DM basis
Crude protein (CP)	28.00
Crude fibre (CF)	5.42
Ether extract (EE)	4.00
Nitrogen free extract (NFE)	52.00
Energy (kcal/g)	3.51

Feed offered was recorded on regular basis and then calculated at the end of the experiment to record feed intake. A group of fifteen numbers of fishes in each pond were batch weighed randomly once in every month till completion of experiment to estimate the average weight and biomass of fish in each pond. The survivability was checked after harvesting the fish after experiment. The water quality parameters were checked regularly and found to be optimum. Blood was collected from tail vein of five fish samples in each pond n=5 for control and treatment to study the immunological parameters.

Non-specific immune parameters like lysozyme, myeloperoxidase, bacterial agglutination, haemagglutination and haemolytic activity were estimated in rohu fed on existing and developed fish feed (CIFA-Carp Grower). For lysozyme assay, a 130 ìl of freshly prepared Lyophilized *Micrococcus lysodeikticus* (Sigma, USA) at a concentration of 0.6 mg/ml (in 0.02 M sodium citrate buffer) was added to a mixture containing 10 ìl fish serum samples and 10 ìl of 0.02 M sodium citrate buffer (Ellis, 1990). After immediately adding bacterial solution, the initial OD was read at 450 nm. The OD of the samples was measured at 450 nm

Fig 2. Sampling of fish in Bhadrak, Odisha during demonstration of CIFA-Carp Grower

after 1 hour of incubation at 24°C. Using a mixture of 20 ìl working standard and 130 ìl *M. lysodeikticus* solution, a standard curve was created. Lysozyme activity was measured in units/ml, with one unit equaling a 0.001/min decrease in absorbance. Myeloperoxidase activity was performed as described by Quade and Roth (1997). In brief, fish serum (15 ìl) was diluted in 135 ìl of Hank's balanced salt solution (Ca^{2+}, Mg^{2+} free) and further, 50 ìl of 20 mM of 3, 32, 5, 52 tetramethylbenzidine and 5 mM of hydrogen peroxide were added in the same well. The mixture was incubated for 2 min at room temperature. The final reaction was stopped by the addition of 4 M sulphuric acid and the optical density (OD) was read at 450 nm using the UV VIS Spectrophotometer, Thermo Spectronic, UK. Bacterial agglutination activity was carried out in a "U" shaped microtitre plate as described by Swain *et al.* (2018). In brief, two-fold serial dilution of 25 ìl serum sample was done in the titre plate with an equal volume of normal saline solution (NSS) in each well. A 25 ìl of formalin killed *Aeromonas hydrophila* (10^7 cells/ml) suspension was then added to each of the wells. After an overnight incubation at 37°C, the titre was calculated as the reciprocal of the highest dilution of serum showing complete agglutination of the bacterial cells. The haemagglutination activity was carried out according to procedures of Blazer and Wolke's (1984). A two-fold serially diluted 25 ìl fish serum sample was inactivated at 45°C for 30 minutes and combined with an equivalent amount of NSS in a "U" shaped microtitre plate. The wells were then filled with a freshly manufactured (25 ìl) 1 percent New Zealand white rabbit red blood cell (RBC) suspension. The titre was estimated as the reciprocal of the greatest dilution of serum demonstrating complete agglutination of RBCs after 2 hours of incubation at room temperature. The hemolytic activity was carried out as described for HA titre (Blazer and Wolke, 1984). But here, plates were kept in room temperature for overnight and the HA titre was presented as the reciprocal of the highest dilution of serum showing complete hemolysis of rabbit RBCs.

The physico-chemical parameters of the water were routinely monitored. The temperature, pH, total alkalinity and total hardness of water were 25-30 ºC, 7.5-8.5, 100-140 ppm, and 100-130 ppm, respectively in both control nad treatment ponds. All the data of the experiment were statistically analyzed by using statistical software (Prism, version 4.0, Graph Pad Software, San Diego, CA, U.S.A). Values were expressed as standard error of the mean (SEM) and P values of <0.05 were considered significant.

RESULTS AND DISCUSSION

The Extrusion technology is the primary technique now-a-days used in aquatic feed industry for fish feed production to maintain high physical and nutritional quality of the feed (Hilton *et al.,* 1981). It is a high-temperature-short-time heating process that minimizes the degradation of food nutrients while improving the digestibility of protein and starches. It has

been reported that, aquatic animals cannot digest starch effectively resulting in excessive excrement which causes physiological problems such as excessive gas, bloating diarrhea and these apart from affecting the growth of the fish also lead to water pollution. Starch in the feed is gelatinized and is effectively utilised by this technology for production of floating fish feed. Floating fish feed comes with a host of advantages in terms of digestion, growth, water protection, zero water pollution, optimized labor usage and zero wastage of raw materials (Hashimoto *et al.,* 2003). The farmer can directly observe the feeding requirements and adjust feeding rates accordingly. It has been experimented that, floating feed could be prepared successfully by inclusion of locally available ingredients. However, maintenance of appropriate temperature, pressure and moisture during extrusion is the key step in production of quality fish feed from locally available feed resources in spite of the fact that, it varies on the type of ingredients and extruder used for floating feed production. The basic concept of extrusion process is high temperature, short time, whereby the high temperature is a direct result of friction or pre-conditioning and steam injection or a combination of both (Levic, 2010) and it has become popular for aqua feed production (FMT, 2005). The feed is floated for 2 to 3 hours which indirectly reduced the wastage of feed as compared to mash or sinking feed. Higher feed utilization as reported for extruded feed was responsible for improved growth performance in growing carp (Das *et al.,* 2018). CIFA-Carp Grower was produced through extrusion technology as sufficient starch was there for gelatinization of starch.

The growth performance of rohu fed with existing feed and CIFA-Carp Grower are presented in table 3. The weight gain of rohu fed with CIFA-carp Grower (treatment) was significantly higher ($P<0.05$) as compared to rohu fed with existing feed (control). The feed conversion ratio (FCR) of the fishes was calculated from feed intake and weight gained (g/day) during the experimental period, and the result indicated the superior FCR ($P<0.05$) of CIFA-Carp Grower as compared to existing mash feed. The survival percentage of fish fed with CIFA-Carp grower was higher ($P<0.05$) compared to fish fed with existing mash feed. The yield and net return was higher by feeding with CIFA-Carp Grower compared to other feed indicating higher profits to farmers adopting the technology. Use of locally available ingredients provides a variety of nutrients for fish in addition to growth promoting effects of some local ingredients. Local ingredients are not only economical but also suitable in Indian agro climatic conditions. Many locally available ingredients were included for production of CIFA-Carp Grower along with conventional ingredients (Das *et al.,* 2016 ;Das *et al.,* 2021). The feed was also supplemented with mineral mixture containing nano selenium and nano zinc which was responsible for higher growth performance, immunity and muscle quality (Swain *et al.,* 2019). There may be balancing of amino

Table 3. Growth and production performance of rohu fed with existing feed (control) and CIFA-Carp Grower (treatment)

Parameters	Control	Treatment	SEM	P value
Initial weight (g/fish)	105	105	5.858	>0.9999
Final weight (g/fish)	890[a]	1088[b]	43.49	0.0061
Weight gain (g/fish)	785[a]	983[b]	38.1	0.0001
Feed intake (g/fish)	2261[a]	1268[b]	191.2	0.0001
FCR	2.879[b]	1.290[a]	0.303	0.0001
Survival (%)	78[a]	82[b]	0.982	0.0255
Yield(q/ha)	52.10[a]	67.00[b]	2.984	0.0004
Net Return (₹/ha)	2,74000[a]	3,70000[b]	21733	0.0002

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

Table 4. Non-specific immunity parameters of fish fed with existing feed (control) and CIFA-Carp Grower

Parameters	Control	Treatment	P value	SEM
Bacterial agglutination(log 2)	2.56[a]	3.10[b]	0.0012	0.1240
Lysozyme (ì g/ml)	9.36[a]	10.30[b]	0.0003	0.1966
Myeloperoxidase (OD)	0.656[a]	0.950[b]	0.0039	0.0694
Haem-agglutination (log 2)	2.70[a]	3.12[b]	0.0075	0.1012
Haemolytic activity (log 2)	2.58[a]	2.81[b]	<0.001	0.0497

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

acids like methionine, lysine and arginine in carp grower compared to feed of control group. It has also been reported that the feed prepared by using more than one protein sources always resulted in better growth of fish due to proper balancing of amino acids (Djissou *et al.,* 2016, Gaylord *et al.,* 2017).

Non-specific immunity parameters of fish like bacterial agglutination, lysozyme, myeloperoxidase, haem-agglutination and haemolytic activity fed on existing feed (control) and CIFA-Carp Grower (treatment) is presented in Table 4. Fish fed on CIFA-Carp Grower had significantly higher levels of lysozyme, myeloperoxidase, haemaglutination, bacterial agglutination, and haemolytic activity than fish fed on existing feed. Increased non-specific immune measures in treatment group of fish indicate that fish fed on CIFA-Carp Grower had higher immunity responses than fish fed on existing feed used by farmers. In this experiment, fish fed on CIFA-Carp Grower had significantly higher levels of lysozyme, myeloperoxidase, haemaglutination, bacterial agglutination, and haemolytic activity than fish fed on existing feed. Increased immune parameters like lysozyme, myeloperoxidase and hem-agglutination are indication of improved immunity responses in fish (Panigrahi *et al.,* 2004; Kim and Austin, 2006). Fish use lysozyme as one of their main defence mechanisms against pathogenic infection (Feng *et al.,* 2011). Myeloperoxidase activity is used to assess neutrophil antimicrobial activity. Increased non-specific immune parameters in fish fed with CIFA-Carp Grower proved that, CIFA-Carp Grower resulted improved disease resistance in fish compared to existing feed used by farmers.

CONCLUSIONS

The results of the demonstration showed that, the weight gain, survivability, feed conversion ratio (FCR) and immunity parameters of grow out rohu fed with CIFA-Carp Grower was higher compared to existing feed practiced by many farmers of eastern region. This is suitable for carp grower to enhance their production and profitability. This feed may be popularized for aquaculture development of the country.

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Growth and Carcass Characteristics of Japanese Quails Fed Diets Containing Varying Levels of Moringa Leaf Meal

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ABSTRACT

The present experiment was conducted to evaluate the effect of incorporation of Moringa Leaf Meal (MLM) in the diet on growth and carcass characteristics of Japanese quails. One hundred and fifty day-old chicks were randomly allotted to five dietary treatment groups with three replicates of 10 birds each for 5 weeks. Moringa leaves were dried, ground and incorporated at 0 (T_1; Control), 1.5% (T_2), 3.0% (T_3), 4.5% (T_4) and 6.0% (T_5) per cent levels, respectively, in iso-caloric and iso-nitrogenous broiler quail diets to meet the requirements as per NRC (1994). Results indicated a significant increase ($P<0.01$) in body weight gain, overall feed intake and improved ($P<0.01$) FCR with incorporation of MLM at 3.0 % in the diet compared to other treatment groups. There was no significant difference in the carcass traits with respect to dressing percentage, meat: bone ratio and percentage of heart, liver, gizzard or giblet. However, incorporation of MLM at 3.0% level had increased carcass yield and ready to cook yield ($P<0.05$) compared to control group. The feed cost/kg gain decreased by ₹ 7.27 in T_3 as compared to the control (T_1). Thus, it is concluded that MLM can be incorporated at 3.0% level in the quail diets for economical production without any adverse effects.

Key words: Carcass characteristics, Growth, Moringa leaf meal, Quail

INTRODUCTION

Poultry production sectors in developing countries are facing magnitude of problems, one of which is an increase in the cost of feed due to high prices of protein and energy sources. Feeds and feeding are an integral part of broiler production that claims between 60-70% of total cost of production and at the same time, it dictates the production strength and quality. This also exerts impact on quail farming. Commercial quail farming is becoming popular in the recent years in most of the Asian countries. Quail farming is economically viable and technically feasible because quails are quite resistant to various diseases, early sexual maturity (6 weeks of age) and easily adapt to various rearing conditions (RSPCA, 2011). Quail meat and egg are known for their high-quality protein, high biological value and low caloric content. Quail eggs are rich in essential minerals, vitamins and the nutritional value is three to four times greater than chicken eggs. Quail meat is reported to be tastier than chicken and promotes body and brain development in children (Igado and Aina, 2010). The high and increasing prices, especially of protein and energy ingredients for animal feeds have compelled researchers to direct their attention to cheap, available and safe alternative sources. The possible alternative source to protein is the leaf meal of tropical legumes. Leaf meals serve as source of protein and also provide some necessary vitamins, minerals and oxycarotenoids (D'Mello *et al.,* 1987). One such leaf meal, which could be of value for poultry feeding, is the leaves of Moringa.

Moringa oleifera belonging to the family Moringaeceae is an effective remedy for malnutrition. Moringa has for long been consumed by humans and every part of the tree, from the roots to the leaves has beneficial properties, which are used as fodder, herbal medicine, spices, food, natural coagulants, nectar to bees, fuel and fertilizer. Moringa contains very high antioxidants and anti-inflammatory compounds. The tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, calcium, iron, vitamin C and carotenoids. Moringa leaves have a traditional application for its antimicrobial and pharmacological properties (Gakuya *et al.,* 2014). These excellent nutritional and medicinal characteristics would

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make it suitable forage for feeding animals (Nuhu, 2010). The leaves of moringa are potentially inexpensive protein for livestock feeding with a protein content ranging from 25.1 to 29.7 per cent (Makkar and Becker, 1996; Kakengi *et al.,* 2003). Moringa leaf meal is reported to improve the growth performance and carcass quality of broilers (Banjo, 2012; David *et al.,* 2012). Hence, the present study was undertaken to evaluate the effect of incorporation of moringa leaf meal in the diet on the growth and carcass characteristics in quails.

MATERIALS AND METHODS

The present experiment was carried out at the Poultry Experimental Station attached to the Livestock Farm Complex, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India. Moringa leaves were collected form trees in the college campus, dried under shade and ground in a Wiley mill to prepare Moringa leaf meal.

One hundred and fifty day-old quail chicks were procured and randomly allotted into 5 groups, each with 3 replicates of 10 chicks. Chicks were wing banded and weight of the chick was recorded. The experiment was carried out for 5 weeks in a completely randomized design (CRD). During the experiment, MLM was included at 0 (T_1; Control), 1.5 (T_2), 3.0 (T_3), 4.5 (T_4) and 6.0 (T_5) per cent levels in iso-caloric and iso-nitrogenous quail diets (Table 1) formulated to meet nutrient requirements as per NRC (1994). All the chicks were housed in battery brooders throughout the experiment. All the cages were provided with uniform brooding facilities, feeders and waterers. Irrespective of the treatments, all the chicks were fed ad-libitum 2 times a day with respective broiler quail diets. Fresh and clean drinking water was made available at all the time. B-complex vitamins were offered in water for 3 days during first week. Except for feeding experimental diets, other managemental practices were uniform throughout the experimental period. The individual body weight of the quails was recorded at weekly interval up to 5 weeks of age. The feed offered and feed leftover was weighed daily to quantify the feed consumption. Weekly feed consumption was recorded replicate wise and feed efficiency was calculated accordingly.

At the end of study period (5th week), two birds per replicate and thus a total of 6 birds per experimental diet were randomly selected, weighed and slaughtered.

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

Parameter	T_1	T_2	T_3	T_4	T_5	Cost/kg (₹)
Maize	50.00	48.67	47.67	46.33	45.33	13.60
DORB	8.67	9.00	9.67	10.33	10.67	10.00
Soybean meal	34.00	33.33	32.67	32.00	31.33	38.00
Fish meal	5.00	5.00	5.00	5.00	5.00	60.00
Moringa leaf meal	0.00	1.50	3.00	4.50	6.00	5.00
DCP	0.20	0.20	0.20	0.20	0.20	26.0
Shell grit	1.25	1.25	1.25	1.25	1.25	2.00
Salt	0.25	0.25	0.25	0.25	0.25	3.50
Trace min mix	0.15	0.15	0.15	0.15	0.15	240
Feed additives	0.55	0.55	0.55	0.55	0.55	1116.00
Total	100	100	100	100	100	
ME* kcal/kg	2900.27	2900.51	2900.09	2900.17	2900.18	
Crude protein	23.99	24.02	24.00	23.99	24.01	
Feed cost/100 kg (₹)	25423	25119	24814	24506	24202	

*Calculated value

The data on dressing percentage, carcass yield, meat bone ratio, ready-to-cook yield and per cent weight of heart, liver, gizzard and giblet were recorded. All the data were analyzed statistically (SPSS, 17th Version) as per Snedecor and Cochran (1994) and comparison of means was done using Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Moringa leaf meal used in the present study contained 93.86, 86.11, 24.61, 8.56, 14.53, 38.41, 13.89 and 0.35 DM, OM, CP, EE, CF, NFE, TA and AIA, respectively. The chemical composition of the experimental diets containing varying levels of MLM is presented in Table 1. Data pertaining to body weight gain, feed intake and feed conversion ratio (FCR) under different dietary treatments are presented in Table 2. The mean body weight gain (g) was significantly higher (P<0.01) in quails fed diets containing 3.0% MLM as compared to control and other dietary treatments. The improved weight gain observed in MLM fed groups can be attributed to higher protein quality of the diets which were efficiently metabolized for growth (Olugbemi *et al.,* 2010) and decreased weight gain at higher inclusion level may be attributed to higher crude fibre content which may impair nutrient digestion and absorption (Onu and Aniebo, 2011). The result of the present investigation was in agreement with earlier reports which indicate increased body weight gain in broilers (Hasan *et al.,* 2016; Khan *et al.,* 2017; Kumar *et al.,* 2018) and quails (Elkloub *et al.,* 2015; Mahmud *et al.,* 2016) as a result of incorporation of MLM in the diet. On the other hand, some researchers (Tesfaye *et al.,* 2013; Al-Bahouh *et al.,* 2017) reported decreased (P<0.05) body weight gain in broiler chicken fed MLM as compared to the control. However, Onunkwo and George (2015) and Elbashier and Ahmed (2016) reported no effect (P>0.05) on body weight gain in broilers fed Moringa leaf meal.

Feed intake increased linearly from T_1 to T_3 and decreased up to T_5 with increased level of inclusion of MLM in the diet and the differences between the treatments were significant (P<0.01). In line with the present findings, El-Tazi and Tibin (2014) reported increased feed intake at 5.0% level, while decreased at 7.0% level. Decreased feed intake with increased level of MLM may be due to the increased bulk and lower metabolizable concentration (Olugbemi *et al.,* 2010) and decreased palatability at higher levels (Kakengi *et al.,* 2003). In contradiction to the present findings, several authors reported significantly (P<0.05) increased (Hassan *et al.,* 2016; Mousa *et al.,* 2017; Kumar *et al.,* 2018) and decreased (Makanjuola *et al.,* 2014; Agashe *et al.,* 2017) feed intakes in broilers fed varying levels of MLM in the diet. On the other hand, Onunkwo and George (2015) and Mahmud *et al.* (2016) reported no effect (P>0.05) on feed intake in broilers fed MLM in the diet.

Inclusion of MLM at varying levels from 0 to 6.0% in the diet had significant effect (P<0.01) on feed conversion ratio in quails with better FCR reported at 3.0% level. The improvement in FCR at 3.0% level may be attributed to rich content of nutrients in Moringa leaf meal (Kakengi *et al.,* 2003), antimicrobial properties of Moringa (Fahey *et al.,* 2001) and adequate utilization of diets by the birds. In line with the present findings, Kumar *et al.* (2018) reported better FCR at 5% level of moringa leaf compared to 10, 15, 20% and control. Similarly, improved FCR was reported by Nkukwana *et*

Table 2. Effect of inclusion of MLM at varying levels on body weight gain, feed intake, FCR and feed cost/kg gain in quails

Parameter	T_1	T_2	T_3	T_4	T_5	SEM	P Value
Body weight gain (g)	170.61^{ab}±1.22	179.49^{cd}±0.76	185.53^{d}±2.86	176.16^{bc}±1.64	166.80^{a}±4.17	1.91	P<0.01
Overall feed intake (g)	555.93^{ab}±1.50	562.93^{bc}±1.14	564.88^{c}±3.05	561.76^{bc}±2.99	551.49^{a}±1.81	1.66	P<0.01
Feed conversion ratio	3.26^{c}±0.03	3.14^{ab}±0.02	3.05^{a}±0.04	3.19^{bc}±0.04	3.31^{c}±0.04	0.03	P<0.01
Feed cost/kg gain (₹)	82.84^{c}±0.82	78.79^{b}±0.47	75.57^{a}±1.04	78.18^{b}±1.02	80.05^{bc}±1.74	0.72	P<0.01

Table 3. Effect of inclusion of MLM at varying levels in the diet on carcass characteristics of quails

Parameter	T_1	T_2	T_3	T_4	T_5	SEM	P Value
Carcass yield (g)	116.17$^{ab\pm}$4.25	120.00$^{ab\pm}$3.10	125.67$^{b\pm}$4.11	121.00$^{ab\pm}$2.25	114.67$^{a\pm}$2.80	1.58	P<0.05
Dressing (%)	64.16±2.52	63.98±0.97	64.38±0.81	61.66±0.91	61.04±1.53	0.67	NS
Ready to cook yield (g)	124.33$^{ab\pm}$4.34	128.63$^{ab\pm}$3.16	134.58^{b}±3.91	129.45^{ab}±2.23	122.58$^{a\pm}$2.77	1.60	P<0.05
Meat: bone ratio	3.67±0.32	3.74±0.11	4.15±0.26	4.15±0.26	3.38±0.08	0.12	NS
Heart (%)	1.50±0.22	1.58±0.27	1.67±0.21	1.53±0.21	1.45±0.26	0.10	NS
Liver (%)	2.33±0.21	2.55±0.22	2.67±0.21	2.50±0.22	2.30±0.19	0.09	NS
Gizzard (%)	4.33±0.21	4.50±0.22	4.58±0.20	4.42±0.27	4.17±0.31	0.11	NS
Giblet (%)	8.17±0.31	8.63±0.19	8.92±0.33	8.45±0.43	7.92±0.59	0.18	NS

al. (2014) and Hassan *et al.* (2016), in broiler chicks and Elkloub *et al.* (2015) in Japanese quails. However, no effect (P>0.05) on feed conversion ratio was reported by several authors (Makanjuola *et al.,* 2014; Mousa *et al.,* 2017) in broiler chicks.

Data pertaining to carcass characteristics are presented in Table 3. Incorporation of MLM at 3.0% level in the diet of quails resulted in increased (P<0.05) carcass yield (g) and ready to cook yield (g) as compared to control and other treatments. These results corroborated with the findings of Kumar *et al.* (2018) who reported that carcass weight (g) was significantly (P<0.05) higher in broilers fed 15% MLM in the diet. On the other hand, Ayssiwede *et al.* (2011) reported no effect (P>0.05) on relative weight of carcass fed MLM up to 24.0% level in the diet of broilers. Incorporation of MLM up to 6.0% level in the diet of quails had no effect (P>0.05) on dressing percentage and meat: bone ratio in the current study. Zanu *et al*. (2012) and Liaqat *et al*. (2016) also reported that feeding broilers with diets containing MLM at varying levels had no effect (P>0.05) on dressing percentage as compared to control. In contradiction, Mousa *et al*. (2017) reported increased (P<0.05) dressing percentage in broilers fed 1.5% MLM in the diet as compared to control. Incorporation of MLM up to 6.0% level in the diet of quails had no effect (P>0.05) on per cent weight of heart, liver, gizzard and giblets which was in agreement with the findings of earlier researchers (Aderinola *et al.,* 2013; Ayo-Ajasa *et al.,* 2016; Liaqat *et al.,* 2016).

The current study indicated that the feed cost/kg gain (₹) decreased by 4.05 in T_2, 7.27 in T_3, 4.66 in T_4 and 2.79 in T_5 group of quails fed diets containing MLM at varying levels as compared to the control (Table 2). The decreased (P<0.01) feed cost/kg gain in T_3 group of quails might be attributed to the better feed efficiency and increased weight gains in quails fed MLM at 3.0% level as compared to the other treatments. Similarly, El-Tazi and Tibin (2014) reported highest profitability ratio (P<0.05) in broiler chicks fed 5.0% MLM in the diet followed by 3.0 and 7.0% as compared to the control.

CONCLUSION

The present study indicated that incorporation of MLM at 3.0% in the diet resulted in increased body weight gain, carcass yield and improved FCR with a concomitant decrease in cost of production. Hence, it would be beneficial to incorporate MLM at 3.0% level in the diet of broiler quails without any adverse effects.

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SHORT COMMUNICATION

Nutrient intake, Digestibility and Rumen Fermentation Parameters in Buffalo Bulls fed Cumbu Napier (COBN-5) Fodder

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ABSTRACT

In the present study, Cumbu Napier (COBN-5) fodder was evaluated for nutrient intake, digestibility and rumen fermentation pattern in buffalo bulls. The average DMI (kg/d) of buffalo bulls was 6.01 and met the requirements for maintenance indicating that the fodder is palatable. The pre cent DCP (8.74) and TDN (56.05) in the fodder consumed met the ICAR standards. The DM, DCP, TDN and ME intake per kg $W^{0.75}$ in buffalo bulls fed *ad libitum* of COBN-5 fodder were 73.03, 6.38, 40.93 and 0.15, respectively. The digestibility co-efficients (%) nutrients in COBN-5 fodder were better as compared to many Napier grass varieties. Positive balance of nitrogen, calcium and phosphorus was observed and sole feeding of the fodder met the requirement of these nutrients n buffalo bulls. Rumen pH values were highest at 0 h and declined to minimum by 6 h post feeding, while TVFA reached peak at 6 h post feeding and NH_3-N reached peak at 2 h post feeding. It was concluded that feeding COBN-5 fodder *ad libitum* to buffalo bulls had no adverse effects.

Key words: COBN-5 fodder, Apparent digestibility, Nutrient utilization, Rumen fermentation

The low productivity of dairy animals in India is attributed to inadequate supplies of quality feeds and fodder. Due to ever-increasing human population, arable land is diverted to production of food and cash crops with little chance for fodder production. There may be many ways to overcome the shortage of forage and one among them is the introduction of high yielding forage varieties (Mahr-un-nisa and Sarwar, 1998). This led to the cultivation of perennial fodders with higher biomass per unit area, an immediate solution to meet the current livestock production. Consequently, high yielding forages including a number of varieties of Napier hybrids have been introduced in many parts of the world, including India. Cumbu Napier (COBN-5) fodder was newly developed hybrid Napier variety by Tamil Nadu Agricultural University (TNAU) at Coimbatore and released for commercial cultivation in 2013 (Babu *et al.,* 2014). It is an inter-specific hybrid between fodder Cumbu IP 20594 (*Pennisetum glaucoma*) and Napier grass FD 437 (*Pennisetum purpureum Schumach*). The characteristic features of COBN-5 include robust tillering perennial grass, high biomass potential round the year, high palatability, high leaf to stem ratio *etc.* Green forages generally meet the requirements of maintenance, however nutritive evaluation of a particular fodder is the need of the hour for their optimum utilization in ruminant feeding. Hence, the present study was undertaken to study the nutrient intake, digestibility and rumen fermentation parameters in buffalo bulls fed COBN-5 fodder.

Six Murrah buffalo bulls (5 years; Avg. BW 357.97 ± 43.36 kg), housed in well ventilated conventional sheds maintained in good hygienic condition were used for the present study. The chopped COBN-5 green fodder was procured from the cultivated fields of Live-stock Farm Complex (LFC), NTR College of Veterinary Science, Gannavaram and fed *ad libitum* to the animals for a period of 66 days. The chopped COBN-5 fodder was offered to animals four times during a period of 24 hours. The animals were weighed at the beginning and ending of the metabolism trial. Every time, weighing was done on two consecutive days before offering feed and water and the average body weight was calculated.

On 58th day of the experimental period, the

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buffaloes were shifted to the metabolism stalls for adaptation (2 days) so as to reach their normal feed consumption. This was followed by metabolism trial of 6 day collection period during which daily feed intake, feed refusals if any as well as faces and urine voided were recorded at 9.00 AM. Representative samples of feed offered, feed residue and faeces voided during collection period were collected and pooled for 6 days, dried and ground in a laboratory Wiley mill through 2 mm screen and preserved in air tight bottles for subsequent analysis. The urine voided by each animal was measured, mixed thoroughly and representative sample (2%) was taken in glass bottle for each animal and stored at 4ºC in a refrigerator after addition of few drops of concentrated H_2SO_4. Samples of fodder and faeces were analyzed for proximate constituents and urine for N according to AOAC (2007) methods and forage fibre constituents (Van Soest *et al.,* 1991). Calcium and Phosphorus in fodder and faeces were estimated following methods of Talapatra *et al.* (1940). Ca and P content of urine samples were determined the methods of determined by Ferro and Ham (1957) and Fiske and Subba Row (1925), respectively.

Table 1. Chemical composition (% DM basis) of COBN-5 fodder

Nutrient	Mean ± SE
Dry matter	17.94±1.09
On % dry matter basis	85.64±1.61
Organic matter	
Total ash	14.36±1.61
Crude protein	13.05±0.51
Ether extract	2.82±0.22
Crude fibre	27.09±4.30
Nitrogen free extract	42.67±1.99
Neutral detergent fibre	67.31±3.03
Acid detergent fibre	40.84±2.41
Hemi-cellulose	26.47±0.44
Cellulose	35.07±2.97
Acid detergent lignin	5.73±2.74
Silica	3.41±0.25
Calcium (%)	0.73±0.20
Phosphorus (%)	0.57±0.10

Three adult rumen fistulated Murrah buffalo bulls (380±9.36 kg BW) maintained on COBN-5 fodder were used to determine the rumen pH, ammonia nitrogen (Livingston *et al.,* 1964) and total volatile fatty acids (Barnett and Reid, 1957). Data obtained were analyzed statistically (Snedecor and Cochran, 1994) using SPSS 17.0 version.

The chemical composition and cell wall constituents of COBN-5 fodder are presented in Table 1. The CP content in the COBN-5 (13.02 %) was similar to the values in Napier grass varieties reported by earlier workers (Garg *et al.,* 2012). The NDF content of COBN-5 fodder in the present study is 67.06 per cent. In comparison, Kebede *et al.,* (2016) reported higher values. The ADL content of fodder in the present study is 6.13 per cent which is in agreement with the reports of Budiman *et al.* (2012). However, lower (Singh *et al.,* 2012) values were reported in a previous study as compared to the value of present study. These differences in proximate composition and cell wall constituents observed in the present study with COBN-5 variety as compared to other bajra×napier varieties might be attributed to the differences in breeding, variety of cultivar used, stage of harvest, type of soil, season, cultivation practices *etc.*

Data pertaining to intake digestibility and nutritive values are presented in Table 2. Perusals of data revealed that sole feeding of COBN-5 fodder supplied adequate amount of DCP and TDN to meet the requirements for maintenance as recommended by ICAR (2013). This indicates that COBN-5 fodder is highly palatable. Abdulrazak *et al.* (1996) and Kariuki *et al.* (1998) reported lower DMI for Napier grass as compared to that of COBN-5 fodder indicating that COBN-5 fodder is more palatable as compared to Napier grass.

Calculated ME (Mcal/d) intake by buffalo bulls in the present experiment was 12.18 and is higher than the earlier report (Garg *et al.,* 2012) in animals fed hybrid Napierxbajra fodders. Increased amount of ME Mcal) in COBN-5 fodder may be attributed to easily soluble and degradable fraction of carbohydrates and protein. The DM, DCP, TDN and ME intakes per $kgW^{0.75}$ in

Table 2. Intake and apparent digestibility (%) of nutrients and plane of nutrition in buffalo bulls fed COBN-5 fodder

Parameter	COBN-5
Dry matter intake (kg/d)	6.01±1.11
Intake of nutrient (g/ kg $W^{0.75}$)	
DM	73.03±1.21
DCP	6.38±1.48
TDN	40.93±1.91
ME (Mcal)	0.15±1.32
Apparent Digestibility of nutrients (%)	
Dry matter	60.62±2.17
Organic matter	62.38±2.30
Crude protein	68.74±1.58
Ether extract	62.60±1.30
Crude fibre	55.72±2.64
Nitrogen free extract	62.74±3.56
Neutral detergent fibre	55.07±1.21
Acid detergent fibre	46.18±2.64
Hemi cellulose	71.30±2.62
Cellulose	47.00±2.10
Nutritive value (%)	
DCP	8.74±2.10
TDN	56.05±2.14

buffalo bulls fed *ad libitum* of COBN-5 fodder were higher than the values recommended in ICAR (2013) standards.

Data pertaining to apparent digestibility of nutrients are presented in Table 2. The apparent digestibility of DM, CP, CF, OM and NFE in the present study was higher than the other Napier varieties reported by earlier workers (Reddy *et al.,* 2009; Wadhwa *et al.,* 2010; Chandra *et al.,* 2012). Apparent digestibility of CP for COBN-5 fodder observed in the present study was similar to that observed in bajra variety (PHBF-1) as reported earlier (Singh *et al.,* 2009). The NDF digestibility of COBN-5 fodder as reported in the present study was similar with the findings of Chandra *et al.* (2012) in Napier fodder. Similarly, the ADF digestibility of COBN-5 fodder corroborated with findings of Wadhwa *et al.* (2010) in Napier grass. The digestibility of cellulose and hemi-cellulose for COBN-5 fodder as observed in the present study were antithetical to the values reported in Napier fodder by Reddy *et al.* (2009) and Wadhwa *et al.* (2010).

Data pertaining to nitrogen, calcium and phosphorus utilization in buffalo bulls fed COBN-5 fodder are presented in Table 3. Calcium and phosphorus intake (g/d) was higher in the present experiment when compared to the other Napier varieties. Further, the calcium and phosphorus requirement of buffalo bills for maintenance (Ranjhan, 1998) was met by feeding *ad libitum* COBN-5 fodder as a sole diet.

Data on rumen fermentation pattern in buffalo bulls fed COBN-5 fodder is presented in Table 4. Time of sampling had a significant ($P<0.01$) effect on pH, ammonia nitrogen and TVFA in strained rumen liquor (SRL) of buffalo bulls fed COBN-5 fodder. The present study revealed that the pH of rumen liquor showed a decreasing trend up to 6 h post feeding in all the buffalo

Table 3. Nutrient utilization in buffalo bulls fed *ad libitum* COBN-5 fodder

Parameter	Nitrogen	Calcium	Phosphorus
Intake, g/d	676.67±11.24	160.00±9.48	148.00±8.12
Outgo, g/d			
Faeces	211.25±22.09	132.10±20.34	104.64±8.22
Urine	63.11±16.78	6.19±0.30	19.20±1.39
Total	274.36±36.73	138.29±20.48	123.84±8.19
Retention			
Retention, g/d	402.31±48.46	21.71±3.96	24.16±14.86
% intake	59.45±1.80	13.57±2.86	16.32±5.74
% absorbed	86.44±1.87	77.81±1.46	55.72±3.81

Table 4. Rumen fermentation parameters in buffalo bulls fed COBN-5 fodder

Parameter	Hours					
	0	2	4	6	8	Mean ± SE
pH	6.52±0.02[d]	6.43±0.01[c]	6.33±0.03[b]	6.21±0.04[a]	6.31±0.03[b]	6.36±0.08
Ammonia Nitrogen (mg/100 ml SRL)	12.50±0.03[b]	16.60±0.03[e]	15.54±0.04[d]	14.00±0.06[c]	12.23±0.05[a]	4.17±1.34
TVFA(meq/L of SRL)	65.75±2.46[a]	78.75±3.01[b]	83.00±2.94[c]	92.50±2.90[e]	87.25±2.59[d]	81.45±7.17

[a,b,c,d,e]values in the rows bearing different superscripts differ significantly; P<0.01

bulls fed COBN-5 fodder and increased thereafter. These findings were in agreement with the values of Srinivas Kumar *et al.* (2011) in buffalo bulls fed Napier fodder. Post prandial decline in pH observed in the present study might be attributed to increased microbial fermentation and accumulation of organic acids in the rumen. The rumen ammonia nitrogen concentration increased linearly up to 2 h post feeding beyond which there was a decline in its concentration. These values are in agreement with the findings of previous workers (Kariuki *et al.,* 2001 in cattle, and Puga *et al.,* 2001 in sheep) fed Napier fodders. The mean TVFA concentration in SRL of buffalo bulls was higher at 6 h of post feeding and lowest was reported at 0 h of feeding, respectively. Similar findings were reported in buffalo bulls fed CO-1 fodder (Srinivas Kumar *et al.,* 2011). However, higher TVFA concentration was reported by Muia *et al.* (2001) in Friesian calves and Puga *et al.* (2001) in sheep than the values in the present study.

CONCLUSION

It was concluded that feeding *ad libitum* COBN-5 fodder to buffalo bulls met the adequacy of nutrients interms of supply of DCP, TDN, Ca and P. Further, feeding COBN-5 fodder as a sole diet to buffalo bulls had no adverse effects nutrient utilization, rumen fermentation pattern and plane of nutrition.

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SHORT COMMUNICATION

Effect of Supplementation of *Tinospora cordifolia* and Ascorbic Acid either Alone or in Combinations on Leucogram of Broiler Chickens

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ABSTRACT

A 42 day feeding trial was carried out to investigate the effect of supplementation of *Tinospora cordifolia* (at graded levels) or ascorbic acid alone and in combinations on Leucogram and H/L ratio using 360 one-day-old broiler chicks in an experiment based 5×2 factorial design and divided into ten (10) dietary treatments groups (T_1-T_{10}) in triplicate of twelve chicks per replicate. The average temperature (31°C) during the research trial was higher than the recommended normothermia zone *i.e.* 18-24°C established for poultry birds, which indicated that poultry birds were in chronic heat stress. Improvement in leucogram and H/L ratio of broilers was observed in the present trial due to supplementation of ascorbic acid or *Tinospora cordifolia* (geloi) alone and in combinations. Supplementation of ascorbic acid or *Tinospora cordifolia* (geloi) may render positive immune response of immune organs in broilers during chronic heat stress.

Key words: Ascorbic acid, DLC, H/L ratio, *Tinospora cordifolia*, TLC

The Indian poultry industry has attained momentum during the last five decades and consequently, it has turned into highly organized and most sophisticated industry from the earliest system of backyard keeping in most of the world. Poorly developed poultry industry in the Western zone of Rajasthan is mainly due to disadvantageous climatic conditions. The average temperature during summer is more than 34°C and during winters it fluctuates in between 12-16°C which results into stress to the poultry birds. The stem of *T. cordifolia* (Geloi) has potential application in food systems as a biologically potent nutraceutical and as an antioxidant, because it can lessen oxidative stress with consequent health benefits (Bhawya and Anilakumar, 2010). *T. cordifolia* (Geloi) has the capacity to strengthen the macrophages thereby it improves general immunity to fight against diseases (Rege *et al.,* 1999; Dhanukar *et al.,* 2000). Ascorbic acid helps to save cells from oxidative damage. Ascorbic acid supplementation augmented productivity, immune responses and survivability under nutritional stress (Zulkifli *et al.,*1996). The addition of ascorbic acid to the diet of bird increased the immune response during heat stress (Zahraa, 2008). Interest behind the incorporation of ascorbic acid in poultry ration is that endogenous synthesis may not be adequate to provide the full need of poultry at all times or the requirement of this vitamin may increase during heat stress (Lin *et al.,* 2006). Thus supplementation of Tinospora and vitamin C in birds exposed to chronic heat stress may be beneficial. Therefore, the present study was designed to evaluate the effect of supplementation of *Tinospora cordifolia* (at graded levels) or ascorbic acid alone and in combinations on leucogram and H/L ratio in poultry.

A 42-days feeding trial was carried out at the poultry farm of College of Veterinary and Animal Science (CVAS), Bikaner. The 360 experimental day-old-broiler chicks were equally and randomly divided into ten (10) dietary treatments groups (T_1-T_{10}), and each dietary group was replicated to three (3) sub-groups (R_1-R_3) to make uniformly in various treatment

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groups. Rations included: T_1-basal ration with no supplementation; T_2-basal ration supplemented with 0.25% *Tinospora cordifolia*; T_3-basal ration supplemented with 0.50% *Tinospora cordifolia*; T_4-basal ration supplemented with 0.75% *Tinospora cordifolia*; T_5-basal ration supplemented with 1.0% *Tinospora cordifolia*; T_6-basal ration supplemented with 0.025% ascorbic acid; T_7-basal ration supplemented with 0.25% *Tinospora cordifolia* plus 0.025% ascorbic acid; T_8-basal ration supplemented with 0.50% *Tinospora cordifolia* plus 0.025% ascorbic acid; T_9-basal ration supplemented with 0.75% *Tinospora cordifolia* plus 0.025% ascorbic acid and T_{10}-basal ration supplemented with 1.0% *Tinospora cordifolia* plus 0.025% ascorbic acid. Good quality of *Tinospora cordifolia* (Geloi) stem was procured from reputed farm of Bikaner (Rajasthan).Thereafter, it was identified and authenticated by the Department of Botany, Government Dungar College, Bikaner (Rajasthan).The commercially available ascorbic acid (99.99% pure), was used. The broiler starter and finisher feed contained 21.37% and 20.32% crude protein (CP), respectively. Broilers were maintained under standard managemental practices regarding brooding, watering, feeding and disease control throughout the research period.

Blood was collected at 42nd day from 2 birds per replicate (6 birds/treatment) at the end of experiment for the estimation of DLC, TLC and H/L ratio. Blood was collected in vacutainer tubes containing ethylenediamine tetra acetic acid (EDTA) for estimation of DLC, TLC and H/L ratio. Differential leucocyte count was carried out as per the standard method described by Jain (1986). Total leukocytes count (TLC) was carried out manually through haemocyometer as per standard method of Benjamin (1978).The data obtained in the research trial were analysed statistically for main effect of *Tinospora cordifolia* (Geloi) or ascorbic acid alone as well as interaction (Geloi x Ascorbic acid) in factorial design (5X2) as per Snedecor and Cochran (2004) and significance of mean differences was tested by Duncan's New Multiple Range Test (DNMRT) as modified by Kramer (1957).

The TLC, DLC and H/L ratio values observed in various treatment groups have been presented in Table 1. The overall mean values of TLC (10^3/cumm) were found to be 22.85, 25.49, 25.17, 25.28, 25.06, 24.50, 27.31, 27.10, 27.22 and 27.13 in T_1 (Control), T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 23.68, 26.40, 26.13, 26.25 and 26.09 (10^3/cumm) in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplementation, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 24.77 (10^3/cumm) in non-supplemented group and 26.65 (10^3/cumm) in ascorbic acid supplemented group. The statistical analysis of data on TLC revealed non-significant effect on geloi supplementation and interaction (Geloi x Ascorbic acid). With respect to ascorbic acid supplementation, TLC was significantly ($P<0.05$) improved in ascorbic acid supplemented group. The findings of study are in line with observation of Dhore *et al.* (2014) who reported significant increase in TLC values on supplementation of vitamin C @ 0.025% in broilers. In addition, present findings are well supported by Sairam *et al.* (2002) who reported *in vitro* immunomodulatory activity of *Emblica officinalis*. On contrary, Elagib and Omer (2012), and Adenkola and Angani (2017) found non-significant values of TLC by supplementation of ascorbic acid in broilers. The non-significant effects of geloi supplementation in TLC are in accordance with findings of Khobragade (2003) who reported no effect of geloi supplementation on TLC in broilers. The increase in TLC in geloi and ascorbic acid either alone or in combination may be due to the positive immune response of immune organs in broilers.

The overall mean values of lymphocyte (%) were found to be 61.85, 66.09, 65.89, 65.84, 65.76, 67.69, 68.18, 68.15, 68.11 and 68.16 in T_1 (Control), T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} treatment groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 64.77, 67.13, 67.02, 66.98 and 66.96% in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplemented groups, respectively. With respect to effect of ascorbic acid supplementation, the mean

values were recorded to be 65.09% in non-supplemented group and 68.06% in ascorbic acid supplemented group. The statistical analysis of data revealed significant (P<0.05) effect of geloi and ascorbic acid supplementation (P<0.01 but remained non-significant for interaction. With respect to geloi supplementation, the highest lymphocyte (67.13%) was observed in treatment group supplemented with 0.25% geloi, which was comparable with 0.50%, 0.75% and 1% geloi supplemented groups, but significantly higher as compared to control group. With respect to effect of ascorbic acid supplementation, an improvement in lymphocyte (%) was revealed in ascorbic acid supplemented group.

The overall mean values of monocyte (%) were found to be 5.00, 5.13, 5.18, 5.20, 5.21, 5.09, 5.10, 5.12, 5.11 and 5.10 in T_1 (Control), T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 5.05, 5.11, 5.15, 5.15 and 5.16 % in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplemented groups, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 5.14% in non-supplemented group and 5.10% in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect of geloi and ascorbic acid supplementation as well as interaction.

The overall mean values of heterophils (%) were found to be 28.82, 24.43, 24.57, 24.58, 24.88, 23.06, 22.29, 22.41, 22.34 and 22.40 in T_1 (Control), T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 25.94, 23.36, 23.49 23.46 and 23.64 % in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplemented

Table 1. Effect of supplementation of geloi and ascorbic acid on leucogram and H/L ratio

Treatment groups	TLC (10^3/cumm)	Lymphocyte (%)	Monocyte (%)	Heterophils (%)	Eosinophils (%)	Basophils (%)	H/L ratio
T_1	22.85	61.85	5.00	28.82	2.09	2.24	0.47
T_2	25.49	66.09	5.13	24.43	2.16	2.19	0.37
T_3	25.17	65.89	5.18	24.57	2.18	2.19	0.37
T_4	25.28	65.84	5.20	24.58	2.21	2.18	0.37
T_5	25.06	65.76	5.21	24.88	2.11	2.05	0.38
T_6	24.50	67.69	5.09	23.06	2.23	1.94	0.34
T_7	27.31	68.18	5.10	22.29	2.35	2.09	0.33
T_8	27.10	68.15	5.12	22.41	2.25	2.06	0.33
T_9	27.22	68.11	5.11	22.34	2.27	2.17	0.33
T_{10}	27.13	68.16	5.10	22.40	2.27	2.08	0.33
SEM	**1.171**	**0.801**	**0.132**	**0.861**	**0.102**	**0.670**	**0.016**
Effect of Geloi							
0 %	23.68	64.77[a]	5.05	25.94[b]	2.16	2.06	0.405[b]
0.25%	26.40	67.13[b]	5.11	23.36[a]	2.25	2.09	0.349[a]
0.50 %	26.13	67.02[b]	5.15	23.49[a]	2.22	2.13	0.351[a]
0.75%	26.25	66.98[b]	5.15	23.46[a]	2.24	2.14	0.351[a]
1 %	26.09	66.96[b]	5.16	23.64[a]	2.19	2.17	0.354[a]
SEM	**0.828**	**0.567**	**0.0930**	**0.609**	**0.072**	**0.473**	**0.012**
Effect of Ascorbic acid							
0 %	24.77[a]	65.09[a]	5.14	25.45[b]	2.15	2.17	0.39[b]
0.025%	26.65[b]	68.06[b]	5.10	22.50[a]	2.27	2.07	0.33[a]
SEM	**0.524**	**0.358**	**0.059**	**0.385**	**0.046**	**0.299**	**0.007**

groups, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 25.45% in non-supplemented group and 22.50% in ascorbic acid supplemented group. The statistical analysis of data revealed significant (P<0.05) effect of geloi and highly significant (P<0.01) effect of acid supplementation, but the interaction was non-significant. With respect to geloi supplementation, the highest heterophils (25.94%) was observed in treatment supplemented with 0% geloi, which was significantly higher than rest of the geloi supplemented groups. With respect to effect of ascorbic acid supplementation, a decrease in heterophils (%) was revealed in ascorbic acid supplemented groups.

The overall mean values of eosinophils (%) were found to 2.09, 2.16, 2.18, 2.21, 2.11, 2.23, 2.35, 2.25, 2.27 and 2.27 in T_1 (Control), T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 2.16, 2.25, 2.22, 2.24 and 2.19 % in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplemented groups, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 2.15% in non-supplemented group and 2.27% in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect of geloi and ascorbic acid supplementation as well as interaction.

The statistical analysis of data of basophils revealed non-significant effect due to supplementation of geloi and ascorbic acid as well as interaction. The results obtained in the present study following ascorbic acid supplementation are in line with Dhore *et al.* (2014), who reported non-significant effect on monocytes and eosinophils and significant effect for heterophils and lymphocytes on supplementation of vitamin C @ 0.025% in broilers. Likewise, Elagib and Omer (2012), and Adenkola and Angani (2017) reported non-significant effect on eosiophils and basophils, while, significant improvement was recorded on heterophils and lymphocytes on supplementation of ascorbic acid @ 350 ppm in broilers. Further, Elkheir *et al.* (2008) also reported significant increase in lymphocytes on inclusion of ascorbic acid supplementation @ 0.025% in broilers. Significant increase in lymphocytes due to supplementation of vitamin C are well supported by Sairam *et al.* (2002) who reported immunomodulatory response of amla on lymphocyte during *in vitro* studies. Response of geloi supplementation as observed in present study are in line with Khobragade (2003) who reported non-significant effect on monocytes, eosinophils and basophils on supplementation of geloi in broilers. In addition, the results are in line with the findings of Sharma and Pandey (2010) who reported significant increase in lymphocytes due to geloi supplementation in broilers. On the contrary, Khobragade (2003) found non-significant values of heterophils and lymphocytes in broilers on supplementation of geloi. Improved DLC may be due to anti-inflammatory and immunomodulatory effect of additives *i.e.* geloi and ascorbic acid as quite evident from phytochemical study of *Tinospora cordifolia* and reported antioxidant activity of ascorbic acid.

The overall mean values of H/L ratio were found to 0.47, 0.37, 0.37, 0.37, 0.38, 0.34, 0.33, 0.33, 0.33 and 0.33 in T_1 (Control), T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 0.405, 0.349, 0.351, 0.351 and 0.354 in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplemented groups, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 0.39% in non-supplemented group and 0.33% in ascorbic acid supplemented group. The statistical analysis of data of H/L ratio revealed highly significant (P<0.01) effect of geloi and ascorbic acid, but remained non-significant for interaction. With respect to effect of ascorbic acid supplementation, an improvement in H/L ratio was revealed in ascorbic acid supplemented group.The H/L ratio in birds is a reliable indicator of stress (Langsdorf and Zydney, 1993). The findings of H/L ratio obtained in the present study are in accordance with the results of Lohakare *et al.* (2005) who reported significant decrease in H/L ratio on ascorbic acid supplementation @ 200 ppm in broilers. The reduction in H/L ratio on supplementation of geloi at graded levels or ascorbic acid alone and various

combinations of both may be due to antistress effect of both through the reduction in the synthesis of adrenal steroid in broilers.

CONCLUSIONS

Improvement in TLC, DLC and H/L ratio of broilers was observed due to supplementation of ascorbic acid or *Tinospora cordifolia* (geloi) alone and in combination. Supplementation of ascorbic acid or *Tinospora cordifolia* (geloi) may positively influence the immune organs in broilers during chronic heat stress.

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CONTENTS

Review

Ruminants

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