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Indian Journal of Animal Nutrition

REVIEW

Role of Carotenoids in Ornamental Fish Nutrition: A Review

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ABSTRACT

A bright colourful fish is the primary attraction of an aquarium. This colouration is due to skin pigments, underlying tissues and body fluids. Carotenoids are the major group of colouring pigments among other similar compounds. These pigments have a significant role in the reproduction, respiration, membrane permeability, light absorption, reflection and the efficiency of the immune system *etc.* Unfortunately, fish, cannot produce the carotenoids themselves and are reliant upon a dietary source. Coloured fish often show faded or degraded coloration especially when the fishes are kept under captivity and also in intensive culture condition. This faded colouration can be improved with feed containing either pure natural /synthetic carotenoids directly or indirectly through natural sources of microbial, plant and animal origin. This review article deals with carotenoids, their types, function, sources and physiological effect on fish in a comprehensive manner.

Keywords: Aquarium, Astaxanthin, Colouration, Carotenoid, Ornamental fish feed

INTRODUCTION

Aquarium keeping is amongst the most popular of hobbies worldwide. In the past 60-70 years ornamental fish keeping were mainly restricted to members of well to do family but now a days it has become ubiquitous and is very popular activities in town and in cities across the world. Many of the fishes which were used for ornamental purpose are small in size and also, they have got attractive colours. Ornamental fish culture has made a great progress from last few centuries. A considerable growth has been seen from last 4-5 decades, not only in culture but also in diversification in the international trade in ornamental fishes.

Indigenous ornamental fishes have a vast potential in the domestics as well as in the international market, but since this sector is mainly done through wild collection so there is always a shortage in the number of fishes and cannot fulfil the demand from wild collection alone until and unless breeding methods of indigenous fishes are evolved. In spite of the fact that this sector has a vast potential for development, there is a problem in developing this multibillion-dollar business. This can be attributed to the lack of knowledge and other information regarding breeding under captivity, the lack of knowledge about the food and feeding habits and behaviours of fishes and also the absence of low cost and good quality feed.

Coloration is one of the most important factors in which the value of fish in the market depend i.e. the better is the colour the best is the price. The colour of the skin of ornamental fish and the colour of the food is the major concern for farmers and traders because it is the colour of the skin which will determine its price in the market. Coloured fish often show faded or degraded coloration especially when the fishes are kept under captivity and also in intensive culture condition.

Many shows and exhibition reveal that the competition in ornamental fish is mainly dependent on achieving vibrant colour. The value of the fish in the market reflects these requirements and hence farmers, researchers and many entrepreneurs and many others are mainly involving themselves in discovering methods of enhancing skin colouration. Colour enhancement by using colour additives is now being studied although very few research papers have been published.

It is also known throughout the animal kingdom that male having the flashiest looks have got the best chance of finding mate. But researchers have found the reasons for this only recently. Males which are flashy

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and brightly coloured are able to use carotenoids as ornaments and they are also stronger and healthier. Carotenoids have been shown to increase not only growth of young fish but also their survivals.

A fish cannot synthesize these pigments, they rely on dietary supply of carotenoids to achieve their natural skin pigmentation. Carotenoids are found in nature the largest group being the fat soluble carotenoids and xanthophylls; the carotenoids mostly found in the green plant material and have α and β form. β-Carotene a precursor of vita- A. Carotenoids having role in the reproduction, respiration, membrane permeability, light absorption reflection and the efficiency of the immune system. Many animals also use colours to communication warning, mating call, feeding signal and camouflage. The predominant carotenoids present in the aquatic environment are lutein (green yellow), zeaxanthin (yellow orange) and (alpha) carotene (light yellow) astaxanthin (red) animal the algae have higher amount of alpha carotene alpha and grass meal have a preponderance of β carotene, this would (spirulina) the algae from the pond would more efficient utilized by fish. Natural Carotenoids are found all section of the living world.

1. Mechanism of coloration in fish

Colouration is due to skin pigments, underlying tissues and body fluids. There are specialized pigments containing cells called 'chromatophores' located beneath the scales which can cause coloration. The most common types of chromatophores are melanophores; these contain black and brown coloured melanin crystals. Carotenoids are not only among the most widespread of naturally occurring group of pigments, but also have the important function.

1.1 Types of chromatophores and properties

Chromatophores	Pigment	Type	Colours
Melanophores	Melanins	Colour	Black, Brown
Xanthophores	Pteridins & Carotenoids	Colour	Red, Orange
Erythrophores	Pteridins & Carotenoids	Colour	Orange, Yellow
Iridophores	Guanine & Other Purines	Reflective	White, Silver, Blue, Other

2. Carotenoids

2.1 Definition

A Carotenoid may be defined as a nitrogen free pigment, consisting wholly or chiefly of carbon atoms united in an uninterrupted sequence of conjugated double bonds. These pigments vary in colour from a bright yellow to a deep red or even a violet or dark blue, the depth of a shade increasing with the number of conjugations in consecutive union and decreasing as the double bonds are saturated. Although carotenoids occur in low concentration the total carotenoids production in nature has been estimated to be over 100 million tons per year with the majority being synthesized via the photosynthetic pathway and subsequently being stored in leaves, algae and zooplankton.

2.2 History

Carotenoid was first discovered in the roots of carrot (*Daucus carota*). The name 'carotene' was derived from the carrot from which it was first isolated. Its wide distribution in nature probably accounts for the subsequent use of the class name, "carotenoids" to include all the pigments of related chemical composition. Carotenoids are responsible for many of the red orange and yellow hues of plant leaves, fruits and flower as well as the colours of some birds, insects, fish and crustaceans. Some familiar examples of carotenoid colouration are the oranges of carrot and citrus fruits, the reds of peppers and tomatoes, the pinks of flamingos, salmon and rainbow colours in ornamental fishes. Some 600 different carotenoids are known to occur naturally and new carotenoids continue to be identified.

3. Carotenoids in fish

Coloration plays a role in social structure as well as defence of fish in the wild. Fish depend a great deal on visionary cues. All but a few species (mainly cave

dwellers) have well-developed eyes. As is the case in many other groups of animals, the body colours of fish are predominantly dependent on the presence of special cells in the skin, called chromatophores. These contain pigments or light scattering or light-reflecting organelles. In biology, any substance that can impart colour to the tissues or cells of animals or plants can be called a pigment. There are four main groups of pigments that can be used to provide colour in these cells: melanins, carotenoids, pteridines, and purines. Melanins are responsible for the dark coloration seen in fishes. Carotenoids are lipid soluble, gives yellow to red colours dominate the other pigments. Pteridines are water-soluble compounds and result in bright coloration. Pteridines play a small role in coloration when compared to carotenoids. In the purine compounds, mostly guanine predominates. A large amount of guanine can be found in the silvery belly skin of most fish species. These molecules can combine with other biomolecules, like proteins, to produce the blue, violet, and green colour ranges seen in fishes. In the flesh (muscle), the carotenoids are the dominant pigment. In lobsters and shrimps, astaxanthin is attached to a protein to produce the carotenoprotein, crustacyanin. This carotenoprotein imparts a blue colour in living crustacea; in the presence of heat the carotenoprotein molecule is cleaved, which subsequently results in the characteristic astaxanthin red colour of cooked lobsters and shrimps. To date, over 600 carotenoids have been identified in nature, varying in colour from yellow to red. Most carotenoids are polyunsaturated hydrocarbons, containing 40 carbon atoms, and comprising of two terminal ring systems. Carotenoids that are composed entirely of carbon and hydrogen are known as carotenes, while those that contain oxygen are termed xanthophylls. Carotenoids are the major pigmenting compounds and cannot typically be synthesized by fish. Whereas, most other pigmenting compounds can be made by the fish. Carotenoids are used in aquaculture feeds to provide the colour associated with consumer products, such as the bright vibrant colours of ornamental fish. The same carotenoid, astaxanthin, found in wild salmon is used in aquafeeds to impart this natural, pink-red colour to farmed salmon fillets. Colour matters, particularly with regards to consumer preference for aquaculture products. As is the case with other carotenoids, salmonids cannot endogenously synthesize astaxanthin; therefore, it must be supplemented in the fish's ration. Research also indicates additional benefits from dietary carotenoids besides coloration. Astaxanthin, for example, has biological functions related to growth, reproduction and tissue health in salmonids and shrimp, possibly due to the compound's strong antioxidant properties.

Only a small number of plants and micro-organisms can synthesize carotenoids. Higher animals, including fish, depend upon the dietary carotenoids. They absorb the carotenoids from the feed and deposit them in their tissues. Astaxanthin accounts for more than 90% of the total carotenoid content found in the flesh of wild salmonids (salmon and trout) and gives salmon flesh its characteristic rich pink-red colour. In the wild, salmonids absorb astaxanthin from the crustaceans they eat. The absorbed carotenoid is transported via blood to the muscles and skin to be deposited. In other fishes and to a limited extent in salmonids, additional carotenoid compounds are the source of other bright colours. Research results indicate that tunaxanthin is a rather common pigment in marine fish and is especially abundant in yellow-coloured fishes. Astaxanthin, by contrast, seems to be dominant in red marine fish. Lutein is also widely found in many marine species. Carotenoids commonly occurring in freshwater fish include beta-carotene, lutein, taraxanthin, astaxanthin, tunaxanthin, alpha-, beta-doradexanthins, and zeaxanthin *etc.*

In nature, plants and micro algae can produce carotenoids. Animals cannot synthesize carotenoids by their own, so they are dependent on plants and algae to obtain these pigments (Britton *et al.,* 1995). The pigments found in fishes are gained from these sources or from their prey that have accumulated in. These absorbed carotenoids are then transported in the blood to the muscle skin where it is deposited. Carotenoids in fishes have been studied and observed that some species contain a variety of carotenoids, where others contain specific ones (Fox, 1957), e.g. the Carotenoid 'tunaxanthin' is especially abundant in yellowtail (*Seriola quinqueradiata*).

3.1 Common carotenoids in fishes

i. Lutein :- greenish-yellow
ii. Tunaxanthin :- yellow
iii. Beta-carotene :- orange
iv. Doradexanthin :- yellow
v. Zeaxanthin :- yellow- orange
vi. Canthaxanthin :- orange- red
vii. Astaxanthin :- red

4. Use of carotenoids in aquaculture

In natural condition fishes obtain carotenoids from variety of sources like micro algae, crustaceans, and insects but in culture conditions this pigmentation is obtained by supplementing or complimenting diets with carotenoid pigments. Various synthetic carotenoids (β-carotenoids, canthaxanthin, zeaxanthin and astaxanthin) and natural sources yeast, bacteria algae plant and crustacean meal have been used as dietary supplement to enhance the pigmentation of the fish and crustacean (Shahidi *et al.,* 1998). Natural carotenoids are usually composed of several carotenoids in various form and various degree of digestibility making their pigmentation efficiency complicated. Supplementation of feed with various natural pigment sources of plant origin for good growth and attractive colorations, carotenoids from carrot gives satisfactory result. Significant deposition of total carotenoids and astaxanthin as well as visual enhancement of the flesh colorations was observed in trout. Swimming performance, courtship behaviour of guppy due to carotenoids differ in different upstream and downstream water.

Salmonidae, cannot synthesize carotenoids and therefore rely on dietary intake under intensive culture condition. Carotenoids are among the most important micro ingredients used in many intensive aquaculture systems. This group of compounds has been linked to many important biological functions associated with their strong antioxidant properties.

4.1 Pigmentation using pure carotenoid pigments

Many researchers have used pure carotenoid pigments in the fish diet to enhance pigmentation. Astaxanthin is added to feed in order to make up for the lack of natural dietary sources of the pigment. Torrissen *et al.* (1989), Fey and Mayers (1980) showed that canthaxanthin at the rate of 0.005% enhances colour in the pearl gourami (*Trichogaster leeri*) and they also exhibited greater iridescence in the chromatophores along the entire integument. Kim *et al.* (1999) found that the number of total carotenoids in the group of bitterlings (Rhodeus*uyrkii)* fed with supplemented carotenoids (astaxanthin, lutein, and β-carotene) were relatively higher than the control group of fish fed with no carotenoid supplementation. To enhance colouration in ornamental fish a combination of synthetic and natural carotenoid pigments can be added at the rate of 0.04 to 2.00% of the diet (Chapmann, 2000).

Astaxanthin is very commonly used supplement as it not only provides pigmentation in farmed animals but also has been found to be essential for their proper growth and survival (Torrissen and Christiansen, 1995). Synthetic astaxanthin is the major form currently being used in fish feeds (McCoy, 1999).

4.2 Pigmentation using natural sources

4.2.1 Animal sources

Many studies have shown that fish can be pigmented by inclusion of crustaceans and crustacean wastes in their diets (Torrissen *et al.,* 1981). Allapichay *et al.* (1984) shown that the rate of Carotenoid deposition in red sea bream *Chrysophrys major* fed with raw Atlantic krill (*Ephausia superba)* and raw mysid (*Neomysis sp.*) was significantly higher and resulted in distinct pigmentation. Atlantic krill (*Ephausia superba*) oil containing astaxanthin diester was shown its significance in pigmenting the flesh of coho salmon (Oncorhynchus *kisutch*) (Arai *et al.,* 1987). But the use of crustacean or crustacean waste brings several problems (Spinelli & Mahnken, 1978). The drying of crustacean or crustacean waste brings significant loss of carotenoid pigments from it (Brinchmann, 1967). Incorporation of large quantities of shrimp meal or waste modifies the mineral composition of diet (Meyers and Rutledge, 1971).

4.2.2 Plant sources

Several workers have utilized carotenoid pigments from the plant sources. Bitzer (1963) used paprika, Savolainen and Gyllenberg (1970) used yeast (*Rhodotorula sanneii)*, Neamtu *et al.* (1976) used

chestnut flowers, Johnson *et al.* (1977) used yeast (Phaffia *rhodozyma*) in salmonids to improve the colour of the flesh. Tsushima *et al.* (1998) demonstrated that paprika was effective for pigmentation in gold fish and its effect was significantly increased by sunlight. The goldfish accumulated capsanthin and oxidized it into 4-keto capsanthin. Nhan *et al.* (2019) evaluated the effect of dietary natural carotenoid sources like sweat potato and gut weed on skin colour enhancement of false clownfish *Amphiprion ocellaris*.

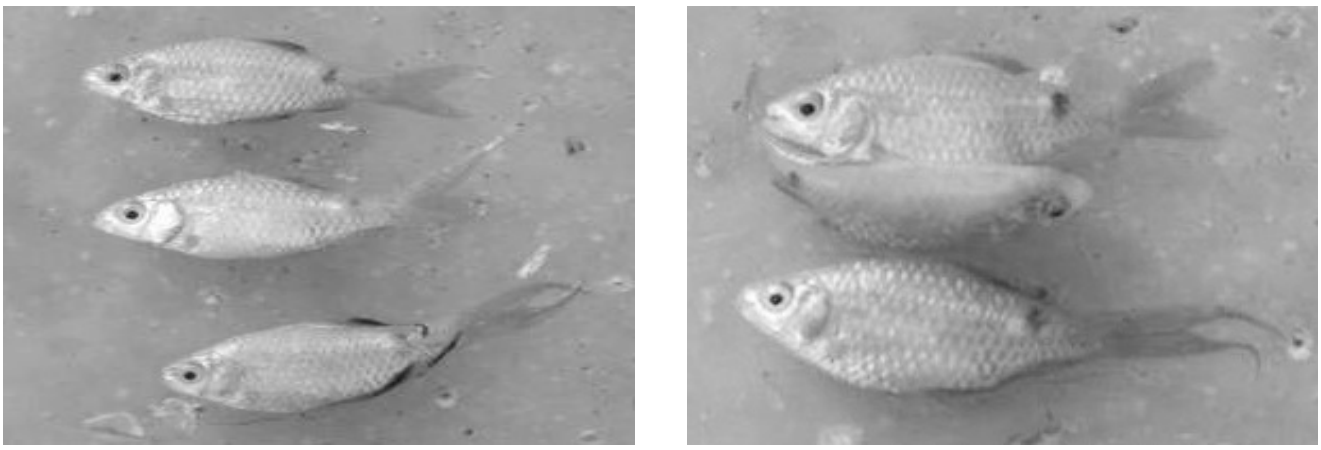
Rose petals

Rose flowers (*Rosa canina*) are available in different colours. Commonly petals of the flowers collectively called as corolla which are rich in carotenoids and polyphenols that provides colour to the flowers which attracts the insects useful for pollination. The main pigments present in the rose flowers are water soluble flavonoids and non-water-soluble carotenoids. Anthocyanin which is abundant in rose flower is part of diverse family of aromatic molecules called flavonoid which is derived from phenyl alanine and malonyl coA (Shirley, 2001). Anthocyanin is a sub class of plant flavonoid responsible for orange to blue colour of flower and other plant organs; these are accumulated in the vacuole. There are three major types of anthocyanin that contribute the colour of flower (Zuker *et al.,* 2005). The flavonoids that are present is rose flower are – cyaniding, pelarogonidin and the flavonols. The flavonols like quercetin and kaempferol cyanidin are responsible for deep red colour pelarogonidin responsible for orange red, quercefin and kaempferol are for white creamy colour. But the appearance of dark red and purple colour in rose flower is due to presence of cyaniding. The blue or purple colour in rose is produced by delphinidin, red or magenta colour by cyanidin & orange, red or pink colour is due to presence of pelarogonidin. Rose flower is primarily composed of structurally simple 3,5 diglucosides (anthocyanin). Pailan *et al.* (2012a, 2012b) used the rose petals meal as a carotenoid source on pigmentation and growth of dwarf gourami (*Colisa lalia*).

Marigold

Marigold is a herb very commonly found all over the world. Marigold is normally used for decorating the gardens. The petals of marigold flower are commercially valuable as a natural source of lutein pigments (yellow orange pigments). It is used in poultry industry as a feed additive to improve the colour of egg yolk to orange and skin yellow. The main pigments present in the marigold flower are xanthophylls and lutein which are present in the form of ester of palmitic and myristic acids. Marigold flower can be used as feed additive either in dried form or as solvent extracts.

Marigold petal meal was used by Boonyaratpalin and Lovell (1977) for the tiger barb (*Puntius tetrazona*) and found the tiger barbs more brightly coloured than the fishes fed with control diet. Ezhil *et al.* (2008) used the marigold as a carotenoid source on pigmentation and growth of red sword tail (*Xiphophorus helleri*). Gocer *et al.* (2006) used marigold flower along with red



Carotenoids gain in fish fed with different experimental diets

pepper and synthetic astaxanthin on pigmentation growth and proximate composition of *Penaeus semisulcatus* and they found marigold as a carotenoid source was as useful as the synthetic astaxanthin for the shrimp. Pailan *et al.* (2015) observed that marigold petal meal at 4% level can be supplemented in the diet of swordtail for improvement of skin coloration through increase in carotenoids concentration in skin without any adverse effect on body composition, growth and feed conversion efficiency.

Other plant-based sources

The plants like paprika (*capsicum annum L.*), red pepper, carrots are also the good source of natural carotenoids for fishes. Paprika was used in salmonids diets by Ellis, (1979). Some dried flower can be used as well (Torissen *et al.,* 1989). Sheriff and Mathew (1996) demonstrated that carrot meal produced gold yellow colour in gold fish. China rose (*Hibiscus rosasinensis*) petals was used as a source of natural carotenoid for gold fish (*Carassius auratus*). Agus *et al* (2019) observed that supplementation of 10% RDFM (Red dragon fruit by product meal) could be added into the diet to enhance skin coloration of koi carp.

4.2.3 Microbial sources of carotenoids

There are several microbes like algae, fungus, yeast and some bacteria are reported to produce carotenoids. Though the synthetic carotenoid is available, there is a renewed interest in microbial sources of carotenoids (Nelis and De Leenheer, 1991).

Algae

Algae can be called as the base of the food chain which is rich in carotenoids. Production of β-carotene by algae *Dunaliella* sp. is well developed tech. (Ben-Amotz 1998, 1999). Red algae *Haematococcus pluvialis* can be used for the commercial product of astaxanthin. Other blue green algae Spirulina can be used as a carotenoid source due to its occurrence in almost all environment. Spirulina has been used as source of carotenoid in rainbow trout and fancy carp (Choubert 1979). It is also used as carotenoid sources for the pigments of the blue gourami (*T. trichopterus)* (Alagappan *et al.,* 2004). It also accelerated the growth, sexual maturity and increase of fertility in fishes like African cichlids and gold fish, which ate a lot of algae (Matsuno *et al.,* 1980). Spirulina is also rich in nutrients like protein (55-70%), carbohydrates (15-25%) (Hudson and Karis, 1974) and pigments like carotenes, chlorophyll and phycocyanin (Choubert, 1979). The cell wall of spirulina sp has been used in fish diets in *C. macrocephalus* and *P. goninotous,* colour was increase with the quantity of spirulina. The undesirable yellow coloration of edible trout flesh can be observed in pond with heavy algal growth. The trout consume varying quantity of algae with their food and their yellow pigment is assimilated in the musculature. Algae destruction with gramoxone is recommended as a counter measure.

5. Absorption of carotenoid in fish

Hata and Hata (1972) have suggested that the occurrence of hydroxyl group within molecular configuration of carotenoid enhances its absorption by the digestive epithelium. Thus astaxanthin, dihydroxy cathaxanthin may be more easily absorbed than canthaxanthin. Pigmentation depends upon verity of factors like dietary pigment source and concentration, period of feeding and continuity of feeding the pigmented diet, size of the fish, temp. of water, developmental stage (sexual maturity), hormonal status of the fish, chemical form of the carotenoid fed, photoperidod regime, dietary lipid/ emulsifier concentration and the availability of natural food organisms. In salmon astaxanthin and canthaxanthin were deposited unchanged but astaxanthin was better retained (Bjerkeng *et al.,* 1990) due to higher digestive retention rate. Efficiency of gastrointestinal absorption of the carotenoid mainly depends on the carotenoid and on the composition of the diet (Van Het Hof *et al.,* 2000). Digestibility coefficient for astaxanthin & canthaxanthin increases with increase in the dietary level, thus, the higher carotenoid in the flesh of rainbow trout. Canthaxanthin pigmented farmed salmon is more yellowish than astaxanthin pigmented wild salmon (Skrede and Storebakken, 1986). Idoxanthin (3,3´, 4´-trihydroxy- β, β -carotene-4-one), an astaxanthin metabolite, may accumulate in considerable amounts in the muscle up to 30% of total carotenoids in large Atlantic salmon (Schiedt *et al.,* 1981). It has a shorter

chromophore than astaxanthin, and appears as more yellow. Accumulation of idoxanthin in the muscle may therefore contribute to a more yellowish hue of the flesh as indicated by Hatlen *et al.* (1995).

6. Functions of carotenoids

The carotenoids have several major biological functions. Astaxanthin plays an important role in salmonid growth and health (Christiansen *et al.,* 1995). Astaxanthin has also been shown to increase the survival of crustaceans (Chien and Jeng, 1992). It has been suggested that *Penaeus semisulcatus*, requires astaxanthin and not retinol, per se (Dall, 1995). Yet, it is important to stress that carotenoids do not replace the requirement for vitamin A in fish diets. The mobilization of carotenoids and their transport from the flesh to the skin and ovaries during maturation has led to the hypotheses that carotenoids have a function in reproduction. Possibly carotenoid functions included: a fertilization hormone, a source of pigments for chromatophores, a function in cellular respiration, protection from light, resistance to elevated temperature and ammonia, and as provitamin A. Carotenoids also have excellent antioxidative characteristics. Cold-water fishes, like salmon, have a high level of polyunsaturated fat in their membranes, and protection of lipid tissue from peroxidation seems to be a metabolic function for astaxanthin (Bell *et al.,* 2000). Astaxanthin has been shown to be one hundred times more effective than vitamin E as an antioxidant (Miki, 1991).

7. Application of carotenoids

7.1 Medicines

Age-related macular degeneration (AMD) is the most common cause of blindness at old age. Carotenoids like lutein and zeaxanthin were found to be associated with reduced risk of AMD. These two carotenoids are obtained primarily from dark green leafy vegetables such as spinach and coloured greens. The lens and cornea of human eye filter out ultraviolet light, but still visible blue light reaches the retina. This near-UV radiation can cause photo damage. Lutein and zeaxanthin enter the eye from the plasma and selectively accumulate in the retina, where, they filter out visible blue light. These carotenoids may also protect against per oxidation of fatty acids in the photoreceptor membrane, and thus protects the blood vessels that supply the macular region.

7.2 Feed additives

Carotenoids play important role in animal health by inactivating harmful free radicals produced in normal cellular activity and in stress. Carotenoids like astaxanthin and β-carotene were also reported for prevention of gastric ulceration of stressed rats (Yoshiyuki *et al.,* 1999). Pure carotenoids or carotenoid-containing preparations are also playing important role as feed additive for pigmentation in aquaculture. Astaxanthin is the major carotenoid used for pigmentation of fishes and salmons. Yeast *Phaffia rhodozyma* is widely used as astaxanthin source in aquaculture industries (Johnson *et al.,* 1977). Biotechnology companies active in the pigment business have devoted considerable research and development efforts to this organism (Nelis and De Leenheer, 1991).

7.3 Fish diets for skin and flesh colour

The characteristic red/pink colour of salmon flesh is perceived by the consumer as one of the most important quality criteria (Baker and Gunther, 2004). Salmonids seem not to deposit other red carotenoids than astaxanthin and canthaxanthin in the flesh to significant amount. Corn gluten, containing lutein, reduces the deposition of astaxanthin or canthaxanthin. It is well documented that astaxanthin is more efficiently deposited in the flesh of rainbow trout compared to canthaxanthin (Storebakken and Choubert, 1991). A similar difference seems not to be evident in Atlantic salmon. Astaxanthin and/or canthaxanthin are added to aqua feeds for salmonids (*Salmo, or Salvelinus spp.*) to obtain astaxanthin level and flesh colour comparable to those of their wild counterparts. The astaxanthin is normally supplemented to diets throughout the whole grow-out phase (100 g to harvest) and at levels between 30 to 100 mg/kg complete feed in order to obtain the desired pigment level, depending on species, fish size dietary lipid level, farming environment and initial astaxanthin level.

7.4 Antioxidant

Carotenoids are free radicals scavenger both in vitro and in vivo (Deshpande *et al.,* 1996). The antioxidative behaviour of carotenes is closely related to their own oxidation. The polyene chain of the molecule is highly reactive, electron-rich system that is susceptible to attack by peroxyl radicals and other electrophilic reagents. It is responsible for the instability of carotenoids toward oxidation and at the same time an important property of the molecule concerning free-radical (Britton *et al.,* 1995). Carotenoid are therefore sensitive to oxygen, light, heat, acid and alkali, particularly combinations of these factors (Britton, 1995). Although majority of the work on antioxidative activity of carotenoids has been done on β-carotene (Yanishlieva *et al.,* 1998), theoretically, all carotenoids with similar conjugated double bond system should have antioxidant properties (Britton *et al.,* 1995). Carotenoids are able to quench free radical species such as singlet oxygen. In addition to quenching reactive species formed by photochemical reaction, carotenoids may also act as chain-breaking antioxidants, although they do not have the characteristic structural features associated with this class of antioxidants (Deshpande *et al.,* 1996). The antioxidant potency of carotenes depends on several factors such as oxygen pressure, intrinsic chemical reactivity of the molecule toward radicals, site of generation and reactivity of the radicals, concentration and mobility in the microenvironment, stability and fate of carotene-derived radicals, and interaction with other antioxidants (Yanishlieva *et al.,* 1998).

In some food and model systems, carotenoids work as pro-oxidants under certain conditions and as antioxidants under other condition depending on their concentration. The balance between pro-oxidant and antioxidant behaviour is very delicate, and the antioxidant behaviour is most pronounced at low oxygen partial pressure (Jorgensen and Skibsted, 1993).

CONCLUSION

The colours resulting from the deposition of carotenoids are considered to be of significant behavioural importance to the animal. However, in addition to their coloration properties, the carotenoids have major biological functions. There are several ways in which the colouration of ornamental fishes can be improved by dietary means and make them more valuable. There is a vast natural resource in earth, which need to be explored and novel carotenoids to be found.

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Effect of Feeding Moringa *(Moringa oleifera)* as Green Fodder on Feed Intake, Milk Yield, Microbial Protein Synthesis and Blood Profile in Crossbred Cows

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ABSTRACT

To evaluate the effect of feeding Moringa *(Moringa oleifera)* as green fodder on feed intake, milk yield, microbial protein synthesis, and blood profile, a study was conducted on twenty lactating crossbred cows in Anand district of Gujarat. Cows were divided into two groups of ten each, based on milk yield (9-10 kg/d), fat content (3.5-3.8%) and stage of lactation (av. 60 days in milk). Cows in control group were fed 3.0 kg each chaffed wheat and pulse straw, 15.0 kg green hybrid Napier grass and 5.0 kg cattle feed (23.0% CP; 4.0% EE), Whereas, cows in experimental group were fed 15.0 kg Moringa green fodder (by replacing 15.0 kg hybrid Napier) for 90 days. Nutrient requirement was adjusted by reducing quantity of cattle feed (3.5 kg). Study revealed that milk yield and fat content increased (P<0.01) by 9.17% and 7.41%, respectively in experimental group as compared to control group. Level of β-carotene improved by 37.76%, while level of cholesterol decreased by 17.60% in cows fed Moringa as green fodder. Feeding of Moringa fodder improved intestinal flow of microbial nitrogen from 112.67 to 159.53 g/d (P<0.01). Serum concentration of non-esterified fatty acid (NEFA) reduced from 0.28 to 0.22 meq/L (P<0.05) whereas, the Ferric Reducing Antioxidant Power (FRAP) increased from 442.73 to 849.44 (P<0.01) in experimental group. Level of serum immunoglobulin, IgM was higher by 22.19% (P<0.05) in experimental cows. Organoleptic properties of milk were not affected by feeding Moringa green fodder. The average net daily income was higher by ₹ 46.21 (P<0.01) by replacing hybrid Napier with Moringa fodder. It is concluded that *Moringa oleifera* is highly nutritious fodder, and palatable; thus can be used as green fodder for lactating cows under field conditions.

Keywords: Crossbred cows, Hybrid napier, Milk yield, Moringa, Organoleptic

INTRODUCTION

One of the major constraints for dairy production in India is the non-availability or fluctuating quantity and quality of the year-round green fodder supply. Farmers feed their animals mostly on crop residues and poor quality straw/hay that are low in nitrogen, high in lignocellulose and poor in minerals and vitamins that leads to low digestibility and reduced voluntary feed intake (Sultana *et al.,* 2014). Utilization of fodder trees and shrubs could be a potential strategy for increasing the quality and availability of feeds for resource-limited dairy farmers. Moringa *(Moringa oleifera)*; originated in the northwest region of India, Pakistan and south of Himalayan mountains is recently being investigated for its fast growth, higher nutritional attributes, and utilization as a livestock fodder crop (Nouman *et al.,* 2014). Moringa tree is a drought-tolerant, fast-growing, multi-purpose and one of the most useful trees due to its medicinal and nutritional properties, and therefore described as a *'miracle tree'* (Yisehak *et al.,* 2011, Ashfaq *et al.,* 2012 and Meel *et al.,* 2018). Moringa grows in all types of soil, naturally drought resistant crop and grows even during the scarcity period of the fodder. The tree has a capacity to produce high quantities of fresh biomass per unit area and produces dry matter yield from 4.2 to 8.3 tonnes per hectare with a cutting frequency of 40 days interval (Wasif *et al.,* 2014, Mariswamy *et al.,* 2017). Moringa leaves contains high levels of crude protein, essential vitamins, minerals and amino acids (Makkar and Becker, 1997; Gidamis *et al.,* 2003). Nevertheless, the potential benefits and actual worth of the tree as inclusion in

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ruminant feeding have not been yet fully exploited (Akinbamijo *et al.,* 2004). Keeping the aforesaid facts in mind, the present investigation was planned to find out the possibilities of utilization of *Moringa oleifera* as green fodder on feed intake, milk production performance, and economics in lactating crossbred cows.

MATERIALS AND METHODS

Present study was undertaken at an organized dairy farm of Sihol village in Anand district of Gujarat. Twenty lactating crossbred cows (450 kg ± 20) in early to mid-stage of lactation were selected. Cows were divided into two groups of ten each, based on milk yield (9-10 kg/d), fat (3.5-3.8%) and stage of lactation (av. 60 days in milk). Cows in control group were fed 3.0 kg each chaffed wheat and pulse straw, 15.0 kg green hybrid Napier and 5.0 kg cattle feed (crude protein: 23%; crude fat: 4%). However, cows in the experimental group were fed 15.0 kg Moringa green fodder, 3.0 kg each of chaffed wheat and pulse straw, and 3.5 kg cattle feed for 90 days. Nutrient requirements of cows were met as per NRC (2001).

The chemical composition of feeds and fodder offered to lactating crossbred cows during the trial period was carried out as per AOAC (2005). The milk samples were collected at weekly intervals from individual cows during both milking time (06:00 and 18:00 hrs). Pooled milk samples (100-150 ml) from each cows were analysed for fat, SNF, protein and lactose contents by an automatic milk analyser (based on ultrasonic principle with accuracy of ± 0.1-0.2%). Blood samples were collected from the jugular veins and analyzed for protein, glucose, triglycerides, creatinine, NEFA, Ferric Reduction Anti-oxidant Power (FRAP) activity (Benzie and Strain, 1996) and immunoglobulin (IgG, IgM, IgA and IgE) by using kit supplied by DiaSys Diagnostic Systems GmbH (Germany).

Spot urine (100 ml) samples were collected from individual cows in the morning and preserved with sufficient quantity of 10% H_2SO_4 to maintain pH below 3.0. The urine samples were assayed for allantoin, uric acid and creatinine (Young and Conway, 1942; Hawk *et al.,* 1976). Purines absorbed and microbial nitrogen (N) supply was calculated from the daily urinary purine derivatives excreted (IAEA, 1997):

- PDC (Purine derivative: creatinine) index = Purine derivative/ Creatinine x $LW^{0.75}$

Whereas LW is live weight in kg

- Intestinal flow of microbial nitrogen (g/d) =Absorbed purine (mmol/d) x 0.727

For calculation, following factors were considered

- microbial digestibility of microbial purines is assumed to be 0.83;
- the N content of purines is 70 mg N/mmol and
- Ratio of purine N to total N in mixed rumen microbes is taken as 11.6:100.

Organoleptic test of milk samples was conducted by a panel of 10 members from different departments. For organoleptic test, milk sample was evaluated two times during the trial, so two observations were recorded. Members were selected to represent the two genders and dairy technology/ non-dairy technology background. Total four samples were evaluated; i) cow milk (control), ii) milk produced from Moringa-fed cow (study sample), iii) cow milk containing 1.0% Moringa extract, and iv) cow milk containing 2.0% Moringa extract. Moringa extract was prepared by mixing chaffed Moringa fodder in water in proportion of 23:100 by weight. The content was heated to 70 ºC, kept closed at room temperature for 15 min, and then filtered through the nylon filter. The filtrate added to milk @ 1.0% and 2.0% for sensory evaluation.

Data were analysed using one-way ANOVA with SPSS package programme (SPSS 9.00 software for Windows, SPSS Inc., Chicago, IL) as per Snedecor and Cochran (1994). When F-test was significant ($P<0.05$), Turkey's test was utilized to compare significant difference ($P<0.05$) among the groups.

RESULTS AND DISCUSSION

Chemical composition of Moringa and Hybrid Napier fodders indicates that the crude protein (18.25 *vs* 9.43%), ether extract (3.42 *vs* 2.01%) and calcium (2.41 *vs* 0.31%) contents were higher in Moringa fodder as compared to Hybrid Napier fodder (Table 1). Moringa green fodder also contained higher amount of trace minerals such as copper (13.15 *vs* 6.83 ppm), zinc

(24.61 *vs* 20.00 ppm), manganese (61.25 *vs* 55.53 ppm) and iron (591.09 *vs* 308.22 ppm).

In our study, non-significant impact on dry matter intake (DMI) was observed between the groups (Table 2). Similar results were also observed by Mendieta-Araica *et al.* (2011), who reported that there were no significant differences among treatments with regard to feed intake when Moringa leaf meal was fed to cows. However, Reyes *et al.* (2006) observed an increase in DMI (8.5 vs 10.2 vs 11.0 kg DM/day) with the supplementation of 2.0 kg and 3.0 kg DM of Moringa, respectively, as compared to control.

Milk yield improved (P<0.01) by 9.17% in experimental group, as compared to control group. The average milk fat in control and experimental groups was 3.82% and 4.11%, respectively, which indicates that the Moringa fodder supplementation in lactating crossbred cows increased (P<0.01) milk fat content. Sarwatt *et al.* (2004) reported that when Moringa leaf meal at 10, 20, or 30% of dry matter was used as substitution for cotton seed cake, resulted in significant improvement of milk yield by 1.4, 0.9 and 0.8 kg/cow/day, respectively. Reyes *et al.* (2006) also observed improvement in milk yield by feeding Moringa in crossbred cows. Significant improvement in total solids content (12.36 *vs* 12.79%; P<0.01) of milk was observed. However, other milk constitutes such as milk protein, lactose and SNF were not affected (P>0.05) by feeding Moringa to crossbred cows.

The daily feeding cost (₹ /head) of ration was lower (156.86 *vs* 149.01; P<0.01) in experimental group. This is mainly due to the lower cattle feed intake in experimental group as compared to control group. However, daily realizable receipt (₹ /head) from the sale of milk was higher by 13.40% (P<0.01) in experimental group as compared to control group. Thus, net daily income of farmers increased by ₹ 46.21 per animal by feeding Moringa as green fodder in lactating crossbred cows.

Microbial N flow to the duodenum is considered as an important and sensitive indicator to optimize the rumen metabolism in dairy animals. Urinary excretion of allantoin has been successfully used to estimate the microbial protein synthesized in the rumen and subsequently digested in the lower gut of ruminants (Dipu *et al.,* 2006). In our study, daily average intestinal flow of microbial N increased from 112.67 to 159.53 g which indicate higher (P<0.01) yield of microbial protein after feeding Moringa fodder (Table 3). Feeding of Moringa fodder in the ration of crossbred cows had resulted in improvement of purine derivative (PD) concentration (P<0.05), PDC index, total PD excreted and absorbed

Table 1. Chemical composition of Moringa and Hybrid Napier fodder (DM basis)

Parameters	Moringa green fodder	Hybrid Napier
CP (%)	18.25	9.43
EE (%)	3.42	2.01
CF (%)	29.03	31.15
Total Ash (%)	9.18	8.84
AIA (%)	0.83	2.33
Ca (%)	2.41	0.31
P (%)	0.28	0.30
Mg (%)	0.67	0.80
K (%)	0.69	2.10
Na (%)	0.117	0.05
Cu (ppm)	13.15	6.83
Zn (ppm)	24.61	20.00
Mn (ppm)	61.25	55.53
Fe (ppm)	591.09	308.22

Table 2. Effect of feeding *Moringa oleifera* on milk yield, milk composition and economics in crossbred cows

Parameter	Control	Experimental	SEM
Feed Intake			
DMI (kg/d)	12.06±0.27	11.81±0.30	0.100
Milk Yield and Milk Composition			
Milk yield (kg/d)	9.89^{a}±0.47	10.80^{b}±0.39	0.127
Total solids (%)	12.36^{a}±0.08	12.79^{b}±0.04	0.044
Fat (%)	3.82^{a}±0.04	4.11^{b}±0.04	0.027
SNF (%)	8.54±0.07	8.68±0.04	0.043
Protein (%)	3.22±0.03	3.30±0.03	0.019
Lactose (%)	4.41±0.03	4.60±0.04	0.023
β-carotene (µg %)	1.63^{a}±0.05	2.24^{b}±0.08	0.051
Cholesterol (mg %)	12.73^{b}±0.37	10.49^{a}±0.18	0.198
Economics			
Feeding cost (₹/day)	156.86^{a}±3.50	149.01^{b}±3.74	1.308
Realizable receipt from sale of milk (₹/head/d)	286.32^{a}±14.21	324.68^{b}±10.95	3.992
Average gross income (₹/head/d)	129.46^{a}±10.81	175.67^{b}±8.33	3.938
Net daily increase in income (₹/head)		+ 46.21	

^{ab} Mean bearing different superscripts in a row differ significantly (P<0.01)

purine (P<0.01), thus, (P<0.01) improved microbial N supply to cows. Microbial protein synthesis in rumen depends upon supply of ammonia, energy and carbon skeleton for amino acid synthesis (Tomar *et al.,* 2010). Most of the carbon skeletons are produced as a result of degradation of carbohydrates into volatile fatty acids. Due to feeding of Moringa fodder, greater availability of energy or protein might have resulted in increased microbial protein synthesis, thereby, improving the performance of lactating crossbred cows. Soliva *et al.,* (2005) reported that Moringa leaves are a good protein source and a convenient substitute of soybean and rapeseed meals for ruminants, and they are able to improve the microbial protein synthesis in the rumen.

Changes in serum lipid profile in cows subjected to the both feeding regimes are presented in Table 4. Levels of serum immunoglobulins; IgG, IgM and IgA (mg/ml) increased from 19.28 to 24.03 (P=0.084), 3.02 to 3.69 (P=0.011) and 0.45 to 0.53 (P=0.487), respectively on feeding Moringa as green fodder. However, the levels of glucose, triglycerides, cholesterol, HDL, LDL and creatinine were not affected (P>0.05) on feeding Moringa. Presence of NEFA in

Table 3. Effect of feeding *Moringa oleifera* fodder on microbial protein synthesis

Parameter	Control	Experimental	SEM
Allantoin (mmol/l)	10.32^{c}±0.44	11.92^{d}±0.51	0.386
Uric acid (mmol/l)	1.16±0.10	1.15±0.10	0.068
Creatinine (mmol/l)	6.82±0.56	6.40±0.36	0.323
Purine derivatives (mmol/l)	11.48^{c}±0.41	13.08^{d}±0.50	0.373
PDC index	175.87±12.97	205.14±10.46	8.891
Intestinal flow of microbial nitrogen (g/d)	112.67^{a}±9.19	159.53^{b}±8.73	8.608
Microbial protein yield (g CP/d)	704.21^{a}±57.46	997.06^{b}±54.57	53.803

$^{a, b}$ Means with different superscript in a row differ significantly (P<0.01); ^{c,d} Means with different superscript in a row differ significantly (P<0.05).

blood is a direct indicator of energy balance and massive fat mobilization, suggesting more energy requirement than supplied in the diet. NEFA can be managed by optimizing the capacity of the liver to dispose of excess NEFA by exporting it back to the blood stream in the form of very low density lipoproteins (VLDL). In this process, the body uses VLDL for availing more usable energy for various body functions and health of the liver is maintained. In our study, mean serum NEFA level reduced to 0.22 mmol/l in experimental group, as compared to control group (0.28 mmol/l).

Level of serum Ferric Reducing Antioxidant Power (FRAP) was also observed significantly higher (442.73 *vs* 849.44; P<0.01) in Moringa fed group. Present results indicates that feeding of Moringa as green fodder in the ration of dairy cows had potential effect on increasing internal antioxidant defense. It is reported that Moringa leaves contains flavonoids such as kaempferol, rhamnetin, isoquercitrin and kaempferitrin, which could significantly contribute in scavenging free radicals or act as free radical terminator (Iqbal and Bhanger, 2006; Pourmorad *et al.,* 2006; Khalafalla *et al.,* 2010 and Satish *et al.,* 2013). Anti-oxidant properties of methanolic extract of Moringa leaves has been observed by Siddhuraju and Becker (2003) and Odukoya *et al.,* (2005). Studies conducted by Asokkumar *et al.* (2008) and Oyedemi *et al.* (2010) also indicated that Moringa can reduce reactive free radicals that might lessen oxidative damage in the tissues through hydrogen peroxide decomposition.

Following are the sensory evaluations of Moringa-fed cow milk based on its organoleptic characteristics:

Observations I:

Sample	Flavour defect		Odour defect		Over all acceptability	
	Yes	No	Yes	No	Yes	No
A	5	5	1	9	8	2
B	10	0	9	1	5	5
C	9	1	5	5	3	7
D	1	9	0	10	10	0

Note: The digits indicate the number of panellists. A- Study sample (Moringa-fed cow milk) B- Sample prepared using 1% Moringa extract, C- Sample prepared using 2% Moringa extract, D- Control (Cow milk). Overall acceptability: D>A>B>C.

Table 4. Effect of feeding *Moringa oleifera* on serum profile in crossbred cows

Parameter	Control	Experimental	SEM
Total protein (g/dl)	6.82±0.15	7.09±0.09	0.094
Albumin (g/dl)	3.51±0.09	3.60±0.05	0.050
Globulin (g/dl)	3.31±0.09	3.49±0.10	0.068
Glucose (mg/dl)	58.98±2.74	61.88±3.08	2.003
Triglyceride (mg/dl)	43.49±0.57	40.89±2.17	1.142
NEFA (meq./L)	0.28^{d}±0.02	0.22^{c}±0.02	0.016
Total Cholesterol (mg/dl)	236.96±11.99	209.25±7.79	8.172
HDL (mg/dl)	144.42±8.45	167.07±8.56	6.183
LDL (mg/dl)	49.65±4.10	32.53±2.87	3.702
IgG (mg/ml)	19.28±1.33	24.03±2.01	1.384
IgA (mg/ml)	0.45±0.10	0.53±0.03	0.052
IgM (mg/ml)	3.02^{c}±0.08	3.69^{d}±0.19	0.147
IgE (mg/ml)	0.07±0.00	0.05±0.00	0.003
Creatinine (mg/dl)	0.98±0.03	1.01±0.08	0.041
Serum FRAP	442.73^{a}±77.54	849.44^{b}±62.81	82.509

[a, b] Means with different superscript in a row differ significantly (P<0.01); [c,d] Means with different superscript in a row differ significantly (P<0.05).

Observations II:

Sample	Flavour defect		Odour defect		Over all acceptability	
	Yes	No	Yes	No	Yes	No
A	2	8	0	10	10	0
B	5	5	1	9	9	1
C	10	0	5	5	3	7
D	5	5	1	9	7	3

Note: The digits indicate the number of panellists. A- Control (Cow milk), B- Study sample (Moringa-fed cow milk), C- Sample prepared using 2% Moringa extract and D- Sample prepared using 1% Moringa extract. Overall acceptability: A>B>D>C.

Based on the above observations, the overall acceptability of milk samples on organoleptic characteristics are considered as: cow milk (control) > Moringa-fed cow milk > cow milk containing 1.0% Moringa extract > cow milk containing 2.0% Moringa extract. Similar results were also observed by Reyes *et al*. (2006a) who reported that organoleptic characteristics, colour, smell and taste were not significantly affected by feeding Moringa in lactating cows.

CONCLUSIONS

Present study concludes that Moringa is palatable and is a highly nutritious fodder with antioxidant properties, which has reflected on the improvement in milk yield, and milk fat; thus improving net daily income of dairy farmers. Therefore, *Moringa oleifera* can be recommended as green fodder in the ration of crossbred cows.

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High Level of Feeding in Addititon to Dam Milk does not Improve Pre-weaning Growth Performance and Kleiber Ratio of Boer × Central Highland Kids

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ABSTRACT

In order to evaluate pre-weaning growth performance and kleiber ratio of Boer crossbred goats under different levels of supplementation an experiment based on A 2×2 ×3 factorial (2 levels of sex, 2 levels of birth type and 3 levels of feed) arrangement of treatments in completely randomized block design was conducted. Thirty six one- month old kids with an average initial body weight of 7.33±0.19 kg were randomly distributed into three dietary treatment groups. The feeding groups were: Dam milk + grazing/browsing only (T_1), dam milk+grazing/browsing+supplementary concnetrates at 1.26% of body weight (T_2), dam milk+ grazing/ browsing+ supplementary concnetrates at 2.10% of body weight (T_3). In addition, birth type (single and twin) and sex (female and male) were also considered. The average milk yield of does was 282, 332 and 394 g/day for groups T_1, T_2, and T_3, respectively. Weaning weight of kids had a positive relationship (r=0.68) with milk yield of their dams. The pre-weaning average daily gain of kids was 85±9.01 g/day, 120.45±9.19 and 113.06±9.0 g/day for groups T_1, T_2, and T_3, respectively. Sex had not a considerable influence on pre-weaning growth performance and kleiber ratio of kids. However, Single born kids exhibited higher weaning weight and average daily gain (ADG) (119.52 g/day vs 92.82 g/day) than twin born kids. Weaning weight, ADG and Kleiber ratio were significantly higher ($P<0.05$) in concnetrtae supplemented group, however, the differences between T_1 and T_2 were non-significant. Thus it can be concluded that conncetrtae supplementation at 1.26% of body weight in addition to dam's milk would improve weaning weight and pre-weaning growth rate of kids. Feeding of supplementary concentrates higher level (2.1% of BW) during pre-weaning period yielded no futher benefits.

Key words: Birth type, Correlation, Growth rate, Kleiber ratio, Milk yield, Sex

INTRODUCTION

The pre-weaning growth of kids is an economically important trait in goat production and it is essential for the post-weaning growth rate and for the successful weaning (Adenaike and Bemji, 2011). The objective of any goat production enterprise is to produce kids that are in good body condition at weaning and subsequent marketing. Rapid growth during the early period can minimize the cost of rearing and thus provide more benefit to the farmer. The birth weight and early growth rate of animals are determined not only by genetic potential but also by maternal and environmental factors (Mandal *et al.,* 2006). Among these environmental factors climate and seasonal differences among different years affect the production of the whole flock, while sex, type of birth, age and weight affect the individual performance (Kuthu *et al.,* 2013). Successful weaning is contingent upon factors including the age and weight of the goat, the modality of weaning, and nutrition prior to weaning (Mor- and-Fehr *et al.,* 1982).

In developing countries, because of the low productivity of indigenous breeds and inadequate environmental circumstances in traditional farming systems in rural areas, solutions must be aimed at genetic improvement (genetic selection and crossbreeding), development of new feeding strategies and improvement of farming systems (Gökdal, 2013). Thus, successful livestock production requires the application of strategies that optimize the use of the

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environment and available nutrient sources so as to capitalize on the production potential of livestock. For the effective use of native pasture, supplementation is needed in order to meet the nutritional requirements of goats, especially for the small producer (Silva *et al.,* 2010).

The Boer goat is a meat purpose breed famous for its rapid growth, excellent meat quality and high fertility (Malan, 2000).This breed originated in South Africa and was ûrst imported to Ethiopia in 2007 by the project entitled "Ethiopian Sheep and Goat Improvement Program (ESGPIP)" funded by USAID. Crossbreeding of Boer goats with indigenous Central Highland goat were aimed to improve their productivity. However, Boer crossbred kids didn't perform well at breeding and evaluation site management condition; the growth rate of kids become decreased after one month of age. Since milk production in goats peaks within 2-3 weeks after parturition and then declines rapidly to a low level by 8-10 weeks after parturition (Rankins and Pugh, 2012), higher growth performance of kids cannot only be sustained by milk supply from their dams. Hence, the aim of the present study was to evaluate pre-weaning growth performance and kleiber ratio of Boer crossbred kids under different levels of concentrate supplementation in addition to their dam milk.

MATERIALS AND METHODS

The study was conducted from April to June, 2016 at Sirinka Agricultural Research Center which is located 508 km away from Addis Abeba. The site is located at an altitude of 1850 m above msl. The rainfall pattern is bimodal, with two-rainfall season, *belg* (Feb./Mar.- April) and *meher* (July- Oct./Nov.) and the mean annual rainfall amount is on average about 950 mm. The area is a moderate warm temperature zone with mean daily temperature ranges from 16 - 21°C.

A total of 45 crossbred Boer does with 50 % blood level assigned to two crossbred Boer bucks with 50% blood level for mating. After kidding 27 does with normal teat and udder were selected with their 36 kids and identification number was given for each kid. A 2×2 ×3 factorial (2 levels of sex, 2 levels of birth type and 3 levels of feed) arrangement of treatments was followed. Thirty six one- month old kids with an average initial body weight of 7.33±0.19 kg were randomly distributed into three dietary treatment groups. The feeding groups were: Dam milk+grazing/browsing only (T_1), dam milk+grazing/browsing+ supplementary concnetrates at 1.26% of body weight (T_2), dam milk+ grazing/ browsing+ supplementary concnetrates at 2.10% of body weight (T_3). In addition, birth type (single and twin) and sex (female and male) were also considered. All groups were suckled their dams three times a day and during full night until being weaned at 3 month of age (natural weaning). All kids were allowed to graze/browse on natural pasture for 6:00 h from 8:00-11:00 in the morning and from 13:00-16:00 in the afternoon separately from their mothers during day time. Throughout the course of experiment kids were kept with their dams during the night in the semi-opened concrete barn. Dams were supplemented with 300 g/ day of concentrate in the evening in addition to 6:00 h grazing. Group feeding was practiced according to the level of feeding two times a day (half in the morning and a half in the evening) for supplemented groups after 7 days adaptation periods.

Average daily gain (g/day) was calculated as the difference between final and initial body weights divided by number of days of feeding experiment. The Kleiber

Table 1. Chemical composition (%) of concentrate mixture

DM	Ash	OM	CP	NDF	ADF	ADL
90	7.8	92.2	28.8	20	11.1	4.4

DM = dry matter; CP = crude protine, OM= organic matter, NDF = neutral detergent fiber; ADF = acid detergent fiber and ADL=acid detergent lignine

ratio (KR), defined as growth rate/(body mass)$^{0.75}$. The feed refusal collected and weighed on group bases for laboratory analysis. All kids were weighed weekly to estimate feed intake and weighted fortnightly on suspended weight balance after they forced to fast overnight.

Average daily milk yield was determined once weekly during the rearing period, by milk difference technique according to Louca *et al.* (1974). Does were kept away from their kids for 12 hours (overnight) and then one teat from the two teats was hand milked in the next morning. So, daily milk yield for 24 hours for the two teats was estimated as the amount of milk for one teat multiplied by four (Alsheikh, 2013).

The concentrate consisted of 55.7% wheat bran, 40% noug cake, 3% limestone and 1.3% salt. Feed samples were analyzed for dry matter (DM) by drying at 105°C for 24 h, ash by ignition in a muffle furnace at 600°C for 6 h, crude protein (CP) by the Kjeldahl procedure (AOAC, 2006), and neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to procedures of Decruyenaere *et al.* (2009).

Data on dry matter intake, body weight, average daily gain, and kleiber ratio were analyzed using the GLM (General Linear Models) procedure implemented in the SAS package (SAS, 2002). Treatment, sex, birth type and their interaction effect were considered as fixed factors. Means were separated by least significant difference when the overall treatment effect was

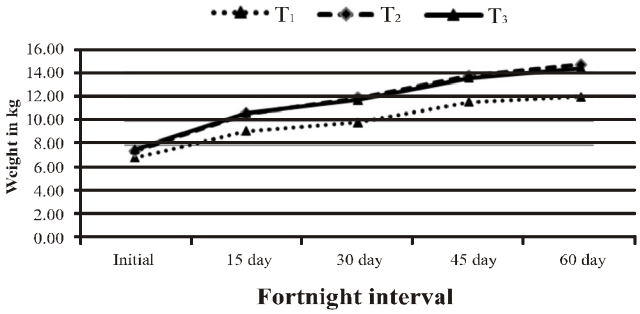

Fig. 1. Pre-weaning body weight of Boer × Central Highland kids from different treatments

significant (P<0.05). Correlations between milk yield of doe and growth performance of kids were obtained using PROC CORR by using standard package(SAS, 2002). Since the interaction between treatment, sex and birth type was non-significant, only the value of main effects were presented and the interacton effect was removed from the model.

The statistical model used was:

$Y_{ijkl} = ì + F_i + B_j + S_k + e_{ijkl}$

Where:

Y_{ijkl} = the feed intake, body weights, average daily weight gain, and kleiber ratio of Boer x Central highland kids

ì = overall mean

F_i = fixed effect of the feeding levels (i= control, 1.26 and 2.1% of their body weight)

B_j = fixed effect of birth type (j=single, twin)

S_k = fixed effect of kid sex (k=male, female)

e_{ijkl}= residual error.

Table 2. Intake, growth rate of crossbred Boer kids and milk yield (g/day) of their dam

Parameters	T_1	T_2	T_3	SEM	P-value
DMI (g/day)	-	93.6	141.3	4.120	<.0001
DMI (% of BW)	-	0.68	1.01	0.049	.0002
Dam milk yield (g/day)	282	332	394	32.58	0.378
Initial weight (kg)	6.92	7.51	7.60	0.19	0.0998
Final(weaning)weight (kg)	12.02^b	14.74^a	14.38^a	0.26	0.0192
Average daily gain (g/day)	85.00^b	120.45^a	113.06^a	5.21	0.038
KR	12.85^b	15.94^a	15.03^{ab}	0.49	0.0247

DMI= dry matter intake, BW= body weight, KR= kleiber ratio, T_1= grazing/ browsing only, T_2= grazing/browsing + supplement 1.26% of their body weight, T_3= grazing/browsing + supplement 2.1% of their body weight; $^{a\,b}$ Means in the same row followed by different letters differ between treatments (P < 0.05)

Table 3. Effect of sex and birth type on weight and average daily gain of kids (n=36)

Items	Sex		Birth type		SEM	P-value	
	Male	Female	Single	Twin		Sex	Birth type
Initial weight (kg)	7.30	7.38	7.68	7.00	0.19	0.8413	0.0948
Final weight (kg)	13.65	13.78	14.85	12.57	0.40	0.8785	0.0122
ADG (g/day)	105.73	106.60	119.52	92.82	5.21	0.9379	0.0221
KR	14.61	14.60	15.45	13.77	0.78	0.8682	0.0692

ADG= Average daily gain, SEM= Standard error of mean, KR= Kleiber ratio

RESULTS AND DISCUSSION

Data pertaing to milk yield of dams, concentrate intake and growth performance of kids are presented in Table 2. The milk yield of Boer x Central Highland goat is found within the range of milk production performance of Ethiopian indigenous goats which varies from 0.28 kg/day for Afar goat (Awigchew *et al.,* 1989) to 1.13 kg/day for Arsi Bale goat (Mestawit *et al.,* 2012). Concentrate intake of kids was increased with the supplementation levels and the highest concentrate intake was observed for kids of group T_3, followed by T_2.

Kid mortality during the period from birth to puberty induces one of the most critical stress periods (Otuma and Osakwe, 2008). Pre-weaning growth performances of kids in different treatment are presented in Table 2. Weaning weight, ADG and Kleiber ratio were significantly higher ($P<0.05$) in concnetrtae supplemented group, however, the differences between T_1 and T_2 were non-significant. The weaning weight as observed in the current study was higher than the value of 12.84 kg (pure Boer), 10.77 kg (Boer x Baladi) and 8.54 kg (Baladi) goat kids fed supplementary concnetrtaes at 1% of their body weight (Salama *et al.,* 2015). Values were also higher than those earlier reported by Belay *et al.* (2015) for the crossbreed Boer goat. This was probably because of strategic supplementation of dams in this experiment during late pregnancy and early lactation.

Results of this experiment demonstrate that conncetrtae supplementation at 1.26% of body weight in addition to dam's milk would improve weaning weight and pre-weaning growth rate of kids. Htoo *et al.* (2015) observed that the ADG of kids from creep feed with alfalfa group was greater than ADG of kids from creep feed without alfalfa and dam milk groups. The current study revealed that kids from T_1 gained least as they relied on their dam milk only. Bhatt *et al.* (2009) reported that pre-weaning parameters such as body weight, ADG and feed conversion ratio were higher in lambs fed creep feeding and milk replacer supplementation. This concurs with our findings and also with other studies (Yiakoulaki *et al.,* 2009; Yiakoulaki *et al.,* 2014), which showed that supplementation of

Table 5. Correlation coefficient (r) of average daily milk yield of does (g/day) and pre weaning weight of kids

	2MWT	WWT	2MADMY	3MADMY	CADMY
2MWT	1.00				
WWT	0.93***	1.00			
2MADMY	0.74***	0.61***	1.00		
3MADMY	0.57**	0.66***	0.68***	1.00	
CMADMY	0.73***	0.68***	0.94***	0.88***	1.00

***= significant at P<0.001, **= significant at P<0.01, 2MWT=2 Month weight, WWT=Weaning weight (3 month weight), CADMY=Cumulative average daily milk yield, 2MADMY=2 month average daily milk yield and 3MADMY=3 month average daily milk yield

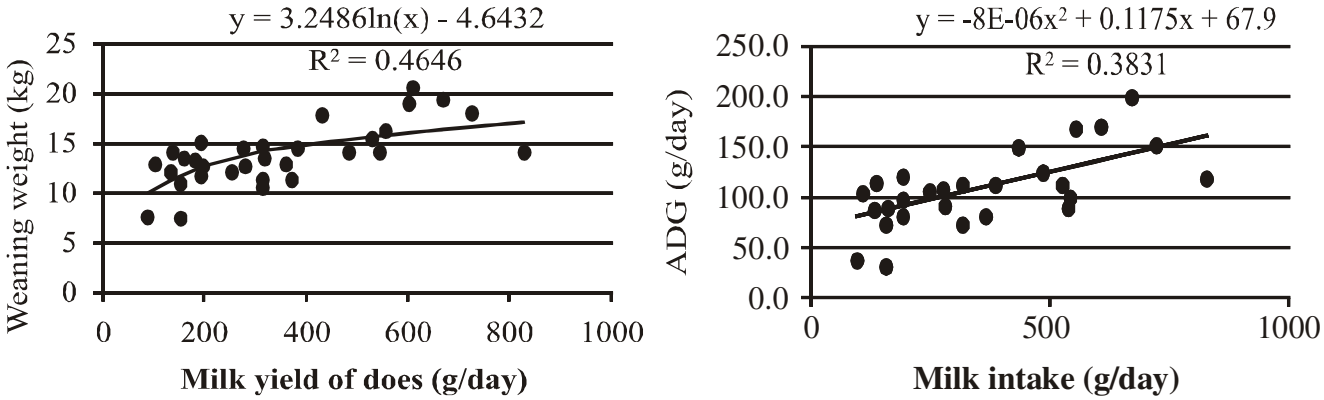

Fig. 2. Regression of milk intake on body weight (left) and weight gain (right) of experimental kids

different pastures during weaning increase ADG of kids and lambs.

The kleiber ratio is the useful indicator of efficiency of feed conversion independent of body size and an important selection criterion for efficiency of growth (Kleiber, 1961; Köster *et al.* 1994). In this study, kids in T_2 had higher kleiber ratio and thus considered efficient users of feed than kids in the other feeding groups. However, KR was not influenced by sex and birth type of kids (Table 3).

Data pertaining to average weaning weight and weight gain of kids for different sex and birth type are presented in Table 3. Weaning weight and pre-weaning average daily gain were not not influenced (P>0.05) by sex of kids. Similar observation had been made by several scholars (Zahraddeen *et al.,* 2008; Belay *et al.,* 2015). However, the weaning weight of single born kids was 2.28 kg heavier than twin kids. Likewise, Belay *et al.* (2015) reported that single born kids were relatively heavier at birth and weaning than twins. Single born kids had greater (P<0.05) pre-weaning average daily gain than twin (119.52±7.45 g/day vs 92.82±7.72 g/day). These results are consistent with other scholars (Madibela *et al.,* 2002; Zeleke, 2007; Belay and Mengistie, 2013). The daily weight gain advantage of kids born single may be linked to pre-and-early post-natal nutrient competition and the less inter-uterine space the twin kids experienced (Zeleke, 2007; Belay and Mengistie, 2013). It is quite clear that multiple born kids need to compete for milk consumption from their dam while single born kids are sole users of milk from their dam.

The relationship between pre-weaning live weight of kids and milk of dams is shown in Table 5. Higher and positive correlation (r=0.68) among weaning weight of kids and average daily milk yield of their dams was observed in this study. Similar strong and positive association was observed by other scholars (Nurfeta *et al.,* 2012; Andualem *et al.,* 2016) for Arsi-Bale goat. This suggests that the live weights and growth rate of kids could be improved by enhancing milk yield of their respective mothers. The regression of milk intake on body weight and weight gain of experimental crossbred kids is presented in Figure 2.

CONCLUSIONS

The study exhibited that supplemention of concnetrtaes at 1.26% of their body weight during pre-weaning period beside dam milk and good pasture could improve the productivity of kids and provide a better profit. Feeding of supplementary concentrates at higher level (2.1% of BW) during pre-weaning period yielded no futher benefits and thus may not be necessary.

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Effect of Dietary Supplementation of Rumen Protected Calcium Salts of Rapeseed Oil and Encapsulated Rapeseed Oil to Lactating Dairy Cows

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ABSTRACT

This study was conducted to evaluate the effect of dietary supplementation of encapsulated and calcium salts of rapeseed oil (CaRSO) on performance of crossbred lactating cows. Eighteen lactating crossbred cows were randomly divided into three experimental groups *viz.* GI, GII and GIII of six animal each. The rumen protected rapeseed oil was prepared as CaRSO and encapsulated RSO. The control group (GI) animals received ration comprising of forages and concentrate mixture without any rapeseed oil. The animals of experimental group received same ration supplemented with either CaRSO (GII) or encapsulated RSO (GIII) at 20 g per kg of milk per day for a period of 90 days. Dietary supplementation of CaRSO and encapsulated RSO tended to increase ($P<0.05$) milk yield. It was observed that supplementation of encapsulated RSO increased ($P<0.01$) milk fat, 4 % fat corrected milk (FCM) and total solids compared to GII, however, responses were comparable to GI. Feed efficiency in terms of MY/DMI (kg/kg) was similar among GI and GII. The solid not fat (SNF) and protein content of milk was not altered by dietary supplementation of rumen protected rapeseed oil. In conclusion, rumen protected encapsulated rapeseed oil seems to have positive effect on milk production and milk fat content in lactating cows.

Keywords: Encapsulation, Feed efficiency, Milk yield, Milk composition, Rapeseed oil, Rumen protected fat

INTRODUCTION

High yielding dairy cows require increased amount of nutrients for increased milk production, however, there will be reduced dietary dry matter intake (DMI), especially during early lactation (Drackley, 1999). Very particularly, there will be increased energy demand during early lactation but energy supply will be less due to decreased DMI which may adversely affect milk production (Sirohi *et al.,* 2010). In early lactating stage, dairy cows meet energy demand from body reserves by mobilizing the body fat reserves which result in negative energy balance and ultimately decreases body weight and milk yield (Drackley, 1999). To overcome this negative energy balance, energy intake must be increased by enhancing the energy density of ration. Both cereal grains and fats are recommended as energy sources to increase the energy density of dairy cow ration. However, there are limitations in excess incorporation of cereal grains that otherwise compromise the rumen function, dietary fat supplement could be considered as an alternate energy source (Saijpaul *et al.,* 2010). However, dietary fat sources can be supplemented in excess of three per cent (on DM basis) in dairy rations. On the other hand, rumen protected fat can be included up to 6-7 per cent of the ration without affecting dry matter digestibility (NRC, 2001) which may result in improved milk and milk fat production in dairy cows (Naik, 2013). There are other studies which indicate that supplementation of rumen protected fat at a level of 4-6 per cent increased the milk production and milk fat in high yielding dairy cows (Purushothaman *et al.,* 2008, Gowda *et al.,* 2013; Kundu *et al.,*2014). The degradability of calcium soaps of long chain fatty acids in rumen was less and digestibility was high in the intestine, it also acts as source of calcium (Elmeddah *et al.,*1991). Like that, encapsulated vegetable oils used as rumen protected fat have high potential to protect the oil from rumen bio-hydrogenation (Gawad *et al.,* 2015). Considering the efficacy of both the methods of protection of oil, it would not be out of context to make a comparative evaluation of these two methods of protection of rapeseed oil. Specific objective of the present experiment was to study the effect supplementing

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rumen protected rapeseed oil on DMI, milk yield and composition.

MATERIALS AND METHODS

The feeding experiment was conducted at Instructional Livestock Farm Complex, College of Veterinary and Animal Sciences, KVASU, Pookode, Kerala. Eighteen crossbred lactating cows were selected from the farm based on their pariety, milk yield and stage of lactation. They were randomly divided into three groups (GI, GII and GIII) with six animals in each group. The experiment lasted for 90 days, following an adaptation period of 15 days. All experimental cows were supplied with chopped green forage (Hybrid Napier) and concentrate mixture (compounded cattle feed) and fed separately to meet their nutrient requirements. In addition, cows in GII and GIII were supplemented with CaRSO and encapsulated RSO, respectively. The quantity of concentrate mixture and supplements required by each lactating cow was adjusted at every fifteen days interval based on their milk production. The animals were housed in well ventilated, shed with automated drinking facilities.

The CaRSO and encapsulated RSO were supplemented at 20 g per kg milk per day, hand-mixed with concentrate mixture and offered during evening. The milking was done daily at 4.30 AM and 3.30 PM and milk yield was recorded daily at each milking by using an electronic weighing balance. The cows were offered concentrate mixture daily, half an hour before and after milking and green forages daily at 8:30 AM, 2:00 PM and at evening 6:00 PM. The rapeseed oil was prepared as rumen protected fat (RPF) by encapsulation method demonstrated by Gawad *et al.* (2015) and calcium salts of rapeseed oil (CaRSO) was prepared as per Naik *et al.* (2007) and Perez (2009).

The daily DMI from feed and roughage was recorded. Individual animal's milk samples (20 ml) were taken weekly and kept at 4° C for further analysis. The monthly pooled milk sample of individual animal was analysed for protein, fat (IS; 1223:2001), SNF and total solids (IS 12333:1997).The samples of concentrates, forages and experimental ration offered were analysed for chemical composition (AOAC, 2016) and presented in Table 1. Milk yield was converted into four per cent fat corrected milk (FCM). Every month, 7 body check points were examined for body condition score (BCS) as elaborated by Mishra *et al.* (2016). The data was analysed statistically according to methods suggested by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The findings of this study indicated that the voluntary DMI was significantly ($P<0.05$) increased in animals supplemented with CaRSO and encapsulated RSO as compared to control group (GI) (Table 2). Earlier researchers like Sirohi *et al.* (2010) reported non-significant increase in intake of DM in lactating crossbred cows. Similarly, Kumar *et al.* (2017) also reported a non-significant increase in DMI (kg/day) of lactating Murrah buffaloes supplemented with prill fat at a level of two per cent of DMI. Other researchers, Naik *et al.* (2009) and Harish *et al.* (2018) reported that supplementation of CaRSO and commercial bypass fat had no significant effect on DMI (kg/day). Significantly ($P<0.01$) lower relative DMI (per cent BW

Table 1. Nutrient composition of concentrates, green forages and experimental rations

Nutrient	Concentrate	Green forage	Experimental ration		
			GI	GII	GIII
Dry matter	86.87±0.43	26.55±0.38	47.93±0.6	51.84±0.17	52.45±0.13
			on % DM Basis		
Crude protein	22.03±0.13	10.52±0.15	15.36±0.05	15.48±0.22	15.69±0.15
Crude fibre	13.27±0.51	36.15±0.26	31.11±0.23	27.79±0.29	29.25±0.33
Ether extract	2.34±0.10	1.43±0.036	2.07±0.05	3.57±0.021	3.14±0.061
Total ash	12.31±0.14	8.19±0.18	5.04±0.01	6.13±0.039	6.07±0.03

Table 2. Effect of supplementation of rumen protected rapeseed oil on body condition score and dry matter intake in lactating cows

Particular	Group I	Group II	Group III	P-Value
Body condition score	3.021±0.06	3.13±0.046	3.00±0.06	0.272
DMI from roughage (kg/day)**	3.33 [b]±0.03	3.41 [a]±0.028	3.27 [b]±0.03	P<0.01
DMI from concentrate mixture (kg/day)**	6.77 [c]±0.05	7.16 [b]±0.055	8.23 [a]±0.05	P<0.01
Total DMI (kg/day)**	10.10±0.06	10.57±0.062 [b]	11.50±0.06 [a]	P<0.01
DMI(kg/100kg BW)**	3.73 [c]±0.03 [a]	2.74 [c]±0.034	3.31 [b]±0.03	P<0.01
DMI (g/kg W $^{0.75}$)**	150.56 [a]±1.15	120.90 [c] 1.176	142.63 [b]±1.15	P<0.01

[a b c] Means with different superscripts in the same row differ significantly; *Significant at 5% level, **Significant at 1% level, [ns] non-significant

and g/kg $W^{0.75}$) were reported in CaRSO supplemented group as compared to encapsulated RSO and control group (Table-2). This corroborates with findings of Mane *et al.* (2017) who supplemented protected fat of palm fatty acids given at level of 10 g/litre of milk in crossbred cattle and observed lower DMI expressed as per cent BW. On the other hand, Ranjan *et al.* (2013) observed significant (P<0.05) increase in DMI (kg/100 kg BW) in lactating Murrah buffaloes fed with bypass fat of palm oil (PO) distillate included at a level of 200 g/day. The response of supplementation of rumen protected fat often varies between studies might be due to palatability, inclusion level (Naik *et al.,* 2009) and its solubility and melting point in the rumen (Purushothaman *et al.,* 2008).

BCS was similar in among all experimental animals (Table 2), comparable to findings of Naik *et al.* (2009) in early lactating cross breed cows fed with calcium soaps of rice bran oil. In contrast to present findings, Kundu *et al.* (2014) and Sharma *et al.* (2016) reported that supplementation of rumen protected prill fat to lactating cows and buffaloes had significantly (P<0.05) improved the BCS. Garg and Mehta (1998) also reported an improved BCS of HF cows supplemented with CaRSO. The additional energy supplied by rumen protected rapeseed oil in this study might have been utilized for increase in MY, thereby causing no change in BCS.

Results of this study clearly demonstrated that added extra energy through CaRSO and encapsulated RSO supplementation might had been used for milk production that was reflected in significant (P<0.05) increase in MY (Table 3) compared to control. The best response, however, was observed in GIII in terms of FCM yield. The feed efficiency (MY/DMI (kg/kg) observed in the present study was better significantly (P<0.05) in CaRSO group than GIII group and similar to control. Purushothaman *et al.* (2008) reported protected fat supplementation caused poorer feed efficiency, whereas, Ranjan *et al.* (2013) reported

Table 3. Effect of supplementation of rumen protected rapeseed oil on milk yield and composition

Particular	Group I	Group II	Group III	P-Value
MY (kg/day)*	10.34 [b] 0.04	10.65 [a]±0.09	10.65 [a]±0.14	0.041
4 % FCM, kg/day **	10.55 [b]±0.05	10.75 [b]±0.10	11.18 [a]±0.15	P<0.01
MY/DMI (kg/ kg)**	1.03 [a]±0.006	1.02 [a]±0.009	0.91 [b]±0.009	P<0.01
Milk composition (%)				
Fat **	4.23 [a]±0.121	3.80 [b]±0.126	4.56 [a]±0.121	P<0.01
Protein [ns]	3.34±0.117	3.36±0.122	3.21±0.117	0.627
SNF [ns]	8.18±0.089	8.12±0.093	8.15±.089	0.895
Total solids**	12.41±0.185 [a]	11.78±0.192 [b]	12.71±0.185 [a]	P<0.01

[a b c] Means with different superscripts in the same row differ significantly *Significant at 5% level, **Significant at 1% level, [ns] non-significant

increased (P<0.05) feed efficiency in treatment group, compared to control group in lactating Murrah buffaloes. Similarly, dietary supplementation of protected fat (Purushothaman *et al.,* 2008; Gowda *et al.,* 2012; Kowalski *et al.,* 1999; Naik *et al.,*2009) resulted in significant (P<0.05) increase in MY in dairy cows. Similar response was also observed in Murrrah buffaloes (Shelke *et al.,*2012; Harish *et al.,* 2016). However, no significant responses were observed in mid lactating cross breed cows (Kundu *et al.,* 2014).

Perusals of the data of milk composition reveals that there was influence of protected fat supplementation on milk fat and total solids content, whereas, SNF and protein content were not affected. In the present study, CaRSO supplementation had significantly (P<0.01) reduced milk fat (per cent) compared to the control and GIII treatment group. These results were in agreement with the studies of Kowalski *et al.* (1999) who reported that CaRSO supplementation decreased milk fat content by 1.9 g/kg in dairy cows as compared to control group. The reasons might be decreased *de novo* synthesis of synthesis of short and medium-chain fatty acids in the mammary gland due to CaRSO supplementation (Kowalski *et al.,* 1999). There was no significant difference in milk fat content of encapsulated RSO (GIII) as compared to control group animals. Similar findings were reported by Ranjan *et al.* (2013), Kundu *et al.* (2014) and Veena *et al.* (2018) in dairy animals. It seems that supplementation RSO did not cause any adverse impact on crude fibre digestibility and acetate to propionate ratio (Ranjan *et al.,* 2013). However, some other studies have reported increased milk fat content due to feeding of protected fat in dairy cows (Naik *et al.,* 2009; Sirohi *et al.,* 2010; Ansar *et al.,* 2014 and Meshram *et al.,* 2016) and buffaloes (Shelke *et al.,* 2012; Harish *et al.,* 2016). It was explained that feeding of protected fat increased availability of fatty acids in the intestine and mammary gland for milk fat synthesis (Shelke *et al.,* 2012).

Milk protein and SNF content were not affected by supplementation of CaRSO and encapsulated RSO protected fat in this study (P>0.05) (Table 3) Similar results were reported by Sirohi *et al.* (2010) and Kundu *et al.* (2014). In contrary, CaRSO supplementation decreased milk protein and SNF content (P<0.05) when Calcium salts of rice bran oil were supplemented (Veena *et al.,* 2018) and increased milk protein values were reported by Ranjan *et al.* (2013). Regarding the total solid content of milk, CaRSO protected fat supplementation in this study shown decreased values (P<0.05) as compared to other groups. But Ranjan *et al.* (2013) and Harsha *et al.* (2016) mentioned that rumen protected fat (prill fat) did not affect the total solid content of milk (P<0.05).

CONCLUSION

From the present study, it may be concluded that supplementation of either encapsulated or calcium salts of rapeseed oil in the diet of lactating cows would increase the average daily milk yield and 4% FCM yield.

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Effect of Dietary Supplementation Area Specific Mineral Mixture along with Hormonal Interventions on the Performances of the Anoestrous and Repeat Breeding Crossbred Cattle

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ABSTRACT

A study was conducted to assess the effect of dietary supplementation of area specific mineral mixture and hormonal therapy on the reproductive performances of anoestrous and repeat breeder crossbred cattle at Nimapara block of Puri district, Odisha, India. A total of 60 animals (40 cows, 20 heifers) were selected and were randomly distributed into four groups of 15 animals each (containing both anoestrus and repeat breeder), experiment was continued for 60 days. Animals of the Group I (control) were fed straw based diet along with concentrates as practiced by the farmers and received no dietary or hormonal intervention. However, animals of group II received dietary supplementation of an area specific mineral mixture at 50 g/animal/d, Hormonal protocols like double synch and estra double synch along with area specific mineral mixture at 50 g/d/animal were tested on animals in group III and IV, respectively. Results revealed significant ($P<0.05$) improvement in growth and reproductive performance in treatment groups as compared to control group. Thus, supplementation of area specific mineral mixture along with hormones gave better results in augmenting reproductive disorder in crossbred animals.

Key words: Cattle, Hormones, Minerals, Reproduction

INTRODUCTION

Minerals play important role in vital metabolic and physiological fucntions including reproduction. Deficiency of essential minerals may result in failure of the homeostasis mechanism, affecting the productive and reproductive potential of animals. Hormonal imbalance affects follicular growth and ovulation which are dependent on the pulsatile secretion of luteinizing hormone (Canfiel and Butler, 1990). Reproductive problems like anestrous, prolonged estrous, conception failure and retained foetal membranes have been associated with deficiency of some essential minerals (Gupta *et al.,* 2005). Moreover, other problems like abortion in cattle and weak calf syndrome have been reported with deficiency of essential minerals (Logan *et al.,* 1990). Deficiency of calcium and phosphorous in cattle of Odisha had been reported (Mohapatra *et al.,* 2012). Treatment with GnRH and $PGF_{2\alpha}$ synchronises estrus, enhances ovulation and maximizes the conception rates and timed estrus behaviour in many dairy herds. A follicular wave can be hormonally programmed for synchronous development (Diskin *et al.,* 2002). Keeping in view the magnitude and complexity of the problem affecting the livelihood security and profitability of the venture, concept of stimulating corpus luteum and follicular development through minerals and hormones were evaluated.

MATERIALS AND METHODS

An on-farm trial was carried out at Nimapara block (20.07º N latitude and 86.02º E longitudes at an altitude of 19.3 m above msl) of Puri district, Odisha, India which lies in the east and south eastern coastal plain agro-climatic zone. The average rainfall of the zone is about 1449.68 mm. General information like breed and age of animals, details of oestrus, treatment after oestrus, age at first calving, calving number, services per conception, date of last calving and other breeding history including anoestrus, postpartum anoestrus, repeat breeding, and feeding practices of dairy cows were collected from the farmers. Status of reproductive organs like the cervix, ovary, and uteri of individual animals was examined par rectum. On the

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basis of survey, 60 animals with reproductive disorders with no physiological and anatomical abnormality were selected and dewormed with broad spectrum anthelmintic (Fenbendazole at10 mg/kg body weight) to rule out the possible effect of worms on reproduction of the animals. These animals were randomly distributed into four different groups of 15 animals (05 anestrous and 10 repeat breeders) each on the basis of their body weights following completely randomized design. Animals in control group (I) were maintained as per the traditional practices of the farmer (straw based diet with locally available concentrate) without any nutritional and hormonal intervention, whereas, the animals in treatment II were fed with area specific mineral mixture @ 50 g/d/animal. The hormonal protocols like double synch and estra double synch along with area specific mineral mixture @ 50 g/d/animal were tested in group III and IV, respectively.

The area specific mineral mixture (ASSM) was prepared as per the reported formulation of Mohapatra *et al.* (2012). The ASSM contains dicalcium phosphate (800 g); wheat flour (200 g); cupric sulphate (200 mg); potassium iodide (1.63 mg); manganous sulphate (400 mg) and zinc sulphate (500 mg). In double synch protocol, prostaglandin $F_{2\alpha}$ (dinoprost tromethamine) and gonadotropin releasing hormone (GnRH) analogue (buserelin acetate) were used twice each on any day of cycle in the following manner (0 day: PGF2α, 2nd day: GnRH, 9th day: PGF2α, 11th day: GnRH). Fixed time artificial insemination was conducted twice on 16 and 24 h of the last GnRH injection. The same protocol was used for both anoestrus and repeat breeding animals (Savalia *et al.,* 2014). In *Estra* double synch protocol, the steps were similar to double synch protocol with replacement of the last GnRH injection with estradiol benzoate in the following manner (0 day: PGF2α, second day: GnRH, ninth day: PGF2α, tenth day: estradiolbenzoate). Fixed time artificial insemination was conducted twice on 48 and 72 h of the last estradiol injection (Biradar *et al.,* 2014).

The body measurements of the animals was recorded at monthly intervaland body weight was calculated using Johnson's formula (1940). About 10 ml of blood was collected from each animal through jugular venipuncture in the morning (before watering and feeding) at 0 and 60 d of the experiment and serum was separated. The serum was kept at -40°C until used. The haemoglobin (Hb) content of the blood samples were estimated by using a Hellige and Sahli's haemoglobin-meter. The packed cell volume (%) determinations of all the blood samples were carried out (Jain, 1986). The concentration of glucose, total protein, albumin, and urea in serum were measured using the Crest Biosystems kit (Mumbai, India). Globulin concentration was determined by subtracting the albumin from the total protein concentration in the serum samples. Serum concentrations of calcium and phosphorus were estimated by using commercial kit (Crest Biosystems, India). Serum concentrations of micro minerals like copper, zinc and manganese were estimated by Atomic Absorption Spectrophotometer (ELICO-SL243, Hyderabad, India).

All the animals were regularly monitored for the onset of heat. The heat was detected by behavioural symptoms (Layek *et al.,* 2011). Animals exhibiting the sign of heat were par-rectally inseminated artificially by the local Veterinary Assistant Surgeon. Pregnancy diagnosis was conducted routinely par rectum at 45 days

Table 1. Body weight changes in experimental animals

Attribute	Groups				P value
	I	II	III	IV	
Initial BW, kg(0 day)	263.60±6.56	258.45±5.21	246.67±5.44	253.33±6.21	0.613
Final BW, kg(60 days)	271.41±7.06	273.17±5.21	261.64±5.42	267.93±6.18	0.740
ADG (g)	130.16[a]±3.51	245.33[b]±9.51	249.50[b]±9.70	243.33[b]±11.30	0.01

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

Table 2. Haematological profile of cross bred cattle under different dietary treatments

Attribute	Group					P value
	Days	I	II	III	IV	
Hemoglobin (g/dl)	0	11.46±0.44	11.78±0.48	11.61±0.23	11.54±0.49	0.220
	60	11.04[a]±0.59	12.77[b]±0.37	11.99[b]±0.07	11.67[b]±0.50	0.04
Packed cell volume (%)	0	26.73±3.00	28.87±2.45	30.20±2.97	29.40±1.57	0.392
	60	26.65±2.73	28.93±337	29.60±3.40	27.90±2.72	0.116

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

post-insemination. Statistical analysis was done by using Software Package for Social Sciences (SPSS) version 17.0 (2008) and one-way analysis of variance (generalized linear model, ANOVA) with comparison among means was made by Duncan's multiple range test (Duncan 1955) with significance level of P≤0.05.

RESULTS AND DISCUSSION

Average daily gain in the treatment groups was higher (P<0.05) than control group whereas there was no significant difference among the 3 treatment groups (Table 1). Trenkle (1976) and Sawant *et al.* (2013) also reported similar findings. The increased body weight gain due to ASMM supplementation might be due to increased nutrient metabolism in supplemented animals. Haemoglobin concentration was higher (P<0.05) in treated groups whereas PCV values were similar in all the groups (Table 2). Increased Hb concentration in mineral supplemented groups was probably due to better interaction of trace minerals and utilization of dietary Fe because of supplementary Cu in the diet (Tiwari *et al.,* 2000).

Serum glucose concentration varied (P<0.05) between control and treatment groups at 60 days of the experiment (Table 3). Similarly, Satapathy *et al.* (2019) observed increased blood glucose levels in anestrous cows supplemented with ASMM at 50g/day/animal. The higher blood glucose concentration in treatment groups might be due to altered molar proportion of volatile fatty acid (VFA) in the rumen with an increase in propionate concentration resulting in increased glucose level in the plasma due to mineral supplementation (Aliarabi and Chhabra, 2006).

Serum concentrations of macro (Ca, P) and micro mineral were more (P<0.05) in treated group than control one (Table 4). Serum concentration of Ca and P at 0 d was below the critical value (Ca:9-12 mg/dl) and

Table 3. Serum biochemical profile of experimental animals under different dietary treatments

Attribute	Group					P value
	Days	I	II	III	IV	
Glucose(mg/dL)	0	49.70±1.61	52.12±3.55	48.69±3.24	49.49±15.02	0.992
	60	42.09[a]±1.85	53.33[b]±2.29	58.69[b]±3.35	59.61[b]±12.65	0.040
Total Protein(g/dl)	0	6.30±0.32	6.49±1.19	5.98±0.66	6.01±0.81	0.963
	60	6.48±0.35	6.65±1.21	6.06±0.66	6.04±0.75	0.927
Albumin(g/dl)	0	3.08±0.06	3.14±0.25	3.23±0.23	3.14±0.20	0.120
	60	3.04±0.05	3.26±0.18	3.20±0.21	3.15±0.21	0.170
Globulin(g/dl)	0	3.86±0.21	3.35±0.96	2.76±0.62	2.87±0.62	0.540
	60	3.79±0.27	3.40±1.02	2.86±0.68	2.88±0.62	0.634
Urea(mg/dl)	0	21.74±3.73	22.87±5.96	21.09±1.06	21.42±3.94	0.785
	60	21.40±4.20	21.52±3.52	22.47±0.87	20.72±3.89	0.037

[ab]Values bearing different superscripts in a row differ significantly (P<0.05)

Table 4. Serum mineral profile of crossbred animals under different dietary treatments

Attribute	Group					P value
	Day	I	II	III	IV	
Ca(mg/dl)	0	6.87±0.43	6.85±0.12	6.96±0.23	6.90±0.32	0.922
	60	6.78[a]±0.36	8.04[b]±0.30	8.15[b]±0.14	7.92[b]±0.37	0.04
P(mg/dl)	0	3.69±0.12	3.90±0.29	4.18±0.33	3.91±0.21	0.277
	60	3.70[a]±0.13	5.96[b]±0.34	5.44[b]±0.29	5.67[b]±0.25	<0.01
Zn(ppm)	0	0.80±0.02	0.87±0.02	0.88±0.04	0.79±0.02	0.130
	60	0.80[a]±0.01	1.52[b]±0.18	1.36[a]±0.03	1.51[b]±0.01	<0.01
Cu(ppm)	0	0.73±0.03	0.76±0.02	0.74±0.02	0.75±0.02	0.329
	60	0.72[a]±0.02	1.24[b]0.04	1.29[b]±0.02	1.17[b]±0.01	<0.01
Mn(ppm)	0	0.32±0.02	0.31±0.02	0.36±0.02	0.34±0.01	0.429
	60	0.33[a]±0.01	0.64[b]±0.03	0.58[b]±0.04	0.57[b]±0.01	<0.01

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

P 4-8 mg/dl) which might be due to the traditional feeding practices. At 60 days of the experiment, both macro and micro minerals concentration improved (P<0.05) in treated animals than control group. The increased serum mineral concentration might be due to extra supplementation of minerals through concentrate and ASMM. Similar results were reported in dairy cattle (Samanta *et al.,* 2005; Agrawalla *et al.,* 2017).

The overall conception rates in different groups were found to be 20, 40, 66.70 and 80% respectively (Table 5). The maximum conception rate was found in ASMM and estra-double synch group (IV). Similarly, increased conception rate was found in ASMM supplemented (Mohapatra *et al.,* 2012) and hormone treated animals (Shah *et al.,* 2003; Lopes *et al.,* 2011). Naikoo *et al.* (2015) also recorded 71.42% estrous induction by combined treatment of mineral and ovosync protocol. The increased conception rate in ASMM and hormone treated group might be due to enhanced LH surge and ovulation rate in animals (Naikoo *et al.,* 2015). Kumar *et al.* (2012) and Agarwalla *et al.* (2017) also reported 45.65 and 40% conception rate, respectively in crossbred cattle supplemented with ASMM at 50 g/day/animal.

CONCLUSION

Supplementation of area specific mineral mixture along with hormonal intervention (double synch and estra double synch) enhanced the growth, serum mineral concentrations and conception rate in repeat breeder and anoestrus animals.

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

Attribute	Group							
	I		II		III		IV	
	An	RB	An	RB	An	RB	An	RB
Total animals	5	10	5	10	5	10	5	10
Animals conceived	1	2	2	4	3	7	4	8
Total animals conceived	03	06	10	12				
Percent of animals conceived	20	40	66.7	80				

AN, Anestrus; RB, Repeat breeders

approach for enhancing reproductive performance in cattle and buffalo" for providing necessary funds and facilities to carry out this research.

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In Vitro Metabolizable Protein and Utilizable Amino Acid Estimation of Ruminant Feed Ingredients

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ABSTRACT

Present study was conducted to estimate *in vitro* metabolizable protein (IVMP) and utilizable amino acid (uAA) content of ruminant feed ingredients like grains *viz.* maize, red sorghum, pearl millet and barley; agro-industrial byproducts *viz.* wheat bran, rice bran, gram churi and maize gluten meal (MGM); oilseed cakes *viz.* groundnut cake-deoiled (DGNC), mustard cake-deoiled (DOMC) and cotton seed cake (CSC) and fodders *viz.* maize green, sorghum green, pearl millet green, berseem fodder, and wheat straw. The samples were incubated in nine replicates. Three replicates followed filtration of incubation mixture and N estimation in the filtrate and residue and ammonia N estimation in the filtrate. Ammonia N was subtracted from total N in filtrate and residue to obtain utilizable crude protein (uCP). Rest of the incubated replicates were freeze dried to obtain residue with CP concentration equivalent to that of uCP, of which half of samples were subjected to amino acid analysis. From uCP, methionine and lysine availability among grains ranged from 1.21 to 2.01% and 2.28 to 4.52%, respectively. The utilizable methionine (% CP) and lysine (% CP) from agro-industrial byproducts was highest from maize gluten meal (MGM; 1.82) and rice bran (5.27), respectively. Methionine (% CP) availability from groundnut cake-deoiled (DGNC; 0.74) and mustard cake-deoiled (DOMC; 0.89) were lower than that of cottonseed cake (CSC; 2.40) and soybean meal (SBM; 2.85). Rest of the sample residues were subjected to hydrolysis with pepsin and pancreatin for estimation of intestinal digestibility of uCP. Intestinal digestibility was multiplied with uCP to obtain *in vitro* metabolizable protein (IVMP). The regression equation developed by regressing estimated MP (IVMP) from calculated MP derived from protein fractions as per CNCP system was IVMP = 0.915× calculated MP + 1.489 (R^2 = 0.934 at P<0.0001, n=39).

Key words: Amino acid, CNCPS protein fractions, Metabolizable protein, Utilizable crude protein

INTRODUCTION

In ruminants, protein is digested by microbes in rumen followed by post ruminal digestion by intestinal and pancreatic enzymes. The absorbed protein at duodenum otherwise, metabolizable protein consists of intestinally digestible microbial protein (DMP) and digestible rumen undegradable protein (DUP). Metabolizable protein (MP) is available to animal for functional purposes after absorption from intestine. Thus, its estimation gives most reliable estimate of protein quality in ruminants. Various MP determination systems (NRC, 2000; 2001, AFRC, 1992) have been proposed. In above systems separate estimation of DMP and DUP was undertaken. To test the usefulness of absorbed protein for specific functions such as growth, lactation, knowledge about amino acid composition of absorbed protein is critical. *In vivo* MP estimation required duodenal canulated animal. Duodenal amino acids flow was estimated in canulated ruminants by Cecava *et al.,* 1988, McCuistion *et al.,* 2004. However, use of duodenal canulated animal is often not feasible due to stringent animal ethic laws, labour and cost on animal experiment. *In vitro* duodenal available amino acid (utilizable amino acid) could be effective alternative to determine utility of protein source to ruminants. An alternative method proposed by Zhao and Lebzien (2000) applying *in vitro* fermentation technique for estimation of utilizable crude protein (uCP) appears more precise. Treatment of uCP residue with pepsin and pancreatin is likely to yield quantity of metabolizable protein more accurately. Considering the paucity of data on IVMP and uCP under Indian context, feedstuffs commonly used in ruminant feeding were quantitatively estimated for *in vitro* metabolizable protein (IVMP) and utilizable amino

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acid composition.

MATERIALS AND METHODS

Dried ground samples of grains *viz.* maize, red sorghum, pearl millet and barley; agro-industrial byproducts *viz.* wheat bran, rice bran, gram churi and maize gluten meal (MGM); oilseed cakes *viz.* groundnut cake-deoiled (DGNC), mustard cake-deoiled (DOMC) and cotton seed cake (CSC) and fodders *viz.* maize green, sorghum green, pearl millet green, berseem fodder, and wheat straw were analyzed for chemical composition. Samples were incubated in nine replicates along with blank. Each group of feed ingredients was incubated in different runs. Each sample in triplicate was processed for utilizable crude protein (uCP) estimation, whereas, remaining six replicates were freeze dried after incubation. From the freeze dried samples, three replicates were analyzed for their amino acid composition to determine the utilizable amino acid (uAA) content in the feed ingredients by using HPLC (AOAC, 2005). Last three replicates were digested further by pepsin and pancreatin (Calsamiglia and Stern,1995) to determine the intestinal digestibility of uCP.

Proximate compositions (AOAC, 2005) and fibre fractions (Van Soest *et al.,* 1991) of the feedstuffs were estimated. NDF was analyzed by using heat stable β-amylase (Number A3306, Sigma Chemical Co.). Acid detergent and neutral detergent insoluble nitrogen (ADIN and NDIN) were estimated as per Licitra *et al.* (1996). Protein fractions *viz.* PA (instantly soluble), PB_1 (instantly degradable), PB_2 (readily degradable), PB_3 (available cell wall protein) and PC (unavailable cell wall protein) were determined as per Cornell net carbohydrate and protein system (Sniffen *et al.,* 1992).

In vitro incubation procedure of Zhao and Lebzien (2000) was adopted to determine uCP content of the feedstuffs. The rumen liquor (RL) from the rumen of fistulated buffalo was collected into thermos flask whose internal temperature was maintained at 39°C before collection. Collection of RL was performed early morning from fistulated animal fed on standard (NDRI) concentrate mixture along with maize green *ad lib* and had free access to water. Collected RL was filtered through 4 layers of surgical gauze and put into thermos and used at earliest for incubation. The following preparations were ready before collection of RL. Buffer I was prepared by dissolving Na_2HPO_4. $12H_2O$ (23.5 g), $NaHCO_3$ (15 g) and NH_4HCO_3 (9.5 g) in 400 ml distilled water. Buffer II was prepared by dissolving NaCl (23.5 g), KCl (28.5 g), $MgCl_2$. $6H_2O$ (6 g) and $CaCl_2$. $2H_2O$ (2.63 g) in 1000 ml distilled water. To 50 ml of buffer II, 400 ml of buffer I was added and the final volume was made to 500 ml. Then, 250 ml of the mixed buffer was diluted with 1000 ml of distilled water and kept at 39°C to maintain the temperature. Feed sample weighing 0.5 gm was taken in 100 ml glass bottle and put in incubator at 39°C before incubation.

For final incubation, 312.5 ml rumen fluid was added to the diluted mixed buffer in a laboratory flask and continuously gassed with CO_2. Then to feed sample, 50 ml of buffered rumen liquor was added and the bottles were sealed with rubber stopper and incubated in water bath maintained at 39°C for 24 h. Blanks without any substrate were incubated along with the samples. After incubation, all the bottles were taken out of the water bath and the pH was measured immediately. Six replicates from each sample and blank were freeze dried. Three replicates of each sample along with blank were filtered through ashless filter paper (no. 42) and washed with distilled water to obtain separate liquid filtrate and solid residue, which was dried in hot air oven.

Utilizable CP sample was freeze dried after 24 h of incubation. Amino acid profile was determined by using High Performance Liquid Chromatography (HPLC) after acid hydrolysis of sample with 6 M HCl at 110°C for 24 hr and further processing as described in AOAC (2005).

From the filtrate 15 ml was taken in glass tube for ammonia N estimation by Kjeldahl distillation method (Melesse *et al.,* 2013). To it, 15 ml of 0.25M phosphate buffer (90 g of $Na_2HPO_4/12.H_2O$/l of distilled water; pH = 11.0 adjusted with NaOH) was added. The pH was adjusted above 10.0 by adding NaOH, since NH_3 release was facilitated at alkaline pH. The liquid sample was then distilled and released ammonia was collected in 3% (w/v) boric acid solution and titrated against 0.05 M hydrochloric acid solution.Nitrogen content of the solid

residue, liquid filtrate and blank were estimated by Kjeldahl distillation method. Residue along with filter paper was collected into a Kjeldahl tube for nitrogen determination. For filtrate, 25 ml of liquid were sampled for determination of N.

Calculation: The utilizable crude protein (uCP) was calculated as

$$uCP\ (\%) = \frac{[NH_3\text{-}N_{blank\ filtrate}(g)+N_{residue}(g)+N_{filtrate}(g)\text{-}NH_3\text{-}N_{sample\ filtrate}(g)] \times 6.25 \times 100}{\text{Sample weight (g)}}$$

After 24 h of incubation the bottle content was freeze dried which has equivalent CP content as that of uCP. The dried sample containing about 15 mg residual N was digested with pepsin followed by pancreatin (Calsamiglia and Stern, 1995) to obtain intestinal digestibility. Utilizable crude protein was multiplied by intestinal digestibility to obtain *in vitro* metabolizable protein (IVMP)

The attributes were compared by analysis of variance using the one-way analysis procedure of SAS System, version 9.3.

RESULTS AND DISCUSSION

Utilizable crude protein (uCP), intestinal digestibility of uCP and *in vitro* metabolizable crude (IVMP) protein content of feed ingredients (% DM) are presented in table 1.

Among grains, uCP (% DM) and MP (% DM) were similar in maize, sorghum and barley. Intestinal digestibility of uCP from grains was variable from 78.63% (barley) to 93.20% (sorghum). Among

Table 1. Crude protein (CP), fibre bound CP, Utilizable CP (uCP), intestinal digestibility of uCP and metabolizable protein (MP) content of common feed ingredients

Feed	CP (%DM)	NDICP (%DM)	ADICP (%DM)	uCP (% DM)	Intestinal digestibility of uCP	MP (%DM)
Grains						
Maize	9.75^{ab}±0.20	1.22^{c}±0.12	0.56^{c}±0.05	9.12^{a}±1.02	86.27^{b}±0.54	7.88^{a}±0.93
Sorghum red	10.25^{ab}±0.25	1.26^{c}±0.06	0.78^{bc}±0.11	7.92^{a}±0.60	93.20^{a}±0.21	7.38^{a}±0.57
Pearl millet	10.34^{a}±0.18	1.66^{bc}±0.03	1.55^{a}±0.01	5.11^{b}±0.28	85.26^{b}±0.64	6.36^{b}±0.27
Barley	9.49^{b}±0.06	2.22^{a}±0.11	0.46^{c}±0.05	9.89^{a}±0.03	78.63^{c}±1.04	8.43^{a}±0.08
Agro-industrial byproducts						
Wheat bran	14.75^{b}±0.19	2.92^{a}±0.12	1.26^{b}±0.06	12.41^{b}±0.50	80.60^{c}±0.54	10.00^{b}±0.42
Rice bran	14.72^{b}±1.11	2.10^{b}±0.24	1.12^{b}±0.11	11.25^{b}±1.52	94.37^{a}±0.48	10.61^{b}±1.40
Gram churi	16.24^{b}±0.17	1.63^{b}±0.19	1.00^{b}±0.15	12.51^{b}±1.16	76.43^{d}±0.38	9.57^{b}±0.93
Maize gluten meal	58.39^{a}±0.37	3.59^{a}±0.02	2.22^{a}±0.10	21.33^{a}±0.14	86.06^{b}±1.51	18.36^{a}±0.20
Oilseed cake						
DGNC	41.76^{b}±0.40	4.21^{a}±0.42	0.74^{b}±0.05	33.25^{a}±0.59	84.85^{a}±0.54	28.21^{a}±0.33
DOMC	35.40^{c}±0.57	1.44^{bc}±0.02	0.30^{c}±0.08	29.11^{b}±0.80	75.75^{b}±1.42	22.07^{b}±1.01
CSC	23.90^{d}±0.52	1.02^{c}±0.04	0.37^{bc}±0.12	19.59^{c}±0.36	75.24^{b}±1.18	14.74^{c}±0.34
SBM	44.38^{a}±0.60	2.14^{b}±0.04	1.16^{a}±0.08	30.61^{ab}±1.03	83.70^{a}±0.67	25.62^{a}±0.86
Fodder						
Maize	11.33^{c}±0.12	6.05^{b}±0.19	1.72^{c}±0.11	9.53^{b}±0.73	75.38^{c}±0.38	7.18^{c}±0.55
Sorghum	9.53^{d}±0.18	3.23^{c}±0.07	0.82^{e}±0.03	7.37^{c}±0.22	81.26^{b}±0.96	5.99^{c}±0.24
Pearl millet	9.58^{d}±0.13	5.97^{b}±0.01	1.17^{de}±0.09	7.47^{bc}±0.03	76.08^{c}±0.40	5.69^{c}±0.03
Berseem	16.89^{a}±0.06	6.92^{a}±0.05	2.97^{b}±0.12	13.70^{a}±0.62	87.70^{a}±0.86	12.03^{a}±0.66
Wheat straw	3.31^{e}±0.28	1.95^{d}±0.02	1.32^{cd}±0.02	2.14^{d}±0.07	39.30^{e}±1.40	0.84^{d}±0.05

agro-industrial byproducts, uCP ranged from 11.25 (rice bran) to 21.33% (maize gluten meal) and intestinal digestibility of uCP varied from 76.43 (gram churi) to 94.37% (rice bran), resulting IVMP ranged from 9.57 (Gram churi) to 18.36% (maize gluten meal). Among oil cakes uCP and IVMP were lowest in CSC (19.59 and 14.74%) and highest in DGNC (33.25 and 28.21%). Intestinal digestibility of uCP of DGNC (84.85%) and SBM (83.70%) was similar and higher compared to those of DOMC (75.75%) and CSC (75.24%). Among the fodders, highest uCP (16.89%) as well as intestinal digestibility (87.70%) was of berseem, thus resulting highest MP (12.03%) from berseem among fodders. Lowest uCP (2.14%) and MP (0.84%) was of wheat straw.

Essential and non-essential amino acid contents of the feed ingredients were expressed as percentage of CP (Table 2). Among grains, pearl millet had highest histidine (3.91%) and phenylalanine (6.81%), whereas, barley had highest arginine (5.35%) content. Red sorghum had the highest valine (4.90%) and leucine (12.87%) content. All the grains had >7.5% leucine and >4.5% of phenylalanine. Maize had the highest methionine (2.06%), cysteine (2.47%) and isoleucine (4.79%) among the grains, whereas, the lysine content was highest in pearl millet (4.11%). Among agro-industrial byproducts, wheat bran had highest % of histidine (5.82%), valine (4.92%), cysteine (2.12%), isoleucine (2.72%) and phenylalanine (8.83%). Phenylalanine was >5% in agro-industrial byproducts. Methionine ranged from 1.37 to 1.69% among the agro-industrial byproducts. Maize gluten meal was richest source of leucine and was least in lysine content (2.27%). In oil cakes, lysine content ranged from 3.13 to 6.77%, arginine ranged from 5.59 - 6.19% except DGNC (1.48%). CSC was richest source of arginine (7.37%) and cysteine (2.29%) among cakes. GNC was a poor source of methionine (0.72%), cysteine (0.22%) and was richest in histidine (4.98%). SBM was highest in leucine (7.98%) and methionine (2.29%). Among the amino acids (% CP), histidine (7.07%), phenylalanine (6.39%) and cysteine (1.99%) were analyzed to be highest in pearl millet fodder. Among fodders methionine was higher in pearl millet (2.17%) and

Table 2. Amino acid content of the feeds (% CP)

Feed	HIS	ARG	THR	VAL	MET	CYS	ILE	LEU	PHE	LYS	ASP	GLU	SER	GLY	ALA	PRO	TYR
Grains																	
Maize	2.42	4.63	4.24	3.31	2.06	2.47	4.79	11.01	5.44	2.06	2.70	7.33	2.47	2.01	0.21	2.31	1.21
Sorghum red	2.32	3.97	3.68	4.90	1.79	1.91	4.06	12.87	4.88	2.44	6.13	6.48	5.22	4.71	4.90	6.06	3.47
Pearl millet	3.91	4.11	1.51	3.31	1.41	0.81	2.51	4.11	6.81	4.11	4.11	6.81	9.31	9.81	2.71	6.91	2.91
Barley	2.91	5.35	2.90	4.45	1.57	2.30	3.40	7.51	4.68	3.66	6.02	7.21	10.01	6.79	6.57	9.21	6.01
Agroindustrial byproducts																	
Wheat bran	5.82	3.12	1.32	4.92	1.52	2.12	2.72	6.02	8.83	3.93	5.73	6.62	6.82	1.82	1.02	4.32	4.82
Rice bran	3.29	2.19	2.09	2.69	1.43	0.63	2.13	2.89	7.49	3.29	4.69	3.29	4.09	1.59	1.69	13.99	2.69
Gram churi	3.47	2.17	1.97	2.37	1.37	0.59	1.99	3.19	5.19	4.49	4.97	3.77	3.87	1.57	1.97	11.79	2.19
Maize gluten meal	3.49	3.89	0.99	1.99	1.69	0.37	2.27	19.17	8.17	2.27	4.59	3.99	9.13	2.13	4.03	4.02	3.02
Oil cakes																	
DOGNC	4.98	1.48	2.08	1.78	0.72	0.22	1.82	2.02	7.63	3.13	6.23	5.12	5.72	1.62	1.82	7.92	1.82
DOMC	1.99	6.19	4.39	4.29	0.63	1.63	4.23	5.19	5.99	4.68	4.48	12.08	2.58	2.99	0.89	2.79	1.79
CSC	2.57	7.37	2.37	4.17	1.37	2.29	3.79	2.29	7.49	5.29	4.27	10.07	2.37	2.27	0.07	1.69	3.29
SBM	2.49	5.59	2.69	4.19	2.29	1.47	3.47	7.98	4.58	6.77	6.79	10.59	2.73	2.33	1.03	2.32	1.32
Fodder																	
Maize	1.58	4.38	3.08	4.88	1.42	0.72	3.42	6.72	3.93	2.73	7.23	8.32	3.42	4.22	1.32	1.02	1.52
Sorghum red	3.49	2.59	1.39	4.29	2.13	1.13	4.23	4.69	3.79	3.68	9.48	7.38	4.28	4.19	1.29	4.99	1.99
Pearl millet	7.07	8.27	1.77	3.87	2.17	1.99	3.09	6.49	6.39	3.49	5.87	6.27	4.87	2.27	2.47	4.39	3.69
Berseem	1.39	5.59	4.89	4.79	1.49	1.47	4.37	6.88	4.28	5.37	8.49	7.99	2.03	7.03	1.13	3.12	1.82
Wheat straw	1.51	1.06	2.55	2.75	1.05	0.99	1.68	3.40	2.17	3.17	10.19	6.79	1.13	1.33	2.53	3.92	1.23

sorghum (2.13%) whereas highest lysine content was of berseem (5.37%). CP in berseem had highest concentration of threonine (4.89%) and valine (4.79%) among fodders.

The utilizable amino acid (uAA) was expressed as percentage of CP (Table 3). Among grains, the availability of arginine, valine and phenylalanine from uCP were 4.40 to 4.52%, 3.75 to 4.93% and 4.02 to 5.15%, respectively. The methionine and lysine availability from grains ranged from 1.21 to 2.01% and 2.28 to 4.52%, respectively and that of cysteine ranged from 1.11 to 2.33%. The utilizable arginine from agro-industrial byproducts was highest in rice bran (4.41%) followed by maize gluten meal (3.82%), wheat bran (3.67%) and gram churi (2.09%). The methionine and cysteine availability were highest from maize gluten meal (1.82%) and wheat bran (2.69%), respectively. The lysine availability from byproducts varied from 2.63% (maize gluten meal) to 5.27% (rice bran). Among oil cakes DGNC had highest histidine (5.26%) and lowest arginine (1.80%) availability at the duodenum. In other cakes the arginine availability at duodenum was much higher and ranged from 5.52% (SBM) to 6.03% (CSC). Methionine availability from DGNC (0.74%) and DOMC (0.89%) were lower than that of CSC (2.40%) and SBM (2.85%). But S containing amino acid cysteine was highest utilizable from DOMC (2.05%). The lysine and phenylalanine availability from the cakes ranged from 3.70% (DGNC) to 6.42% (SBM) and 4.60% (SBM) to 7.12% (DGNC), respectively. The utilizable histidine was highest from pearl millet (5.46%) followed by sorghum (4.51%) fodder, whereas that of valine and threonine was highest from berseem. Methionine availability at duodenum was highest from sorghum fodder (2.01%) followed by wheat straw (1.79%) whereas that of cysteine was highest from wheat straw (1.72%) and lowest from maize fodder (0.37%). The utilizable lysine from fodders varied from 2.58 to 5.59%.

The uCP of feeds analyzed by Zhao and Lebzien (2002) are in corroboration with present findings. The uCP (%DM) of maize, barley, MGM, rapeseed meal, CSC and SBM estimated from *in vitro* incubation with rumen liquor of cattle was 15.4, 16.4, 21, 23.3 and 28.9 whereas that estimated with rumen liquor of sheep was 14.4, 14.2, 17.2, 25.2 and 24.2, respectively (Zhao and Lebzien, 2002). Higher RDP and RUP was reported in MGM (Prusty *et al.,* 2013a), which might be the possible cause of higher uCP in MGM. The small intestinal digestibility of feed ingredients *viz.* cotton seed meal, rapeseed meal, SBM, maize gluten feed analyzed by *in vivo* methods ranged from 73.7-90.4%, 64.6-76.5%, 96.6-98.4% and 55-72.1% (Moloney *et al.,* 2001), are in corroboration with present findings. Similar MP of maize grain, CSC and wheat bran has been reported by Das *et al.* (2014) using nylon bag technique (AFRC, 1992). Taghizadeh *et al.* (2008) reported lower MP of maize (3.51%) and barley (4.85%) and higher MP of cottonseed meal (23.22%) compared to present findings by using *in situ* degradability method to estimate MP. Calsamiglia and Stern (1995) reported higher (85-90%) post ruminal digestion of SBM and maize gluten meal. The variations might be due to variation in processing methods for oil cakes or difference in rumen microbial population. In agreement to present findings, Promkot and Wanapat (2003) found higher intestinal digestibility of rumen residual CP of SBM compared to CSC. The uCP (%DM) of wheat straw analyzed from *in vitro* incubation with rumen liquor of cattle and sheep was 6.3 and 6.7%, respectively (Zhao and Lebzien, 2002). MP of fodders *viz.* maize, sorghum and berseem analyzed by Das *et al.* (2014) by *in sacco* incubation technique corroborated with present report.

In Cornell net carbohydrate and protein system (CNCPS), CP of feeds is classified according to rate of degradability (Prusty *et al.,* 2013b; Mondal *et al.,* 2008) into different fractions. Findings of the present study were validated from regression of IVMP from calculated MP derived from CNCPS protein fractions (Table 1). Rumen degradable protein (RDP) and rumen undegradable protein (RUP) based on the protein fractions were calculated (NRC, 2000). The MCP availability was obtained by multiplying 0.85 with the RDP. MCP contribution towards metabolizable protein (digestible microbial protein, DMP) was 64 percent

(NRC, 2001). Intestinal digestibility of RUP (NRC, 2001; Das *et al.,* 2014; De Boer *et al.,* 1987) was applied to RUP to obtain digestible undegradad protein (DUP). Thus, calculated MP was estimated by adding up DMP and DUP. Significant relationship between calculated MP and IVMP was established (Fig. 1). The developed regression equation was IVMP = 0.915× calculated MP + 1.489. The correlation coefficient between the calculated and *in vitro* analyzed MP was 0.97 with R^2 value of 0.93 at P< 0.0001 level of significance. Thus IVMP could be effectively estimated by pepsin and pancreatin hydrolysis of *in vitro* incubated sample. In case of unavailability of required set up for conducting *in vitro* incubation procedure, CNCP fractions also could give an idea of the availability of protein from a feed. Significant multiple regression relationship between the uCP and the CNCPS crude protein fractions, i.e. PB1, PB2, PB3 and PC of feeds was observed by Zhao and Cao (2004) which supported current findings.

Unlike present findings, NRC (1984) suggested maize to be a relatively good source of sulfur AA and a poor source of lysine. Previous report (Ejeta *et al.,* 1987) of higher lysine% and lower tyrosine% of pearl millet than sorghum support present finding. Lower isoleucine/ leucine ratio in pearl millet than that of sorghum and maize (Ejeta *et al.,* 1987) was in support of present findings. As per McDonald *et al.* (2010) arginine, lysine, methionine, cystine and tryptophan were the main

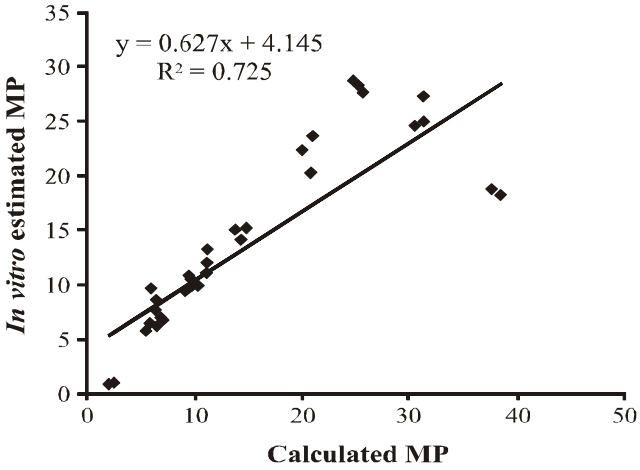

Fig. 1. Regression of in vitro MP on CNCPS protein fraction calculated MP

Table 3. Utilizable amino acid content of the feeds for buffaloes (% CP)

Feed	HIS	ARG	THR	VAL	MET	CYS	ILE	LEU	PHE	LYS	ASP	GLU	SER	GLY	ALA	PRO	TYR
Grains																	
Maize	2.31	4.52	4.43	3.75	2.01	1.78	3.29	10.18	4.82	2.28	3.86	7.13	3.67	1.95	0.44	4.11	2.45
Sorghum red	2.43	4.44	3.41	4.93	1.86	1.34	3.27	9.34	4.02	3.07	6.42	2.49	9.05	4.51	0.86	5.89	6.92
Pearl millet	5.95	4.40	1.98	4.14	1.21	2.33	2.16	3.54	5.15	4.52	4.73	8.69	11.77	12.89	2.50	5.87	3.88
Barley	3.42	4.41	2.60	4.61	1.61	1.11	3.60	7.62	4.14	3.28	6.95	7.62	5.34	4.07	6.01	9.70	6.21
Agro-industrial byproducts																	
Wheat bran	5.99	3.67	1.15	5.67	1.53	2.69	2.70	6.03	8.55	3.40	6.14	8.01	6.48	1.06	2.70	4.73	5.13
Rice bran	5.13	4.41	1.59	2.60	1.74	1.88	1.45	2.24	6.55	5.27	5.37	3.40	3.33	1.81	1.52	12.16	3.47
Gram churi	4.18	2.09	1.23	2.03	1.66	1.18	1.78	2.46	5.60	4.79	4.73	3.32	4.40	1.48	1.29	10.97	2.46
Maize gluten meal	3.83	3.82	1.65	2.60	1.82	0.61	2.70	13.40	7.95	2.63	4.96	4.32	9.22	2.82	4.40	4.51	3.05
Oil cakes																	
DOGNC	5.26	1.80	2.40	1.70	0.74	0.46	1.26	1.74	7.12	3.70	6.50	6.14	5.86	1.44	2.36	7.26	1.76
DOMC	2.20	5.62	4.65	4.69	0.89	2.05	4.00	4.50	6.83	4.59	4.49	10.59	1.47	2.70	1.77	4.09	1.19
CSC	3.30	6.03	2.66	4.19	2.40	1.10	3.73	3.06	6.79	5.19	5.22	9.39	1.90	1.80	0.59	3.40	4.21
SBM	3.60	5.52	1.30	4.99	2.85	1.44	1.32	7.28	4.60	6.42	7.47	9.70	3.09	1.28	1.26	3.27	1.38
Fodder																	
Maize	2.93	4.47	3.55	4.47	1.52	0.37	3.47	6.64	3.31	2.58	9.28	7.00	1.27	4.82	1.28	2.38	1.92
Sorghum	4.51	1.92	0.86	4.30	2.01	1.15	4.15	4.59	3.79	5.59	9.26	7.78	4.73	3.44	1.15	5.94	2.49
Pearl millet	5.46	7.87	1.12	3.46	1.10	1.39	1.86	6.16	7.49	5.20	5.57	6.35	4.45	2.35	2.46	2.21	3.70
Berseem	1.95	5.10	4.98	4.80	1.12	1.20	4.27	6.95	4.45	4.80	8.05	7.32	2.90	7.51	1.07	3.63	1.90
Wheat straw	2.57	1.81	2.32	3.66	1.79	1.72	2.89	4.78	2.70	4.40	9.23	6.48	1.87	2.23	2.22	3.60	2.04

limiting indispensable amino acids in maize and sorghum. As per Merchen and Titgemeyer (1992) maize gluten meal was a poor source of lysine and an excellent source of sulfur AA and leucine which corroborates well with the present findings. Though lower concentration of cysteine was reported in present study, higher methionine content in maize gluten meal resulted in higher concentration of sulphur amino acid in MGM. There are reports indicating high level of valine, isoleucine, leucine, arginine and phenylalanine in groundnut meal-deoiled (Maneemegalai and Prasad, 2011). In the present study, however, content of only phenylalanine was higher in DGNC. Thanaseelaan (2013) observed that rape seed meal (RSM) was a better source of lysine and methionine as compared to GNC (NRC, 1994). In the present study it was observed that DOMC was a better source of lysine than GNC, whereas, similar methionine content was reported in both the cakes.

Much less work has been done regarding the utilizable AA composition of ruminant feedstuffs. Zhao and Lebzien (2002 found a linear relationship between the utilizable crude protein (g/kg) and the uAA (g/kg) estimated from *in vitro* incubations. Similar to present results, intestinal digestible lysine and methionine of 6.7 and 2.7g per kg of SBM were observed in a study by Borucki Castro *et al.* (2007). Sampath *et al.* (2003) reported that UDP fraction of groundnut cake, CSC, maize gluten meal (40%CP), bajra grain and rice polish contained 0.85 and 0.25; 1.65 and 0.34;1.33 and 0.88; 1.48 and 0.11, 0.95 and 0.17% of lysine and methionine, respectively. Less variation was observed in the composition of amino acid in the feed CP and uCP. Findings of Boisen *et al.* (2000) also suggested reduced variation in the concentrations of individual amino acids in the duodenum related to original feed composition. In a study, Merchen and Titgemeyer (1992) found higher proportion of threonine and isoleucine in the duodenal digesta of animals fed on alfalfa fodder. It was suggested that the profile of EAA at duodenal level could be altered by appropriate supply from the supplemental sources. Thus, amino acid composition of protein at duodenum level might depend on variations in the microbial population as well as dietary protein.

CONCLUSIONS

The data generated would be useful in determining the protein quality and requirement of specific amino acid for ruminants. Critical analysis of the variation in amino acid profiles among different classes of feedstuffs *viz.* energy rich (grain), protein rich (oil ckaes), fibrous (fodders), fillers (agro-industrial byproducts, wheat straw) would be useful in precise ration formulation for livestock. The less variation in the amino acid composition of CP and uCP signifies that supplementation of specific amino acid could be modified through diet of animals.

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Effect of Feeding Fresh Banana Plant Waste and its Silage on Dry Matter Intake, Nutrient Digestibility and Rumen Fermentation Parameters in Osmanabadi Kids

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ABSTRACT

This study was conducted to evaluate the possibility of incorporating banana plant waste either fresh or as silage in the diet of small ruminants. To study the effect of fresh banana plant waste and its silage on the performance of Osmanabadi kids, 15 growing kids were randomly allocated into three groups (n=5) on body weight (16.0 kg) and age (8-11 months) basis. The fresh banana plant waste silage (FBPWS) was fed to one of the three treatment groups (T_3) and compared with groups fed fresh banana plant waste (FBPW; T_2) and sorghum straw (T_1) as source of roughage with concentrate mixture in the ratio of 55.8:47.2 (T_1), 25.3:74.7 (T_2) and 38.9:61.1 (T_3). The DM and CP (%) content of FBPW and FBPWS were 8.60 and 8.84; 29.97 and 5.44 respectively, while silage had pH of 5.05. The DMI in T_2 (0.54%) and T_3 (0.98%) groups were significantly ($P<0.01$) lower than T_1 group (1.57%). When these roughage sources were supplemented with CFM, it supported daily gain (g/d) of 2.33 and 14.14 in T_2 and T_3 groups which indicated that these were maintenance type of roughages. There was no significant difference between treatments in any of the nutrient digestibility parameters. The DCP and TDN content (%) in T_1, T_2 and T_3 groups were 4.43 and 66.24; 9.96 and 73.50; 6.21 and 69.55 respectively. The pH values of rumen fluid of three groups were within the normal range of variations, whereas, TVFA levels were different among the treatment groups. Total nitrogen, TCA precipitable- N and soluble nitrogen levels were similar among groups. It is concluded that FBPW and FBPW silage due to their low DM level could not be a sole source of roughage in the diet of ruminants and require to be supplemented with either good quality hay or straw and CFM to meet the nutrient requirements to support growth.

Keywords: Fresh banana plant waste, Banana plant waste silage, Gain, Kids

INTRODUCTION

Among several fruit plants wastes used as animal feed, banana plant by-products like stem and leaves may be one of the major substitutes of roughages after harvesting fruits (Bhimsen *et al.,* 2014). Presently the by-products of banana plants are thrown out as waste on roadsides, fields and allowed to rot away or sometimes burnt in the field (Amaranth and Balakrishnan, 2007). Banana plant wastes cannot be stored for longer period due to higher moisture level and in order to increase the self life for efficient utilisation of wastes, silage making is preferred. The moisture level can be reduced to desired level by adding any dry roughages and cereal grain powder to enhance fermentation process to obtain good quality silage. Hence, attempt was made to convert it into silage and evaluate for its nutritional significance in Osmanabad goats in comparison with fresh banana plant waste feeding.

MATERIALS AND METHODS

The banana plant wastes were collected, chopped manually and dried under sun for 5 days to reduce the moisture to 73%. Then banana plant waste (85%) was mixed with sugarcane bagasse (13%) as a water absorbent material and ground sorghum grain (2%) as a source of carbohydrate and the DM content of mixed material was 36.65%. The same was filled in silo pit (above ground level, 8 cu m, capacity of 5.6 tons). After 6 weeks, the containers were opened and characteristics of silage were studied for their physical characteristics like pH, colour, texture, and odour. Fifteen Osmanabadi kids (8-11 months age; 16 kg BW) were divided into three groups of 5 kids each in completely randomised design and designated with following treatments; free choice feeding of sorghum

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stover and concentrate feed mixture (CFM) (T_1) serves as control group, free choice feeding of fresh banana plant waste and CFM (T_2) and free choice of banana plant waste silage and CFM (T_3). All the kids were housed individually in metabolic cage and provided with similar management practices. All the kids were dewormed with fenbendazole and were vaccinated against FMD, ET, PPR and HS as per standard schedule. The animals were fed as per requirement of NRC (1981). During the experimental period, daily feed intake and weekly body weight gain were recorded. All 15 kids were subjected for metabolism trial for 5 days at the end of the experiment. The rumen liquor was collected before the start and end of the experiment by passing stomach tube into the rumen from all kids at two hours post feeding and pH was recorded immediately using electronic digital pH meter and rumen fluid was stored at –20°C for estimation of NH_3-N, TN, TCA-N and soluble nitrogen (Gloppe and Hvidsten, 1995) and TVFA's according to Barnet and Reid (1956). The samples of feed ingredients, concentrate feed mixture (CFM), fresh banana plant waste (FBPW), fresh banana plant waste silage (FBPWS) and faecal matter were subjected to proximate analysis (AOAC,1995) and forage fiber fraction (Van Soest *et al.,* 1991). Data obtained were subjected to analysis of variance using the General Linear Model procedures of Statistical Analytical System (SAS, 2012).

RESULTS AND DISCUSSION

The physical characteristics like colour of silage was brownish yellow and no mould growth was seen, but pH of the silage was 5.05. The level of volatile fatty acids (75 mmol/kg) was well comparable with the good silage and the NH_3-N level was slightly higher (4.66 mg/dl) than the normal value.

DM (%) content of FBPW and FBPWS was similar to the values (Table 1) reported by Marie-Magdeleine (2010). The CP (8.84% DMB) in FBPW was reduced to 5.44 % when it was ensiled with 2% sorghum grain powder and 13 % sugarcane bagasse and value was lower than the values (14 to 15%) reported by Hambade and Patel (2001), Khattab *et al.*(2000) and Rahman and Huque (2002). The NDF and ADF values were well comparable with values obtained both for fresh and silage in the study conducted by Ally and Kunjikutty (2003) and Rahman and Huque (2002). The ADL content of FBPW and FBPWS was higher (9.39 and 9.72) than values reported (5.25%) by Reddy and Reddy (1991) for fresh banana plant waste and its silage (3.61%) by Khattab *et al.* (2000).

Significantly lower (P<0.01) DMI from FBPW

Table 1. Chemical composition (% DM) of fresh banana plant waste (FBPW), its silage (FBPWS), sorgum stover (SS) and concentrate mixture (CFM) fed to experimental kids

Particular	FBPW	FBPWS	SS	CFM
Organic matter	85.22	84.48	90.61	93.96
Crude protein	8.84	5.44	2.73	16.69
Crude fibre	35.34	37.76	35.08	3.56
Ether extract	1.27	1.15	1.75	2.31
Nitrogen free extract	39.77	40.13	51.05	71.40
Total ash	14.78	15.52	9.39	6.04
Acid insoluble ash	2.74	3.60	3.65	1.06
Neutral detergent fibre	69.13	73.58	76.86	23.59
Acid detergent fibre	43.11	52.72	52.18	9.37
Cellulose	29.87	36.18	39.99	5.79
Hemicellulose	26.02	20.86	24.68	14.22
Acid detergent lignin	9.39	9.72	5.60	6.88

Note: FBPW- Fresh banana plant waste, FBPWS- Fresh banana plant waste silage, SS-sorgum stover, CFM- concentrate feed mixture

(T_2) and FBPWS (T_3) groups was attributed to higher moisture level when compared to DMI from sorghum stover (T_1) (Table 2). The DMI from CFM was not differed among the groups as its quantity was restricted. The difference in total DMI among the groups was due to variation in intake of FBPW and FBPWS. The roughage and concentrate ratio in T_1, T_2 and T_3 were 55.8: 47.2, 25.3:74.7 and 38.9: 61.1, respectively. As per cent of body weight, kids in T_2 group had very low DMI than T_3 groups (0.54 v/s 0.98%) which was significantly (P<0.01) lower than T_1 group (1.57%). On the contrary, Reddy and Reddy (1991) noticed DMI of 1.61% of live weight in bulls and Gupta *et al* (2001) observed 1.26 % DMI in bullock when fed whole banana plant alone. The significant variations in DMI did not cause variation in CP intake due to difference in the CP level of feedstuffs but total CPI in all groups was higher than the maintenance requirement of kids. Significantly higher (P<0.01) total intake of OM, CF, EE, NDF and ADF was observed in T_1 group followed by T_3 and T_2 groups. The difference in the nutrient intake was attributed to the variations in the DMI. The results were well corroborated with the findings of Ginni (2014).

The daily gain of 2.33 g/d in T_2 group indicated that nutrients received by kids were just sufficient for maintenance, the gain in T_3 group was little better than T_2 group (Table 3) which indicated that FBPW and FBPWS might be used as maintenance type of roughage with little supplementation. There was no significant difference among treatments in any of the nutrient digestibility parameter (Table 3). These values were higher than the values reported by Hambade and Patel (2001), similar to the values reported by Ally and

Table 2.Mean daily DM intake and nutrients intake of experimental kids

Parameter		T_1	T_2	T_3	SEM	P-Value
DMI, g/d	Rough	266.63^a	81.85^b	153.67^c	8.30	<0.01
	CFM	238.29	242.21	241.59	1.21	0.39
	Total	504.92^a	324.05^b	395.25^c	8.91	<0.01
%BW	Rough	1.57^a	0.54^b	0.98^c	0.05	<0.01
	CFM	1.43	1.57	1.53	0.06	0.63
	Total	3.00^a	2.11^b	2.51^{ab}	0.10	<0.05
CPI, g/d	Rough	7.28	7.28	8.36	0.39	0.43
	CFM	39.77	40.42	40.32	0.20	0.39
	Total	47.05	47.66	48.68	0.48	0.39
%BW		0.28	0.31	0.31	0.01	0.65
OMI, g/d		465.49^a	297.33^b	356.81^c	8.01	<.0.01
%BW		2.77^a	1.94^b	2.26^{ab}	0.09	<.0.01
CFI, g/d		102.02^a	37.55^b	66.62^c	2.94	<.0.01
%BW		0.60^a	0.25^b	0.42^c	0.02	<.0.01
EEI, g/d		10.17^a	6.64^b	7.35^b	0.15	<.0.01
%BW		0.06^a	0.04^b	0.04^b	0.02	<.0.01
NFEI, g/d		306.26^a	205.49^b	234.16^b	4.53	<.0.01
%BW		1.82^a	1.34^b	1.48^{ab}	0.06	<.0.05
NDFI, g/d		261.14^a	113.72^b	170.06^c	6.39	<.0.01
%BW		1.55^a	0.74^b	1.08^c	0.05	<.0.01
ADFI, g/d		161.45^a	57.98^b	103.64^c	4.24	<.0.01
%BW		0.95^a	0.38^b	0.66^c	0.03	<.0.01

P<0.05, P<0.01, Means with different superscripts in a row differ significantly

Kunjikutty (2003) and Ginni (2014) in Osmanabadi kids. No significant difference was observed between the groups in mean nitrogen intake and dung nitrogen outgo but there was a significant difference (P<0.01) in urine nitrogen outgo, whereas no difference was noticed in total nitrogen outgo among the groups. However, all the kids in the treatment groups were on positive nitrogen balance which was evident in terms of marginal gain of daily body weight (Table 3).

DCP (%) in T_2 group was significantly higher (P<0.01) than T_3 and T_1 because of higher digestibility of CP (67.48%), while there was no significant difference in TDN and ME levels in the experimental diets. However, intake of DCP, TDN and ME among the groups were statistically significant (P <0.01). The findings of this study were similar to the values reported by Bhuyan *et al.* (1989) and Ally and Kunjikutty (2003) and Ginni (2014).

The intension of analysing rumen fluid parameters of the experimental kids was to know the trend of rumen ecology related to the constant feeding of FBPW and its silage in comparison with usual roughage feeding. On observation, pH values of rumen of the kids were within the normal range under different treatments

Table 3. Mean body weight, daily gain, nutrient digestibility and density in experimental kids

Parameter	T_1	T_2	T_3	SEM	P-Value
Body Weight					
Initial (kg)	16.00	16.00	16.02	2.61	0.99
Final (kg)	17.25	16.10	16.61	2.66	0.79
Gain (g/d)	29.76	2.33	14.14	26.69	0.30
Nutrient digestibility, %					
DM	65.01	74.53	71.22	2.10	0.21
OM	70.09	78.10	75.02	1.90	0.26
CP	46.46[a]	67.48[b]	50.40[b]	1.63	<0.01
CF	63.67	69.86	67.29	2.05	0.48
EE	64.25	69.11	78.58	3.06	0.19
NFE	76.09	82.25	82.25	2.08	0.40
NDF	56.82	53.19	52.46	3.11	0.83
ADF	63.32	52.62	54.06	2.40	0.24
Nitrogen balance (g/d)					
Nitrogen intake	7.57	7.5	7.78	0.20	NS
Faeces-N Out go	3.95[a]	2.66[b]	3.92[a]	0.34	<0.01
Urine-N Out go	1.18[b]	2.98[a]	2.36[a]	0.38	<0.01
Total-N-out go	5.13	6.15	6.28	0.52	NS
N balance	2.44	1.35	1.50	0.51	NS
Nutrient density					
DCP, %	4.34[a]	9.96[b]	6.21[c]	0.26	<0.01
TDN, %	66.24	73.50	69.55	1.19	0.30
DOMD, %	64.62	71.71	67.73	1.79	0.30
ME, MJ/kg	10.00	11.10	10.50	0.28	0.32
DCPI, g/d	21.86[a]	32.06[b]	24.53[a]	0.71	<0.01
TDNI, g/d	335.14[a]	236.36[b]	274.81[b]	8.56	<0.01
MEI, MJ/d	5.06[a]	3.57[b]	4.15[b]	0.13	<0.01

P<0.05, P<0.01, Means with different superscripts in a row differ significantly.

Table 4. Rumen parameters: pH, total volatile fatty acids(TVFAs), ammonia nitrogen(NH_3-N),totalnitrogen(TN), TCA precipitable nitrogen(TCA-N) and soluble nitrogen(SN) in phase-I

Parameter	T_1	T_2	T_3	Mean	SEM	P-Value
pH						
Initial	6.79[a]	6.71	6.65[a]	6.72[a]	0.05	NS
Final	6.29[b]	6.32	6.16[b]	6.26[b]	0.05	NS
TVFA's, m mol/dl						
Initial	12.11	10.92	10.36[a]	11.13[a]	0.16	<0.01
Final	13.25	11.99	9.03[b]	11.42[a]	0.16	<0.01
NH_3-N, mg/dl						
Initial	26.70	22.69[a]	28.11[a]	25.83[a]	0.51	<0.05
Final	29.72	13.15[b]	17.67[b]	20.18[b]	0.51	<0.01
TN, mg/dl						
Initial	96.96	99.52	96.32	97.6[a]	1.01	NS
Final	104.41	79.31[b]	99.39[a]	94.37[b]	1.01	<0.01
TCA-N, mg/dl						
Initial	50.70	54.72[a]	59.24[a]	54.89[a]	1.27	NS
Final	50.20	27.11[b]	25.10[b]	34.14[b]	1.27	<0.01
Soluble –N, mg/dl						
Initial	46.26	44.80	37.08	42.71[a]	1.69	NS
Final	54.21	52.21	74.30[b]	60.24[b]	1.69	<0.01

P<0.05, P<0.01, Means with different superscripts between columns differ significantly.

even though the proportion of roughage component was only 25% of total DMI in T_2 group. Similar pH values were reported in ruminants fed with banana plant waste and its silage in various experiments (Reddy and Reddy 1991; Rahman and Haque 2002; Mahanta and Pachauri, 2005; Ginni,2014). Significant difference in the level of TVFA between the treatments was observed due to the dietary variation and nutrient intake in kids. The values reported in this study were far lower than the values obtained by Rahman and Haque (2002).

Highly significant (P<0.01) level of total nitrogen in T_1 group between the treatment at the final of the experiment in T_1 and T_2 might be due to variation in the DCP intake. TCA precipitable nitrogen is an indication of insoluble or unavailable nitrogen and no significant difference among the treatments was observed. The TCA precipitable nitrogen level was lower in T_2 and T_3 than in T_1 group which indicated that available nitrogen was more in banana plant waste and banana plant waste silage when compared to sorghum (Table 4). No significant difference was observed among the treatments in the level of soluble nitrogen.

CONCLUSIONS

From the results it is evident that FBPW can supply nutrients enough to meet maintenance requirements of goats. In order to achieve higher performance FBPW based diet need concentrate supplementation along with feeding of good quality hay.

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Body Heat Storage and Physiological Responses of Peripartuient Karan Fries and Sahiwal Cows During Summer and Winter Season

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ABSTRACT

Ten each of periparturient Karan Fries and Sahiwal cows were selected separately during summer and winter season. These animals were equally divided (five each) into two groups i.e. high and low yielding on the basis of their earlier lactation milk yield. The cows were maintained on standard conditions of feeding and management followed at the Institute farm. The physiological responses (RR, PR, T_{re} and T_{skin}) and climatic variables (T_{db}, T_{wb}, T_{max}, T_{min}, RH and THI) were recorded -45, -30, -15, and 15, 30, and 45 with respect to day of parturition (day 0) and the heat storage of these animals were calculated. The physiological responses and heat storage were significantly ($P<0.05$) higher during summer compared to winter season. The magnitude of increase in physiological responses were higher ($P<0.05$) in high and low yielding Karan Fries cows compared to both the groups of Sahiwal cows during both the seasons. The mean values of heat storage increased by 6.20 and 1.80% in high and low yielding Sahiwal cows during summer season, whereas, in high and low yielding Karan Fries cows the increase was 6.70 and 5.40%, respectively, on the day of calving from the pre-calving values (45th day). During winter season the values of heat storage, increased by 2.60 and 4.40% in high and low yielding Sahiwal cows, whereas, the respective increase in high and low yielding Karan Fries cows was 6.10 and 6.04%, respectively, on the day of calving from the pre calving values (45th day). The overall mean values showed significantly ($P<0.05$) higher values of heat storage during summer, in high yielding and in KF cows compared to winter season, low yielder and Sahiwal cows respectively. Based on the results, it can be stated that high yielding KF cows are more sensitive compared to Sahiwal cows during summer season. Therefore, KF cows needs to be provided better microenvironment and improved managemental practices for normal physiology and productive performances.

Keywords: Heat storage, Karan Fries, Periparturient, Sahiwal, Temperature humidity index

INTRODUCTION

Mammalians have the ability to maintain homoeothermic condition in order to maintain their core body temperature within narrow limits despite wide fluctuations in ambient temperature. High ambient temperature and relative humidity are the primary factors that cause heat stress in dairy animals. Increased respiration rate is the first reaction, when animals are exposed to temperature above the thermoneutral zone (Moran, 1999). Silanikove (2000) suggested that recording the respiration rate appears to be the most accessible and easiest approach for evaluating the degree of heat stress in farm animals (low: 40 – 59, medium high: 60 – 79, high: 80 – 120, and severe stress: >150 breaths per minute in cattle). The outermost surface of the skin, fur, or feathers and the shell of an animal, is the transducing surface across which the environment interacts with the internal physiology of the animal (Gebremedhin *et al.,* 2007). Banerjee (2008) found a positive correlation between physiological responses and antioxidant status during climatic chamber exposure study. Mayengbam (2008) reported an increased in rectal temperature during climatic chamber exposure at 40 and 45°C with 50% RH and during summer stress in crossbred cattle. Thankachan (2007) reported increased in skin temperature of Murrah buffaloes at the end of four hours exposure in climatic chamber. Dandage (2009) also observed similar results in Sahiwal and Karan Fries cattle. Thermal stress is a unique and complex mechanism that causes alterations of the normal physiological mechanisms, which elicits a stressful response that often reflects in the failure to

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achieve genetic potential for production traits (Dobson and Smith, 2000). The skin on the limbs seems to be much more active in heat dissipation as compared with that of the hide of the trunk. Singh *et al.* (2017) reported an increase in skin temperature with the increase of ambient temperature. Increased heat storage in Brahman x Hereford-Shorthorn and Shorthorn was observed, when air temperature exceeded rectal temperature (Finch, 1985). Keeping all above facts in mind, the present study was planned to find out the changes in physiological parameters and heat storage in zebu and crossbred periparturient cows during summer and winter season and correlate them with temperature humidity index.

MATERIALS AND METHODS

Ten each of periparturient Karan Fries and Sahiwal cows were selected separately during summer and winter season from Livestock Research Centre of ICAR- NDRI, Karnal. Further, these animals were equally divided (five each) into high and low yielding group during both the seasons on the basis of their earlier lactation milk yield. All the pregnant cows were fed a ration consisting of roughages (berseem, oats, maize or jowar fodder) as per the availability at farm. Throughout the experimental period cows were given concentrate mixture at 8.30 AM at the rate of 1 kg per cow per day, then 1.50 kg per cow per day 15 days before calving. After calving, the cows were given concentrate mixture @ 1 kg per 2.50 kg of milk production. Concentrate mixture consisted of 28% maize, 10% groundnut cake, 13% mustard cake, 15% wheat bran, 11% rice polish, 15% soyabean deoiled, 5% bajra, 2% mineral mixture and 1% salt. Fresh tap water was available for drinking to all the animals round the clock. The physiological parameters *viz.* respiration rate (RR), pulse rate (PR), rectal temperature (RT), skin temperature (ST) were recorded at fortnightly intervals i.e. -45, -30, -15, and 15, 30, and 45 with respect to parturition (day 0) and the heat storage of the animals were calculated. The environmental variables (T_{db}, T_{wb}, T_{max}, T_{min}, RH and THI) were also recorded on the day of sampling throughout the study. The THI varied from 79.31-85.24 during summer and 61.67-74.35 during winter season.

Respiration rate was recorded from visual observation of inward and outward abdominal movement. One complete inward and outward movement of abdomen was counted as one respiration. The respiration rate is expressed as breaths per minute.

Pulse rate was counted by observing the pulsation of middle coccygeal artery at the base of the tail and the results are expressed as pulse rate per minute.

Rectal temperature was recorded using digital thermometer by keeping the thermometer in contact of rectal mucosa for about 2 minutes.

Peripheral skin temperature of the animals at different anatomical sites, *viz.* forehead, ears, neck, shoulder region (proximal scapula) and flank regions were recorded using non-contact tele-thermometer by keeping it 2-3 inches away from the desired surface site.

Increment in body heat storage due to heat exposure was calculated from the changes in rectal temperature and skin temperature. The body heat storage was calculated using the following formula.

Heat storage (KJ) = 3.47x BW (kg) ($0.8\Delta T_{re} + 0.2\ \Delta T_{skin}$) Where,

T_{re} and T_{skin}= Rectal temperature and Skin temperature respectively

3.47 kJ kg^{-1} $°C^{-1}$ is the Specific heat of tissues

BW = Body weight (kg)

Data obtained during the study were analyzed statistically for mean, standard error of means and multiple comparisons using SPSS.

RESULTS AND DISCUSSION

During summer season the mean values of respiration rate in high and low yielding Sahiwal cows were 24.60± 0.96 and 23.40±1.08 breaths/min on 45th day of prepartum and the values increased to 27.60±0.46 and 25.00±1.17 breaths/min, respectively, on the day of calving. In high and low yielding Karan Fries cows the respiration rate were 23.40±1.08 and 25.00±1.17 breaths/min. on 45th day of prepartum and the values increased to 30.00±0.57 and 28.80±0.33 breaths/min,

respectively on the day of calving (Table 1). Silanikove (2000) indicated respiration rate as most accessible and easiest approach for evaluating the degree of heat stress in farm animals. Bianca (1965) showed a linear relationship among environmental temperature and respiration rate. Aggarwal and Upadhyay (1998) also reported similar high RR in Karan Fries compared to Sahiwal cows after 4 hrs of direct sun exposure. Respiration rate was higher in KF cattle compared to Sahiwal during different seasons, but the magnitude of increase in RR was higher during summer season compared to winter season (Chandra *et al.,* 2013). The mean values of respiration rate in high and low yielding Sahiwal cows increased by 12.20 and 6.80%, and 28.20 and 15.20% in Karan Fries cows, respectively on the day of calving from the pre calving values (45th day) during summer season (Table 1).The respiration rate differed significantly (P<0.01) among Sahiwal and Karan fries cows. Gaughan (2002) concluded that a decreasing respiration rate with rising ambient temperature is not always indicative of an animal coping with the hot conditions, but may indicate a failure to cope. Koubkova *et al*. (2002) found maximum respiratory rate at the beginning of exposition, and then there was a progres-sive fall, though the ambient temperature was at the constant level of 32°C. During winter season, the mean values of respiration rate of high and low yielding Sahiwal cows were 18.60±0.88 and 18.00±0.94 breaths/min on 45th day of prepartum and the values increased to 22.60±0.36 and 23.20±0.33 breaths/min on the day of calving, respectively (Table 1). In high and low yielding Karan Fries cows the respiration rate were 21.20±0.52 and 21.60±0.22 breaths/min. on 45th day of prepartum and the values increased to 25.80±0.33 and 24.20±0.33 breaths/min, respectively on the day of calving during winter season. The mean values of respiration rate of Sahiwal and KF cows increased by 21.50 and 28.80% and 21.60 and 12% in high and low yielding groups on the day of calving from the pre calving values (45th day), respectively during winter season (Table 1). The least square mean values of RR of Sahiwal and Karan Fries cows, during summer and winter season and in high and low yielding groups were 21.64±0.92 and 24.17±0.71 breaths/min; 25.26±0.99 and 20.54±0.64 breaths/min and 23.46±0.79 and 22.35±0.84 breaths/min, respectively and differed significantly (P<0.05) among them (Table 2).

During summer season the mean values of pulse rate in high and low yielding Sahiwal cows were 70.40±0.67 and 71.00±1.10 beats/min on 45th day of prepartum and the values increased to 74.20±0.52 and 75.40±0.96 beats/min, respectively on the day of calving. In high and low yielding Karan Fries cows the pulse rate were 74.00±0.40 and 70.60±1.08 beats/min on 45th day of prepartum and the values increased to 78.20±0.52 and 77.00±0.40 beats/min on the day of

Table 1. Respiration rate (breaths/min) of periparturient Sahiwal and Karan Fries cows during summer and winter season (Mean ± SEM)

Season	Summer				Winter			
Breed	Sahiwal		Karan Fries		Sahiwal		Karan Fries	
Days	HY	LY	HY	LY	HY	LY	HY	LY
-45	24.60^{b}±0.96	23.40^{b}±1.40	23.40^{b}±1.08	25.00^{b}±1.17	18.60^{b}±0.88	18.00^{b}±0.94	21.20^{b}±0.52	21.60^{b}±0.22
-30	26.00±0.40	20.60^{b}±1.28	26.20^{b}±1.78	25.00^{b}±1.10	19.20^{b}±0.59	18.60^{b}±0.83	21.80^{b}±0.66	21.00^{b}±0.49
-15	25.60±0.36	22.60^{b}±0.96	27.20^{b}±1.00	26.80^{b}±0.52	19.20^{b}±0.87	19.20^{b}±1.07	21.20^{b}±0.44	21.00^{b}±0.57
0	27.60^{a}±0.46	25.00^{a}±1.17	30.00^{a}±0.57	28.80^{a}±0.33	22.60^{a}±0.36	23.20^{a}±0.33	25.80^{a}±0.33	24.20^{a}±0.33
15	25.80^{b}±0.52	22.00^{b}±1.02	26.40^{b}±1.56	27.80^{b}±0.44	18.60^{b}±0.92	18.20^{b}±1.18	21.80^{b}±0.91	21.00^{b}±0.40
30	25.00^{b}±1.02	20.80^{b}±0.82	25.60^{b}±1.46	25.60^{b}±1.85	17.60^{b}±1.00	18.80^{b}±0.52	21.20^{b}±0.72	20.60^{b}±0.67
45	26.40^{b}±0.83	21.60^{b}±1.31	27.00^{b}±1.02	25.60^{b}±1.46	18.60^{b}±0.46	18.40^{b}±0.54	22.60^{b}±0.54	21.40^{b}±0.61

The columns with similar superscripts do not differ significantly

Table 2. Respiration rate (breaths/min) during periparturient period with respect to breed, season and yield

Effects	Days	-45	-30	-15	0	15	30	45	Mean ± SEM
Breed	Sahiwal	21.15	21.10	21.65	24.60	21.15	20.55	21.25	21.64^{a}±0.92
	Karan Fries	22.80	23.50	24.05	27.20	24.25	23.25	24.15	24.17^{b}±0.71
Season	Summer	24.10	24.45	24.55	27.85	25.50	24.25	25.15	25.26^{a}±0.99
	Winter	19.85	20.15	20.15	23.95	19.90	19.55	20.25	20.54^{b}±0.64
Group	High Yielding	21.95	23.30	23.30	26.50	23.15	22.35	23.65	23.46^{a}±0.79
	Low Yielding	22.00	21.30	22.40	25.30	22.25	21.45	21.75	22.35^{b}±0.84

Overall least square means with similar superscripts do not differ significantly from each other for particular effects

calving, respectively during summer season (Table 3). The mean values of pulse rate in high and low yielding Sahiwal cows increased by 5.30 and 6.10% and in Karan Fries, the values increased by 5.60 and 9%, respectively on the day of calving from the pre calving values (45th day), respectively during summer season. The mean values of pulse rate differed significantly (P<0.01) among both the groups of Sahiwal and Karan Fries cows. Our results are in accordance with the finding of Chandra *et al.* (2013), who observed higher PR during summer and hot humid season over the spring season. During winter season, the mean values of pulse rate in high and low yielding Sahiwal cows were 60.40±1.61 and 63.00±1.74 beats/min on 45th day of prepartum and the mean values increased to 71.6±0.46 and 71.00±0.63 beats/min, respectively on the day of calving. In high and low yielding Karan Fries cows the pulse rate were 66.40±1.34 and 63.60±1.08 beats/min on 45th day of prepartum, respectively and the values increased to 72.20±0.33 and 71.80±0.77 beats/min, respectively on the day of calving (Table 3). The mean values of pulse rate in high and low yielding Sahiwal cows increased by 18.00 and 12.60% and in Karan fries the values increased by 8.70 and 12.80%, respectively on the day of calving from the pre calving values (45th day) during winter season. The mean values of pulse rate differed significantly (P<0.01) in both the groups of Sahiwal and Karan Fries cows. The least square mean values in Sahiwal and Karan Fries; during summer and winter season and in high and low yielding groups were 67.21±1.03 and 69.90±1.02 beats/min; 72.60±0.88 and 64.62±1.17 beats/min and 68.81±0.85 and 68.40±1.20 beats/min, respectively. The least square mean values of PR were higher by 4.10% in Karan Fries cows

Table 3. Pulse rate (beats/min) of periparturient Sahiwal and Karan Fries cows during summer and winter season (Mean ± SEM)

Season	Summer				Winter			
Breed	Sahiwal		Karan Fries		Sahiwal		Karan Fries	
Days	HY	LY	HY	LY	HY	LY	HY	LY
-45	70.40^{b}±0.67	71.00^{b}±1.10	74.00^{b}±0.40	70.60^{b}±1.08	60.40^{b}±1.61	63.00^{b}±1.74	66.40^{b}±1.34	63.60^{b}±1.08
-30	69.80^{b}±0.82	71.60^{b}±1.54	75.20^{b}±0.18	73.20^{b}±1.73	60.80^{b}±1.78	63.40^{b}±1.73	66.00^{b}±1.44	63.80^{b}±1.21
-15	70.20^{b}±0.52	72.00^{b}±1.47	75.60^{b}±0.73	72.20^{b}±1.34	61.60^{b}±1.51	63.20^{b}±1.63	65.80^{b}±1.15	64.40^{b}±0.36
0	74.20^{a}±0.52	75.40^{a}±0.96	78.20^{a}±0.52	77.00^{a}±0.40	71.60^{a}±0.46	71.00^{a}±0.63	72.20^{a}±0.33	71.80^{a}±0.77
15	69.80^{b}±0.33	71.20^{b}±0.52	74.60^{b}±1.15	74.80^{b}±1.15	61.80^{b}±0.77	61.40^{b}±1.46	66.20^{b}±1.78	66.80^{b}±1.31
30	70.20^{b}±0.66	70.00^{b}±1.06	75.00^{b}±0.63	70.80^{b}±1.66	61.60^{b}±1.00	62.80^{b}±1.53	64.40^{b}±0.78	65.00^{b}±1.33
45	71.00^{b}±0.40	70.80^{b}±1.04	73.80^{b}±0.72	70.80^{b}±1.37	61.20^{b}±0.59	61.40^{b}±0.88	64.20^{b}±1.04	61.60^{b}±1.46

The columns with similar superscripts do not differ significantly

Table 4. Pulse rate (beats/min) during periparturient period with respect to breed, season and yield

Effects	Days	-45	-30	-15	0	15	30	45	Mean ± SEM
Breed	Sahiwal	66.20	66.44	66.82	73.11	66.10	66.22	66.11	67.21^{a}±1.03
	Karan Fries	68.65	69.61	69.52	74.81	70.60	68.81	67.62	69.90^{b}±1.02
Season	Summer	71.50	72.50	72.51	76.23	72.61	71.50	71.61	72.60^{a}±0.88
	Winter	63.35	63.52	63.83	71.71	64.12	63.51	62.10	64.62^{b}±1.17
Group	High Yielding	67.80	68.01	68.31	74.13	68.11	67.80	67.60	68.81^{a}±0.85
	Low Yielding	67.05	68.05	68.00	73.81	68.63	67.20	66.21	68.40^{a}±1.20

Overall least square means with similar superscripts do not differ significantly from each other for particular effects

compared to Sahiwal cows, 12.30% during summer season compared to winter season and 0.59% in high yielding compared to low yielding groups (Table 4).

During summer season the mean values of rectal temperature in high and low yielding Sahiwal cows were 38.80±0.10 and 38.80±0.06°C on 45th day of prepartum and the values increased to 39.40±0.10 and 39.40±0.09°C, respectively on the day of calving. Whereas in high and low yielding Karan Fries cows the rectal temperature were 39.40±0.09 and 39.40±0.13°C on 45th day of prepartum and the values increased to 39.90±0.02 and 39.80±0.03°C, respectively on the day of calving (Table 5). During the present study higher RT was observed during summer compared to winter season in both the breeds of cattle. The magnitude of increase in RT was higher in KF than Sahiwal cows. Similar observations have also been reported by Chandra *et al.* (2013) and Kumar and Singh (2018) i.e. higher magnitude of increase in rectal temperature in Karan Fries cows during summer seasons compared to winter among different breeds of cattle. Bernabucci *et al.* (2002) also reported similar effects of hot season in transition dairy cows. Soly and Singh (2001) reported an increase in rectal temperature as the ambient temperature increased in both the group of animals maintained inside and outside the shelter. Silanikove (2000) stated that rectal temperature is an indicator of thermal balance and may be used as an effective tool to quantify the harshness of the thermal environment. Amakiri and Funsho (1979) observed a diurnal rhythm of core body temperature in domestic animals which depends mainly on the climatic conditions. The mean values of rectal temperature in high and low yielding Sahiwal cows increased by 1.50 and 1.54% and in Karan Fries the values increased by 1.20 and 1.01%, respectively on the day of calving from the pre calving

Table 5. Rectal temperature (°C) of periparturient Sahiwal and Karan Fries cows during summer and winter season (Mean ± SEM)

Season	Summer				Winter			
Breed	Sahiwal		Karan Fries		Sahiwal		Karan Fries	
Days	HY	LY	HY	LY	HY	LY	HY	LY
-45	70.40^{b}±0.67	71.00^{b}±1.10	74.00^{b}±0.40	70.60^{b}±1.08	60.40^{b}±1.61	63.00^{b}±1.74	66.40^{b}±1.34	63.60^{b}±1.08
-30	69.80^{b}±0.82	71.60^{b}±1.54	75.20^{b}±0.18	73.20^{b}±1.73	60.80^{b}±1.78	63.40^{b}±1.73	66.00^{b}±1.44	63.80^{b}±1.21
-15	70.20^{b}±0.52	72.00^{b}±1.47	75.60^{b}±0.73	72.20^{b}±1.34	61.60^{b}±1.51	63.20^{b}±1.63	65.80^{b}±1.15	64.40^{b}±0.36
0	74.20^{a}±0.52	75.40^{a}±0.96	78.20^{a}±0.52	77.00^{a}±0.40	71.60^{a}±0.46	71.00^{a}±0.63	72.20^{a}±0.33	71.80^{a}±0.77
15	69.80^{b}±0.33	71.20^{b}±0.52	74.60^{b}±1.15	74.80^{b}±1.15	61.80^{b}±0.77	61.40^{b}±1.46	66.20^{b}±1.78	66.80^{b}±1.31
30	70.20^{b}±0.66	70.00^{b}±1.06	75.00^{b}±0.63	70.80^{b}±1.66	61.60^{b}±1.00	62.80^{b}±1.53	64.40^{b}±0.78	65.00^{b}±1.33
45	71.00^{b}±0.40	70.80^{b}±1.04	73.80^{b}±0.72	70.80^{b}±1.37	61.20^{b}±0.59	61.40^{b}±0.88	64.20^{b}±1.04	61.60^{b}±1.46

The columns with similar superscripts do not differ significantly

values during summer season (Table 5). The overall mean values of rectal temperature were significantly (P<0.05) different in both the groups of Sahiwal and Karan Fries cows. The results of the present study corroborate well with the findings of Robertshaw (1985) who found significantly higher diurnal variation in rectal temperature in open environment compared to under shade, irrespective of seasons. Zhang *et al.* (1994) and Mader *et al.* (1999) also reported the similar trend in RT of the animals exposed to natural environmental conditions. During winter season, the mean values of rectal temperature in high and low yielding Sahiwal cows were 37.90±0.09 and 38.20±0.02°C on 45th day of prepartum and the values increased to 38.70±0.09 and 38.80±0.04°C, respectively on the day of calving. Whereas in high and low yielding Karan Fries cows, the rectal temperature were 38.70±0.06 and 38.50±0.07 °C on 45th day of prepartum and the values increased 39.10±0.05 and 39.20±0.02°C on the day of calving, respectively during winter season. Zhang *et al.* (1994) found a circadian rhythm in rectal temperature of beef calves exposed to hot and cold stress conditions. The mean values of rectal temperature in high and low yielding Sahiwal cows increased by 2.10 and 1.50% and in Karan Fries the values increased by 1.03 and 1.80%, respectively on the day of calving from the pre calving values (45th day) during winter season. The mean values of rectal temperature were significantly (P<0.01) higher in high yielding groups of Sahiwal and Karan Fries cows as compared to low yielding groups of both the breeds. The least square mean values in Sahiwal and Karan Fries cows; summer and winter season and in high and low yielding cows were 38.60±0.05 and 39.10±0.07°C; 39.30±0.09 and 38.40±0.03°C and 38.80±0.10 and 38.80±0.09°C, respectively (Table 6). The least square mean values of RT were higher by 1% in Karan Fries cows and 2.20% in summer season compared to Sahiwal cows and winter season respectively.

During summer season, the mean values of skin temperature in high and low yielding Sahiwal cows were 38.00±0.66 and 37.30±0.32°C on 45th day of prepartum and the values increased to 38.80±0.30 and 38.70±0.13°C, respectively on the day of calving. In high and low yielding Karan Fries cows the skin temperature were 38.10±0.25 and 38.50±0.14°C on 45th day of prepartum and the mean values of skin temperature increased to 39.00±0.18 and 38.90±0.08°C, respectively on the day of calving (Table 7). Results of the present study are in agreement with that of Chandra *et al.* (2013) who reported the significantly (P<0.05) higher skin temperature in Karan Fries cows among different breeds of cattle during different seasons, but the mean values of ST (38.5°C) was higher during summer season. Somagond *et al.* (2019) reported elevated skin temperature in response to increased environmental temperature and humidity in buffaloes. Ashour (1993) found an increase in skin temperature due to high ambient temperature that leads to increased heat storage in the body of calves. The mean values of skin temperature in high and low yielder Sahiwal cows increased by 2.10 and 3.70% and in Karan Fries the values increased by 2.30 and 1.03%, respectively on the day of calving from the pre-calving values (45th day) during summer season. The overall mean values of skin temperature were significantly (P<0.05) different among

Table 6. Rectal temperature (°C) during periparturient period with respect to breed, season and yield

Effects	Days	-45	-30	-15	0	15	30	45	Mean ± SEM
Breed	Sahiwal	38.40	38.50	38.50	39.10	38.60	38.70	38.50	38.60^{a}±0.5
	Karan Fries	39.00	39.00	39.00	39.50	39.10	39.00	38.80	39.10^{b}±0.07
Season	Summer	39.10	39.10	39.20	39.60	39.40	39.40	39.00	39.30^{a}±0.09
	Winter	38.30	38.40	38.30	39.00	38.20	38.30	38.30	38.40^{b}±0.03
Yield	High Yielding	38.70	38.70	38.80	39.30	38.80	38.80	38.70	38.80^{a}±0.10
	Low Yielding	38.70	38.70	38.80	39.30	38.80	38.80	38.60	38.80^{a}±0.09

Overall least square means with similar superscripts do not differ significantly from each other for particular effects

Table 7. Skin temperature (°C) of periparturient Sahiwal and Karan Fries cows during summer and winter season (Mean ± SEM)

Season	Summer				Winter			
Breed	Sahiwal		Karan Fries		Sahiwal		Karan Fries	
Days	HY	LY	HY	LY	HY	LY	HY	LY
-45	38.00^{b}±0.66	37.30^{b}±0.32	38.10^{b}±0.25	38.50^{b}±0.14	20.10^{b}±0.44	20.70^{b}±0.37	21.90^{b}±0.23	21.90^{b}±0.51
-30	38.10^{b}±0.32	37.30^{b}±0.71	37.80^{b}±0.22	37.80^{b}±0.32	21.30^{b}±0.47	20.50^{b}±0.24	22.10^{b}±0.15	21.50^{b}±0.64
-15	38.50^{b}±0.40	37.80^{b}±0.30	38.70^{b}±0.15	38.30^{b}±0.17	20.90^{b}±0.53	21.90^{b}±0.46	22.70^{b}±0.32	22.50^{b}±0.52
0	38.80^{a}±0.30	38.70^{a}±0.13	39.00^{a}±0.18	38.90^{a}±0.08	23.70^{a}±0.32	22.90^{a}±0.70	24.90^{a}±0.44	24.10^{a}±0.25
15	38.60^{b}±0.28	38.40^{b}±0.10	38.40^{b}±0.16	38.50^{b}±0.15	21.30^{b}±0.54	20.80^{b}±0.30	22.10^{b}±0.26	21.50^{b}±0.57
30	38.00^{b}±0.93	38.30^{b}±0.13	38.40^{b}±0.22	38.40^{b}±0.51	20.90^{b}±0.48	20.30^{b}±0.28	21.00^{b}±0.53	21.70^{b}±0.18
45	37.60^{b}±0.57	37.60^{b}±0.14	38.20^{b}±0.33	37.70^{b}±0.05	20.90^{b}±0.24	20.80^{b}±0.36	22.40^{b}±0.28	21.60^{b}±0.20

The columns with similar superscripts do not differ significantly

high and low yielding Sahiwal and Karan Fries cows. Singh and Upadhyay (2009) reported increase in skin temperature with the increase in ambient temperature. Similar findings have been reported by Sailo *et al.* (2017) in crossbred dairy cattle and Dandage (2009) in Sahiwal and Karan Fries cattle. During winter season the mean values of skin temperature in high and low yielding Sahiwal cows were 20.10±0.44 and 20.70±0.37°C on 45th day of prepartum and the values increased to 23.70±0.32 and 22.90±0.70°C, respectively on the day of calving (Table 7). In high and low yielding Karan Fries cows the skin temperature were 21.90±0.23 and 21.90±0.51°C on 45th day of prepartum and the values increased to 24.90±0.44 and 24.10±0.25°C on the day of calving, respectively during winter season (Table 7). The mean values of skin temperature in high and low yielding Sahiwal cows increased by 17.90 and 10.60% and in Karan Fries the values increased to 13.60 and 10.10%, respectively on the day of calving from the pre calving values (45th day) during winter season. The overall least square mean values in Sahiwal and Karan Fries; during summer and winter season and in high and low yielding groups were 29.60±0.39 and 30.30±0.29°C; 38.20±0.29 and 21.70±0.39°C and 30.10±0.36 and 29.90±0.32°C, respectively (Table 8). The overall least square means of ST increased by 2.30% in Karan Fries compared to Sahiwal cows and 76% during summer than winter season (Table 8).

During summer season the mean values of heat storage in high and low yielding Sahiwal cows were 2206.90±33.0 and 2271.20±37.2 kJ/h on 45th day of prepartum and the mean values increased to 2344.30±21.8 and 2314.10±17.5 kJ/h, respectively on the day of calving. In high and low yielding Karan Fries cows the heat storage were 2576.60±79.40 and 2401.80±56.0 kJ/h on 45th day of prepartum and the

Table 8. Skin temperature (°C) during periparturient period with respect to breed, season and yield

Effects	Days	-45	-30	-15	0	15	30	45	Mean ± SEM
Breed	Sahiwal	29.01	29.30	29.70	31.00	29.80	29.30	29.20	29.60^{a}±0.39
	Karan Fries	30.12	29.80	30.60	31.70	30.20	29.90	30.00	30.30^{b}±0.29
Season	Summer	38.00	37.80	38.30	38.90	38.50	38.30	37.80	38.20^{a}±0.29
	Winter	21.20	21.30	22.00	23.90	21.40	21.00	21.40	21.70^{b}±0.39
Yield	High Yielding	29.50	29.80	30.20	31.60	30.10	29.60	29.80	30.10^{a}±0.36
	Low Yielding	29.60	29.30	30.10	31.10	29.80	29.70	29.40	29.90^{a}±0.32

Overall least square means with similar superscripts do not differ significantly from each other for particular effects

Table 9. Heat storage (kJ/h) in periparturient Sahiwal and Karan Fries cows during summer and winter season (Mean ± SEM)

Season	Summer				Winter			
Breed	Sahiwal		Karan Fries		Sahiwal		Karan Fries	
Days	HY	LY	HY	LY	HY	LY	HY	LY
-45	2206.90^{b}± 33.0	2271.20^{b}± 37.2	2575.60^{b}± 79.4	2401.80^{b}± 56.0	1794.80^{b}± 37.0	1774.70^{b}± 35.0	2071.30^{b}± 70.5	1924.20^{b}± 48.7
-30	2261.80^{ba}± 32.5	2283.90^{ba}± 46	2626.70^{ba}± 72	2447.00^{ba}± 47	1814.50^{ba}± 31	1818.70^{ba}± 27	2100.00^{ba}± 67	1962.90^{ba}± 50.8
-15	2318.90^{a}± 22.3	2326.20^{a}± 30.5	2684.40^{a}± 75.2	2494.30^{a}± 55.3	1850.70^{a}± 16.7	1855.90^{a}± 29.2	2131.60^{a}± 8.1	1976.60^{a}± 42.8
0	2344.30^{a}± 21.8	2314.10^{a}± 17.5	2748.80^{a}± 67.5	2532.80^{a}± 53.7	1841.90^{a}± 26.3	1854.20^{a}± 18.8	2197.60^{a}± 54.5	2040.60^{a}± 49.6
15	2209.80^{c}± 22.1	2201.10^{c}± 16.9	2469.10^{c}± 258.4	2249.10^{c}± 41.3	1753.90^{c}± 23.0	1770.10^{c}± 29.1	1922.70^{c}± 45.3	1750.90^{c}± 37.4
30	2180.00^{c}± 15.1	2163.50^{c}± 12.8	2416.70^{c}± 58.4	2212.40^{c}± 22.2	1745.50^{c}± 23.9	1762.70^{c}± 29.6	1893.30^{c}± 45.6	1723.20^{c}± 39.4
45	2158.50^{c}± 36.1	2163.40^{c}± 19.0	2383.00^{c}± 58.5	2166.50^{c}± 33.1	1737.80^{c}± 24.1	1760.70^{c}± 28.6	1878.40^{c}± 43.8	1741.10^{c}± 30.7

The columns with similar superscripts do not differ significantly

mean values increased to 2748.80±67.50 and 2532.80±55.30 kJ/h, respectively on the day of calving during summer season. Finch, (1985) reported maximum heat storage in Brahman x Hereford-Shorthorn and Shorthorn cattle, when air temperature exceeded rectal temperature. The mean values of heat storage in high and low yielding Sahiwal cows increased by 6.20 and 1.80% and in Karan Fries the values increased by 6.70 and 5.40%, respectively on the day of calving from the pre calving values (45th day) during summer season (Table 9). The mean values of heat storage were significantly (P<0.01) different in high and low yielding groups of Sahiwal and Karan Fries cows during summer season compared to winter season. The results of the present study are in agreement with that of Finch *et al.* (1984) who showed linear increase in heat storage as the intensity of solar radiation increased. During winter season the mean values of heat storage in high and low yielding Sahiwal cows were 1794.80 ±37.00 and 1774.70±35.00 kJ/h on 45th day of prepartum and the values increased to1841.90±26.30 and 1854.20±18.80 kJ/h on the day of calving, respectively. In high and low yielding Karan Fries cows the heat storage were 2071.30±35.00 and 1924.20±48.70

Table 10. Heat storage (kJ/h) during periparturient period with respect to breed, season and groups

Effects	Days	-45	-30	-15	0	15	30	45	Mean ± SEM
Breed	Sahiwal	2011.90	2044.70	2087.90	2088.60	1983.70	1962.90	1955.10	2019.30^{a}±26.52
	Karan Fries	2243.20	2284.10	2321.70	2380.00	2098.00	2061.40	2042.20	2204.40^{b}±52.30
Season	Summer	2363.90	2404.90	2456.00	2485.00	2282.30	2243.10	2217.90	2350.40^{a}±40.80
	Winter	1891.20	1924.00	1953.70	1983.60	1799.40	1781.20	1779.50	1873.20^{b}±38.02
Group	High Yielding	2162.20	2200.70	2246.40	2283.10	2088.90	2058.90	2039.40	2154.20^{a}±43.59
	Low Yielding	2093.00	2128.10	2163.30	2185.40	1992.80	1965.40	1957.90	2069.40^{b}±35.24

Overall least square means with similar superscripts do not differ significantly from each other for particular effects

kJ/h on 45th day of prepartum and the values increased to 24.90±0.44 and 24.10±0.25 kJ/h, respectively on the day of calving (Table 9). The mean values of heat storage in high and low yielding Sahiwal cows increased by 2.60 and 4.40% and in Karan Fries the values increased by 6.10 and 6.04%, respectively on the day of calving from the pre calving values during winter season. The mean values of heat storage were significantly (P<0.01) different among high and low yielding groups of Sahiwal and Karan Fries cows (Table 10). The overall least square mean values in Sahiwal and Karan Fries; summer and winter season and high and low yielding groups were 2019.30±26.52 and 2204.40±52.30 kj/h; 2350.40±40.80 and 1873.20±38.02 kJ/h and 2154.20±43.59 and 2069.40±35.24 kJ/h, respectively (Table 10). The overall least square mean values increased by 9.1% in Karan Fries cows compared to Sahiwal cows. The heat storage was higher by 25.40% during summer season compared to winter season whereas it was 4.10% higher in high yielding groups than low yielding groups of both the breeds (Table 10).

CONCLUSIONS

The rise in ambient temperature influenced physiological reactions (respiration rate, pulse rate, rectal temperature and skin temperature) at different magnitude in both the breeds. The physiological responses and heat storage were higher in KF compared to Sahiwal cows and during summer than winter season. The magnitude of increase in physiological reactions and heat storage was found to be higher in high yielders ascompared to low yielders during both the season. The results indicating that KF and high yielders are sensitive to heat stress. Therefore, KF cows / high yielder groups of both breed needs to be provided better microenvironment and improved managemental practices for normal physiology and productive performances.

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Rumen Protected Choline along with Green Tea Extract Maintain Glucose Homeostasis in Transition Karan Fries Cows

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ABSTRACT

The aim of this study was to determine the effect of supplementation of rumen protected choline (RPC) and green tea extract (GTE) on glucose homeostasis in transition cows. Thirty-two pregnant Karan Fries (KF) cows were randomly allocated into three groups on the basis of most probable production ability (MPPA) and parity. In control group, cows were fed basal diet. In T_1 group, each cow was fed 55g of RPC/day along with basal diet; cows in T_2 group were fed on basal diet supplemented with 3g of GTE/day and in T_3 group, 55 g of RPC and 3 g of GTE/day along with basal diet was fed. The duration of experiment was 30 days before calving to 60 days after parturition. Non significant difference was observed across the groups for plasma glucose concentration. The concentration of insulin and IGF-1 increased significantly ($P \leq 0.01$) in T_1, T_2 and T_3 as compared to control in days' dependant manner around parturition. In conclusion, feeding of RPC and GTE in combination maintained glucose homeostasis during transition period in Karan Fries cows.

Key words: Green tea extract, Glucose, Insulin, IGF-1, Rumen protected choline, Transition Karan Fries cows

INTRODUCTION

High-yielding dairy cows enter a state of negative energy balance (NEB) around calving when the energy demand for maintenance and lactation exceeds that of dietary energy intake (Bauman and Currie, 1980). Around 46 % of maternal glucose taken up by the uterus is utilised by the foetus (Bell, 1995). Additionally, a cow producing 30 kg of milk per day uses at least 2 kg of blood glucose to synthesize lactose (Bell, 1995). So, to maintain the heavy glucose demand, the liver synthesizes all of this glucose from propionate and amino acids. Again, just after calving the energy requirement of body increases approximately 2.5 times (Grummer, 1995). As cows begin to use large amount of energy to drive milk production, a lag occurs in DMI, and they find themselves physically unable to increase DMI enough to meet their needs (Grummer, 1995). It is well known that fat accumulation by the liver, during the transition period, inhibits liver glucose production (Grummer, 1995). Choline, a component of phospholipid and methyl donor, takes part an important role in VLDL synthesis and thereby fat transport from liver. Dietary supply of choline in post parturient dairy cows may be inadequate, even though choline can be synthesized by the animals (Pires and Grummer, 2008). Methionine and lysine are the 2 most limiting AA for milk production in dairy cattle (NRC, 2001). The demand for choline as a methyl donor is probably the main factor determining how rapidly choline deficiency induces a disease state (Zeisel *et al.* 1991). Therefore, supplementation of choline could spare a portion of methionine needed to meet daily choline needs, which would leave a larger supply of methionine for milk production.

Green tea contains polyphenols (Major catechin present in green tea is epigallocatechin-3-gallate [EGCG]), tannins and caffeine (Khan and Mukhtar, 2019). Some of these components have been shown to enhance the basal and insulin-stimulated glucose uptake of adipocytes (Wu *et al.,* 2004) and inhibit intestinal glucose uptake by inhibiting the sodium-dependent glucose transporter of intestinal epithelial cells (Kobayashi *et al.,* 2000). Very few studies have been reported till date, regarding the effect of RPC and GTE on glucose and insulin correlation in transition dairy cows. So, the present study has been designed to study the effect of supplementation of RPC and GTE on glucose homeostasis in transition KF cows.

MATERIALS AND METHODS

This study was conducted in the Livestock Research Centre (LRC) unit of National Dairy Research Institute (NDRI), Karnal, India. The RPC was

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purchased from Kemin animal nutrition, India, which was prepared by spray freeze drying technology, in the form of encapsulation with fatty acids. The GTE was purchased from Sarthak Herbs, Karnal. Ethical permission was granted for the experiment by the Institutional Animal Ethical Committee (IAEC) of Indian Council of Agricultural Research-NDRI constituted as per article 13 of the CPCSEA rules, laid down by the Govt. of India (Regd no-1705/GO/al/13 CPCSEA) dated 3/7/2013.

Thirty-two pregnant KF cows (30 days before calving) were selected from the herd with MPPA of around 4000 l milk production, in their second to fourth lactation stage. Nutrient requirement of experimental cows were met by feeding basal diet consisted of concentrate mixture, green fodder and wheat straw as per the guidelines of NRC (2001). Ingredients and nutrients composition is depicted in Table 1.

In control group, cows were fed basal diet. In T_1 group, each cow was fed 55g of RPC/day along with basal diet; In T_2 group, each cow was fed on basal diet supplemented with 3g of GTE/day and in T_3 group, 55 g of RPC and 3 g of GTE/day along with basal diet was fed. The treatment was given 30 days before calving to 60 days after calving. The blood samples were collected at weekly intervals by jugular vein puncture into vacuutainer tubes (Becton Dickinson, Rutherford, NJ). The serum was separated by centrifugation of the blood samples at 2400 rpm for 15 min and stored in vials at -20 °C until further analysis. Estimation of blood glucose was done by using kit purchased from Recombigen Laboratories Pvt. Ltd. New Delhi, India. Insulin was estimated in serum samples by using Bovine Insulin ELISA kit (Cat. No: E0015Bo, Bioassay Teachnology Laboratory, Shanghai, China). IGF-1 was estimated in serum samples by using Bovine Insulin-like Growth Factors 1 ELISA kit (Cat. No. E0016Bo, Bioassay Technology Laboratory, Yanghu Dist. Shanghai, China).

Data were represented as mean ± SE. One-way analysis of variance (ANOVA) (using GRAPHPAD PRISM software) was adapted to find out significant difference between groups and day of treatments and to interpret the effect of dietary treatment on various parameters.

RESULTS AND DISCUSSION

The mean plasma glucose concentration in experimental KF cows during transition period is presented in Table 2. No significant difference was found

Table 1. Physical and chemical composition of concentrate mixture

Ingredients	Parts (%)
Maize grain	33
Groundnut Cake (GNC)	21
Mustard Cake	12
Wheat Bran	20
Deoiled Rice Bran (DORB)	11
Mineral Mixture	2
Common Salt	1
Total	100

Nutrient percent	Concentratem mixture	Sorghum	Maize	Oats	Sugar graze	Wheat straw
DM	89.87	24.61	14.90	24.29	26.33	90.11
OM	92.57	91.41	92.60	90.81	90.66	91.43
CP	19.93	9.54	9.82	9.80	11.11	2.93
EE	3.75	1.85	2.1	1.44	2.10	1.35
NDF	25.63	58.75	50.45	64.42	59.55	78.40
ADF	15.42	33.63	28.45	40.61	36.91	51.20
Ash	7.29	8.55	7.25	9.13	9.34	8.52

Table 2. Effect of RPC and GTE supplementation on serum glucose concentration (mg/dl)

Days of calving	Group				SEM	P-value
	C	T_1	T_2	T_3		
Days	**C**	**T_1**	**T_2**	**T_3**		
-30	58.00±2.42	59.37±2.15	60.62±2.48	60.50±1.47		
-15	55.87±2.35	56.50±2.16	58.32±2.23	57.25±1.67		
-7	53.12±2.25	54.37±2.00	55.10±1.96	53.00±1.98		
0	48.25±2.13	50.75±1.84	48.04±1.54	49.25±1.50		
7	45.87±1.69	47.75±1.53	47.04±2.09	48.25±1.49		
15	48.62±1.91	50.50±1.99	50.42±1.77	53.00±1.55		
30	51.87±1.52	52.75±1.88	53.02±1.38	54.50±1.40		
45	53.62±1.34	55.00±1.81	53.98±1.33	56.25±1.11		
60	55.25±1.11	56.00±1.60	56.63±1.23	57.37±0.99		

across the groups for plasma glucose concentration. Similar results were also obtained by many workers (Chung *et al.,* 2009; Zom *et al.,* 2011; Leiva *et al.,* 2015) in RPC supplemented transition cows. However, Sheikh *et al.* (2014) found significant increase (P≤0.05) in plasma glucose concentration in transition KF cows when they were fed 60 g of RPC per day. Various reports documented that green tea supplementation lowers blood glucose level (Tsuneki *et al.,* 2004). In T_3 group numerically higher concentration of glucose in spite of supplementation of GTE indicates overshadowing effect of RPC on GTE's glucose lowering ability, that enabled maintenance of a balance gluconeogenic state in transition cows to meet the high energy demand.

Significant difference (P≤0.05) was noted between control and treatment groups from seven days before parturition up to day of parturition and significant (P≤0.01) difference was seen from 15 days after parturition up to 30th days of parturition, whereas no significant difference was recorded between T_1, T_2 or T_2, T_3 or T_1, T_3 in plasma insulin level (Table 3).

Leiva *et al.,* (2015) reported significantly higher (P≤0.01) concentration of insulin in transition HF cows, those were given 50 and 100 g of RPC before and after calving. In early lactation, there is decrease in insulin concentration which is considered as a part of energy metabolism, allowing an animal to mobilize the fat deposit. In one study, EGCG dose-dependently improved insulin resistance in NAFLD mice not only by reducing body weight but also through enhancing the insulin clearance by hepatic insulin degrading enzyme (IDE) (Gan *et al.,* 2015). So to meet the energy demand,

Table 3. Effect of RPC and GTE supplementation on insulin (pmol/L) of KF cows

DAYS	C	T_1	T_2	T_3
-30	17.98±0.75	20.61±0.84	19.59±0.71	20.28±0.49
-15	17.32±0.73	19.82±0.75	18.64±0.71	19.63±0.46
-7*	16.46^{a}±0.69	18.73^{b}±0.66	17.94^{ab}±0.66	19.13^{b}±0.49
0*	14.75^{a}±0.62	17.01^{b}±0.71	16.05^{ab}±0.70	17.23^{b}±0.52
7	14.61±0.657	17.29±0.71	15.37±1.90	17.19±0.57
15**	15.33^{a}±0.60	18.49^{b}±0.76	16.66^{ab}±0.65	18.03^{b}±0.39
30**	16.08^{a}±0.47	18.50^{b}±0.70	17.40^{ab}±0.62	19.07^{b}±0.49
45	17.18±0.51	18.35±0.45	18.15±0.59	18.99±0.68
60	17.65±0.44	18.96±0.59	18.48±0.52	19.64±0.57

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

Table 4. Effect of RPC and GTE supplementation on IGF-1 (ng/ml) of KF cows

DAYS	C	T_1	T_2	T_3
-30	126.07±5.79	130.28±6.69	131.29±7.27	134.39±7.54
-15	114.44±5.79	120.26±6.29	119.27±7.58	126.16±7.72
-7	104.50±5.35	112.01±6.26	109.49±7.86	121.56±7.44
0*	70.23$^{a\pm}$3.75	87.01^{ab}±5.89	86.32^{ab}±6.43	98.06^{b}±7.55
7**	58.79^{a}±3.48	74.03^{ab}±5.37	71.37^{ab}±6.36	90.93^{b}±7.48
15**	67.45^{a}±3.07	90.56^{b}±6.23	86.61^{b}±6.09	111.33^{c}±7.14
30**	90.12^{a}±4.78	112.68^{b}±5.36	104.75^{ab}±6.05	134.73^{c}±7.31
45**	95.77^{a}±4.26	115.32^{b}±5.36	108.64^{ab}±6.00	136.87^{c}±7.81
60**	99.64^{a}±4.38	122.49^{b}±5.20	113.27^{ab}±6.17	141.29^{c}±7.61

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

glucose need to be utilized by cells and increase in concentration of insulin by RPC and GTE helped the cells to achieve that successfully to avoid NEB.

From day of parturition to 60 days of parturition, all the treatments significantly (P≤0.05) increased IGF-1 concentration from control (Table 4). In T_1, IGF-1 concentration was significantly higher (P≤0.01) than control from 15th day post-partum to 60th day of post-partum in our study. However, Leiva *et al*. (2015) detected no treatment effect (P≥0.68) for IGF-1 in RPC treated cows during transition period. Wimmer *et al*. (2015) showed that flavonoid Epigallocatechin 3-gallate (EGCG), found in the popular beverage green tea demonstrated similar effects as the endogenous hormones IGF-1 and insulin in the ability to suppress action of the atrophy-promoting transcription factor.

Cows under NEB have limited hepatic expression of GH receptor 1A triggered by low circulating concentrations of insulin (Butler *et al.,* 2003, 2004). This phenomenon uncouples the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis which reduces the synthesis of IGF-1 by the liver. Reduced concentrations of IGF-1 in blood have been associated with diminished follicle sensitivity to LH, growth and steroidogenesis (Lucy *et al.,* 1992; Butler *et al.,* 2004). Conversely, the increase in circulating concentrations of insulin as energy balance improves seems to be one of the signals to re-establish GH receptor expression in the liver and restore IGF-1 synthesis in dairy cows (Butler *et al.,* 2003).

CONCLUSION

Insulin and IGF-1 are not only involved in energy homeostasis, but also involved in growth and maturation of follicles as well as in maintaining the healthy reproductive tract. Hence, both RPC and GTE could be supplemented in the diet of transition cows to enhance the plasma concentration of insulin and IGF -1, which have a stimulatory effect on production and reproduction of animals.

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Influence of Dietary Mannan-oligosaccharide and α-Tocopherol on Intestinal Microbiology and Histomorphology in Broiler Chickens

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ABSTRACT

Day-old Vencobb broiler chicks (240) were randomly assigned to 4 dietary treatment groups having 3 replicates of 20 birds each to study the effect of mannan oligosaccharide (MOS) and α-tocopherol (AT) on intestinal microbiology and histomorphology. The trial lasted for 7 weeks. The chicks of the control group (T_1) were fed a basal diet. The birds in group T_2 were fed control diet as in T_1 + α-tocopherol at 400 IU per kg of feed; whereas, those in group T_3 were fed control diet as in T_1 + mannan-oligosaccharide at 0.4% and in group T_4 control diet was supplemented with α-tocopherol at 400IU per kg feed + mannan-oligosaccharide at 0.4% in feed. The results of study revealed that supplementation of MOS and AT in the diet of birds showed significant ($P<0.05$) lowering in cfu count of pathogenic bacteria, *E.coli* and *Salmonella spp.* as compared to the control group. The effect on the histomorphology of the birds also showed a significant ($P<0.05$) increase in the villi height (VH) and surface area (SA) of different parts of the intestine on supplementing the feed with MOS and AT. Thus, it was concluded that supplementation of MOS and AT improved the gut health and also had a positive influence on the microarchitecture and integrity of the intestine.

Keywords: Alpha-tocopherol, Broiler, Histomorphology, Intestinal microbiology, Mannan- oligosaccharide

INTRODUCTION

Past few years, with the ban on the use of sub-therapeutic level of antibiotics in poultry feed to prevent disease or promoting growth has resulted into an increasing interest in alternative safe feed additive having similar action (Waldroup *et al.,* 2003). These may include acidifiers, probiotics, prebiotics, enzymes, vitamins and immuno-modulators. Prebiotics, an alternative to antibiotics is defined by Gibson and Roberfroid (1995) are non-digestible food ingredients that selectively stimulate the growth and activity of one or a limited number of bacteria residing in the colon mainly that improves host health and performance. In view of this, mannan oligosaccharide (MOS), a prebiotic, derived from the cell wall of yeast, *Sacchromyces cerevisiae* is regarded as safe and cheap compound (Ferket, 2004). It has the ability of inhibiting colonization of enteric pathogens by blocking bacterial adhesion to gut lining (Spring *et al.,* 2000; Duncan *et al.,* 2005; Yang *et al.,* 2008a, 2008b, 2008c; Paul *et al.,* 2013), as well as maintain the integrity and lining of the intestinal tract of poultry healthy, thus, safeguarding the animal health status and performance (Ferket, 2002; Loddi *et al.,* 2004; Kocher., 2005; Hooge, 2006). The avian gastrointestinal tract harbours diverse and dynamic population of microorganism living in symbiotic relationship with their host which is important for host nutrition, metabolism and performance (Sohail *et al.,* 2010). But this balance is very delicate and under any stress condition changes towards more of pathogenic type. This led towards finding of such functional foods which maintain this balance and at the same time maintain the integrity of the intestinal wall (Patterson and Burkholder, 2003; Sohail *et al.,* 2011). Intestinal histomorphology is described on the basis of villi height (VH), villi width (VW), crypt depth (CD)

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and surface area (SA).The ratio of villi height (VH) and crypt depth (CD) gives the gut health index (Pluske, 1997) and if the ratio is low, the intestinal environment is more favourable to nutrient absorption and *vice-versa.* At the same time antioxidant vitamins such as vitamin E in the broilers diet enhanced histology of terminal ileum (Yoo *et al.,* 2016). The present study was conducted to see the effect of supplementing Mannan-oligosaccharide (MOS) and α-tocopherol (AT) in the diets of broiler chickens on intestinal microbiology and histomorphology.

MATERIALS AND METHODS

A total of 240 day-old Vencobb broiler chicks of either sex were randomly assigned to 4 treatment groups of 3 replicates of 20 chickens for a period of 7 weeks. The starter and finisher ration contained 23 and 20 percent crude protein and 2800 and 2900 kcal ME/kg feed respectively. The chicks were fed with standard basal diet as per BIS (1992). Ingredient composition of broiler starter and finisher diets are presented in Table 1. The control group T_1 was fed a basal diet. The birds in group T_2 were fed control diet as in T_1 + α-tocopherol @ 400IU per kg of feed ;whereas, those in group T_3 were fed control diet as in T_1 + mannan-oligosaccharide at 0.4% and in group T_4 control diet was supplemented with α-tocopherol at 400IU per kg feed + mannan-oligosaccharide at 0.4% in feed. Proper mixing of the feed supplement was ensured by initially mixing a small amount of feed by hand and then mixing into complete feed. The birds were raised in well ventilated house with proper lightening and were supplied with *ad-libtum* water during the experimental period.

Feed samples were analyzed for proximate principles (AOAC 1990). Nitrogen free extract was calculated by difference. Ca and P content of feed was also determined.

At the end of 7th week, two chicks from each pen replicate were sacrificed for determining the gut microbial population and histomorphology The intestine was taken out from each and the contents were collected from each part (duodenal, jeju-ileal, cecal) aseptically and labeled as per the group. Samples were weighed (1 g) and transferred to sterile tubes and homogenized with sterile 0.9% normal saline solution (1:1). The solutions were mixed on vortex and serial dilutions were made up to sixth dilution. Then 0.1 ml of each dilution was poured and spread uniformly on Mac Conkey agar and Eosin Methylene Blue Agar (EMB). Plates were incubated at 37°C for 48 hours. Bacterial

Table1. Ingredients and chemical composition (%) of broilers starter and finisher's diet

Feed ingredient	Starter phase				Finisher phase			
Groups	**T_1**	**T_2**	**T_3**	**T_4**	**T_1**	**T_2**	**T_3**	**T_4**
Yellow Maize	52.75	52.75	52.75	52.75	60.00	60.00	60.00	60.00
Soyabean meal	35.00	35.00	35.00	35.00	27.5	27.5	27.5	27.5
Fish meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Mineral mixture	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
α-tocopherol(AT)	-	400 IU	-	400 IU	-	400 IU	-	400 IU
MOS	-	-	0.4%	0.4%	-	-	0.4%	0.4%
Chemical composition %								
CP%	23.61	23.61	23.61	23.61	21.14	21.14	21.14	21.14
ME, kcal/kg	2806.85	2806.85	2806.85	2806.25	2882.25	2882.25	2882.25	2882.25
Ca	1.222	1.222	1.222	1.222	1.108	1.108	1.108	1.108
P	0.556	0.556	0.556	0.556	0.548	0.548	0.548	0.548

T_1= Basal diet; T_2= Basal diet + AT (400 IU); T_3= Basal diet + MOS (0.4%),T_4= Basal diet + MOS (0.4%) + AT (400 IU).

colonies were counted by pour plate method (Quinn *et al.,* 1992). The average number of colonies were multiplied by reciprocal of the dilution factor and expressed as cfu/g of contents. Colony forming units (CFU) were defined as being distinct colonies measuring at least 1mm in diameter. The colonies were detected for E.coli and Salmonella bacteria. The Mac conkey agar medium having lactose as one ingredient and E. coli being rapid lactose fermenting bacteria gives dark pink donut shaped surrounded with dark pink area of precipitated bile salts. Salmonella species do not ferment lactose or produce acid, their colonies appears grey on EMB agar.

For determining the villus height (VH), width (VW), crypt depth (CD), parts of the small intestine were sectioned from birds of each group and were fixed in formaldehyde 10% (v/v), buffered (pH 7.0) (Ricca Chemical Company, Arlington, TX) and then dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Five-micrometer thick sections were cut with a microtome and mounted on slides. Four discontinuous paraffin-embedded sections per broiler intestinal sample were processed for evaluation. These sections were de-paraffinized in xylene, dehydrated in ethanol, and stained with haematoxylin and eosin. The slides were scanned by light microscope and examined for determining the morphometric indices as villus height (VH) from the tip of the villus to crypt, width (VW) from one wall to next, and crypt depth (CD) from the base of the villi to the submucosa (Awad *et al.* 2008). For this morphometric investigation was made on 20 intact villi and 30 crypts chosen from the intestinal segments of broiler chickens. Villus height and Villus width data were used to calculate villus surface area (SA) [$2\pi \times (W/2) \times L$], where W= villus width and L= villus length (Sakamoto *et al.* 2000).

All the data were analyzed statistically using statistical packages for social science (SPSS), 17th Version as per Snedecor and Cochran (1980) and comparison of means was done using Duncan's multiple comparison tests.

RESULTS AND DISCUSSION

Dietary treatments had significant effect on reduction of pathogenic bacteria in all the three segments of intestine (Table 2). The average cfu count of *E.coli* and *Salmonella spp*. in the intestine were significantly (P<0.05) low in T4 group as compared to others which corroborates well to earlier reports (Finucane *et al.,* 1999b; Spring *et al.,* 2000; Iji *et al.,* 2001; Loddi *et al.,* 2004; Yang *et al.,* 2008a, 2008b, 2008c; Hrangkhwal *et al.,* 2013). The two supplements had a positive effect on modulating the gut ecosystem by lowering pathogenic colonization. Using prebiotics helps in to reduce pathogenic bacteria by interference with attachment via the type-1 fimbriae found on many gram negative bacteria as E.coli and Salmonella (Ferket, 2004).

The results of gut histomorphology of all the three parts of the intestine had been presented in table 3. The results showed significant (P<0.05) increase in VH and SA of jejunum followed with ileum and duodenum in all the three treatment groups as compared to control one. The dietary supplements affected the structure of the small intestine in broilers. Intestinal morphology is the main indicator of gut health which is characterized in particular to enhanced villus height and crypt depth. The

Table 2. The effect of supplementation of mannan-oligosaccharide and α-tocopherol individually and in combination on study of gut microbiology in broiler chicken

Attribute	E. coli, Cfu/ml				Salmonella, Cfu/ml			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Duodenal	1.77^{ab}±0.01	1.79^{b}±0.01	1.78^{b}±0.01	1.74^{a}±0.01	1.96^{b}±0.01	1.97^{b}±0.01	1.97^{b}±0.01	1.63^{a}±0.02
Jeju-ileal	1.64^{b}±0.01	1.67^{b}±0.01	1.56^{a}±0.01	1.55^{a}±0.01	1.97^{c}±0.01	1.97^{c}±0.01	1.94^{b}±0.01	1.58^{a}±0.01
Cecal	1.67^{c}±0.01	1.65^{c}±0.01	1.37^{b}±0.01	1.27^{a}±0.01	1.99^{d}±0.01	1.95^{c}±0.01	1.76^{b}±0.01	1.61^{a}±0.01

*Values with similar superscript (row wise- a, b, c, d) did not differ significantly (P<0.05)

Table 3. The effect of supplementation of mannan-oligosaccharide and α-tocopherol individually and in combination on study of gut histology in broiler chicken

	Jejunum				Ileum				Duodenum			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
VH (µm)	248.6[a]	255.56[ab]	293.33[b]	282.2[ab]	180.0[a]	180.00[a]	271.1[b]	255.5[b]	168.9[a]	173.3[a]	215.55[b]	206.6[b]
	±5.93	±4.44	±20.37	±9.69	±10.18	±13.33	±4.44	±8.01	±2.22	±3.85	±9.69	±3.85
VW (µm)	6.67[a]	12.22[b]	13.33[b]	12.22[b]	11.11[a]	32.22[b]	62.22[c]	28.89[b]	57.78[a]	46.67[a]	53.33[a]	49.11[a]
	±0.01	±0.78	±0.01	±1.11	±1.11	±1.11	±2.22	±2.22	±2.22	±3.85	±3.85	±8.17
CD(µm)	86.67[a]	86.67[a]	133.33[b]	68.89[a]	115.55[b]	48.89[a]	133.33[b]	55.55[a]	37.76[a]	66.67[b]	35.55[a]	95.56[c]
	±6.67	±6.67	±24.04	±2.22	±9.69	±5.88	±0.01	2.22	±5.86	±3.85	±2.22	±8.01
SA (µmm²)	781.51[a]	1468.8[b]	1842.1[c]	1615.36	952.47[a]	2717.85[b]	7940.71[d]	3460.98[c]	4591.38[a]	3816.84[a]	5449.64	4786.76[a]
	±1.84	±1.24	±1.27	±1.04	±1.52	±1.01	±2.42	±1.55	±1.18	[a]±3.7	±6.36	±8.58

*VH= villus height; VW= villus width; CD= crypt depth; SA= villus surface area; **Values with similar superscript (row wise- a, b, c, d) did not differ significantly (P<0.05).

lengthening of villi may be the source of increased total luminal villus absorptive area resulting in increased digestive enzyme action and higher nutrient transport. Thus, from the table it was evident that there was positive effect of the two supplements on improving the structural integrity of the gastrointestinal tract which was in agreement to previous reports (Iji *et al.,* 2001, Zikic *et al.,* 2008, Hrangkhawl *et al.,* 2013, Yoo *et al.,* 2016). The two in combination showed positive interaction in maintaining the intestinal integrity and uniformity.

CONCLUSIONS

On the basis of these observations, it was concluded that the dietary supplementation of MOS and AT in the broilers diet had a positive influence on reducing the pathogenic bacteria in the intestinal tract. Supplementation also enhanced the intestinal microarchitecture resulting in more efficient absorption and assimilation of nutrients. This may be beneficial augmenting performance, health and welfare of chickens.

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Effect of Varying Nutrient Density of Diets on Productive Performance in Dahlem Red Layers

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ABSTRACT

This study was conducted to optimize the nutrient requirement for Dahlem Red (DR) birds. Five diets were formulated i.e., one basal diet (BD) with optimum (100%) nutrients, two diets with lower (97.5 % and 95%) and two diets with higher (102.5% and 105%) nutrients as compared to the BD. A total of 240 number of DR layers of 23 weeks were selected and divided into 5 groups, each having 8 replicates and each replicate had 5 birds. The experiment was continued up to 70 weeks. Feed intake was higher (P<0.05) in groups fed the diet containing 95 and 97.5% of nutrient of the BD. The egg production and egg weight were higher (P<0.05) in groups fed the diet containing higher nutrient (102.5% and 105%) as compared to those birds fed lower nutrient dense diets (97.5 % and 95%) during the 23-42 weeks. The feed efficiency was better (P<0.05) among the groups fed diet containing higher nutrient density diets as compared to those groups fed lower nutrient density diets during the 23-42 and 59-70 weeks. However, egg components and egg quality parameters did not differ among the groups. Therefore, it seems that the diet with 102.5% nutrients would be more remunerative as the higher egg production and better feed efficiency was eobserved.

Keywords: Egg production, Egg quality parameters, Nutrient density

INTRODUCTION

Dahlem Red (DR) is a brown egg-laying chicken that is extensively used for producing variety suitable for the backyard chicken. The pullet attains sexual maturity at about 22-23 weeks of age and produces about 230 eggs/year with an average egg weight of 54 g at 40 weeks (Prakash *et al.,* 2014). The optimum performance of poultry is achieved through optimum nutrient intake. The poultry inclined to meet energy needs first and assumes all other essential nutrients are adequate. Therefore, CP and other nutrients should be in proportion with the ME (NRC, 1994). Feeding the diet with suboptimal nutrients hinders the performance and increase the production cost. Therefore, it is essential to determine the optimum nutrient requirements for maximizing the performance of layers, where all the nutrients are changed proportionately. It has been reported that the nutrient requirement of the brown egg-laying layers is suggested to be 10% greater than those of the white egg-laying layers (NRC, 1994). Considering the climatic variables the nutrient requirements studied for DR in temperate environment may not be applicable in tropical regions like India. The present study was therefore conducted to study the effect of various concentrations of ME, CP and amino acids (lysine and methionine) on productive performance, egg components and egg quality parameters in DR birds.

MATERIALS AND METHODS

Experimental diets, feeding and productive parameters: A total of 240 DR (23 weeks) layers were distributed into 5 groups, each having 8 replicates with 5 birds in each replicate. The hens were reared in individual cages under the open side housing system. Five experimental diets were formulated i.e., basal diet (BD) (100%) as per NRC (1994), two diets with lower (97.5 % and 95%) and two diets with higher (102.5% and 105%) nutrients of the BD for feeding the experimental birds up to 70 weeks (Table 1). Daily feed intake and egg production was recorded throughout the experiment.

Eight eggs were randomly chosen from each treatment during the last three consecutive days at the end of the experiment to determine the shell weight,

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shell thickness and Haugh unit (Egg Multi testers EMT-5200, Japan) and yolk colour. The cleaned eggshells were dried for 24 h, weighed and expressed as percent of whole egg. The shell thickness was measured at three different locations (middle, broad and narrow ends) using a micrometer gauge (Mitutoyo Code, 7027, Japan) and mean value was taken as thickness. The variations in data of different parameters were analysed using the general linear model procedure. The model included different dietary treatments as source of variation. Treatment means were compared using Tukey's test. The data of egg mass (EM), egg production (EP), egg weight (EW) and age of the birds (weeks) were analysed for linear regression analysis. The model included the EM, EP, EW were dependent variable and week as independent variable using SAS 9.2.

RESULTS AND DISCUSSION

Feed intake was higher (P<0.05) among the groups fed the diet containing lower nutrient density (95 and 97.5%) compared to those groups fed the diet containing optimum (100%) or higher (102.5 and 105%) nutrient (Table 2). Nevertheless, the feed intake among the different groups did not vary during the 43-70 weeks of age. The higher feed intake is generally seen with decreasing the energy content of the diet (Wu *et al.,* 2007, Panda *et al.,* 2012, Prakash *et al.,* 2014). Therefore, when energy content of the diet is altered, the concentration of other nutrients should also be changed proportionately to optimize the intake of other essential nutrients. Nevertheless, the nutrient requirement also depends on other factors, such as strain, body weight and egg production (Harms *et al.,* 1999, Gomez and Angels, 2009).

Table 1. Ingredient composition of the experimental diets

Ingredients (%)	Diets (% of standard)				
	95	**97.5**	**100**	**102.5**	**105**
Maize	58.7	61.2	63.6	66.0	65.8
Soybean meal	8.87	13.0	18.1	23.0	22.9
Sunflower cake	20.0	15.2	7.6	0.06	0.00
DORB	2.39	0.00	0.00	0.00	0.00
Salt	0.40	0.40	0.40	0.40	0.39
Dicalcium phosphate	0.78	0.94	1.15	1.36	1.37
Shell Grit	8.56	8.64	8.72	8.80	8.92
DL-methionine	0.126	0.132	0.141	0.15	0.15
Premix[¥]	0.40	0.40	0.40	0.40	0.38
L-lysine HCL	0.217	0.144	0.051	0.04	0.08
Nutrient composition					
ME (kcal/kg)	2538	2605	2673	2739	2804
CP (%)	14.53	15.00	15.2	15.6	16.0
Lysine (%)	0.72	0.74	0.76	0.78	0.80
Methionine (%)	0.37	0.38	0.39	0.40	0.41
Ca (%)	3.31	3.39	3.48	3.56	3.64
NPP (%)	0.30	0.31	0.32	0.33	0.34

[¥]Supplied per kilogram of diet: 1 mg of thiamin, 2 mg of pyridoxine, 0.01 mg of cyanocobalamine, 15 mg of niacin, 10 mg of pantothenic acid, 10 IU of α-tocopherol, 10 mg of riboflavin, 0.08 mg of biotin, 2 mg of menadione, 2.75 mg of retinol acetate, 0.03 mg of cholecalciferol, 650 mg of choline, 8 mg of copper, 45 mg of iron, 80 mg of manganese, 60 mg of zinc, 0.18 mg of selenium, 50 mg of monensin sodium and 800 mg hydrated sodium calcium alumino silicates.

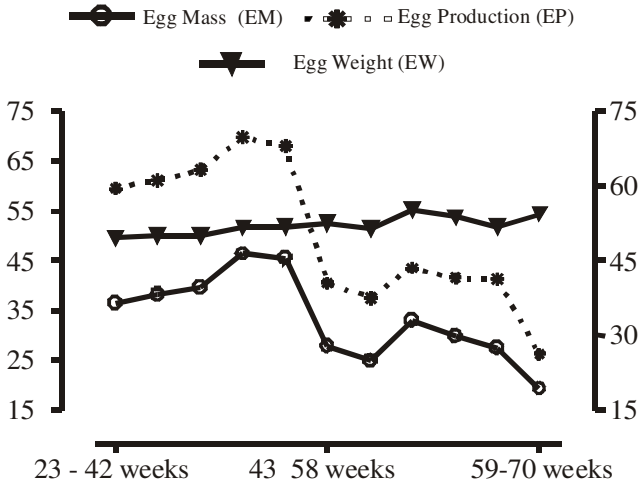
Table 2. Effect of feeding diets varying in nutrient density on feed intake, egg production, egg weight, FCR (feed conversion ratio) and egg mass in Dahlem red layers

Particulars	Diets (% of standard)					SEM	P value
	95	97.5	100	102.5	105		
Feed intake (g/d)							
23 - 42 weeks	98.67[a]	95.93[ab]	94.93[b]	91.99[bc]	90.91[c]	1.25	0.05
43 - 58 weeks	92.24	87.32	89.93	85.01	90.32	3.01	0.14
59 - 70 weeks	89.79	83.88	82.68	76.97	86.14	2.35	0.54
Hen day egg production							
23 - 42 weeks	59.51[b]	61.17[b]	63.13[ab]	69.67[a]	68.05[a]	1.64	0.03
43 - 58 weeks	40.45	37.58	43.54	41.55	41.19	2.24	0.88
59 - 70 weeks	26.20	28.50	28.42	29.72	34.89	2.60	0.34
Egg weight							
23 - 42 weeks	49.48	49.93	50.09	51.60	51.80	1.72	0.41
43 - 58 weeks	52.47	51.45	55.12	53.65	51.68	2.01	0.18
59 - 70 weeks	54.31[b]	55.03[ab]	56.23[a]	57.78[a]	57.36[a]	0.91	0.04
FCR (kg/dozen eggs)							
23 - 42 weeks	1.608[a]	1.508[ab]	1.441[b]	1.228[b]	1.238[b]	0.001	0.03
43 - 58 weeks	2.086	2.168	1.799	1.831	2.037	0.02	0.07
59 - 70 weeks	3.029[a]	2.567[ab]	2.483[ab]	2.151[b]	2.066[b]	0.001	0.05
Egg mass (g/hen/d)							
23 - 42 weeks	36.42[c]	38.12[bc]	39.60[b]	46.38[a]	45.65[a]	1.04	0.05
43 - 58 weeks	27.84	24.87	33.07	29.90	27.50	1.47	0.08
59 - 70 weeks	19.32[c]	21.58[bc]	22.46[b]	24.81[ab]	28.70[a]	1.14	0.04

The egg production was higher (P<0.05) in groups fed the diet containing higher nutrients (102.5% and 105%) as compared to those birds fed lower nutrient dense diets (97.5 % and 95%) during the 23-42 weeks. However, the egg production did not vary among the various groups during the later stages of the experimental period. The egg weight was higher (P<0.05) among the groups fed higher nutrient density diets compared to those groups fed lower nutrient density diets towards the end of the experiment. Though the difference was insignificant, the marginal increase in the egg weight was recorded in groups fed high

Table 3. Effect of feeding diets varying dietary nutrient density on egg components and egg quality parameters in Dahlem red layers

Diets (% of standard)	Egg components (%)			Shell thickness (mm)	Haugh unit	Yolk colour
	Albumen	Yolk	Shell			
95	61.29	29.69	9.02	0.42	71.25	6.25
97.5	61.74	28.92	9.34	0.40	81.50	6.50
100	60.42	29.79	9.79	0.40	72.25	7.25
102.5	59.87	30.64	9.49	0.42	75.75	6.75
105	64.17	26.94	8.89	0.42	74.25	6.75
SEM	2.82	0.71	0.23	0.01	1.37	0.25
P value	0.53	0.59	0.80	0.86	0.12	0.53



EM=51.69-0.47 × week; R^2=0.55 (P<0.01)
EP=87.82-0.975 × week; R^2=0.73 (P<0.01)
EW=45.09+0.184 × week; R^2=0.85 (P<0.01)

Fig. 1. Relationship between egg mass, egg weight and egg production

nutrient density diets as compared to those groups fed lower nutrient density diets during the 23-58 weeks of age. The feed conversion ratio and egg mass were improved (P<0.05) among the groups fed diet containng higher nutrient density diets as compared to those groups fed lower nutrient density diets during the 23-42 and 59-70 weeks. It has been reported that the decline in nutrient density decrease the feed efficiency and egg production (Panda *et al.,* 2012, Prakash *et al.,* 2014). However, egg components and egg quality parameters did not differ among the groups (Table 3).

It was recorded that the egg production and egg weight or egg mass were well related with the age of the birds (weeks). Based on the relationships, regression equation were derived to predict the egg mass, egg production and egg weight from the age of the laying (23-70 weeks) Dahlem Red birds (Fig 1). It has been reported by Shafey (1996) that the age of the hen is positively correlated with the production of higher egg mass.

CONCLUSION

Therefore, it is concluded that the diet with higher nutrient (102.5%) would be more beneficial higher egg production and better feed efficiency was recorded in the present experiment.

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Optimization of Dietary Protein Requirement for the Growth, Survival and Feed Utilization of *Osteobrama belangeri* (Valenciennes, 1844) Fingerling

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ABSTRACT

In order to optimize dietary protein requirement of *Osteobrama belangeri* fingerling an experiment was conducted by using six semi-purified diets with graded protein levels (20, 25, 30, 35, 40 and 45 %) using casein and gelatin as main protein source. Six treatments in triplicates (18 circular fiber reinforced plastics tanks; 1000 l capacity each) were used to conduct the experiment for 75 days in the wet laboratory. Ten fingerlings with mean body weight of 4.23±0.08g were stocked in each tank and fed twice daily. The mean weight gain and specific growth rate was recorded highest in fish fed with 25% protein diet. Similarly, net biomass gain, body weight gain and daily weight gain was highest in fish fed with 25% protein diet. 30% and 35% protein diets gave 100% fish survival. The lowest feed conversion ratio (FCR) and highest feed conversion efficiency (FCE) was also recorded in 25% protein diet. Moisture, crude protein, crude lipid, crude ash contents of *O. belangeri* did not differ significantly among the treatments. Thus, growth performance and feed utilization was optimum in fingerling of *O. belangeri* while they were fed semi-purified diet containing 25% protein.

Key words: Feed utilization, Growth, *Osteobrama belangeri*, Protein requirement, Semi-purified diets

INTRODUCTION

Osteobrama belangeri, also known as Pengba, is a highly esteemed fish in India. Formerly, it was widely distributed in rivers and lakes but has got extinct in the wild in some parts of India due to construction of barrage in the early 1990's in Manipur state of India (Dinesh and Mema, 2012; Singh *et al.,* 2016). Despite its culture potential, systematic attempts were not made to culture and propagate this species in India. Among the other carps available *O. belangeri* priced almost double than any other carps consumed by the people and thus has high commercial value.

Intensified culture of fish needs provision of nutritionally balanced diet. So, for obtaining optimum growth for the fish, it is important to know the optimum protein requirement while formulating a balanced diet, because protein is the major nutrient promoting growth and other metabolic activities (Abdel-Tawwab *et al.,* 2010). Very limited information is available on the nutritional requirement of *O. belangeri*. Therefore, this study was carried out to determine the effects of graded dietary protein levels on growth performance, feed utilization and carcass composition of *O. belangeri.*

MATERIALS AND METHODS

The present experiment was carried out for 75 days during February to May, 2017 in the wet laboratory of the College of Fisheries, Central Agricultural University, Lembucherra, Tripura, India. The experiment consisted of 18 circular fiber reinforced plastics tanks (FRP) (1000 l each) of six treatments in triplicate groups and the experiment was conducted by following completely randomized design. The tanks were equipped with sponge filter for providing aeration and filtration of water. About 320 (mean weight of 4.23g) pengba fish were collected from the college pond and kept in two circular FRP tanks for 3 days for conditioning.

The experimental diets consisted of six different protein levels *viz.* 20% (T_1), 25% (T_2), 30% (T_3), 35% (T_4), 40% (T_5) and 45% (T_6). The moisture free ingredients were grounded, sieved, weighed and thoroughly mixed in a container and added water at 300 to 400 ml kg^{-1} diet. The ingredients composition of the diets is shown in Table 1. Dissolved gelatin were

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Table 1. Composition of diets (% dry matter basis)

Ingredients	Protein levels (%)					
	20%	25%	30%	35%	40%	45%
[1]Fish meal	17	17	17	17	17	17
Corn flour	16	16	16	16	16	16
[1]Broken wheat	20	20	20	20	20	20
[2]Casein	9	14	19	24	29	34
[2]Gelatin	2	3	4	5	6	7
[2]Dextrin	30	24	18	12	6	0
[2]CMC (Carboxymethylcellulose)	1	1	1	1	1	1
[3]Vegetable oil	3	3	3	3	3	3
[4]Vitamin + mineral	2	2	2	2	2	2

[1]Obtained from College of Fisheries, CAU, Lembucherra, Agartala, India; [2]Himedia Laboratories Ltd., Mumbai, India; [3]Ruchi Soya Pvt. Ltd., Raighad, India; [4]Agrimin Forte, Mumbai, India; T_1, T_2, T_3, T_4, T_5 and T_6 corresponds to 20%, 25%, 30%, 35%, 40%and 45% crude proteinin diets, respectively.

added and again mixed it properly; vegetable oil, Carboxymethylcellulose (CMC) and vitamin-mineral premix were also added. Using pelletizer, 2 mm pellet size moist feed were prepared and dried in an oven at 50-55 ºC. The dried diets were then stored in air-tight containers with proper labeling until used. The proximate composition of all the used feed ingredients (Table 2) and the experimental diets (Table 3) and whole body composition of the experimental fish (Table 7) were measured according to the standard procedure of (AOAC, 2005).

Feeding was done twice a day at 2-3% of the total biomass in the morning (10 AM) and afternoon (4 PM). The average fish weight was recorded before stocking. The sampling of fish and water quality parameters were done fortnightly. Growth and mortality of the fish were recorded during sampling. Water samples were taken from each FRP tank during sampling and water quality parameters were analyzed according to the procedure given by (APHA, 2005).

The water quality parameters including dissolved oxygen (DO) and temperature were measured using Optical DO Probe (ProODO™, YSI Environmental). The digital pH meter (HI 991001, HANNA) was used to measure pH. Total ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) and orthophosphate (PO_4-P) levels in water were measured by Continuous Flow Analyzer equipped with auto sampler (SA 1100, SKALAR). Alkalinity, total hardness and free carbon dioxide were analyzed by titremetric methods (APHA, 2005).

Sampling of fish was done fortnightly and adjusted

Table 2. Proximate composition of feed ingredients (%)

Parameters	Fish meal	Broken wheat	Corn starch	Casein	Gelatin
% DM					
Dry matter	12.19±0.41	9.25±0.29	5.95±0.12	8.92±0.14	5.88±0.07
Crude protein	48.38±0.46	11.83±0.09	0.37±0.03	77.73±0.56	93.03±0.20
Crude fat	7.75±0.06	4.31±0.16	0.10±0.008	0.16±0.01	0.06±0.01
Crude fiber	0.92±0.03	2.96±0.45	0.41±0.09	0.45±0.27	0.21±0.01
Ash	28.69±0.19	27.22±0.52	0.20±0.02	8.38±0.28	0.59±0.002
NFE*	2.04±0.72	68.91±1.32	92.93±0.12	4.32±0.83	0.20±0.15

Values are expressed as mean± S.E. (n=3); NFE* (Nitrogen free extract)

Table 3. Proximate composition of experimental diets (%)

Parameters	Protein levels (%)					
	20%	25%	30%	35%	40%	45%
Moisture	4.48±0.25	4.85±0.42	5.66±0.37	6.38±0.007	7.15±0.08	6.94±0.004
% DM						
Ash	12.23±0.18	11.85±0.47	13.19±0.10	14.22±0.20	12.71±0.13	13.59±0.29
Crude protein	19.28±0.29	25.34±0.30	29.77±0.15	35.95±0.25	38.06±0.39	44.38±0.31
Crude lipid	1.79±0.12	1.41±0.09	1.66±0.16	1.01±0.04	1.03±0.03	1.10±0.09
Crude fibre	1.47±0.09	1.48±0.27	1.28±0.20	1.03±0.03	1.61±0.30	0.99±0.004
NFE	60.44±0.30	56.04±0.73	48.41±0.77	42.38±0.38	38.42±0.39	32.97±0.41
Digestible energy (kcal 100^{-1}g diet)	33.61±0.38	33.43±4.56	32.77±2.74	31.85±0.47	31.92±0.79	31.93±1.68

Values are expressed as mean± S.E. (n=3); T_1, T_2, T_3, T_4, T_5 and T_6 corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets respectively; Digestible energy (Kcal 100^{-1}g) = (4 x Crude protein %) + (9 x Lipid %) + (4 x Carbohydrate %)

the quantity of feed according to the total biomass after each sampling. To evaluate the growth performance, following parameters were used

Body weight gain (%) = $[(w_t - w_0)/w_0] \times 100$

Where, w_0 and w_t are initial and final live weight of the fish.

Specific growth rate (SGR) (% day^{-1}) = [(In B_{Wf} – In B_{Wi}) /culture period] ×100

Where, B_{Wi} and B_{Wf} were initial and final body weights of the fish.

Survival (%) = [Total number of fish harvested / Total number of fish stocked] ×100

Daily weight gain (g day^{-1}) = [Total final weight – total initial weight] /culture period.

The following parameters were applied to evaluate the feed utilization performance of the experimental fish

Apparent FCR = Amount of dry feed intake (g) / fresh weight gain in fish (g)

Apparent FCE (%) = 1 / FCR×100;

Apparent protein efficiency ratio (PER) = Fresh weight gain in fish (g)/ Amount of protein fed (g)

Apparent protein conversion efficiency (PCE %) = protein gained (g)/ protein consumed *100.

The data was analyzed by using statistical method with Statistical Package for Social Sciences (SPSS, version 22.0). One way-ANOVA was performed to determine the differences between mean values and compared by Duncan's Multiple Range test (Duncan, 1955) at $P<0.05$ level. The broken line regression model was applied to find the optimum value of protein requirement using NLIN procedure (Robbins *et al.,* 1979) of SAS software (SAS, 1996) version 9.3 of the SAS system for windows (SAS Institute Inc., Cary, NC, USA). Approximate sampling errors for L, U and R were computed using the matrix of sums of squares and products of first derivatives and Se^2, given the least squares estimates of each parameter which were shown in Table 7 and Fig. 1, respectively.

RESULTS AND DISCUSSION

The values of different parameters of the water quality including temperature (23.6-29.9 ºC), dissolved oxygen (5.02-10.13 mg l^{-1}), pH (5.5-7.68), alkalinity

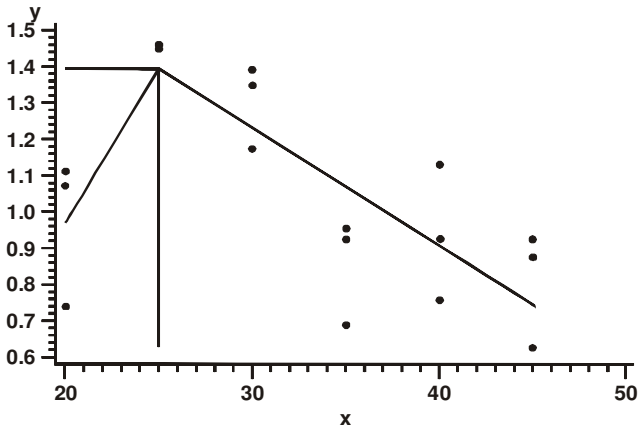

Fig. 1. The broken line curve for SGR (optimum value) at 24.6303

Table 4. Growth performance of the experimental fish

Parameter	Protein levels (%)						P value
	20%	25%	30%	35%	40%	45%	
Initial mean weight (g)	4.41±0.12	4.3±0.05	4.03±0.06	4.25±0.14	4.23±0.01	4.18±0.13	0.195
Mean final weight (g)	9.2^{ab}±0.56	12.6^{c}±0.57	10.8^{bc}±0.44	8.5^{a}±0.74	8^{a}±0.72	7.2^{a}±0.068	0.002
Net weight gain (g)	4.7^{a}±0.67	8.3^{b}±0.1	6.8^{b}±0.44	3.8^{a}±0.6	3.5^{a}±0.71	3.2^{a}±0.73	0.002
Specific growth rate (%/day)	1.03^{a}±0.12	1.53^{b}±0.02	1.39^{b}±0.07	0.88^{a}±0.07	1^{a}±0.11	0.88^{a}±0.09	0.012
Body weight gain (%)	109^{a}±17.6	193^{b}±7.91	168.7±12.01	90.64^{a}±11.64	82.55^{a}±16.54	95.56^{a}±19.9	0.031

Mean value having different superscript in the same row differs significance (P<0.05) (mean± S.E.) (n=3); T_1, T_2, T_3, T_4, T_5 and T_6 corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets, respectively.

(40-122 mg l^{-1}), hardness (34-94 mg l^{-1}) and total ammonia (0.14-0.46 mg l^{-1}) were in acceptable range without indicating any remarkable pattern during the whole duration of the experiment.

Date pertaining to growth performances of *O. belangeri* during the culture period are presented in Table 4. The initial mean weights of pengba for different treatments ranged between 4.03 to 4.41 g whereas variations from 7.2 to 12.6 g mean weights were observed in the final sampling. The highest mean weight gain (12.6±0.57 g) was observed in 25% protein diet which exhibited better performance compared with other treatments. The specific growth rate (% day^{-1}) of the fish were recorded at diminishing order from T_2, T_3, T_1, T_5, T_4 and T_6 groups respectively. The highest specific growth rate (1.53±0.02) was measured in group fed with 25% protein diet and the lowest value (0.88±0.07) was exhibited by fish fed on 40% protein diet.

Dietary protein is considered as important in fish nutrition and feeding, thus adequate dietary protein supply is required to improve their overall health and growth Lovell (1989) and Shang *et al.* (2018). Davies and Gouveia (2010) reported feed mixtures with 25% to 45% of raw protein are required by the fish. Numerous studies on dietary protein requirement have been conducted for different important aquaculture fish species by many workers, Mohanta *et al.* (2004); Chakraborty and Mirza (2007); Paul *et al.* (2009) and Paul and Giri (2015) and they found out that the dietary protein requirement for fish varied from one species to another species, which is due to their feeding habit, size of fish and water temperature. The protein requirement of fish decreases with the increasing size and age of fish NRC (2011). In another study, the dietary protein requirement for Indian major carps was stated to be 35% for spawn and fry and 25% for grown out and broodstock BIS (2013).

Table 5. Yield parameters of the experimental fish

Parameter	Protein levels (%)						P value
	20%	25%	30%	35%	40%	45%	
Initial biomass (g)	44.16±14	43.0±0.6	40.0±1.3	42.5±0.16	42.33±1.36	41.83±0.44	0.195
Final biomass (g)	92.0^{ab}±5.6	126.0^{c}±1.57	107.0^{bc}±3.7	81.0^{a}±7.4	86.0^{a}±6.6	77.0^{a}±7.2	0.003
Net biomass gain (g)	47.83^{a}±6.7	83.0^{b}±1.04	67^{b}±4.3	38.83^{a}±6.05	43.67^{a}±6.8	35.5^{a}±6.04	0.001
Daily biomass gain (g day^{-1})	2.39^{a}±0.007	2.42^{ab}±0.007	2.41^{b}±0.008	2.39^{ab}±0.005	2.34^{ab}±0.002	2.35^{ab}±0.009	0.195
Survival (%)	90^{ab}±3.33	90^{ab}±3.33	100^{b}±00	100^{b}±00	80^{ab}±5.77	70^{a}±8.81	0.048

Mean value having different superscript in the same row differs significance (P<0.05) (mean± S.E.) (n=3); T_1, T_2, T_3, T_4, T_5 and T_6 corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets, respectively.

The mean weight gain was increasing with increasing level of dietary protein up to optimum level followed by decreasing trend in the present study. The tropical fishes generally require moderate temperature between 25-32 ºC (Solomon and Ezigbo, 2010). The growth of Indian major carps was affected when the temperature was lower than 20 ºC and which has been considered to be lower for fish to perform metabolic and other activities. The difference in temperature would hinder the intake of feed by fish and hence their growth rate. This outcome was demonstrated in an experiment conducted under controlled system in aquarium Zenebe *et al.* (2003). The present study is in agreement with the work of Bahnasawy *et al.* (2009) who reported that the weight gain increased significantly with increasing level of dietary protein from 17% to 30% with no significant increase with the diet of 35% protein in Nile tilapia. Lall and Tibbetts (2009) have revealed that the dietary protein requirement for many herbivorous and omnivorous fishesranged from 25% to 30% and is also in agreement with the results of the present study. Also, the optimum protein requirements for bighead carp (*Aristichthys nobilis*) were recorded as 30% (Santiago and Reyes, 1991).

Significant difference was found in SGR of pengba among the treatments. Although fish fed with 25 and 30% proteingave same statistical value the maximum SGR found in the fish fed with 25% (1.53±0.02) protein diet in comparison to other treatments. Similar results were reported by several authors including Nandeesha *et al.* (1994) on stunted yearlings of *L. rohita*, Swamy (2004) on stunted fingerlings of *Cirrhinus mrigala*, Kumar *et al.* (2011) on stunted fingerlings of *L. rohita* and Ramaswamy *et al.* (2013) on stunted fingerlings of *Catla catla*. Kumar *et al.* (2011) found that 25% protein diet as the best for specific growth rate of stunted fingerlings of *L. rohita* which is in agreement with the present study. Ramaswamy *et al.* (2013) also revealed that 25% protein diet gave best SGR values on stunted fingerlings of *C. catla*. Martinez-Palacios *et al.* (2007); Lee and Kim (2009) showed that specific growth rate is a good indicator of protein quality and decrease as fish increase in size. The decrease in growth rate of fingerlings of pengba with increasing level of protein above the satisfactory level in the present study is similar to those reported for catla by Dars *et al.* (2010). Therefore, 25% protein containing semi-purified diet gave the better growth performance of pengba. The growth rate was significantly decreased beyond the requirement level, especially at 35% protein diet. The reason behind this is that every fish species and fish size has a specific protein limit for the growth of fish and after that additional higher protein level could not be utilized efficiently. Wilson (1989) as well as El-Sayed and Teshima (1991) also found that dietary protein requirements decreased with increasing fish size and age.

Table 6. Feed utilization parameters of the experimental fish

Parameter	Protein levels (%)						P value
	20%	25%	30%	35%	40%	45%	
Apparent feed conversion ratio (FCR)	2.37^{b}±0.07	1.28^{a}±0.06	1.78^{ab}±0.12	1.84^{ab}±0.16	2.18^{ab}±0.5	2.02^{ab}±0.31	0.127
Apparent feed conversion efficiency (FCE %)	43.2^{a}±4.8	68.1^{b}±4	56.6^{a}±3.73	55.01^{a}±4.62	50.12^{a}±9.38	51.1^{a}±6.8	0.022
Apparent protein efficiency ratio (PER)	0.087^{a}±0.012	0.14^{b}±0.002	0.12^{b}±0.006	0.09^{a}±0.012	0.078^{a}±0.01	0.077^{a}±0.01	0.005
Apparent protein conversion efficiency (PCE %)	35.16±9.2	57.87±7.15	55.85±7.8	45.58±10	40±2.62	31.76±9	0.313

Mean value having different superscript in the same row differs significance (P<0.05) (mean± S.E.) (n=3); T_1, T_2, T_3, T_4, T_5 and T_6 corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets, respectively.

Salim and Sheri (1999) also reported decreased growth rate of *Labeo rohita* after protein levels exceeded the optimal requirement. The reason behind this was the inability of the fish body to utilize the protein in the diet after reaching the optimum protein level in the diet. Li *et al.* (2000) observed a significant growth response in channel catfish (*Ictalurus punctatus*) when examined with different protein rich artificial diet and is in conformity with the result of the present experiment.

The mean initial and final biomass of different treatments and other yield parameters including net gain in biomass, daily biomass gain and survival percentage for all the treatments have been shown in Table 5. Overall survival (%) of the fish varied from about 80 to 100% in different treatments. No significant difference (P>0.05) was recorded in overall survival (%) among the treatments. The final biomass measured was highest for treatment T_2 (126±1.57 g) followed by T_3 (107±3.7 g) whereas, lowest value was found in treatment T_6 (77±7.2 g). Accordingly, T_2 showed significantly higher net gain in biomass (83.03±1.04 g) as compared to T_3, T_1, T_5, T_4 and T_6. Similarly, relatively higher mean daily biomass gain was recorded for T_2 (2.42±0.007 g day^{-1}) as compared to rest of the treatments and T_5 recorded lowest mean daily weight gain (2.34±0.002 g day^{-1}). Kumar *et al.* (2011) worked on stunted fingerlings of *L. rohita* and Ramaswamy *et al.* (2013) on stunted fingerlings of *C. catla* and found that 25% protein level in diets gave the best weight gain of fish.

The feed utilization parameters including apparent FCR, apparent PER, apparent FCE and apparent PCE were measured for all treatments and are presented in Table 6. The FCR value did not differ significantly (P>0.05) among the treatments, but the lowest (1.28) and highest (2.37) FCR values were measured in T_2 and T_1, respectively. The highest value of FCE (60.1%) was observed in T_2 and lowest FCE (43.2%) in T_1. The PCE also showed no significant variations among treatments. The highest PCE (57.87 %) was recorded in T_2 followed by T_3 (55.85%) and the lowest in T_6 (31.76 %). Similarly, the apparent PER was highest in T_2 (0.14), followed by T_3 (0.12), while the lowest apparent PER value of 0.077 was observed in treatment T_6.

It may be notable that pengba is an omnivorous fish with inclination to consume macrophytes and inclusion of high protein in diet might have problem in feed utilization and leads to poor growth and feed utilization. The apparent FCR, PER and PCE showed no significant difference (P>0.05) among the treatments. Apparent FCR value decreased with increasing dietary protein level up to 25% diet and thereafter, increased continuously except in 45% dietary protein group. Similar FCR values were also observed by Khattab *et al.* (2000) but Paul *et al.* (2009) observed the best FCR value in fish fed 30% dietary protein for *Labeo bata*

Table 7. Proximate composition of the experimental fish (g kg^{-1} dry matter)

Parameter	Protein levels (%)						P value
	20%	25%	30%	35%	40%	45%	
Moisture	69.49±0.69	69.9±0.45	70.0±0.56	67.9±0.61	69.37±0.68	69.59±0.61	0.432
% DM							
Ash	8.8±0.51	8.59±0.39	8.41±0.43	8.85±0.22	8.9±0.42	9.1±0.16	0.505
Protein	54.42^{ab}±0.91	56.73^{b}±0.47	55.31^{ab}±0.82	54.25^{ab}±0.89	53.02^{a}±0.26	54.1^{a}±0.78	0.032
Lipid	34.85±0.23	35.59±0.21	35.46±0.25	34.41±0.36	34.82±0.11	35.44±0.27	0.169
Fiber	0.28±0.041	0.29±0.038	0.36±0.037	0.38±0.058	0.35±0.031	0.33±0.059	0.210
NFE (Nitrogen free extract)	4.62^{bc}±0.92	3.69^{a}±0.86	2.81^{ab}±0.96	3.21^{ab}±0.49	5.21^{c}±0.19	4.32^{bc}±0.78	0.009

Mean value having different superscript in the same row differs significance (P<0.05) (mean± S.E.) (n=3); T_1, T_2, T_3, T_4, T_5 and T_6 corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets, respectively.

Table 8. Parameter estimates for broken line regression model for SGR

Parameter	Estimate	Approx Std Error	Approximate 95% Confidence Limits	
L	1.4029	0.0690	1.2558	1.5500
U	-0.0200	.	.	.
V	-0.0323	0.00631	-0.0458	-0.0189
R	24.6303	0.5854	23.3825	25.8781

fry. Therefore, relatively lowest FCR was observed in T_2 (1.28), fed with 25% diet. On the other hand, relatively higher apparent protein efficiency ratio (PER) was observed (0.14) in T_2 followed by T_3 (0.12). Present results is in agreement with Ramaswamy *et al.* (2013), who studied the effects of dietary protein levels on growth, survival, protein efficiency ratio, feed conversion ratio and specific growth rate for *C. catla* during grow-out phase and reported optimal dietary protein level of 25%. Li *et al.* (2006) observed that 24% and 36% protein diet provided the same growth and feed conversion efficiency. Then they finally recommended that 28% protein containing diet would be useful for the growth of channel catfish fingerlings.

The mean final proximate composition of *O. belangeri* in different treatments is given in Table 7. The moisture, crude ash, crude lipid and crude fibre content of *O. belangeri* did not show any significant variations (P>0.05) among the treatments. However, significantly (P>0.05) higher protein level was recorded in T_2 (56.73±0.47%) compared to other treatments and the lowest protein level of pengba (53.02±0.26%) was obtained in treatment T_5. The nitrogen free extract was found significantly (P<0.05) higher in T_5 (5.21±0.19%) followed by T_1 (4.62±0.92%) and the lowest value in T_3 (2.81±0.96%).

There was no higher significant differences (P>0.05) reported in the proximate compositions of experimental fish except crude protein and nitrogen free extract. Minor variations were observed in mean values of moisture, ash, crude lipids and crude fibre. But, the changes in whole body proximate composition were less consistent. The highest crude protein content was found in 25% protein fed group while lowest ash was obtained in 20% protein fed group. Similar results were also reported by Mohanta *et al.* (2013); Ma *et al.* (2014) and Wang *et al.* (2017). Lipid levels of *O. belangeri* under all the treatments were found to be markedly higher as compared to other carps in the range of 34.82- 35.59%. Proximate composition of fish is influenced by various factors including age, sex, maturity, geographical location, and experimental feeding conditions. The result of the present study showed that pengba is a fatty fish. The protein and lipid contents of the fish were found to be increased with increasing level of optimum protein inclusion in the diet, which is also in agreement with study conducted by Paul *et al.* (2009).

Approximate sampling errors for the two-slope broken-line model of L, U and R were calculated using the matrix of sums of squares and products of first derivatives and S_e^2, given the least squares estimates of each parameter (Table 8 and Fig. 1). The optimum value using broken line regression for SGR is 24.63 % protein for *O. belangeri* fingerlings. From regression analysis [$Y = 1.4029–0.0200\ X\ (246.303 – x) –0.0323X\ (x – 246.303)$; $R^2 = 0.96$, where Y = SGR and x = protein level in the diet], it was found that the requirement of protein of *O. belangeri* is 24.63 %.

CONCLUSIONS

The results of the present study suggests that under laboratory condition the dietary protein level of 25% gives the optimum performance in terms of growth, survival, feed utilization and whole body/carcass composition of *O. belangeri*. From broken line regression analysis for specific growth rate, it was found that the optimum protein requirement of *O. belangeri* is 24.63%.

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Effect of Dietary Inclusion of Graded Levels of Toasted Guar Meal (TGM) at Different Energy Efficiency on Egg Quality and Serum Parameters of White Leghorn Layers

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ABSTRACT

An experiment was undertaken in a completely randomized design to study the effect of supplementation of toasted guar meal (TGM) at graded concentrations on the performance and egg quality parameters of White Leghorn (WL) layers. A total of 224-layer chickens (Babcock, BV 300) were randomly distributed into 56 replicates with 4 birds per each colony cage in well ventilated platform 2 tier cage layer house. Seven experimental diets with graded levels of (0, 6, 12 and 18 %) and two energy efficiency (50 % and 60 % of GE as ME) of TGM were prepared having similar concentrations of protein and ME. Each diet was fed *ad libitum* to 8 replicates from 27 to 42 weeks of age. The results showed that feed intake, feed conversion ratio, egg quality (egg density, yolk index, yolk color, Haugh unit score, shell weight, shell percentage, shell thickness, shell strength) parameters and serum biochemical profile (albumin, protein and cholesterol) were not affected by incorporating TGM up to 18 % (180 g/kg diet) except albumen index which was significantly ($P<0.05$) higher at 18 % inclusion compared to other groups. From the results of the above study, it was concluded that TGM can be included in WL layer diets up to 18% without affecting egg quality and serum parameters.

Key words: Energy efficiency, Haugh unit, Shell quality, Serum protein, Toasted guar meal

INTRODUCTION

Soya bean meal (SBM) is conventionally used as a source of protein in poultry diet. However, the shortage and escalating cost of this prime protein source makes poultry farming uneconomical in many developing countries. Continuous efforts are, therefore, is on in search of viable alternate protein feed ingredients for SBM. Guar (*Cyamopsis tetragonoloba*) is a drought tolerant legume primarily cultivated for culinary preparations. To produce gum (galactomannan) guar seeds are split, which yields protein rich germ fraction and low protein husk fraction as by-products. Guar meal (GM) is a combination of these two fractions, which contains similar amount of CP and less expensive than SBM (Rama Rao *et al.* 2014). The bitter taste and presence of anti-nutritional factors (trypsin inhibitors) limit the use of GM in poultry feeds. Guar gum is a highly viscous galactomannan polysaccharide, its dietary inclusion increases the intestinal viscosity and hampers the nutrient digestion and absorption and performance in chicken (Lee *et al.* 2003a). Further, Nidhina and Muthukumar (2015) found that, heat treatment significantly ($P<0.05$) reduced the emulsifying and foaming properties of industrial GM, which may help in better utilization of GM compared to raw meal. Guar gum present in the meal is heat liable, therefore heat treatment (autoclaving) was reported to improve the energy value of GM (Nagpal *et al.* 1971). Rama Rao *et al.* (2015) reported that toasted GM could be safely included in White Leghorn (WL) layer diets up to 15 % without affecting performance. Therefore, the present study was designed to investigate the effect of supplementation of toasted guar meal (TGM) at graded concentrations with two energy efficiencies of TGM on the egg quality and serum parameters of White Leghorn (WL) layers.

MATERIALS AND METHODS

The research work was carried out at Poultry Experimental Station, LFC, College of Veterinary Science, Rajendranagar, Hyderabad and Directorate of

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Poultry Research, Rajendranagar, Hyderabad. The experiment was conducted on 224 commercial layers (BV 300) of 27 weeks of age having uniform body weight and egg production, which were randomly distributed in to seven experimental groups with eight replicates per group and each replicate contained four birds. Six experimental diets were formulated and ingredient and nutrient composition is shown in the Table 1. The TGM (toasted at 120°C for 35 min) was incorporated in three diets at 6, 12 and 18 % in WL layers considering the ME as 50% of GE. Similarly in another three diets, TGM was incorporated at 6, 12 and 18% considering the ME as 60% of GE and one diet was the reference diet based on Maize-SBM.

The layers were fed respective diets for four laying periods, of 28 days each. Eggs produced by each replicate were collected on the daily basis and all eggs laid during last three consecutive days of every period were collected to assess the egg quality parameters. Internal egg quality parameters were evaluated by utilizing 2 eggs from each replicate during the end of each period. In total, 16 eggs per treatment were utilized for measurement of egg quality traits (*viz.*, shell weight (g), shell thickness (mm), Haugh unit score, egg

Table 1. Ingredient and nutrient composition (%) of layer experiment diets

Ingredients	Control	TGM 50% ME			TGM 60% ME		
		6%	12%	18%	6%	12%	18%
Maize	61.99	61.37	60.72	60.06	59.61	57.21	54.79
Soya DOC 45%	24.41	18.17	11.81	5.37	17.76	10.99	4.15
Guar meal	0.00	6.00	12.00	18.00	6.00	12.00	18.00
De-oiled rice bran	1.08	1.96	2.90	3.94	4.10	7.22	10.40
Salt	0.380	0.38	0.38	0.38	0.38	0.38	0.38
Di-calcium phosphate	1.38	1.41	1.44	1.48	1.40	1.42	1.44
Stone grit	10.25	10.22	10.19	10.16	10.24	10.22	10.20
DL- methionine	0.13	0.13	0.13	0.13	0.13	0.13	0.13
L- lysine HCL	0.00	0.00	0.03	0.10	0.00	0.04	0.12
L-threonine	0.00	0.0214	0.044	0.0678	0.022	0.046	0.072
Choline chloride 50%	0.05	0.05	0.05	0.05	0.05	0.05	0.05
AB_2D_3K[2]	0.02	0.02	0.02	0.02	0.02	0.02	0.02
B- complex[3]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Toxin binder	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Trace mineral pre mix[1]	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition*							
ME (kcal/kg)	2650	2631	2612	2593	2602	2554	2506
Protein (%)	16.50	16.50	16.50	16.50	16.50	16.50	16.5
Calcium (%)	3.700	3.700	3.700	3.700	3.700	3.700	3.700
Available phosphorus (%)	0.330	0.330	0.330	0.330	0.330	0.330	0.330
Lysine (%)	0.874	0.825	0.800	0.800	0.820	0.800	0.800
Methionine (%)	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Sodium (%)	0.163	0.162	0.161	0.160	0.161	0.160	0.159

Trace mineral mix[1] provided per kg diet: manganese, 120mg; Zinc, 80mg; Iron, 25mg; Copper,10mg; Iodine, 1mg and Selenium, 0.1mg.Vitamin premix[2] provided per kg diet: Vitamin A, 20000IU; Vitamin D_3, 3000IU; Vitamin E, 10mg; Vitamin K, 2mg;[3] Riboflavin, 25mg; Vitamin B_1, 1mg; Vitamin B_6, 2mg; Vitamin B_{12}, 40mcg and Niacin, 15mg.

density, shell strength, shell percentage, albumen index and yolk index) were measured during the four laying periods. Serum albumin, protein and cholesterol were determined by using the Erba Chem-5 Plus V2 Clinical Chemistry Semi Auto Analyzer with commercially available diagnostic kit (M/S Excel Diagnostics Pvt. Ltd., Hyderabad, India) methods.

The data was analyzed by using general linear mode procedure of statistical analysis by applying factorial method of ANOVA using SPSS 15th version. Difference was compared with Duncan's multiple range test at P<0.05.

RESULTS AND DISCUSSION

Feed intake/hen/day (FI) was significantly (P<0.05) lower in birds fed 50% energy efficiency of TGM from 27-42 wks of age. Inclusion of graded level of TGM did not significantly influence the FI. However FI was lower in birds fed TGM at 6 % level of inclusion. The interaction between varying energy efficiencies of TGM and graded (6, 12 and 18 %) inclusion levels of TGM did not influence (P>0.05) the FI. Numerically lower FI was observed in birds fed TGM at 6 % and 50 % energy efficiency (Table 2). The results are in agreement with the findings of Rama Rao *et al. (*2015) who reported that FI was not affected by the level of GM in the diet. Ehsani and Torki (2010) also reported no significant effect on FI by dietary GM supplementation. In general, progressive increase in feed intake with reduction in dietary ME level is expected (Rama Rao *et al.* 2014). The lack of significant response in these parameters due to variation in dietary energy concentration could be due to either age of the bird,

Table 2. Effect of inclusion of graded levels and varying metabolisable energy of TGM on albumin and yolk parameters of commercial layers from 27th- 42nd weeks of age

Treatment	Energy efficiency of TGM	Inclusion level of TGM (%)	Feed intake (g/b/day)	Feed conversion ratio	Haugh unit score	Albumen index	Yolk index	Yolk colour
1	0%	0	107.3	118.1	67.97	5.092	33.46	4.406
2	50%	6	103.8	115.4	70.25	5.453	34.05	4.564
3	50%	12	105.2	116.7	71.19	5.427	33.85	4.506
4	50%	18	102.6	116.9	70.91	5.800	35.72	4.500
5	60%	6	104.7	120.4	67.74	5.270	34.63	4.656
6	60%	12	106.1	123.1	67.50	5.370	33.87	4.463
7	60%	18	108.1	117.5	70.37	5.976	34.00	4.630
Energy efficiency of TGM								
0	0%		107.3[a]	118.1	67.97[b]	5.092	33.46	4.406
1	50%		103.9[b]	116.3	70.78[a]	5.560	34.54	4.523
2	60%		106.3[a]	120.3	68.54[ab]	5.539	34.17	4.583
TGM (%)								
0	0		107.3	118.1	67.97	5.092[b]	33.46	4.406
1	6		104.3	117.9	69.00	5.362[b]	34.34	4.610
2	12		105.6	119.9	69.34	5.399[b]	33.86	4.484
3	18		105.3	117.2	70.64	5.888[a]	34.86	4.565
SEM			0.625	0.908	0.427	0.078	0.253	0.026
P-Value								
ME			0.075	0.046	0.019	0.901	0.499	0.289
TGM			0.683	0.516	0.318	0.024	0.339	0.185
ME*TGM			0.284	0.464	0.378	0.679	0.212	0.413

Values bearing different superscripts within the column are significantly (P<0.05) different

body weight or static gut capacity. Alteration in feed intake with dietary ME concentration is largely noticed in broiler or layer chicks. The GI tract volume or capacity is in dynamic mode, in growing birds, while in mature birds (lying phase), the intestinal capacity is fixed and flexibility in gut volume is very limited. Therefore, dietary variation and nutrient concentration influenced the feed intake in growing birds but not in mature laying hens.

Feeding graded levels of TGM (6, 12 and 18 %) had no effect (P>0.05) on the FCR from 27 to 42 wks of age and numerically lower FCR was noticed in 50 % energy efficiency of TGM. whereas the interaction between varying energy efficiencies of TGM and graded (6, 12 and 18 %) inclusion levels of TGM did not influence (P>0.05) the FCR (Table 2). Rama Rao *et al.* (2015) reported no significant difference in feed efficiency by inclusion of TGM up to 10 % in layer diets but FCR was poor at 15 % inclusion. Abdul Mohsen (2014) reported that inclusion of GM up to 10 % in laying hens did not show significant (P>0.05) effect on FCR.

There was no significant effect of varying energy efficiency of TGM on the albumen index, inclusion of TGM at 18 % showed significantly (P<0.05) higher albumen index from 27-42 weeks of age; however, there was no interaction effect between energy efficiency of TGM and TGM inclusion level (Table 2). The Haugh unit score was not significantly affected by inclusion of graded levels of TGM and

Table 3. Effect of inclusion of graded levels and varying metabolisable energy of TGM on shell quality of commercial layers from 27th- 42nd weeks of age

Treatment	Energy efficiency of TGM	Inclusion level of TGM (%)	Egg density	Shell weight (%)	Shell strength (N)	Shell weight (g)	Shell thickness (mm)
1	0%	0	1.072	8.914	21.86	5.093	0.366^{b}
2	50%	6	1.067	9.173	25.15	5.021	0.370^{ab}
3	50%	12	1.067	8.999	23.55	5.122	0.376^{ab}
4	50%	18	1.069	9.301	25.28	5.053	0.373^{ab}
5	60%	6	1.068	9.160	24.62	5.075	0.381^{a}
6	60%	12	1.067	9.029	25.73	5.061	0.373^{ab}
7	60%	18	1.068	9.120	27.02	5.124	0.376^{ab}
Energy efficiency of TGM							
0	0%		1.072	8.914	21.86	5.093	0.366
1	50%		1.068	9.158	24.66	5.065	0.373
2	60%		1.068	9.103	25.79	5.087	0.377
TGM (%)							
0		0	1.072	8.914	21.86	5.093	0.366
1		6	1.067	9.166	24.88	5.048	0.376
2		12	1.067	9.014	24.64	5.091	0.374
3		18	1.068	9.211	26.15	5.089	0.374
SEM			0.001	0.056	0.501	0.027	0.001
P-Value							
ME			0.985	0.653	0.301	0.718	0.174
TGM			0.929	0.384	0.478	0.796	0.911
ME*TGM			0.908	0.753	0.551	0.614	0.090

Values bearing different superscripts within the column are significantly (P<0.05) different

varying energy efficiency of TGM during the experiment. Ehsani and Torki (2010) reported that layers fed diet with GM up to 7 % inclusion should affect no Haugh unit score; similarly Gutierrez *et al.* (2007) also observed no significant effect on Haugh unit score by supplementing guar by- products (2 and 5 %) in high production laying hen diets. Shahbazi (2012) reported that GM supplementation up to 7 % in layer diets did not influence the Haugh unit score; similarly Rama Rao *et al.* (2015) observed no significant difference in Haugh unit score in layers fed TGM up to 15 % compared to control group fed SBM diet. Similar results were also reported by Sagar *et al.* (2017).

Inclusion of TGM at graded levels did not influence the yolk index and yolk color, similarly the interaction between energy efficiency of TGM and inclusion levels of TGM was not significant (Table 2). Ehsani and Torki (2010) reported that layers fed with GM up to 7 % inclusion should no affect on yolk index, while Shahbazi (2012) reported that GM supplementation up to 7 % in layer diets did not influence the yolk index.

Inclusion levels of TGM and energy efficiency of TGM did not show any significant (P>0.05) effect on shell weight, shell percentage, shell thickness, shell strength and egg density from 27 to 47 wks of age (Table 3). These results are in agreement with Ehsani and Torki (2010) and Shahbazi (2012) who reported that layers fed with GM up to 7 % inclusion showed no affect on shell quality parameters. Similarly Rama Rao *et al.*

Table 4. Effect of inclusion of graded levels and varying metabolisable energy of TGM on serum parameters of commercial layers from 27th- 42nd weeks of age

Treatment	Energy efficiency of TGM	Inclusion level of TGM (%)	Serum protein (g/dl)	Serum albumin (g/dl)	Serum cholesterol (mg/dl)
1	0%	0	4.875	1.948	155.2
2	50%	6	4.759	1.815	174.8
3	50%	12	4.901	1.983	208.8
4	50%	18	4.369	1.736	192.0
5	60%	6	4.804	1.790	184.6
6	60%	12	4.639	1.655	197.5
7	60%	18	4.191	1.959	139.4
Energy efficiency of TGM					
0	0%		4.875	1.948	155.2
1	50%		4.676	1.845	191.9
2	60%		4.545	1.801	173.8
TGM (%)					
0	0		4.875	1.948	155.2
1	6		4.781	1.803	179.7
2	12		4.770	1.819	203.2
3	18		4.280	1.848	165.7
SEM			0.174	0.056	8.008
P-Value					
ME			0.727	0.722	0.302
TGM			0.466	0.954	0.213
ME x TGM			0.942	0.188	0.334

Values bearing different superscripts within the column are significantly (P<0.05) different

(2015) observed no significant difference in shell quality parameters in layers fed TGM up to 15 %. Similar results were also reported by Sagar *et al.* (2017).

Serum biochemical parameters like serum albumin, serum protein and serum cholesterol were not affected by (P>0.05) supplementation of graded levels of TGM and varying energy efficiency of TGM in commercial layers (Table 4). Shahbazi (2012) reported increased serum cholesterol concentration in layers fed diet containing GM (5 %). However, other studies have reported that the high viscosity of guar gum may contribute to decreasing plasma cholesterol (Favier *et al.* 1998).

CONCLUSION

From the results of the present study, it was concluded that TGM can be safely and economically included in White Leghorn (WL) layer diets up to 18% without affecting Feed intake, FCR, external and internal quality of eggs.

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Evaluation of Performance of Rajasri Birds Under Different Management Situations

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ABSTRACT

A study was conducted to evaluate the performance of Rajasri bird reared under different systems. The control (T_1) group was reared under intensive system and were fed *ad lib*, while second (T_2) and third (T_3) groups were maintained under semi-intensive system recieving 20 and 40 percent of *ad lib.* feeding, respectively. The remaining two treatment groups were reared under scavenging, one group at farm ((T_4) and the other at farmer's backyard (T_5), respectively. Significantly higher ($P<0.05$) body weight was noticed in control group as compared to other groups at 40 and 60 weeks of age. The mean hen day production was better with control group and least with 20% and 40% *ad lib.* groups. Physical verification revealed that crop contents at 20 and 60 weeks respectively comprised of grains (35.88 and 52.02%), green forages (15.06 and 15.39%), kitchen waste (24.26 and 6.33%), animal food (1.44 and 3.84%) and other items (23.36 and 22.41%). Dry matter (DM) content of the crop contents was 36.34 and 36.04% at 20 and 60 weeks of age, respectively. Content of nutrients (on DM basis) such as crude protein (CP), ether extract (EE), total ash, crude fibre (CF) and nitrogen free extract (NFE) at 20 and 60 weeks of age were 9.68 and 10.59; 6.53 and 3.57; 31.18 and 23.80; 18.05 and 28.67; and 34.58 and 33.38%, respectively. It can be concluded that significantly higher hen day egg production could be attained in Rajasri birds reared under intensive system offering *ad lib.* feeding compared to other systems of rearirng.

Key words: Crop contents, Chemical composition, Rajasri, Scavenging system

INTRODUCTION

Modern poultry production is rapidly progressing towards vertical integration. The scale of operations of poultry farms has gone up. The number of small and marginal poultry farms both in urban and rural areas is reducing and availability of country chicken is gradually coming down. After commercialization of poultry production, the trend and availability of eggs and chicken meat was completely reversed. As a result, about 75% of total poultry produce is available for 25% of country's population residing in urban/semi urban areas (Prasad, 2004). These two vital aspects have provided an excellent base for production of backyard poultry suitable for backyard rearing. Rajasri, a prolific egg laying chicken variety has been developed by PV Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad to boost the backyard poultry production. Rajasri birds were capable of producing 140-150 eggs per year under scavenging system (Srinivas *et al.,* 2017). It is better to know the type of feed available in the field for making necessary suggestions in supplementing deficient nutrient, if any. Hence, the present study was undertaken to evaluate the physical and chemical composition of crop contents of Rajasri birds reared under scavenging and other systems of management.

MATERIALS AND METHODS

Five hundred day-old chicks were reared up to 6 weeks of age in the nursery. At 7th week of age 400 growers were randomly distributed into 5 treatments groups each having 4 replicates and each replicates comprising of 20 females each (Table 1). The control (T_1) group was reared under intensive system and were fed *ad lib*, while second (T_2) and third (T_3) groups were maintained under semi-intensive system recieving 20 and 40 percent of *ad lib.* feeding, respectively. The remaining two treatment groups were reared under scavenging, one group at farm ((T_4) and the other at farmer's backyard (T_5), respectively. Body weight was recorded at 40 and 60 weeks of age. Egg production was recorded from 20 to 60 weeks of age, divided in to 10 laying periods of 28 days each. Birds were

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Table 1: Experimental design

Treatment	System of rearing	Feeding pattern
T_1	Intensive deep litter	*Ad lib.* Feeding, control
T_2	Semi-intensive deep litter	20% of *ad lib.*+ lucerne meal (500 g)
T_3	Semi-intensive deep litter	40% of *ad lib.* + lucerne meal (500 g)
T_4	Extensive (on the farm)	Scavenging
T_5	Extensive (Backyard)	Scavenging

sacrificed in two lots, first time at the age of 20 weeks and the second at the age of 60 weeks and sampling was done as per the procedure outlined by Rashid *et al.* (2004). A total of 40 birds were slaughtered at each sampling time, 8 birds from each treatment and 2 birds from each replicate. Birds were slaughtered at farmer's households in the evening after their return from scavenging. Necropsy of each bird was conducted; crop contents were collected and were subjected for physical examination. The gizzards were also opened and the contents were collected.

The crop contents were separated into 5 categories namely grains (whole paddy, rice, broken rice, maize and seeds of grasses), green forages (different grasses, plant materials, herbs *etc.*), kitchen waste (cooked rice, cooked pulses, vegetable trimmings, vegetable leaves), animal protein sources (insects, ants, earth worms, snails and flies) and others (feathers, hairs and unidentified items). Each group was weighed quantitatively to the nearest gram and the percentage contributed was calculated. The crop contents were subsequently dried in hot air oven at 26.6 °C, individually ground and analysed for their proximate composition according to AOAC (1990) procedures. The generated data were analysed by Statistical Package for the Social Sciences (SPSS for windows, V10; SPSS Inc., Chicago, IL, USA). Significance was determined at $P<0.05$.

RESULTS AND DISCUSSION

At 40 and 60 weeks of age the body weight of the birds of control group was significantly ($P<0.05$) higher than all the treatment groups (Table 2). Lowest ($P<0.05$) body weight was recorded in semi intensive deep litter and in scavenging at farmers backyard treatments. Ali (2002) recorded least growth rate in scavenging than intensive or semi-intensive systems of management. This may be attributed to the extent of feed available, which could be utilized for deposition of protein and or fat in the body, besides meeting the requirements of egg production. The best mean hen day egg production (Table 3) was recorded in birds of *ad lib.* group (54.07%) compared to other groups because the birds were fed *ad lib.* with layer mash. Ali (2002) had shown that egg production was significantly higher in *ad lib.* fed birds as compared to those reared under scavenging system supplemented with 30 and 60 g of feed or reared on scavenging alone. Liveability was

Table 2. Body weight (g) at 40 and 60 weeks of age and liveability (21-60 weeks) of Rajasri birds reared under different systems of management

Group	Body weight (g)		Livability (%)
	40 wks	60 wks	21-60 wks
T_1	1796[a]	1907[a]	90.20
T_2	1442[bc]	1522[b]	90.79
T_3	1543[b]	1613[b]	91.25
T_4	1382[c]	1541[b]	87.06
T_5	1455[bc]	1564[b]	82.97
SEM	36.26	35.51	1.363
P value	0.001	0.001	0.278

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

Table 3. Hen day egg production (%) of Rajasri birds from 20 to 60 weeks of age

Group	Laying period (28 days each)										Treatment mean
	1	2	3	4	5	6	7	8	9	10	
T_1	35.60^{a}	68.14^{a}	58.76^{a}	50.95^{a}	58.04^{a}	58.75^{a}	64.11^{a}	50.48^{b}	44.35^{a}	51.49^{a}	54.07^{a}
T_2	0.00	2.79^{c}	27.90^{c}	32.88^{c}	21.13^{b}	28.44^{d}	32.08^{d}	22.89^{e}	22.26^{c}	18.92^{e}	20.93^{e}
T_3	0.00	3.46^{c}	36.05^{b}	37.85^{b}	35.34^{c}	36.26^{c}	41.69^{bc}	29.03^{d}	27.52^{c}	24.11^{d}	27.13^{d}
T_4	0.00	22.54^{b}	32.76^{b}	29.39^{dc}	37.18^{c}	37.45^{c}	38.95^{c}	47.80^{c}	37.68^{b}	40.94^{c}	32.47^{c}
T_5	0.25^{b}	27.45^{b}	37.00^{b}	28.49^{d}	40.45^{d}	40.66^{b}	43.43^{b}	54.09^{a}	41.20^{ab}	45.22^{b}	35.83^{b}
SEM	3.263	5.563	2.516	1.960	2.740	2.339	2.525	2.872	2.070	2.911	2.603
P value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

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

recorded low in backyard system of rearing as compared to other systems of management which may be attributed to predators and accidents apart from normal cause of death. Similarly, Czech *et al.* (2004) and Pennycott (2004) reported highest mortality in case of backyard reared birds.

Data pertaining to green forages and other component of crop contents as recorded at 20 and 60 weeks of age are presented in Table 4. Whereas, higher grain content (52.02 %) was noticed during 60 weeks, it was lower at 20 weeks of age. Kitchen waste was higher (24.25%) at 20 weeks and lower (6.33%) at 60 weeks of age. The animal protein sources were less (1.44 %) during 20 weeks than 60 weeks (3.84%) of age indicating change in appetite according to increasing demand for protein during its growth phase.

The data indicated (Table 5) that DM values of crop and gizzard contents varied largely both during 20 and 60 weeks of age for all the treatment groups. DM content was highest in control T_1, T_2 and T_3 groups. In the scavenging group (T_4 and T_5), DM content ranged between 35-36 % indicating the moisture content of 64-65%. High moisture content might be due to more greens, herbage and kitchen waste in diets of scavenging group. In earlier works, the DM of crop contents was shown as 34.4% by Gunaratne *et al.* (1993) and 42.9% by Mwalusanya *et al.* (2002). The CP value of crop contents was highest in control group both at 20 and 60 weeks of age (17.83 *vs.* 17.79%), as these birds were fed *ad lib*. Lower CP values as observed in 20% and 40% *ad lib.* groups might be due to restriction of mash feeding. The highest CF was observed in scavenging groups, attributable to higher forage and kitchen waste contents in their diets, followed by 20% and 40% *ad lib.* groups and least in control group. Based on the other nutrients intake, there was variation in NFE intake. Present findings were found to be varying marginally from the findings of Ukli (1992), who observed 8.18, 1.61, 9.44, 9.18 and 57.9% CP, EE, CF, total ash and NFE, respectively. Prawirokusumo (1988) recorded 11.3, 8.13 and 9.74% CP, EE and CF, respectively. Similarly, Gunaratne *et al.* (1993) reported 9.4, 9.2, 5.4 and 16%, CP, EE, CF and total ash, respectively in the crop contents of scavenging hens.

Table 4. Physical composition of crop contents at 20 and 60 weeks of age in scavenging Rajasri bird

Scavenging group	Total crop contents (g)		Different components of crop contents (%)									
			Grain		Green		Kitchen waste		Animal proteins		Others	
Age (Weeks)	20	60	20	60	20	60	20	60	20	60	20	60
R_1	16.75	19.09	72.16	44.30	12.31	16.85	1.39	6.49	0.73	4.29	13.41	28.07
R_2	19.42	21.35	33.51	30.98	25.72	29.37	16.66	4.50	2.28	4.24	21.83	30.92
R_3	16.64	26.56	29.65	66.60	9.62	2.89	6.80	8.39	0.33	6.08	53.60	16.03
R_4	22.06	28.29	8.20	66.21	12.60	12.45	72.17	5.92	2.41	0.76	4.62	14.65
Mean	18.72	23.82	35.88	52.02	15.06	15.39	24.26	6.33	1.44	3.84	23.36	22.41

Table 5. Nutrient composition (%DM) of crop contents during 20 and 60 weeks age of Rajasri birds

Group	Dry matter		Crude protein		Ether extract		Total ash		Crude fiber		Nitrogen free extract	
Age (Weeks)	20	60	20	60	20	60	20	60	20	60	20	60
T_1	41.97	42.77	17.83	17.79	1.23	1.76	16.71	19.59	9.64	8.07	59.61	57.81
T_2	41.00	40.68	7.66	10.33	1.32	0.43	24.09	25.49	13.32	30.63	53.62	33.13
T_3	40.87	39.67	8.27	11.61	2.70	0.82	26.77	17.48	13.91	32.97	48.36	37.13
T_4	35.72	35.68	8.89	10.88	2.50	0.34	28.93	22.24	17.68	30.17	42.02	36.38
T_5	36.34	36.04	9.68	10.59	6.53	3.57	31.18	23.80	18.05	28.67	34.58	33.38

Similar results were also reported by Rashid *et al.* (2005), Momoh *et al.* (2010) and Goromela *et al.* (2007).

CONCLUSIONS

From this study it can be concluded that significantly higher body weight and hen day egg production were observed in Rajasri birds reared under intensive system offering *ad lib.* feeding compared to others. The birds reared under scavenging were getting low level of protein than required. The full potential of the Rajasri birds can be exploited by supplementing the diet with locally available protein rich sources.

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Enzyme Supplementation of Commercial Feed Diluted with Copra Meal for Laying Hens

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ABSTRACT

The study investigated the effect of enzyme supplementation of commercial feed diluted with copra meal in laying hens. Two hundred 126 d old Shaver Star-Cross pullets were fed commercial feed alone or diluted with 2 levels (20 and 30%) of copra meal (CM) with or without enzyme in 4 replicates of 10 birds each. Feed intake, hen-day production and egg quality measurements formed the response criteria. Results showed no significant differences (P<0.05) among the treatments in terms of feed intake, hen-day production, egg weight, egg mass and FCR (feed: egg). Egg surface area was lower (P<0.05) on the control compared to the dilution diets. There were no effects of diet on egg shell thickness, Haugh unit and yolk colour (P>0.05). Eggs produced on the control had higher shape index compared to the 30% CM without enzyme (P<0.05). Cost of per kg feed reduced linearly with the dilution. Feed cost per unit egg produced was lower on 30% dilution with enzyme compared to the 20% without (P<0.05). In conclusion, diluting commercial feed with CM up to 30% does not compromise egg production and egg quality traits. This will reduce production cost and add value to CM. There is need for more research into higher rates of dilution, enzyme source and concentration.

Keywords: Copra meal, Enzyme, Egg quality traits, Laying performance,

INTRODUCTION

Commercial poultry production in the South Pacific region is constrained by high feed cost (Diarra, 2017). In the region, feed makes up to 80% of the total cost of production (Ayalew, 2011). Traditional protein sources such as soybean, fishmeal and meat and bone meal are expensive in the region; thus, there is need to research on strategies to maximise the utilisation of alternative ingredients. Copra meal, a moderate source of protein, is readily available in the region. Several nutritional characteristics, including high fibre and deficiency of essential amino acids, limit the efficient utilisation of CM in poultry feeding (Sundu *et al.,* 2009). The fibre of CM is primarily in the form of non-starch polysaccharides (NSP) which are not broken by digestive enzymes (Thorne *et al.,* 1990; Olude *et al.,* 2008; Choct, 2015). Amino acid and enzyme supplementation (Panigrahi, 1992; Pluske *et al.,* 1997; Moorthy and Viswanathan, 2006; Diarra *et al.,* 2014; 2015; 2018), pelleting, crumbling and soaking (Sundu *et al.,* 2005) and diet dilution (Pandi, 2005) improved the feeding value of CM for poultry. Pandi (2005) observed that village broilers grown to 53 weeks can utilise commercial feed diluted with 40% CM. Studies reporting the effect of commercial feed dilution with CM and enzyme supplementation for laying hens are however, still scanty. This study investigated the potential of commercial layer feed diluted with CM and enzyme supplementation for laying chickens with the hypotheses that (i) the birds will utilise higher levels of CM in commercial feed; and (ii) enzyme supplementation will improve the utilisation of the diluted feed by the hens.

MATERIALS AND METHODS

The experimental site was the Poultry Unit of the Vanuatu Agriculture College Livestock-Based Integrated Farm, Luganville, Vanuatu. The dietary treatments consisted of a commercial layer feed alone and the commercial feed diluted with 2 levels (20 and 30%) of CM (on protein basis) with and without enzyme. Challenzyme 1309A, a complex enzyme manufactured by Beijing Challenge in China was the enzyme of choice. Challenzyme 1309A has the following eight enzyme activities (U/g): β-glucannase

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800, xylanase 15,000, β-mannanase 100, α-galactosidase 100, proteases 800, amylase 500, pectinase 500, and cellulase 300.

The control commercial feed contained protein 190 g/kg, fat 50 g/kg, fibre 42 g/kg and ash 55 g/kg. Copra meal used in the experiment was analysed to contain per kg dry matter: crude protein 211 g, crude fibre 164 g, total NSP 523 g, lysine 4.7 g and methionine 2.9 g. The animal ethic committee of the University of the South Pacific approved the experimental protocol.

Two hundred 126 d old Shaver Star-Cross point of lay pullets were individually weighed and randomly allocated to one of 5 treatments groups in a completely randomized design with 4 replicates each. The birds were housed in an opened-sided floor pen with wood shavings as litter materials. Feed and water were provided *ad libitum* throughout the experimental period of 84 days. The lighting programme consisted of 17 hours light and 7 hours dark.

Weighed quantities of feed were fed and the left over weighed to account for feed intake by difference. Eggs laid were recorded per pen and hen-day production (HDP) calculated as:

$$HDP = \frac{\text{Eggs Produced}}{\text{Hens in the house}} \times 100$$

Sample of eggs were weighed weekly per pen using a stainless steel IP68 waterproof digital electronic scale. Egg mass was calculated as the product of eggs produced by mean egg weight and feed conversion ratio (FCR) was derived as the ratio of feed consumed to egg mass. Fortnightly, five eggs were randomly selected per pen for egg quality measurements. Egg length and width, shell thickness and albumen height were taken using a digital Varnier caliper (0.02mm). Egg shape index was calculated according to (Anderson *et al.,* 2004) as:

$$\text{Shape index} = \frac{W}{L} \times 100$$

Where W is the width of the egg at the widest point and L, the length of the egg.

Egg surface area was calculated according to Nasr *et al.* (2012) as:

$$\text{Egg surface area} = 3.9782 \times W^{0.7056}$$

Where W is the egg weight in grams.

Immediately after taking the width and length, the egg was broken into a smooth surfaced glass. The height of the albumen was taken at 3 points (plateau of the thickest part and 2 other points on the plane surface) using a tripod spherometer and Haugh unit calculated as:

$$\text{Haugh Unit} = 100 \text{ Log}(h - 1.7w^{0.37} + 7.6)$$

(Eisen *et al.,* 1962)

Where h is the mean of the three albumen height measurements (in millimetres) and w, the egg weight in grams.

Yolk colour was determined using the Roche Yolk Color Fan.

Data on egg production performance and quality measures were treated to analysis of variance (ANOVA) using the General Liner Models (GLM) procedures of the Minitab 18 Statistical Software (Minitab Inc, USA). Treatment means were compared using the Tukey pair-wise test and significant differences reported at 5% probability level.

RESULTS AND DISCUSSION

The results of egg production of the hens are presented in Table 1. There was no significant dietary effect ($P>0.05$) on any of the egg production parameters observed (feed intake, hen-day production, mean egg weight, egg mass and FCR). These results are consistent with those of Moorthy and Viswanatha (2010) who reported similar feed intake in 147 to 364 d old Single Comb White Leghorn layers fed CM at 20% level of inclusion. In contrast, Diarra *et al.* (2014) and Diarra *et al.* (2018) observed higher feed intake in CM-based diet compared to the control diet. Diarra *et al* (2014) also reported that Shaver Brown laying hens (126 to 196 d old) fed enzyme supplemented CM diets ate more feed than the control based on fishmeal. The authors attributed this to improved breakdown of fibre by enzyme and increased energy availability. Ochetim (1992) observed similarity in feed intake between two maize copra meal-based diets (33% CM) containing

Table 1. Egg production of Shaver Star Cross laying hens fed commercial feed alone or diluted with copra meal with and without enzyme

Variables	Control	Diets (% CM inclusion; on protein basis)				SEM	P-Values
		ECM no enzyme		ECM with enzyme			
		20	30	20	30		
Daily feed intake (g/bird)	153.30	151.33	157.82	154.36	147.98	4.19	0.681
Hen-day production (%)	58.33	41.74	53.12	56.23	44.24	4.77	0.104
Mean egg weight (g)	53.35	53.21	52.95	53.65	52.71	0.81	0.937
Egg Mass (kg)	21.54	15.28	19.40	20.85	16.12	1.83	0.113
FCR (Feed: egg)	3.42	4.56	3.79	3.50	4.33	0.37	0.213

[a,b] Means that do not share a letter are significantly different (P=0.05); SEM: Standard error of mean.

either meat and bone meal (MBM) or fishmeal (FM) as protein sources in 140 to 504 d old laying hens and attributed this to similar nutrient characteristics between MBM and FM. In an earlier study, Wignjosoesastro *et al.* (1972) observed linear increases in feed intake in White Leghorn layers fed increasing levels of CM from 10 to 40%. Egg-type birds may tolerate higher levels of CM in the diet due to their lower energy requirement compared to meat-type birds.

The results of hen-day production agree with earlier findings. Hen-day production was similar in Shaver Brown laying hens fed 20% CM with and without enzyme and the control commercial diet (Diarra *et al.,* 2014). Ochetim (1992) also found no effect on 33% CM with MBM or FM on hen-day production of Shaver Brown hens. In an earlier study, Panigrahi (1989) observed that 10 and 20% CM does not negatively affect egg production in Shaver Star Cross 288 hens, but egg production decreased as inclusion level increased to 40% level. In contrast, Moorthy and Viswanathan (2010) reported reduction in hen-day production at 20% CM level in soybean meal compared to fishmeal based diets, probably due to the combined effect of NSP in soybean and CM. These findings suggest that ingredient composition of the basal diet is a major factor affecting the utilisation of CM by poultry. Diarra *et al.* (2018) reported higher hen-day production on 20% CM inclusion level with enzyme supplementation compared to the diet without enzyme and the control diet in laying hens. This trend in laying performance may be due to similarity in nutrient characteristics of the dietary treatments on one hand and possible longer digesta

Table 2. Egg quality of Shaver Star Cross laying hens fed commercial feed alone or diluted with copra meal with and without enzyme

Variables	Control	Diets (% CM inclusion; on protein basis)				SEM	P-Values
		CM without enzyme		CM with enzyme			
		20	30	20	30		
Exterior qualities							
Egg surface area (cm)	71.11[b]	88.39[a]	87.67[a]	88.03[a]	87.82[a]	0.74	0.000
Shell thickness (mm)	1.05	1.50	1.05	1.05	0.98	0.05	0.428
Egg shape index (%)	80.36[a]	78.71[ab]	77.46[b]	79.14[ab]	78.94[ab]	0.48	0.032
Interior qualities							
Haugh Unit	93.52	97.06	96.30	93.57	101.32	1.89	0.069
Yolk colour	8.98	8.60	8.85	9.10	9.03	0.31	0.817

[a,b]Means that do not share a letter are significantly different (P=0.05); SEM: Standard error of mean.

retention of the CM based diets resulting in increased nutrient absorption on the other hand. Diarra *et al.* (2014) also attributed improved laying performance and heavier eggs to increased nutrient absorption resulting from longer retention of CM-based diets in the small intestine.

Similar to previous reports, dietary treatment did not affect mean egg weight and mass in this study (P>0.05). Diarra *et al.* (2018) observed no difference in mean egg weight amongst CM-based diets with and without enzyme and the control feed. Contrary to these findings however, Diarra *et al.* (2014) reported heavier eggs and egg mass in hens fed 20% CM without supplemental enzyme compared to the supplemented group. There was no treatment effect on feed conversion ratio in this study. Similar to this result, inclusion of CM at 20% in Leghorn hens (Moorthy and Viswanatha, 2010) and Shaver Brown layers (Diarra *et al.,* 2014) had no effect on FCR. In a recent study, Diarra *et al.* (2018) also found no effect of 20% dietary CM on the efficiency of feed utilisation in Shaver Brown hens from 133 to 217 d old compared to the control diet. Increasing CM level to 33% in isocaloric diets produced similar FCR between diets based on MBM or FM as protein sources (Ochetim, 1992). These findings suggest that both the composition of the basal diet and energy concentration affect the utilisation of CM by poultry.

The results of egg quality traits presented in Table 2, showed no effect of dietary treatment on eggshell thickness (P>0.05). Our results are in agreement with those of Diarra *et al.* (2018) who reported no effect of enzyme supplementation on shell thickness in laying hens fed 20% CM. Egg shape index was higher (P<0.05) on the control diet compared to 30% CM-based diet without enzyme, but did not differ between the control and other test diets as well as among the test diets (P>0.05). All eggs had shape index above the 76 considered round (Sarica and Erensayin, 2009 cited in Duman *et al.,* 2016). Egg shape index is a very important quality measure as round eggs are more likely to break during transportation because they do not fit well in egg cartons. Egg surface area was lower (P<0.05) in the control compared to the CM-based diets (P<0.05) but did not differ among CM-based diets (P>0.05). Egg surface area predicts shell quality characteristics, and egg interior parameters and influences the amount of oxygen, carbon monoxide and water vapour exchanged between the egg and external environment. The reason for larger surface area of eggs produced on CM-based diets was not clear and needs further investigations.

Haugh unit and yolk colour were similar across the dietary treatments (P>0.05). All eggs had Haugh unit of around 90, which is above the 70 ranked 'A' by the USDA (1984). Xanthophyll is the primary natural yellow pigment in the egg yolk (Barbosa *et al.,* 2011). The similarity in yolk colour suggests that the inclusion of CM did not influence the pigment content of the diets. Panigrahi (1989) observed increased in pale yellow yolks when CM level was increased to 40% in a maize-based diets, suggesting low pigment content of CM. The commercial feed used in this study was wheat-based. The low xanthophyll contents of wheat

Table 3. Feed cost of production of Shaver Star Cross laying hens fed commercial feed alone or diluted with copra meal with and without enzyme

Variables	Control	Diets (%CM inclusion; on protein basis)				SEM	P-Values
		CM without enzyme		CM with enzyme			
		20	30	20	30		
Cost /kg feed (US$)	0.99	0.85	0.78	0.85	0.78	NA	NA
FCR (feed: egg)	3.42^{ab}	4.56^{a}	3.79^{ab}	3.50^{ab}	4.33^{b}	0.370	0.026
Feed cost (US$)/kg egg	3.39^{ab}	3.89^{a}	2.97^{ab}	2.98^{ab}	2.28^{b}	0.257	0.011

^{ab}Means that do not share a letter are significantly different (P=0.05); SEM: Standard error of mean; NA: Not analysed

and CM may explain the trend of yolk colour.

Data pertaining to feed cost of egg production presented in Table 3 showed a linear reduction in the cost of feed with the level of dilution. This is due to the lower market price of CM. Feed conversion ratio (feed: egg) and feed cost per unit egg produced were reduced on 30% dilution with enzyme compared to 20% without enzyme. This could be due to increased enzymatic hydrolysis and nutrient availability for egg production.

CONCLUSIONS

From these results, we conclude that diluting commercial layer feed with CM (on protein basis) up to 30% had no adverse effect egg production. Enzyme supplementation may not be required at this level of dilution. The dilution will reduce cost of egg production and add value to copra meal in the study area. There is need for further research on higher dilution rates, enzyme sources and levels.

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Optimization of Calcium and Phosphorus Levels in the Diet of *Cirrhinus mrigala* (Hamilton, 1822) Fingerlings

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ABSTRACT

An experiment was carried out to evaluate the optimum levels of calcium and phosphorus in the diet of mrigal, *Cirrhinus mrigala*. Ten treatments diets supplemented with additional calcium (Ca) and Phosphorus (P) in ratios 0:0 (C), 5.0:2.5 (D1), 5.0:3.75 (D2), 5.0:5.0 (D3), 7.5:2.5 (D4), 7.5:3.75 (D5), 7.5:5.0 (D6), 10:2 (D7), 10:3.75 (D8), 10:5.0 (D9), respectively for 90 days in triplicates were tested. Different concentrations of phosphorus as di-hydrogen-orthophosphate (0.25, 0.375 and 0.5 $100g^{-1}$ of diet) and calcium as calcium chloride (0.5, 0.75 and 1.0 g 100 g^{-1} of diet) were added to the basal diet. The Ca and P content of the holding water were 62.64±2.60 mg l^{-1} and 0.04±0.001 mg l^{-1}, respectively. Growth performance increased significantly ($P<0.05$) with 3.75 g kg^{-1} of phosphorus and 7.5 g kg^{-1} of calcium, but decreased beyond this concentration. Total ammonia excretion and reactive phosphate production was significantly higher in the fish of groups fed below and above this concentration. Fingerlings showed deficiency symptoms such as reduced growth, low intestinal enzymatic activity when fed on diets other than D5, clearly revealing that the concentration of 7.5 g kg^{-1} of calcium and 3.75 g kg^{-1} of phosphorus in the diet is optimum for enhancing growth in *C. mrigala*.

Keywords: Ammonia excretion, Calcium, *Cirrhinus mrigala*, Growth performance, Phosphorus

INTRODUCTION

Successful and sustainable aquaculture/fish culture is the need of time and it depends upon the provision of nutritionally adequate, environment friendly and economically viable supplementary compounded feeds. Growth, health and reproduction of fish are primarily dependent upon the adequate supply of nutrients both in terms of quantity and quality, irrespective of culture system in which they are grown. The natural feed has to be supplemented with balanced supplementary compounded diets for high survival and growth. The ultimate aim of feeding fish in aquaculture is to achieve maximum protein deposition with minimum inputs of feed at minimum cost. Fish nutrition is a challenging area of research and usual traditional fish diets i.e. rice bran, ground nut oil cake; do not contain enough nutrients required for good health. To reduce pollution and increase fish growth, a nutritionally balanced diet is necessary for improving not only fish growth but also to get fully nutritional fish flesh. Aquaculture is expanding in a period of environmental awareness and is, therefore, subject to regulations designed to limit its effect on the environment.

Feed containing primarily of plant ingredients are relatively low in available P (Cho and Bureau, 2001), therefore, the optimization of phosphorus required for fish growth is essential (Mgbenka and Vgwu, 2005). In order for world aquaculture production to continue to increase, it will be necessary to reduce discharge of wastes especially phosphorus (P), from fish farms to the aquatic environment. The ultimate source of P in aquaculture effluent is feeds. Any excess amount of P in diet above the optimum requirement for fish will be excreted by the fish. It is, therefore, critical to know precisely the dietary requirement of P in order to minimize excess P in diet without risking P deficiency in cultured fish. The dietary requirement of P has been studied for various fish and other animal species using fry or juveniles and indicators such as weight gain, feed efficiency level in various tissues, bone density, bone-breaking strength and enzyme activities (NRC, 1993). Calcium is an essential component constituent of all living cells involved in osmoregulation in some

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aquatic animals (Podoliak and Holden, 1966) and is known to be received from surrounding water and feed provided (Hossain and Furuichi, 2009). It has an interesting effect on the absorption or utilization of other divalent heavy metal by plants, fish and mammals as the toxicity of lead, copper, beryllium, cadmium and vanadium salts to fish becomes considerably less in the hard (calcium containing) waters than in soft or distilled water and deficiency may lead to skeletal deformities in fish larvae owing to the disruptions in developmental process (Cahu *et al.,* 2003).

The requirements of calcium and phosphorus for the fish should be considered together as the metabolism of two elements appears to be intimately connected; the greater part of the phosphorus of the body is associated with calcium in bones. Some information on the dietary requirements of calcium and phosphorus for optimum growth and metabolism in fish are available (Cowey, 1976). Hossain and Yoshimatsu (2014) have discussed the essentiality of optimum Ca:P and dietary Ca requirement in fishes. Keeping up a dietary Ca/P proportion should be considered, otherwise overabundance Ca or P may cause issues in the fish body or be remained unabsorbed and stacked in culture condition. Owing to the importance of calcium and phosphorus, the present study had been undertaken to provide an improved diet for the cultivation of a candidate fish species, *Cirrhinus mrigala.*

MATERIALS AND METHODS

The experiment was conducted under laboratory conditions at Aquaculture Research Unit of Kurukshetra University, Kurukshetra (25±1ºC) in glass aquaria (60x30x30 cm). Each aquarium was filled with dechlorinated tap water and then stocked with 10 fingerlings (weighing 0.24± 0.02 g and 2.85±0.09 cm in length) procured from "Jyotisar Fish Seed Farm", Jyotisar, Kurukshetra. Ten experimental isoenergetic and isonitrogenous diets (C, D1-D9) with 40% crude protein were formulated using processed soybean as the major protein source. Different concentrations of phosphorus as di-hydrogen-orthophosphate (0.25, 0.375 and 0.5 $100g^{-1}$ of diet) and calcium as calcium chloride (0.5, 0.75 and 1.0 g 100 g^{-1} of diet) were added to the basal diet. Diet C with no supplementary Ca or P served as the control. Experimental diets were supplemented with additional calcium (Ca) and phosphorus (P) in ratios 0:0 (C), 5.0:2.5 (D1), 5.0:3.75 (D2), 5.0:5.0 (D3), 7.5:2.5 (D4), 7.5:3.75 (D5), 7.5:5.0 (D6), 10:2 (D7), 10:3.75 (D8), 10:5.0 (D9), respectively and fed daily at 4% body weight in two instalments at 8:00 and 16:30 hours for 90 days in triplicates. The amount of feed was adjusted fortnightly following a bulk weighing of each group of fish. The Ca and P content of the holding water were 62.64±2.60 mg l^{-1} and 0.04±0.001 mg l^{-1}, respectively. Dietary ingredient and proximate composition of formulated diets are given in Table 1.

Fish were exposed to the diet for 3 h during each ration; thereafter, the uneaten feed was siphoned out, stored and weighed for calculating feed conversion ratio (FCR). Faecal matter voided by the fish was collected each morning by siphoning approximately 14 h after the removal of the uneaten food. Faeces were oven dried (60ºC) for subsequent analysis. Growth and digestibility were estimated following standard procedure (Garg *et al.,* 2002a).

During experimentation, the water samples were collected from all the experimental aquaria to analyze temperature, dissolved oxygen (DO), pH, electrical conductivity, total alkalinity, total ammonia (NH_4-N) and orthophosphate following APHA (1998) to investigate the influence of compounded feeds on quality of holding water at regular intervals of time.

At the end of 90 days of feeding trials, fingerlings were offered same diet in sufficient quantity, made available for 2h for the feed to be consumed. Then, fixed levels of water were maintained and excess of feed was removed. After that, water samples from each aquaria/tub were collected at two hours intervals to estimate the excretory levels of total ammonia (N-NH_4) and reactive orthophosphate (o-PO_4) following APHA (1998), and were calculated following Sumagaysay-Chavoso (2003).

$$\text{Total ammonia excretion} = \frac{NH_4 - N\ (Mg1^{-1})\ \text{in aquarium water}}{\text{Fish weight (Kg) per L of water}}$$

$$\text{Reactive phosphate excretion} = \frac{o - PO^4\ (Mg1^{-1})\ \text{in aquarium water}}{\text{Fish weight (Kg) per L of water}}$$

Table 1. Ingredient and proximate composition of experimental diets with different levels of calcium and phosphorus (g Kg^{-1} of diet)

Ingredients	C	D1	D2	D3	D4	D5	D6	D7	D8	D9
Calcium	0	5.0	5.0	5.0	7.5	7.5	7.5	10.0	10.0	10.0
Phosphorus	0	2.5	3.75	5.0	2.5	3.75	5.0	2.5	3.75	5.0
Ingredient composition										
Groundnut cake	650.0	650.0	650.0	650.0	650.0	650.0	650.0	650.0	650.0	650.0
Rice bran	42.0	34.5	33.3	32.0	32.0	30.8	29.5	29.5	28.3	27.0
Processed soybean*	266.0	266.0	266.0	266.0	266.0	266.0	266.0	266.0	266.0	266.0
Wheat flour	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0
Chromic oxide(Cr_2O_3)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Calcium**	0	5.0	5.0	5.0	7.5	7.5	7.5	10.0	10.0	10.0
Phosphorus***	0	2.5	3.75	5.0	2.5	3.75	5.0	2.5	3.75	5.0
Mineral mixture****	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Proximate composition										
Crude protein (%)	$39.85^{A}\pm$ 1.36	$39.58^{A}\pm$ 1.15	$40.34^{A}\pm$ 2.10	$39.85^{A}\pm$ 1.36	$39.21^{A}\pm$ 0.79	$40.42^{A}\pm$ 0.49	$40.85^{A}\pm$ 0.80	$39.98^{A}\pm$ 1.16	$41.31^{A}\pm$ 1.43	$39.85^{A}\pm$ 1.36
Crude fat (%)	$9.10^{A}\pm$ 0.26	$8.16^{A}\pm$ ±0.38	$8.19^{A}\pm$ 0.63	$8.58^{A}\pm$ 0.33	$7.78^{B}\pm$ 0.11	$8.44^{A}\pm$ 0.29	$8.78^{A}\pm$ 0.16	$9.65^{A}\pm$ 0.38	$8.56^{A}\pm$ 0.47	$9.10^{A}\pm$ 0.26
Crude fiber (%)	$6.23^{A}\pm$ 0.06	$6.38^{A}\pm$ 0.36	$6.08^{A}\pm$ 0.03	$6.38^{A}\pm$ 0.36	$6.35^{A}\pm$ 0.33	$5.74^{A}\pm$ 0.31	$5.74^{A}\pm$ 0.31	$6.77^{A}\pm$ 0.17	$6.14^{B}\pm$ 0.10	$6.23^{B}\pm$ 0.06
Total ash (%)	$6.62^{A}\pm$ 0.39	$6.49^{A}\pm$ 0.60	$6.45^{A}\pm$ 0.18	$6.19^{A}\pm$ 0.07	$6.71^{A}\pm$ 0.24	$6.51^{A}\pm$ 0.41	$6.57^{A}\pm$ 0.36	$7.51^{A}\pm$ 0.21	$7.45^{A}\pm$ 0.26	$6.60^{B}\pm$ 0.39
Moisture (%)	7.41± 0.20^{A}	7.26± 0.44^{A}	6.66± 0.14^{A}	7.86± 0.11^{A}	7.67± 0.06^{A}	7.26±0. 0.22^{A}	6.89 0.08^{A}	7.68± 0.23^{A}	8.27± 0.21^{A}	7.41± 0.20^{A}
Nitrogen free extract (%)	$29.30^{A}\pm$ 1.42	$30.74^{A}\pm$ 2.70	$30.46^{A}\pm$ .67	$29.95^{A}\pm$ 1.03	$30.80^{A}\pm$ 1.15	$30.46^{A}\pm$ 0.56	$29.62^{A}\pm$ 0.61	$26.58^{A}\pm$ 1.25	$25.65^{A}\pm$ 1.61	$29.30^{A}\pm$ 1.42
Gross energy (kJ g^{-1})	$17.93^{A}\pm$ 0.09	$17.85^{A}\pm$ 0.12	$17.99^{A}\pm$ 0.24	$17.90^{A}\pm$ 0.09	$17.62^{A}\pm$ 0.06	$18.12^{A}\pm$ 0.15	$18.20^{A}\pm$ 0.05	$17.65^{B}\pm$ 0.06	$17.78^{B}\pm$ 0.06	$17.93^{A}\pm$ 0.09
Feed phosphorus (%)	$1.48^{D}\pm$ 0.11	$1.66^{C}\pm$ 0.21	$1.80^{B}\pm$ 0.04	$1.96^{A}\pm$ 0.22	$1.55^{B}\pm$ 0.14	$1.76^{A}\pm$ 0.04	$1.53^{B}\pm$ 0.17	$1.8^{A}\pm$ 0.10	$1.90^{A}\pm$ 0.03	$1.98^{A}\pm$ 0.11

*Soybean was hydrothermically processed in an autoclave at 121° C (15 lbs for 15 minutes) to eliminate antinutrient factors (Garg *et al.,* 2002b); **Calcium chloride ($CaCl_2.2H_2O$) ***Potassium dihydrogen orthophosphate (KH_2PO_4); ****Each kg has nutritional value: copper 312 mg, cobalt 35 mg, magnesium 2.114g, iron 979 mg, zinc 2 mg, iodine 15 mg, DL-methionine 1.920 g, L-lysine monohydrochloride 4.4 g, calcium 30%, phosphorous 8.25%; All values are Mean ± S. E. of mean; Means with different letters in the same row are significantly ($P<0.05$) different (Duncan's Multiple Range test).

At the termination of the experiment, from each treatment, fishes were obtained and kept on an ice tray. Intestine of fish were extirpated and processed for determination of protease (Walter, 1984) and amylase activity (Sawhney and Singh, 2000).

The moisture content, ash, and protein content of the diets and the fish carcasses were analyzed according to AOAC (1995): moisture content after drying in an oven at 104ºC until constant weight; ash content by incineration in a muffle furnace at 600ºC for 24 hours; crude protein by the Kjeldahl method.

Significant differences among treatment groups were tested by Analysis of variance (ANOVA) followed by Duncan's multiple range tests (Duncan, 1955). Statistical significance were settled at a probability value of $P<0.05$. All statistics were performed using suitable SPSS Version 16.0.

RESULTS AND DISCUSSION

This experiment was designed to provide information on growth responses, survival rate, carcass composition of fingerlings of *C. mrigala* when fed on diets supplemented with nine different concentrations (%) of calcium (Ca) and phosphorus (P). The aim was to determine optimum calcium and phosphorus levels required for the optimum growth of experimental fish. All groups of fingerlings accepted the respective experimental diets satisfactorily and maintained normal behaviour throughout the experimental period.

The growth responses of the *C. mrigala* fed on experimental diets (D1-D9) and control diet are shown in Table 2. Survival was high in all treatments. Growth performance in terms of live weight gain (g) was high in diet D5 where phosphorus was added at 3.75 g kg^{-1} and calcium at 7.5 g kg^{-1}. Growth (%) gain in BW, growth per day (%) in BW, SGR were significantly higher in fingerlings fed on D5 diet (phosphorus was added at 3.75 g kg^{-1} and calcium at 7.5 g kg^{-1}). Further, the growth increased with increase in the dietary P supplementation i.e. from 2.5 g kg^{-1} to 3.75 g kg^{-1}, and thereafter growth declined, similarly growth increased with increase in Ca levels i.e. from 0.5 to 0.75% and thereafter a decline was observed. The deficiency signs observed in this experiment were reduced growth, low digestibility and high feed conversion ratio in diets containing other ratios. In the present studies it was observed that phosphorus should be supplemented at 0.375 g $100g^{-1}$ (3.75 g kg^{-1}) and Ca should be supplemented between 0.5 and 0.75 g $100g^{-1}$ (5.0 and 7.5 g kg^{-1} of diet).

Zhang *et al.* (2006) reported that a phosphorus deficiency produced lower bone ash in Japanese seabass, while Ketola (1975) found that supplementation of organic phosphorus in the diet significantly increased bone ash content for Atlantic

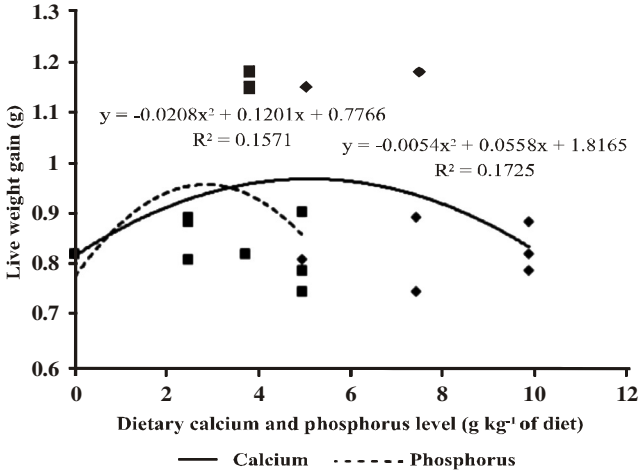

Fig. 1. Polynomial fit curve to show dietary calcium and phosphorus requirement fitting to data of live weight gain

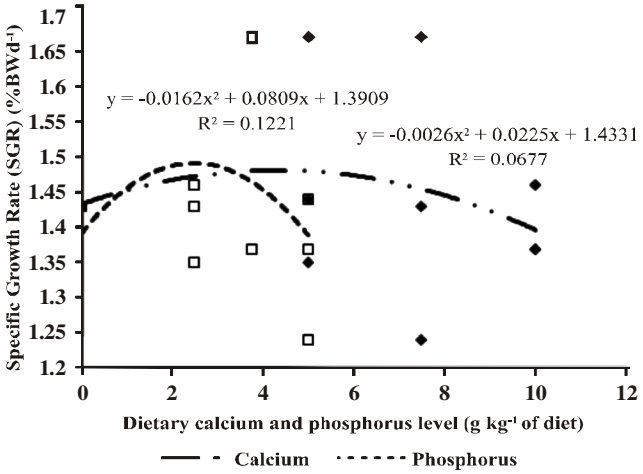

Fig. 2. Polynomial fit curve to show dietary calcium and phosphorus requirement fitting to data of specific growth rate

Table 2. Growth performance and intestinal enzyme activity of *C. mrigala* fingerling fed on different levels of phosphorus and calcium supplementation

Ingredients	C	D1	D2	D3	D4	D5	D6	D7	D8	D9
Calcium	0	5.0	5.0	5.0	7.5	7.5	7.5	10.0	10.0	10.0
Phosphorus	0	2.5	3.75	5.0	2.5	3.75	5.0	2.5	3.75	5.0
Initial weight (g)	$0.31^{A}\pm$ 0.02	$0.34^{A}\pm$ 0.01	$0.32^{A}\pm$ 0.01	$0.31^{A}\pm$ 0.02	$0.33^{A}\pm$ 0.01	$0.32^{A}\pm$ 0.01	$0.34^{A}\pm$ 0.01	$0.32^{A}\pm$ 0.01	$0.33^{A}\pm$ ±0.01	$0.32^{A}\pm$ 0.01
Final weight (g)	$1.13^{C}\pm$ 0.01	$1.15^{B}\pm$ 0.01	$1.48^{A}\pm$ 0.03	$1.24^{B}\pm$ 0.13	$1.22^{B}\pm$ 0.17	$1.51^{A}\pm$ 0.02	$1.09^{C}\pm$ 0.13	$1.20^{A}\pm$ 0.02	$1.16^{B}\pm$ 0.01	$1.12^{B}\pm$ 0.05
Live weight gain (g)	$0.82^{C}\pm$ 0.03	$0.81^{C}\pm$ 0.02	$1.15^{A}\pm$ 0.04	$0.90^{B}\pm$ 0.14	$0.89^{B}\pm$ 0.15	$1.18^{A}\pm$ 0.02	$0.75^{C}\pm$ 0.23	$0.88^{A}\pm$ 0.01	$0.82^{B}\pm$ 0.01	$0.79^{B}\pm$ 0.05
Survival rate (%)	100^{A}	100^{A}	89.96^{A}	90.48^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}	95.24^{A}
Growth (%) gain in BW	$268.1^{B}\pm$ 14.67	$239.12^{BC}\pm$ 13.78	$355.42^{A}\pm$ 28.97	$264.86^{B}\pm$ 43.45	$267.43^{B}\pm$ 36.97	$362.75^{A}\pm$ 12.56	$223.90^{C}\pm$ 31.34	$273.86^{A}\pm$ 7.70	$245.0^{B}\pm$ 10.31	$246.50^{B}\pm$ 17.51
Growth per day (%) in BW	$1.26^{B}\pm$ 0.06	$1.20^{BC}\pm$ 0.02	$1.41^{A}\pm$ 0.04	$1.26^{B}\pm$ 0.10	$1.25^{B}\pm$ 0.07	$1.42^{A}\pm$ 0.01	$1.11^{C}\pm$ 0.17	$1.27^{A}\pm$ 0.01	$1.21^{B}\pm$ 0.02	$1.19^{B}\pm$ 0.05
Specific growth rate (SGR) (% BW d^{-1})	$1.43^{B}\pm$ 0.10	$1.35^{C}\pm$ 0.04	$1.67^{A}\pm$ 0.06	$1.44^{B}\pm$ 0.15	$1.43^{B}\pm$ 0.11	$1.67^{A}\pm$ 0.04	$1.24^{C}\pm$ 0.17	$1.46^{A}\pm$ 0.02	$1.37^{B}\pm$ 0.03	$1.37^{B}\pm$ 0.05
Feed conversion ratio (FCR)	$1.18^{B}\pm$ 0.04	$1.31^{A}\pm$ 0.03	$1.05^{C}\pm$ 0.04	$1.30^{A}\pm$ 0.09	$1.42^{B}\pm$ 0.27	$1.04^{C}\pm$ 0.01	$1.76^{A}\pm$ 0.25	$1.24^{A}\pm$ 0.02	$1.31^{A}\pm$ 0.02	$1.33^{A}\pm$ 0.08
Gross conversion efficiency (GCE)	$0.84^{B}\pm$ 0.03	$0.75^{BC}\pm$ 0.01	$0.95^{A}\pm$ 0.03	$0.80^{B}\pm$ 0.12	$0.74^{BC}\pm$ 0.13	$0.94^{A}\pm$ 0.01	$0.67^{C}\pm$ 0.21	$0.80^{A}\pm$ 0.01	$0.75^{A}\pm$ 0.01	$0.75^{A}\pm$ 0.05
Protein efficiency ratio (PER)	$0.02^{A}\pm$ 0.01	$0.01^{B}\pm$ 0.01	$0.02^{A}\pm$ 0.01	$0.02^{A}\pm$ 0.01	$0.02^{A}\pm$ 0.01	$0.02^{A}\pm$ 0.00	$0.01^{B}\pm$ 0.01	$0.02^{A}\pm$ 0.00	$0.02^{A}\pm$ 0.01	$0.01^{A}\pm$ 0.01
Apparent protein digestibility (APD %)	$76.8^{C}\pm$ 0.15	$82.5^{B}\pm$ 0.16	$86.3^{A}\pm$ 0.35	$83.2^{B}\pm$ 0.7	$85.1^{B}\pm$ 0.32	$87.2^{A}\pm$ 0.5	$80.1^{BC}\pm$ 0.2	$79.3^{A}\pm$ 0.2	$77.6^{AB}\pm$ 0.3	$72.3^{C}\pm$ 0.04
Specific protease activity[1]	$3.82^{B}\pm$ ±0.17	$3.87^{B}\pm$ 0.32	$4.70^{A}\pm$ 0.29	$4.1^{B}\pm$ 0.27	$4.03^{B}\pm$ 0.08	$5.53^{A}\pm$ 0.52	$3.69^{C}\pm$ 0.45	$4.00^{A}\pm$ 0.06	$3.58^{B}\pm$ 0.09	$3.54^{B}\pm$ 0.33
Specific amylase activity[2]	$3.55^{B}\pm$ 0.24	$3.46^{B}\pm$ 0.09	$4.53^{A}\pm$ 0.21	$4.27^{B}\pm$ 0.23	$4.19^{B}\pm$ .09	$4.73^{A}\pm$ 0.13	$4.16^{B}\pm$ 0.18	$3.69^{A}\pm$ 0.07	$3.49^{B}\pm$ 0.07	$3.44^{B}\pm$ 0.07

[1]mg of tyrosine liberated mg of protein $^{-1}$ h^{-1}; [2]mg of maltose liberated mg of protein $^{-1}$ h^{-1}; All values are Mean±S.E of mean; Means with different letters in the same row are significantly ($P<0.05$) different (Duncan's Multiple Range test).

salmon. The dietary phosphorus levels greatly affected the ash, calcium and phosphorus contents of the bones of chum salmon (Watanbe *et al.,* 1980). Robinson *et al.* (1987) found that 0.50 g $100g^{-1}$ of phosphorus was adequate for normal bone mineralization based on calcium levels in bone ash. In contrast, Rodehutscord (1996) was unable to confirm any necessity for phosphorus for maximum mineralization of rainbow trout. Rodgers (1984) performed experiments with brook trout using labelled calcium, showed that ambient calcium concentration had a pronounced effect on calcium dynamics. In the present study mineral contents (Ca and P) of bone and scale were not separately monitored, however, carcass ash content and phosphorus appeared to be positively correlated with growth performance. These results correlate well with the dietary calcium and phosphorus contents.

No significant difference was observed in APD of diets. On the other hand, FCR value was significantly lower in fingerlings fed on D5 diet. Growth rate was high in the group of fingerlings fed on diet D5 supplemented with Ca and P at 7.5 g kg^{-1} and 3.75 g kg^{-1} of diet. No significant ($P<0.05$) variation was observed in GCE and protein efficiency ratio of all diets. Polynomial fit curves denote the relationship of different growth parameters with different levels of calcium and phosphorus (Fig. 1 and 2). Present studies on *C. mrigala* indicate that increasing dietary calcium level (beyond 0.75 g $100g^{-1}$) had a negative effect not only on growth and digestibility (APD) but also on other physiological parameters like specific activities of protease and amylase enzymes observed in the present study. Nakamura (1982) also found that increasing dietary calcium had a negative relationship with amount of phosphorus absorbed. On the other hand, Sakamoto and Yone (1973) showed that some calcium was necessary in diets containing high levels of phosphorus because the uptake of the calcium from water was not sufficient to meet the requirements of growth in red sea bream *Crysophrys major*. Maximum growth of catfish was obtained in experiments where diets contained 1.5% calcium and considered by Andrews *et al.* (1973) that the absence of a growth-response relationship at levels below 1.5% calcium indicated a requirement of at least this level in the diet. Robinson *et al.* (1987) found that weight gain and feed conversion rate was optimum for good growth at 0.70% dietary calcium in *O. aureus*, but this did not clearly reflects bone or scale mineralization.

Garg *et al.* (2002b) have further reported that hydrothermal treatment only slightly reduces phytase phosphorus (from 36.14 to 34.2 μg g^{-1}). Hence, the phosphorus must be supplemented into the diet at optimum level, due to the function of P in the bone structure, an increasing supply there of significantly increases the P content of the bone and thus its mineralization (Ogino *et al.,* 1979). However, much of the phosphorus in commercial fish diets may be released into the environment (Wiesmann *et al.,* 1988) and this is influenced both by the availability of dietary phosphorus and the high levels of phosphorus encountered in feeds because of the high levels found in animal proteins such as fishmeal. The gross requirement of P is significantly affected by its availability in the intestines. The relative availability varies greatly with fish species, diet composition and form of P (Schwartz, 1995). Keeping in view the above points when in the present studies diets D1 to D9 supplemented with varying phosphorus levels were fed to the *C. mrigala* fingerlings it was found that optimum phosphorus level is 0.375 per cent. The results of the present investigation were in accordance with the results observed in Japanese seabass (*Lateolavrax japonicus*) (Zhang *et al.,* 2006), black seabream (*Sparus macrocephalus*) (Shao *et al.,* 2008) and Chinese sucker (Yuan *et al.,* 2011).

Intestinal digestive enzyme activity of two enzymes i.e. protease and amylase were determined. Data revealed that specific activity of digestive enzymes was high in diet D5 supplemented with Ca and P at 7.5 g kg^{-1} and 3.75 g kg^{-1} of diet followed by control (Table 2). A definite relationship of dietary Ca-P ratio and the growth performance as also shown by polynomial fit curves (Fig. 1, 2, 3) of this fish species and their nutritive physiology in terms of specific activities of digestive enzymes (protease and amylase) was observed in the present study. Studies by Paul *et al.* (2004) confirmed these results in *C. mrigala*. However,

Table 3. Effect of fish fed with different levels of phosphorus and calcium supplementation on water quality characteristics

Ingredients	C	D1	D2	D3	D4	D5	D6	D7	D8	D9
Calcium	0	5.0	5.0	5.0	7.5	7.5	7.5	10.0	10.0	10.0
Phosphorus	0	2.5	3.75	5.0	2.5	3.75	5.0	2.5	3.75	5.0
Dissolved oxygen	$5.16^{A}\pm$	$5.00^{A}\pm$	$5.05^{A}\pm$	$5.09^{A}\pm$	$5.90^{A}\pm$	$5.78^{A}\pm$	$5.89^{A}\pm$	$5.07^{A}\pm$	$5.95^{A}\pm$	$5.94^{A}\pm$
DO) (mg l^{-1})	0.07	0.09	0.08	0.07	0.09	0.07	0.07	0.06	0.07	0.05
pH	$8.15^{A}\pm$	$8.21^{A}\pm$	$8.21^{A}\pm$	$8.22^{A}\pm$	$8.17^{A}\pm$	$8.22^{A}\pm$	$8.20^{A}\pm$	$7.86^{A}\pm$	$8.21^{A}\pm$	$8.13^{A}\pm$
	0.01	0.30	0.02	0.01	0.03	0.04	0.01	0.31	0.02	0.02
Conductivity (µ mho cm^{-1})	$572.4^{A}\pm$	$545.8^{A}\pm$	$548.04^{A}\pm$	$533.3^{A}\pm$	$567.84^{A}\pm$	$579.1^{A}\pm$	$520.19^{B}\pm$	$566.32^{A}\pm$	$566.32^{A}\pm$	$576.6^{A}\pm$
	13.10	13.43	16.05	15.55	9.59	23.34	26.78	13.84	13.84	23.75
Carbonates (mg l^{-1})	$14.77^{B}\pm$	$14.21^{B}\pm$	$17.75^{A}\pm$	$20.42^{A}\pm$	$18.21^{A}\pm$	$18.64^{A}\pm$	$18.66^{A}\pm$	$19.55^{A}\pm$	$19.54^{A}\pm$	$18.22^{A}\pm$
	6.95	2.48	1.62	4.97	2.48	4.01	2.66	4.64	3.88	2.22
Bicarbonates (mg l^{-1})	$128.87^{A}\pm$	$163.93^{A}\pm$	$143.53^{A}\pm$	$135.98^{A}\pm$	$138.64^{AB}\pm$	$140.88^{A}\pm$	$145.31^{A}\pm$	$146.18^{A}\pm$	$137.73^{AB}\pm$	$131.54^{AB}\pm$
	30.05	12.73	34.52	38.71	34.73	37.17	34.29	27.86	43.05	28.28
Chloride (mg l^{-1})	$31.77^{A}\pm$	$33.31^{A}\pm$	$31.98^{A}\pm$	$32.41^{A}\pm$	$33.97^{A}\pm$	$33.75^{A}\pm$	$34.19^{A}\pm$	$33.52^{A}\pm$	$31.54^{A}\pm$	$31.77^{A}\pm$
	2.49	1.87	1.14	2.30	2.15	2.49	2.08	2.52	2.28	2.49
Total Ammonia excretion	$1647.76^{A}\pm$	$1163.03^{B}\pm$	$708.33^{D}\pm$	$1036.73^{C}\pm$	$812.26^{C}\pm$	$607.16^{D}\pm$	$1213.80^{B}\pm$	$1408.46^{D}\pm$	$1747.20^{B}\pm$	$1810.10^{A}\pm$
(mg kg^{-1} BW day^{-1})	19.58	22.19	22.20	24.57	18.73	19.82	22.57	19.11	32.24	16.0
Total phosphate production	$756.73^{A}\pm$	$514.43^{B}\pm$	$320.06^{C}\pm$	$458.03^{BC}\pm$	$434.93^{B}\pm$	$258.73^{C}\pm$	$465.83^{B}\pm$	$816.5^{B}\pm$	$816.5^{B}\pm$	$824.5^{A}\pm$
(mg kg^{-1} BW day^{-1})	21.96	17.41	19.08	19.96	19.96	20.60	21.00	23.19	23.19	29.59

All values are Mean±S.E of mean; Means with different letters in the same row are significantly (P<0.05) different; (Duncan's Multiple Range test);
[1]mg of tyrosine liberated mg of protein $^{-1}$ h^{-1}; [2]mg of maltose liberated mg of protein $^{-1}$ h^{-1}; All values are Mean±S.E of mean; Means with different letters in the same row are significantly (P<0.05) different (Duncan's Multiple Range test).

Table 4. Proximate composition of fish carcass fed on different levels of phosphorus and calcium supplementation

Proximate	Initial value	C	D1	D2	D3	D4	D5	D6	D7	D8	D9
Moisture (%)	71.27^{A}±	68.22^{A}±	67.85^{A}±	67.29^{A}±	67.71^{A}±	67.62^{A}±	67.35^{A}±	67.72^{A}±	67.52^{A}±	67.19^{A}±	68.07^{A}±
	0.05	0.02	0.02	0.05	0.03	0.02	0.02	0.03	0.51	0.01	0.01
Crude protein (%)	8.08^{D}±	12.33^{A}±	12.07^{B}±	15.10^{A}±	10.08^{C}±	12.69^{B}±	16.63^{A}±	9.81^{C}±	12.58^{A}±	9.47^{B}±	8.16^{B}±
	0.04	0.17	0.03	0.15	0.14	0.24	0.08	0.11	0.14	0.14	0.20
Crude fat (%)	2.83^{C}±	6.83^{A}±	3.80^{B}±	5.00^{AB}±	3.75^{B}±	3.78^{B}±	5.08^{A}±	3.63^{B}±	3.75^{B}±	3.62^{B}±	3.58^{B}±
	0.05	0.04	0.03	0.15	0.06	0.02	0.05	0.06	0.07	0.05	0.02
Total ash (%)	3.52^{C}±	3.91^{B}±	4.03^{A}±	4.33^{A}±	4.28^{A}±	4.30^{B}±	4.58^{A}±	4.15^{C}±	4.30^{A}±	4.11^{A}±	3.97^{B}±
	0.01	0.03	0.02	0.03	0.01	0.02	0.03	0.02	0.03	0.03	0.02
Nitrogen free	14.28^{A}±	8.7^{C}±	12.23^{B}±	8.28^{C}±	14.17^{A}±	11.60^{B}±	6.34^{C}±	14.67^{A}±	11.84^{B}±	15.61^{A}±	16.20^{A}±
extract (%)	0.11	0.19	0.09	0.15	0.04	0.31	0.14	0.10	0.20	0.16	0.17
Gross energy (kJ g^{-1})	5.46^{C}±	7.10^{A}±	6.43^{B}±	6.96^{A}±	6.28^{B}±	6.48^{B}±	7.02^{A}±	6.27^{B}±	6.48^{A}±	6.23^{B}±	6.11^{B}±
	0.003	0.02	0.01	0.04	0.01	0.01	0.01	0.01	0.02	0.13	0.02

All values are Mean±S.E of mean; Means with different letters in the same row are significantly (P<0.05) different; (Duncan's Multiple Range test)

according to which diets having 1.9 kg^{-1} Ca and 7.5 kg^{-1} P obtained best growth.

The data on water quality characteristics pertaining to all the dietary treatments is presented in Table 4. No significant variations were observed in water quality parameters. In general, significantly (P<0.05) low values in total ammonia excretion and reactive phosphate production (mg kg^{-1} BW d^{-1}) were recorded in fish fed on diet D5 supplemented with Ca and P at 7.5 g and 3.75 g kg^{-1} of diet (Fig. 3). The excretion of wastes into the effluent water remained significantly (P<0.05) low when fish were fed on diet supplemented with Ca/P ratio between the range 1.33 to 2.00. Peak values in total ammonia excretion occurred at 6 h post feeding, whereas the level of soluble phosphate in the aquaria water was high initially, at 2 h post feeding and later on values declined. These results are in broad agreement to those of Kalla and Garg (2003) and Jana *et al.* (2006). The excretion patterns observed in the present trial, however, differ from those obtained by Sumagaysay-Chavoso (2003) in milkfish fed on formulated and natural food-based diets in that the excretory levels in his studies were very low and obtained two peaks (6 h and 18 h) post feeding for total ammonia nitrogen (TAN) and only one peak, at 21 h post feeding, for o-PO_4.

Phosphorus metabolism is closely tied to that of calcium. Any excess minerals in the diet above the

Fig. 3. Post prandial excretory patterns of total ammonia and total orthophosphate (mg kg^{-1} Body weight per day of fish) in holding water for fish *C. mrigala* in different dietary treatments containing varying levels of calcium and phosphorus

optimum requirement for fish will be excreted by the fish in the water. It is, therefore, critical to know precisely the dietary requirement of P and Ca in order to minimize excess P in diet without risking P deficiency in cultured fish. It has been further confirmed that ability of *C. mrigala* to benefit from additional calcium and P in the diet in the ratio between 1.33 (Ca 5.0:P 3.75) – 2.0 (Ca 7.5:P 3.75) for optimum growth and efficient feed utilization.

The carcass composition with respect to proximate nutrients of *C. mrigala* on the basis of feeding trial is shown in Table 4. The accumulation of carcass crude protein (%) was significantly (P<0.05) higher in the carcass of fishes fed on diet D5. Crude fat was significantly higher in control group. No significant variation (P<0.05) were observed in moisture (%) and total ash content (%) of fishes fed on different diets, whereas, NFE (%) was significantly (P<0.05) high in D9 fed group as compared to control. However, there were no significant (P<0.05) variations in gross energy (kJ g^{-1}) in the experimental diets fed group.

The results showed that significantly (P<0.05) higher growth performance in terms of weight gain, SGR, growth % gain in BW was observed in the group of fishes fed on diets supplemented with Ca and P at 7.5 and 3.75 g kg^{-1} of diet (D5). FCR values were also low in D5. Results of present studies have further revealed that fish fed on diets supplemented with Ca-P ratio in the range of 1.33-2.0 had significantly (P<0.05) higher carcass protein, fat, ash and energy and a lower percentage of moisture in comparison with fish fed on other diets. Guerreiro *et al.* (2004) reported whole-body Ca uptake in gilthead sea bream (*Sparus aurata*) larvae during short-term adaptation to altered salinities improved when the Ca concentration in the rearing water was increased. Our studies are at par with other similar studies (Paul *et al.,* 2004) in *C. mrigala* fingerlings. Chavez-Sanchez *et al.* (2000) and Wang *et al.* (2003) showed strong positive correlation between calcium and phosphorus in the diet and ash content in the carcass of the fish, demonstrating the role of these components in mineralization. Similar results were observed by Sugiura *et al.* (2004) in several fish species.

CONCLUSIONS

Supplementation of 3.75 g kg^{-1} of phosphorus and 7.5 g kg^{-1} of calcium was found to be optimum for improved growth performance of *Cirrhinus mrigala* fingerlings.

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SHORT COMMUNICATION

Effect of Supplementation of Acidifiers with Probiotic on Performance of Broiler Chicken

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ABSTRACT

An experiment was conducted to study the effect of supplementation of acidifiers with probiotic on performance and nitrogen retention of broiler chicken. A total of 300 broilers were reared for six weeks with dietary treatments, T_0 (control diet), T_1 (control plus sodium-diformate at 0.2%), T_2 (control plus sodium diformate at 0.2% plus probiotic at 0.02%), T_3 (control plus blends of acidifiers at 0.2%), T_4 (control plus blends acidifiers at 0.2% plus probiotic at 0.02%). Each treatment consisted of sixty birds with four replicates containing fifteen birds in each replication. The weekly body weight, body weight gain, feed conversion efficiency was found to be better in all treatment groups as compared to control. However, weekly feed consumption, was found to be unaffected. Also, there was increase in dry matter digestibility and nitrogen retention in all treatment groups as compared to control. It was concluded that the suppleemntation of blends of acidifiers at 0.2% in combination with probiotic @ 0.02% improved the growth performance and nutrients utilization of broiler chicken rearing.

Key words: Acidifier, Broilers, Probiotic, Sodium diformate, Nitrogen retention

Following the ban on use of antibiotics as growth promoters by European Union since 2006, animal nutrition scientist are actively engaged in research focusing on suitable alternative to antibiotics use in animal feeding. Acidifiers and probiotics are alternatives to antibiotics growth promoters in poultry. Most organic acids have antimicrobial as well as growth-promoting properties and also its use could stimulate the natural immune response (Lohakare*et al.,* 2005). Probiotics are either single blend of live microbial culture which elevate health benefits to the host. *Saccharomyces is an example of probiotics and is also* known to offer a good quality protein and B complex vitamins. Hence, the present study was conducted to assess the effect of supplementation of acidifiers and probiotic alone or in combination in broilers.

A total of 300 broilers were reared for six weeks with dietary treatments (table 1), T_0 (control diet as per BIS, 2007), T_1 (control plus sodium-diformate @ 0.2%), T_2 (control plus sodium diformate @ 0.2% plus probiotic @ 0.02%), T_3 (control plus blends of acidifiers @ 0.2%), T_4 (control plus blends of acidifiers @ 0.2% plus probiotic @ 0.02%). Each treatment consisted of sixty birds with four replicates containing fifteen birds in each replication. The Probiotic contained encapsulated *Saccharomyces cerevisiae* @ 1 x 10^{10} CFU/g. Blends of acidifiers consisted of buffered organic acids like Calcium propionate, sodium formate, fumaric acid, sorbic acid and citric acid in equal quantity. The birds were reared on deep litter system and standard managemental practices were followed during the entire experimental period. Individual body weight of each bird was recorded at weekly interval and bodyweight gain was calculated as mean of each replicate. The cumulative feed intake was recorded weekly and the feed conversion ratio (FCR) was calculated. The economics of broiler production was worked out by considering the prices of input prevalent at the time of experiment in the market. Input considered were the cost of day old chicks, feeds, vaccines, medicines and other miscellaneous items which

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were also considered as uniform for all the treatment groups.Net profit per kg body weight of bird was determined by subtracting total cost of production per bird from selling cost and dividing it by average body weight of treatment. A metabolic trial of 5 day collection period was conducted at the end of experiment and apparent metabolizability of nutrients was determined. The data collected during the study was analyzed statistically as per Snedecor and Cochran (1994) and depicted in table 2.

The body weight gain was found to be higher in all the treatment groups, as compared to control. Similar results were obtained by Thirumeignanam *et al.* (2006). The *Saccharomyces cerevisiae* is considered as rich source of protein, minerals and B-complex vitamins. The performance exhibited by experimental birds in T_4 group may be because of presence of *Saccharomyces cerevisiae* as yeast culture, which play a beneficial role as natural growth promoter (Van Leeuwen *et al.,* 2005). However Vale *et al.*(2004) found non-significant body weight at the end of 6th week on diet containing different levels (0, 0.25, 0.50, 1.0 and 2 %) of mixture of organic acid (70% formic acid and 30% propionic acid).Sheikh *et al.* (2011) reported that

Table 1 Composition of broiler ration

Ingredient	Pre-Starter					Starter					Finisher				
	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
Maize	46.2	46.2	46.2	46.2	46.2	49	49	49	49	49	54	54	54	54	54
Soyabean cake (DOC)	43.5	43.5	43.5	43.5	43.5	40.6	40.6	40.6	40.6	40.6	35.1	35.1	35.1	35.1	35.1
Soya oil	5.57	5.57	5.57	5.57	5.57	6.3	6.3	6.3	6.3	6.3	6.92	6.92	6.92	6.92	6.92
L-Lysine	0.01	0.01	0.01	0.01	0.01	-	-	-	-	-	-	-	-	-	-
DL-Methionine	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
LSP	1.13	1.13	1.13	1.13	1.13	1.15	1.15	1.15	1.15	1.15	1.1	1.1	1.1	1.1	1.1
DCP	2.01	2.01	2.01	2.01	2.01	1.86	1.86	1.86	1.86	1.86	1.79	1.79	1.79	1.79	1.79
Trace-min mix	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin mixture	0.30	0.30	0.30	0.30	0.30	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30	0.30	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat*	0.10	0.10	0.10	0.10	0.10	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Toxin binder*	0.10	0.10	0.10	0.10	0.10	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium diformate*	-	0.2	0.2	-	-	-	0.2	0.2	-	-	-	0.2	0.2	-	-
Sodium diformate* (price ₹ per kg)		210	210	-	-	-	210	210	-	-	-	210	210	-	-
Probiotic*	-	-	0.02	-	0.02	-	-	0.02	-	0.02	-	-	0.02	-	0.02
Probiotic** (price ₹ per kg)			2400		2400			2400		2400	-	-	2400	-	2400
Acid Mixtures*	-	-	-	0.2	0.2	-	-	-	0.2	0.2	-	-	-	0.2	0.2
Acid Mixtures** (price ₹ per kg)				130	130	-	-	-	130	130	-	-	-	130	130
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CP (%)	23	23	23	23	23	22	22	22	22	22	20	20.1	20.1	20.1	20.1
ME (kcal/kg)	3000	3000	3000	3000	3000	3100	3100	3100	3100	3100	3200	3200	3200	3200	3200

*Over and above

Table 2 Growth Performance of broilers

Parameters	Phases	T_0	T_1	T_2	T_3	T_4
Body weight gain**	Pre-starter	93.09^{a}±0.49	93.23^{a}±3.43	93.72^{a}±2.77	93.97^{a}±3.08	94.02^{a}±2.82
	Starter	746.54^{a}±9.32	755.68^{ab}±13.31	768.47^{ab}±18.36	796.8^{bc}±17.06	822.67^{c}±1.53
	Finisher	2162.05^{a}±35.06	2223.02^{ab}±41.58	2256.5^{abc}±16.88	2317.37^{bc}±46.34	2367.45^{c}±61.23
Feed consumption**	Pre-starter	98.25^{c}±1.18	97^{bc}±0.71	95^{bc}±1.78	93.25^{ab}±1.49	89^{a}±2.08
	Starter	1107.5^{a}±15.44	1100.25^{a}±8.29	1114.25^{a}±14.03	1105.25^{a}±16.6	1098^{a}±19.41
	Finisher	3850.25^{a}±122.29	3814.25^{a}±48.09	3871.5^{a}±73.94	3852.33^{a}±60.41	3810.75^{a}±106.85
FCR**	Pre-starter	1.06^{a}±0.01	1.05^{a}±0.05	1.02^{a}±0.03	1.00^{a}±0.03	0.95^{a}±0.04
	Starter	1.63^{a}±0.05	1.63^{a}±0.09	1.63^{a}±0.05	1.57^{a}±0.04	1.56^{a}±0.04
	Finisher	2.25^{a}±0.11	2.1^{a}±0.26	2.02^{a}±0.18	1.94^{a}±0.16	1.95^{a}±0.21
DM digestibility, %		62.54^{a}±0.86	64.52^{ab}±0.83	65.01^{bc}±0.75	66.16^{bc}±0.46	66.81^{c}±0.53
N Retention, %		69.25^{a}±1.91	71.37^{b}±2.09	72.45^{bc}±2.29	73.54^{cd}±1.65	75.05^{d}±2.03
Cost of feed (₹/kg)		32.04	32.06	32.24	32.26	32.71
Net Profit, ₹/kg		8.53	11.21	11.24	13.56	14.88

due to pH reducing properties and direct antimicrobial effect, acidifiers might have resulted in inhibition of intestinal bacteria leading to reduced bacterial competition with the host for available nutrients, which might have improved protein and energy digestibility, resulting in better performance of broiler chicken. The FCR was better in supplemented groups as compared to control and corroborates with Garcia *et al.* (2007) and Bozkurt *et al.* (2009). The DM digestibility and N retention in all treatment groups were found to be higher as compared to control. The results corroborates well with those of Thirumeignanam *et al.* (2006), Garcia *et al.* (2007), Ao *et al.* (2009) and Ramigani *et al.* (2015) who reported that organic acid significantly increased DM digestibility and nitrogen retention. Feed cost per kg gain was highest for T_4 group. Thus, it could be deduced that supplementation of acidifiers with probiotic showed better performance in terms of growth parameters, dry matter digestibility, nitrogen retention in broiler chicken. It was concluded that the feeding of blends of acidifiers at 0.2% in combination with probiotic at 0.02% would be economical in broiler rearing.

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Final Announcement

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Non-Ruminants

Short Communication

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