DIETARY SUPPLEMENTATION OF FEED ACIDIFIERS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS IN BROILER CHICKEN

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Dietary supplementation of feed acidifiers on growth performance and carcass characteristics in broiler chicken"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **“Dietary supplementation of feed acidifiers on growth performance and carcass characteristics in broiler chicken”** is a record of research work done independently by **Axsa P. Thomas (13-MVM-27)** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Axsa P. Thomas (13-MVM-27),** a candidate for the degree of **Master of Veterinary Science in Animal Nutrition**, agree that this thesis entitled **"Dietary supplementation of feed acidifiers on growth performance and carcass characteristics in broiler chicken"** may be submitted by **Dr. Axsa P. Thomas (13-MVM-27)** in partial fulfillment of the requirement for the degree.

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Mannuthy ***Axsa P. Thomas***

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DEDICATION

This thesis is dedicated to

MY FATHER and MOTHER

My first teachers, who taught me to trust in god, believe in hard work and that so much could be done with little

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A constant source of knowledge and inspiration, encouraged me to believe in myself and never give up

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The author of my life

Introduction

INTRODUCTION

Poultry industry has emerged as the most dynamic and fast expanding segment of animal production in India. The demand for poultry products especially poultry meat has consistently increased over the years. In India, poultry production is projected to have increased from less than 1.0 million tons in 2000 to 3.4 million tons in 2014 (BAHS, 2010). Higher levels of production and better feed efficiency, with minimum expenditure are the needs of the modern broiler industry, which could be achieved to a greater extent by the efficient use of different feed additives. Antibiotic growth promoters were being used for the past five decades to improve the production performance of poultry. However, the generalized use of antibiotics as feed additives had been severely restricted recently due to their residual effect on meat and also due to the emergence of antibiotic resistant strains of bacteria in humans. This has compelled the researchers to use other non-therapeutic alternatives as feed additives like organic acids, probiotics, prebiotics, enzymes, herbs, essential oils, immunostimulants etc. to maintain the health and to optimize the production performance of birds.

Feed acidifiers are low molecular weight organic acids having specific antimicrobial activity and they exert their action both in feed and gastrointestinal tract. In gastrointestinal tract the action occurs in the upper part and the lowered gut pH allow most of these organic acids to exist in the undissociated form, which is lipophilic in nature. They diffuses across the cell membranes of bacteria or moulds and causes dissociation of the acid inside the cell, affecting the microbial activity either by acidification of the microbial cytoplasm or by cytoplasmic accumulation of the dissociated acids to the toxic level.

Various organic acids (acetic, citric, formic, isobutyric, lactic and propionic acid) were screened regarding their use as feed additives in broiler production. Dietary acidifiers like propionic acid and formic acid inhibit growth of pathogenic bacteria and maintain beneficial micro flora. Propionic acid occurs endogenously as a by-product of normal intermediate metabolism and its residues in meat or egg are expected to be negligible. Supplementation of adequate levels of formic and propionic acid in diet can also increase villous height, which can increase permeability of mucosal cell of intestine, thus improving the rate of digestion and utilization of nutrients in the gut. Subtle changes in the levels of organic acids added to poultry diet can also have a profound effect on the quality of meat produced. Acidifier blends (mixture of organic acids) could improve the overall health status of birds by reducing intestinal infections or by decreasing the inflammatory process at intestinal mucosa.

Although, the addition of organic acids can beneficially affect gut microbial load, their optimum dosage needs clarification. It is also necessary to study the effect of different feed acidifiers on growth performance and carcass quality in broiler chicken. Moreover, systematic studies comparing the effect of propionic, formic or their blends in broilers are scanty in literature. Hence, the present research work was carried out to study the effect of dietary supplementation of propionic and formic acid as feed acidifiers on growth performance, nutrient utilization, gut microbial load, carcass characteristics and cost of production in broiler chicken.

Review of Literature

2. REVIEW OF LITERATURE

2.1 FEED ACIDIFIERS

Feed additives containing low-molecular weight organic acids were referred to as acidifiers. They inhibit the growth of pathogenic intestinal microflora by lowering the pH of the feed, gut and microbial cytoplasm. Feed acidifiers enhance the growth performance and carcass quality of broiler chicks by activation of proteolytic enzymes through a reduction in pH. They could also stimulate feed consumption and improve digestibility of nutrients by reducing harmful gut microbes that compete with host nutrients.

Acidifiers are broadly classified based on their chemical nature into organic and inorganic forms and they are available either as free form or as inorganic salts for commercial use. The short chain acids (C1 to C7) associated with antimicrobial activity are either simple monocarboxylic acids (formic, acetic, propionic etc.) or carboxylic acids with hydroxyl group (lactic, malic, citric etc.). Along with their effects on animal performance, many organic acids are also known to be effective as food and feed preservatives (Dibner and Buttin, 2002).

2.2 PROPIONIC ACID

Propionic acid is a naturally occurring carboxylic acid and it was first described in 1844 by Johann Gottlieb, who found it among the degradation products of sugar (Haque *et al*., 2009). In the pure state, it occurs as a colorless corrosive liquid having a sharp odor. Biologically it was produced as propionyl-CoA, the anaerobic end product from the bacteria *Propionibacterium sp*.

2.2.1 Effect of supplementation of propionic acid on

2.2.1.1 Growth performance

Izat *et al.* (1990b) conducted an experiment in broiler chicken using an experimental diet containing 0.4 per cent propionic acid and concluded that the body weight gain of broiler chicken was significantly higher (2255 g vs. 2137 g) in supplemented group when compared to unsupplemented group at 49 days of age. In turkey poults, addition of propionic acid at 0.60 and 1.25 per cent levels in the diet resulted in significantly ($p<0.05$) higher body weight and weight gain in the experimental group than control group (Roy *et al*., 2002). Khosravi *et al.* (2008) compared the performance of organic acid and antibiotics in broiler chicken and they observed a significant ($p<0.05$) increase in mean body weight gain when fed with 2 per cent propionic acid than those supplemented with antibiotic during the first (65.45 vs. 64.18 g) and second (157.94 g vs. 157.65 g) fortnights. However, they observed a lowered body weight gain (214.29 g vs. 222.18 g) in organic acid fed group at third fortnight compared to antibiotics fed group.

In a similar study, Al-Kassi and Mohssen (2009) reported an increased live weight gain (2445.4 g) when broiler chicks were fed with diet containing 0.2 per cent propionic acid compared to control diet fed (2186.8 g) group. Brzoska *et al.* (2013) conducted a study to determine the effect of varying levels of propionic acid and its salts along with butyric acid (ammonium propionate (E295) – 17.50, propionic acid (E280) – 12.5, ammonium propionate (E284) – 4.2 and butyric acid (E236) – 20.70 per cent) on growth and post slaughter parameters in chicken. They also observed that the dietary levels of 0.3, 0.6 or 0.9 per cent acidifiers in the diet significantly ($p<0.01$) increased the growth rate and live weight gain of chickens during entire growth period of 42 day. The live weight gains of the chickens were 65 g, 86 g and 89 g per bird respectively which corresponded to an increase of 2.7 to 3.7 per cent than the control value.

On contrary to the above findings, Vogt and Matthews (1981) conducted a study to determine the effect of dietary supplementation of propionic acid at 0.5, 1 and 2 per cent levels and noted no significant effect on the growth performance of supplemented groups when compared to control birds.

However, Cave (1984) conducted an experiment in broiler chicken and observed a significant reduction in the weight gain (192 vs. 597 g) when fed with 5 per cent propionic acid in the diet compared to control group. Similarly, Paul *et al.* (2007) supplemented diet with calcium salt of propionic acid at 0.3 per cent level in broiler chicken and reported a significant reduction in body weight and body weight gain (501 and 460 g respectively) compared to those birds fed control diet (523 and 482 g respectively) at 21 days of age.

2.2.1.2 Feed intake and feed conversion ratio

Cave (1978) hypothesized that propionic acid had a significant role in the satiation regulatory system in broilers, since intraperitoneal injection of the organic acid suspended the feed intake for 0.5 to 1.5 hrs. In another study Cave (1984) reported that when propionic acid was included in the feed up to 10 per cent levels, a dose dependent depression of feed intake was also observed at 5 per cent level (485 g vs. 1155 g) from 0 to 28 days compared to control birds. In a second experiment, the same author compared the effects of propionic acid in feed and drinking water. A significant depression in feed intake (455 g vs. 1184 g) and body weight gain (192 g vs. 597 g) were observed after 28 days when 5 per cent propionic acid was added into the feed. Similarly, a reduction in feed intake (497 g vs. 1184 g) and weight gain (234 g vs. 594 g) was also observed when propionic acid was added at 2 per cent level in drinking water. Pinchasov and Elmaliah (1994) conducted a study using female broiler chicks fed diets containing different levels of metabolizable energy supplemented with 1.0 and 2.0 per cent propionic acid, and they observed a significant reduction in the feed intake in a dose dependent manner compared to those birds fed control diets. However, they could not find any significant effect on the feed conversion ratio. Paul *et al*. (2007) reported a significant reduction in the feed intake (3044 g) when birds were fed diet supplemented with propionic acid at 0.3 per cent level compared to those fed control diet (3232 g) at 42 days of age.

On contrary to the above observation, Khosravi *et al.* (2008) recorded a better ($p<0.05$) feed conversion ratio (1.88 vs. 2.18) in birds fed with 2 per cent propionic acid in the diet compared to control group. However, Venkatasubramani *et al.* (2014) observed no significant effect on the feed intake when propionic acid was supplemented at 0.1 and 0.15 per cent levels in broiler diet. But they observed a significant improvement in feed conversion ratio for birds fed propionic acid at 0.1 per cent (1.80) level than those fed the basal diet (1.86).

2.2.1.3 Nutrient utilization

Izat *et al*. (1990b) reported that addition of 0.4 per cent of propionic acid in the diet of broiler chicken had no effect on the nutrient utilization compared to control diet. Jin *et al*. (1998) reported that supplementation of organic acids could prevent the development of harmful bacteria, increase digestibility of protein and prevent break down of protein to nitrogen. Their finding also corroborates with the observation made by Khosravi *et al*. (2008). They evaluated the performance of broiler chicken on different diets containing probiotic, organic acid and antibiotic and showed that birds supplemented with 2 per cent propionic acid in the diet had a significantly ($p<0.05$) higher protein efficiency ratio (2.61 g vs. 2.27 g) than control group. However, they could not find any significant effect on the utilization of other nutrients such as dry matter, crude fibre and ether extract.

Venkatasubramani *et al*. (2014) evaluated the effect of dietary supplementation of varying levels of propionic acid (0.1 and 0.15 per cent) on nutrient utilization in broiler chicken and observed that the crude fibre (35.05 vs. 34.88 per cent) and ether extract (73.12 vs. 70.55 per cent) utilization was numerically higher at 0.15 per cent level than those fed 0.1 per cent level. Moreover, a lower utilization of ether extract was (70.55 vs. 72.30 per cent) also observed at 0.1 per cent level than those fed control diet.

2.2.1.4 Intestinal microflora

In a study conducted to determine the antibacterial effect of organic acids in broiler chicken, noted that the minimum inhibiting concentration (MIC) of propionic acid on pathogenic anaerobic bacteria was 0.25 per cent, when birds were fed on the diet supplemented with acidifier based product (Luprosil) containing 53.5 per cent propionic acid (BASF, 1998). Kwon and Ricke (2003) supplemented 3 per cent buffered propionic acid in the diet of broiler chicken and observed a maximum inhibitory effect on the growth of *Salmonella* and other intestinal anaerobic microbes when the gut pH was lowered from seven to five.

The poultry diet which is rich in protein and mineral sources had a strong buffering capacity that could change the required acidic condition in stomach and intestine to alkalinity leading to growth of harmful micro flora. Dietary acidification with organic acids found to reduce the population of harmful bacteria that had better growth in alkaline condition by decreasing the gut pH (Nuria *et al*., 2004).

Al-Kassi and Mohssen (2009) reported a significant ($P<0.05$) reduction in the total viable count (9.73 vs. 10.22 $\log_{10}$ cfu per g) and coliform count (5.33 vs. 5.76 $\log_{10}$ cfu per g) when fed with 0.2 per cent propionic acid in the diet compared to control group.

2.2.1.5 Carcass characteristics

Izat *et al*. (1990b) conducted a study in female broilers by supplementing 0.8 per cent of acidifier based product (Luprosil) containing 53.5 per cent propionic acid in their diet and observed a significant ($p<0.05$) improvement in carcass dressing percentage (62.3 vs. 61.3 per cent) compared to control birds or birds fed with 0.2 per cent buffered propionic acid. Brzoska *et al*. (2013) reported significant difference in dressing percentage, which was higher for broiler birds fed with 0.3 per cent propionic acid in the diet (73.38 per cent) compared to those birds fed diets with either 0.6 and 0.9 per cent propionic acid or those fed control diet (72.94, 70.43 and 70.95, respectively). Propionic acid when used as growth promoter could improve the dressing percentage in broiler birds by better utilization of all nutrients especially protein (Haque *et al*., 2009).

Khan and Nagra (2010) observed a reduction in abdominal fat due to supplementation of propionic acid at 0.4 per cent level in commercial broiler diet. Similar results were also recorded in broiler chicks by Khosravi *et al*. (2012). They observed a significant ($p<0.05$) reduction in abdominal fat (1.76 vs. 1.93 per cent) in birds fed with organic acid compared to control group. However, they could not find any effect on thigh, breast and carcass yield by propionic acid supplementation.

2.2.1.6 Blood parameters

Khosravi *et al*. (2008) reported that supplementation of propionic acid at 2 g per kg feed significantly increased HDL cholesterol (78.20 vs. 70.06 mg per dl), total protein (2.616 vs. 2.24 g per dl), albumin (1.694 vs. 1.48 g per dl), globulin (0.922 vs. 0.766 g per dl) and decreased the LDL cholesterol in blood compared to those birds fed control diet.

On contrary to above observation, Brzoska *et al*. (2013) reported no significant difference between blood serum parameters in broiler chicken including glucose, total protein, triglycerides, total cholesterol and HDL cholesterol when the birds were fed with varying levels of propionic acid (0.3, 0.6 and 0.9 per cent) compared to control group. In a study, Venkatasubramani *et al*. (2014) supplemented varying levels of propionic acid (0.1 and 0.15 per cent) in broiler diet and observed a reduction in serum total cholesterol at 0.15 per cent level (126.35 mg per dl) compared to those fed control diets (128.53 mg per dl). They also reported an increased serum total cholesterol concentration (136.99 mg per dl) at 0.1 per cent level of addition of propionic acid in the diet.

2.3 FORMIC ACID

Formic acid also known as methanoic acid is the simplest carboxylic acid with the chemical formula HCOOH or HCO_2H. It was first described by an English naturalist John Ray in 1617 (Johnson, 1803). Later formic acid was synthesised from hydrocyanic acid by a French chemist Joseph Gay-Lugal. It is a colourless liquid with pungent penetrating odour and found in the venom of ants. Formic acid was used as a preservative and antibacterial agent in livestock and poultry feeds.

2.3.1 Effect of supplementation of formic acid on

2.3.1.1 Growth performance

Patten and Waldroup (1988) found that the addition of 0.72 per cent calcium formate improved weight gain (581 g vs. 578 g) in broiler chicks at 21 days of age. In another experiment the same authors added different levels of calcium formate (0.5, 1.0 and 1.5 per cent) in the diet and observed increased body weight at 0.5 and 1.0 per cent (1.72 kg and 1.73 kg respectively), while a reduction in body weight was noted at 1.5 per cent (1.60 kg vs. 1.68 kg) level compared to basal diet fed birds. They concluded that addition of calcium formate at levels greater than 0.72 or 1.0 per cent could significantly reduce the weight gain in broiler chicken.

On contrary to above observations, Izat *et al*. (1990a) reported that the addition of 1 per cent formic acid or 1.45 per cent calcium formate did not improve live weight of broiler chicken. Similarly, Hernandez *et al*. (2006) could not observe any significant effect on the growth performance of broiler chickens when formic acid was supplemented to the diet at 0.5 and 1 per cent levels.

However, Bozkurt *et al*. (2009) reported that formic acid at 1 per cent level improved growth rates by 4.2 to 5.1 per cent during starter period and 1.9 to 2.5 per cent for the entire experimental period. The same authors also reported an increase in body weight gain (2.251 kg) in acidifier supplemented group compared to birds fed control (2.207 kg) diet.

Ghazalah *et al*. (2011) recorded significant difference ($p<0.05$) in the body weight gain of broiler birds fed diet supplemented with varying levels of formic acid (0.25 and 0.5 per cent) and concluded that birds fed diet containing 0.5 per cent formic acid had highest body weight gain (1689.7 g) followed by those fed diet with 0.25 per cent formic acid (1638.2 g), compared to those fed the control diet (1457.5 g). Luckstadt and Theobald (2012) reported that salts of formic acid (diformate) supplementation in the diet at different levels (0.1 and 0.3 per cent) improved growth performance in broilers and highest weight gain was recorded in birds fed with 0.3 per cent sodium diformate (2.37 kg) compared to those fed with control diet (2.26 kg). They also observed a better feed conversion ratio for 0.1 per cent diformate inclusion against the control (1.74 vs. 1.89), and 0.3 per cent inclusion of diformate (1.74 vs. 1.81). Similarly, Mishra *et al*. (2013) conducted a study in broiler chicken to evaluate the effect of dietary supplementation of formic acid at varying levels (1.0, 1.5 and 2.0 per cent) and recorded an improved live weight gain with 1.5 and 2 per cent formic acid addition at both 21 (770 g and 778 g, respectively) and 42 days (2425 g and 2395 g, respectively) of age.

2.3.1.2 Feed intake and feed conversion ratio

Bozkurt *et al*. (2009) observed that the supplementation of 0.1 per cent of formic acid in broiler diet significantly ($p<0.05$) improved the feed conversion ratio during both 21 (1.48 and 1.76, respectively) and 42 days (1.54 and 1.82) of age compared to those fed the control diet. Ghazalah *et al*. (2011) demonstrated the effect of different organic acids (formic, fumaric, acetic and citric acids) in Arbo-Acres broiler chicks and observed a better feed conversion ratio for birds supplemented with 0.5 per cent formic acid in their diet. Dietary supplementation of formic acid as diformate significantly ($p<0.01$) improved feed conversion ratio by 7.6, 12.0 and 11.4

per cent at 0.1, 0.3 and 0.5 per cent levels, respectively compared to the negative control group (Luckstadt and Theobald, 2011).

Increasing the dietary levels of formic acid upto 1.5 per cent significantly improved the feed conversion ratio at 42 days compared to birds fed control diet (1.77 vs 1.88) as reported by Mishra *et al*. (2013).

On contrary to the above observations, Venkatasubramani *et al*. (2014) reported reduction in total feed intake in those birds supplemented with formic acid at 0.1 and 0.15 per cent levels (3465 g and 3369 g) when compared to control fed group (3620 g).

In another study Ologhobo *et al.* (2015) observed a reduction in feed intake (4.42 vs 4.54 kg) at 42 days of age in broiler birds when fed 0.8 per cent formic acid along with 0.08 per cent DL-methionine than the control group. They also reported an improvement in feed conversion ratio for the supplemented group than the control (1.42 vs. 1.73) and concluded that both the formic acid and methionine sources had significant influence on utilization of nutrients. Moreover, supplementation of methionine enhanced the action of formic acid, thus resulting in a better weight gain and feed conversion ratio.

2.3.1.3 Nutrient utilization

Selle *et al*. (2004) demonstrated the effects of potassium diformate on nutrient utilization in broiler birds at dosages from 0.3 to 1.2 per cent till 35 days of age and found higher value of apparent metabolizable energy at 0.3 and 1.2 per cent levels of supplementation (14.33 and 14.2 vs 13.73 MJ per kg DM) compared to control birds. They also observed similar improvement in nitrogen retention for 0.3 and 1.2 per cent dietary addition of potassium diformate. However, no such effect was observed on utilization of DM, crude protein and ether extract. Gracia *et al*. (2007) found that supplementation of formic acid at 0.5 and 1 per cent levels in finisher diet of broiler birds improved utilization of dry matter (67.8 and 68.8 per cent) and crude protein (72.5 and 73.5 per cent) compared to those fed a basal diet.

Ghazalah *et al*. (2011) compared the effect of different levels of formic acid (0.25, 0.5 and 1.0 per cent) in broilers and reported better utilization of crude protein in diets containing 0.5 per cent formic acid. He also noticed that dietary formic acid at 0.25, 0.5 and 1 per cent levels improved availability of metabolizable energy (3.50, 3.53 and 3.48 vs. 3.40 kcal per g) compared to the control diet.

On contrary to the above observations, Hernandez *et al*. (2006) reported that supplementation of formic acid at 0.5 and 1.0 per cent levels in broiler diet did not affect the total tract digestibility of both crude protein and dry matter at 42 days of age. A similar result was also reported by Venkatasubramani *et al*. (2014). They could not observe any significant effect in nutrient digestibility when birds were fed with formic acid at 0.1 and 0.15 per cent levels. They hypothesized that the non-significant effect of organic acids on digestibility could be due to low level of organic acids (0.1 and 0.15 per cent of diet) used in their study.

2.3.1.4 Intestinal microflora

Acikgoz *et al*. (2011) conducted a study on male broiler birds to demonstrate the effect of formic acid administration in drinking water and recorded a reduction in the total viable count in the digesta of experimental birds than control birds (5.84 vs. 6.17 $\log_{10}$ cfu per g).

On contrary, Ghazalah *et al*. (2011) compared the effect of formic acid with basal diet on gut health and observed that caecal contents of the birds fed 0.5 per cent formic acid had higher counts of coliforms (8.87×10^{10} vs. 8.26×10^{10} cfu per ml) and anaerobic bacteria (9.26×10^{10} vs. 8.93×10^{10} cfu per ml) and lower *E.coli* count (2.45×10^{10}vs. 2.52×10^{10} cfu per ml) than control group.

Ologhobo *et al*. (2015) conducted an experiment in broiler chicken using a diet containing 0.8 per cent of formic acid along with 0.08 per cent DL- methionine and observed a significant reduction in total viable count and coliform count (1.60 to 6.26 log CFU per ml digesta) in the digesta of birds supplemented with formic acid and DL- methionine than that of control diet (12.60 to 33.20 log_{10} CFU per ml digesta).

2.3.1.5 Carcass characteristics

Gracia *et al*. (2007) compared the effect of diets with and without supplementation of formic acid in broiler chicken and reported a reduction in the growth performance of birds fed with formic acid at 1 per cent level than control birds and observed that the supplementation of formic acid did not have any significant effect on the carcass characteristics such as carcass weight, yield of breast and thigh muscles. Khan and Nagra (2010) observed a reduction in abdominal fat when formic acid was added at 0.8 per cent level in the diet of commercial broiler birds.

Mishra *et al*. (2013) noted that the yield of dressed carcass significantly ($p<0.05$) increased when the concentration of formic acid was increased in the diet. They conducted an experiment in broiler chicken with dietary addition of formic acid at 1.0, 1.5 and 2.0 per cent levels. The per cent of dressed carcass yield was significantly higher for 2 per cent (72.10) compared to 1.5 per cent (71.80), 1 per cent (70.70) and control fed group (70.55). Moreover, lower abdominal fat content was also observed at 1.5 and 2 per cent levels of formic acid supplementation (1.44 and 1.42 g per 100 g live weight, respectively).

2.3.1.6 Blood parameters

Hernandez *et al*. (2006) observed that serum cholesterol was not altered by the addition of formic acid up to 1.0 per cent in the diet of broiler diet.

Dietary supplementation of formic acid at 0.25, 0.5 and 1 per cent levels showed significantly ($p<0.05$) higher blood calcium (10.66, 12.14 and 12.26 mg per dl, respectively) and inorganic phosphorus (2.29, 3.45 and 2.18 mg per dl, respectively) concentration than control group, which had 10.59 mg per dl for calcium and 1.38 mg per dl for inorganic phosphorus (Ghazalah *et al*., 2011). In the same experiment, they also noted that the total protein and globulin content of the blood serum were significantly higher compared to control group (3.48, 4.42 and 4.61 vs. 2.80 for total protein and 2.46, 3.45 and 3.63 vs. 1.20 for globulin, respectively) for different levels of formic acid supplementation.

Venkatasubramani *et al*. (2014) observed a reduction in the total serum cholesterol in broiler chicken when formic acid was supplemented at two different levels (0.1 and 0.15 per cent) than those fed control birds.

2.4 BLEND OF ORGANIC ACIDS (MIXTURE OF PROPIONIC ACID AND FORMIC ACID)

Organic acids such as propionic acid and formic acid have broader antimicrobial activity and they act synergistically when used as blends in poultry nutrition (Huyghebaert, 1999). Acids with a pKa value between three and five were commonly used as additive in broiler chicken. Preparing acidifier blends by combining organic acids of low pKa value with another having high pKa value could be more effective. But the magnitude of antimicrobial effects varies from one mixture of acids to another depending upon the concentrations used. Various organic acid blends (acetic and citric, formic and propionic, fumaric and sorbic, lactic and propionic acid etc.) were screened to identify their promising effect as feed acidifiers in the diet of broiler birds.

2.4.1 Effect of supplementation of organic acid blend on

2.4.1.1 Growth performance

Addition of formic acid and propionic acid mixture (85 per cent and 15 per cent) at 1 per cent level to the broiler chicken ration during the starter period did not affect weight gain (Visek, 1978). In a similar study, Kaniawati *et al*. (1992) reported that mixture of formic acid and propionic acid (80:20) added at 1 per cent level to the ration of broiler chicken did not affect the live weight.

Vale *et al*. (2004) studied the performance of male broiler chickens receiving mixture of organic acids (70 per cent formic acid and 30 per cent propionic acid) at four different levels (0.25, 0.50, 1.0 and 2.0 per cent) in the diet from day old to 42 days of age and stated that live weight of birds on day 21 was affected ($p<0.05$) by the inclusion of acidifiers in the diet. Addition of organic acid at 0.5 per cent level showed a slight reduction in live weight of birds by about 36 g. Moreover, supplementation of 2 per cent levels showed higher reductions in body weight by around 80 g (3.5 per cent of live weight and weight gain) than the control group. However, the inclusion of 0.25 per cent organic acids did not show any significant effect on live weight.

Thirumeignanam *et al*. (2006) found that blend of organic acids (propionic, citric, sorbic and fumaric acids) in broiler diet at 0.1 per cent level increased the body weight gain. Similar results were also observed by Hernandez *et al*. (2006), Ghazalah *et al*. (2011) and Saki and Eftekhari (2012) who used various blends of organic acids for their study on broiler diets.

Improved body weight gain and feed intake were observed in broilers fed diets containing blend of formic and propionic acids at 0.6 per cent of diet (Khan and Nagra, 2010). Fallah and Rezaei (2013) conducted a study in broiler chicken to demonstrate the effects of dietary supplementation of blend of organic acids and their salts (propionic acid, formic acid and their ammonium salts). They observed significant ($p<0.05$) increase in the final body weight of birds fed with blend of organic acids and their salts (2320 g) compared to birds fed control diet (2250 g).

Hrangkhawl *et al.* (2013) studied the effect of blend of organic acid salts (calcium propionate and ammonium formate each at 0.15 per cent level) with and without the addition of a prebiotic (0.2 per cent mannan oligosaccharides) in the diet of broiler chicken and reported a significant ($p<0.05$) difference between the body weight of supplemented birds than those fed control diets. Highest body weight was recorded in birds supplemented with blend of organic acid with the addition of prebiotics (1996 g) followed by acidifier blend without prebiotics (1963 g) and lowest weight was observed in control birds (1908 g).

2.4.1.2 Feed intake and feed conversion ratio

Vale *et al*. (2004) reported that dietary supplementation of a mixture of formic acid and propionic acid (70:30) at 0.25 and 0.5 per cent inclusion levels increased feed intake by about 117 g and 53 g per chicken, respectively, from day old to 42 days of age, when compared with the control group. However, addition of the organic acid blend at 2 per cent level decreased feed intake by 9 per cent from day one to 21 days, 3.3 per cent from 22 to 42 days, and 5.5 per cent from day one to 42 days, in comparison to the basal diet.

Paul *et al*. (2007) conducted a comparative study in broiler chicken using blend of organic acid salts (ammonium formate and calcium propionate each at 0.15 per cent level) and an antibiotic growth promoter (virginiamycin at 0.5 g per kg diet) and observed that the cumulative feed intake after the third week (789 g vs. 828 g) and sixth week (3380 g vs. 3495 g) were significantly higher ($p<0.05$) in the antibiotic supplemented group compared with the acidifier supplemented groups. However, the feed conversion ratio at the third week (1.52 vs. 1.63) and sixth week (1.65 vs. 1.75) of age was significantly better ($p<0.05$) in the acidifier supplemented group compared to antibiotic supplemented group. Though, the body weight gain of birds was similar between treatments, acidifier supplementation improved the feed conversion ratio in supplemented birds.

On contrary, Isabel and Santos (2009) had reported no significant improvement in the feed conversion ratio in birds fed diet containing ammonium salts of organic acid blend (0.5 per cent propionic acid and 0.2 per cent formic acid) and they could not observe any significant difference on growth performance in supplemented birds than those fed control diet.

In a study to determine the efficacy of blend of organic acids, Al-Kassi and Mohssen (2009) reported a significantly ($p<0.05$) higher value for feed conversion ratio (2.02 vs.1.97) when broiler chicks were fed diet supplemented with 0.3 per cent blend of organic acid (propionic acid and formic acid) than those fed control diet.

A significant increase ($P<0.05$) in the feed intake (4520 g) for broiler chicken was reported by Fallah and Rezaei (2013) when the birds were fed diets supplemented with blend of propionic acid, formic acid and their salts (formic acid – 17.4, propionic acid – 12.4, ammonium formate – 14.1, ammonium propionate – 8.4 per cent and a non-ionic filling material – 47.4 per cent) compared to control diet (4370 g).

However, Venkatasubramani *et al*. (2014) observed a lowered feed intake (3316 g) in broiler birds supplemented with blends of formic and propionic acids at 0.1 per cent level in the diet compared the to control diet fed group (3620 g).

2.4.1.3 Nutrient utilization

In a study to demonstrate the effect of supplementation of organic acid mixture on protein utilization in commercial broiler chicken, Gheisari *et al*. (2009) supplemented protected organic acid mixture (formic acid, propionic acid and their ammonium salts) at three dietary levels (0, 0.2 and 0.4 per cent) and found no significant improvement in the protein utilization. Moreover, there was no significant effect on digestibility of dry matter, crude fibre and ether extract.

Similarly, Venkatasubramani *et al*. (2014) reported that the addition of formic acid and propionic acid each at 1 per cent level in the diets of broiler birds did not result in improved digestibility of various nutrients.

2.4.1.4 Intestinal microflora

Hinton and Linton (1988) demonstrated that under experimental conditions, 0.6 per cent of formic acid and propionic acid blend was effective in preventing intestinal colonization with harmful anaerobic microorganisms and *Salmonella spp*. from contaminated feed. In another study, Ulrich (2006) showed that liquid organic acids were more effective than salts of these compounds (0.36 per cent of formic acid and 0.16 per cent of propionic acid) in decreasing the intestinal pH and *Salmonella typhimurium* populations.

Dietary supplementation with 0.2 and 0.4 per cent of organic acid mixture (combination of formic, propionic and their ammonium salts) lowered the population of coliforms (10.03×10^4 and 8.88×10^4 cfu per ml) compared to birds fed control diet (14.56×10^4 cfu per ml) (Gheisari *et al*., 2009). Al-Kassi and Mohssen (2009) observed that addition of 0.3 per cent mixture of formic acid and propionic acid reduced the coliform count in the gastrointestinal tract of supplemented birds compared to control group (5.26×10^4 and 5.76×10^4 cfu per ml, respectively). Moreover, addition of propionic and formic acid along with its salts (0.1 and 0.2 per cent levels respectively), further reduced the coliform count to 5.20×10^4 cfu per ml.

2.4.1.5 Carcass characteristics

Al-Kassi and Mohssen (2009) reported a significant ($p<0.05$) increase in dressing percentage of birds supplemented with 0.3 per cent mixture of formic acid and propionic acid (71.3 per cent) than those fed control diet (68.1 per cent). Similarly, Panda *et al*. (2011) observed better dressing percentage in broilers fed ammonium salts of formate and propionate in the diet.

On the other hand, Isabel and Santos (2009) in their study reported a reduction in the breast weight (18.47 vs. 18.89 per cent) and abdominal fat (1.92 vs. 1.95 per cent) as per cent of carcass weight in broiler birds fed with a blend of organic acids (0.5 per cent propionic acid and 0.2 per cent formic acid) in the diet compared to those fed control diet. Similarly, Khan and Nagra (2010) observed a reduction in abdominal fat due to the addition of formic (0.8 per cent) and propionic acid (0.4 per cent) in commercial broilers.

2.4.1.6 Blood parameters

Fallah and Rezaei (2013) reported a significant ($p<0.05$) reduction in the total cholesterol (3.88 vs. 4.36 mmol per L) and triglycerides (0.68 vs. 0.95 mmol per L) in broiler birds fed diet supplemented with blend of organic acids and their ammonium salts (formic acid–17.4, propionic acid–12.4, ammonium formate–14.1, Ammonium propionate–8.4 per cent and non-ionic filling material–47.7 per cent) than that of control birds at 42 days of age. On contrary, Venkatasubramani *et al*. (2014) observed no significant effect on serum total cholesterol when birds were fed diet supplemented with blend of formic acid and propionic acid each at 1 per cent level.

2.5. LIVABILITY

Bozkurt *et al*. (2009) supplemented formic acid at 0.1 per cent in broiler diet and observed higher mortality of bird at both 21 days (0.75 per cent) and 42 days (2.75 per cent) of age than that of birds fed control diet. In another study with Arbor-Acres broiler, Al-Kassi and Mohssen (2009) supplemented birds with formic acid, propionic acid and their blend at 0.1, 0.2 and 0.3 per cent levels respectively, and found that mortality rate was higher for control birds (13.2 per cent) than organic acid supplemented groups. They also observed a lower mortality rate (5 per cent) in birds when fed with 0.1 per cent formic acid in the diet.

Silimarly, Isabel and Santos (2009) reported that mortality rates were reduced in broiler chicken receiving mixture of calcium salts of organic acids (0.5 percent propionic acid and 0.2 per cent formic acid) than the control birds (2 and 4 per cent respectively).

Ghazalah *et al*. (2011) stated that mortality rates were reduced to zero in birds fed on diets containing 0.5 and 1.0 per cent formic acid, but a mortality of about 4 per cent was observed at 0.25 per cent dietary addition of formic acid. Brzoska *et al*. (2013) reported that the addition of propionic acid and its salts at 0.3 and 0.6 per cent levels in diet reduced mortality rates to zero. However, they also noted that the higher dose of the acidifier (0.9 per cent) slightly increased chicken mortality to 0.59 per cent, which was four times lower than the value obtained for the control group.

Materials and Methods

3. MATERIALS AND METHODS

An experiment was conducted for a period of 42 days in the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy to study the effect of dietary supplementation of feed acidifiers on growth performance, nutrient utilization, gut microbial load and carcass characteristics in broiler chicken.

3.1 EXPERIMENTAL BIRDS

Two hundred, day-old straight run commercial broiler chicks (Ven Cobb) procured from Costal Krishna breeding farm and hatcheries, Kunathumkara P. O, Ollukkara, Thrissur, Kerala formed the experimental birds. The birds were allotted to four treatment groups (T1, T2, T3 and T4) with five replications of ten chicks each.

3.2 EXPERIMENTAL RATIONS

The birds were fed with standard broiler pre-starter ration (23 percent crude protein and 3000 kcal ME per kg of feed) up to one week of age, starter ration (22 percent crude protein and 3100 kcal ME per kg of feed) up to three weeks of age and finisher ration (20 percent crude protein and 3200 kcal ME per kg of feed) up to six weeks of age as per BIS (2007) specifications.

The four experimental rations formulated were:

T1 – Standard broiler ration as per BIS (2007) specifications – Control.

T2 – Control + propionic acid (2 g per kg of feed)

T3 – Control + formic acid (2 g per kg of feed)

T4 – Control + propionic acid (2 g per kg feed) + formic acid (2 g per kg feed)

The ingredient composition of the four different broiler pre-starter, starter and finisher rations are presented in Tables 1a, 1b and 1c respectively.

3.3 EXPERIMENTAL METHODS

3.3.1 Disinfection

The experimental pens were properly cleaned, white washed and disinfected with Kohrsolin – TH liquid (active content being Glutaraldehyde (10 g per 100 ml) with dilution of 10 ml per L water) one week before the start of the experiment. The feeders, waterers and other equipments were also properly cleaned and disinfected with Kohrsolin-TH liquid with a dilution of 5 ml per litre of water.

3.3.2 Housing and management

The birds were maintained in deep litter system of management during the experimental period of six weeks. Wood shavings were used as the litter material. Each replicate were housed in separate pens in the same shed with facilities for feeding and watering. All birds were maintained under identical management conditions. Feed and clean drinking water was provided *ad libitum* in all the pens throughout the experimental period.

3.3.3 Vaccination

Day old chicks were wing banded, weighed individually before housing and vaccinated against Ranikhet disease using Lasota F_1 vaccine on seventh day by intra ocular route and on 28^{th} day through drinking water. The birds were vaccinated against Infectious Bursal Disease (IBD) on the 14^{th} day by intra ocular route and on the 21^{st} day through drinking water.

3.4 EXPERIMENTAL DESIGN

The chicks were randomly divided into 20 replicates of ten chicks each. The groups were allotted randomly to four dietary treatments such as T1, T2, T3 and T4 with five replicates in each treatment.

3.5 BODY WEIGHT

The body weights of individual birds were recorded at weekly intervals from day one to end of experiment to study the growth pattern under different dietary treatments.

3.6 FEED CONSUMPTION

Feed intakes of the birds were recorded replication wise at weekly intervals. From these data, the average feed in take per bird per day was calculated.

3.7 FEED EFFICIENCY

Feed efficiency (kg of feed consumed per kg body weight gain) was calculated in each replicate based on the data on body weight gain and feed consumed.

3.8 PROTEIN EFFICIENCY RATIO, ENERGY EFFICIENCY RATIO AND PRODUCTION EFFICIENCY FACTOR

Protein intake (g) of the birds was calculated at weekly intervals based on the data on feed intake and protein content of feed. The Protein Efficiency Ratio (PER) was calculated as grams of weight gain per grams of protein intake (Osborne *et al*., 1919).

$$\text{PER} = \frac{\text{Weight gain, g}}{\text{Protein intake, g}}$$

The Energy Efficiency Ratio (EER) was calculated based on the data on weight gain and total ME intake (Cheng *et al*., 1997).

$$\text{EER} = \frac{\text{Weight gain, g}}{\text{Total ME intake, kcal}}$$

The Production Efficiency Factor or European Efficiency Factor (PEF or EEF) was calculated using the formula given below (Lemme *et al*., 2006)

$$PEF = \frac{\text{Final body weight (kg)} \times \text{livability per cent}}{\text{Age in days} \times \text{feed conversion ratio}} \times 100$$

3.9 METABOLISM TRIAL

A metabolism trial of three day duration was conducted after the feeding trial using one bird from each replicate selected randomly. Birds were housed in individual metabolism cages with facilities for feeding, watering and excreta collection to determine the digestibility of nutrients and percentage retention of minerals in experimental rations. Water was provided *ad libitum*. Excreta were collected for three consecutive days over 24 hour period using total collection method as described by Summers *et al.* (1976). Excreta collected daily from each bird was weighed and representative samples were taken after through mixing. These samples were placed in double lined polythene bags, labelled and kept in deep freezer at-20^0 C until further chemical analysis. The total amount of feed consumed was also recorded.

3.10 CHEMICAL ANALYSIS

The chemical composition of experimental rations and excreta were determined as per the standard procedures (AOAC, 2012). Minerals such as Ca and P were analysed by conventional volumetric methods (AOAC, 2012). From the data obtained on total feed intake and outgo of nutrients during the metabolism trial, digestibility of nutrients, nitrogen retention and availability of Ca and P were calculated.

3.11 SLAUGHTER EXPERIMENT

At the end of experimental period of 42 days, four birds from each treatment were fasted overnight and slaughtered to study the carcass traits.

3.12 SERUM BIOCHEMICAL STUDIES

Blood samples were collected in clean, dry sterile centrifuge tubes during slaughter without anticoagulant and then centrifuged at 3200 rpm for 10 minutes. Serum obtained was pipetted out and stored at -20^{0}C for the estimation of Ca (Christian *et al*., 1967), inorganic P (Bernhart and Wreath, 1955) using blood analyser (Phospho molybdate method), total proteins (Jong and Vegeter, 1950) (Biuret Method), total cholesterol (Lie *et al*., 1976) (Enzymatic calorimetric method), HDL cholesterol (Haar *et al.*, 1978) (PT. MG. Acetate method) and triglycerides (GPO-POD method) using the kits supplied by Agappe diagnostics, Agappe Hills, Ernakulam-683562, Kerala.

3.13 FAECAL MICROBIAL POPULATION

3.13.1 Collection and processing of samples

Fresh faecal samples were collected randomly towards the end of feeding trial from birds belonging to the four dietary treatment groups. The samples were processed and subjected to microbiological analysis on the same day of collection. Nine grams of samples were homogenized in 90 milliliter of phosphate buffered saline (PBS) and this form the initial test sample. Further tenfold serial dilution was prepared by transferring one milliliter of inoculum in nine milliliter of the diluents. All aseptic precautions were taken during collection and processing of samples.

3.13.2 Total viable count

Total Viable Count (TVC) of all samples were estimated by pour plate technique, as described by Morton (2001). From the selected tenfold dilution of the each sample, one milliliter of inoculum was transferred into duplicate petridishes of uniform size. To each of the inoculated plates about 10-15 milliliter sterile molten standard plate count agar (HiMedia) maintained at 45^0C was poured and mixed with inoculum by gentle rotary movement i.e., clockwise, anticlockwise, forward and backward. The inoculated plates were left at room temperature and allowed to solidify and were incubated at 37^0C for 24 h. At the end of incubation, plates showing colonies between 30 and 300 were selected and counts were taken with the help of a colony counter. The number of colony forming units (cfu) per mg per ml of sample was calculated by multiplying the mean colony count in duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu per g or ml.

3.13.3 Coliform count

Coliform counts per milliliter of samples were estimated according to the procedure described by Kornacki and Johnson (2001). From the selected tenfold dilution, 0.1 milliliter of inoculum was inoculated into duplicate plates of Violet Red Bile Agar (VRBA) (HiMedia) and was uniformly distributed with a sterile ‘L’ shaped glass rod. The inoculated plates were incubated at 37^0C for 24 h. At the end of incubation, purplish red colonies with a diameter of at least 0.5 mm, surrounded by a reddish precipitation zone were counted as coliforms. The number of organisms was estimated by multiplying the mean count in duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu per g or ml.

3.14 LIVABILITY

The mortality of birds if any, from different treatment groups was recorded and postmortem examination was conducted in each case to find out the cause of death.

3.15 ECONOMICS OF PRODUCTION

Economics of incorporation of propionic acid, formic acid and blend of propionic and formic acid in the ration of birds were estimated by calculating cost per kg live weight gain for each treatment groups.

3.16 STATISTICAL ANALYSIS

Data collected on various parameters were analyzed statistically using One way ANOVA method as per described by Snedecor and Cochran (1994). Means were compared by Duncan's Multiple Range Test (DMRT) using statistical package for social studies (SPSS 20.0v, 2011) software.

Table 1a. Ingredient composition of experimental pre -starter rations, %

Sl. No.	Ingredient	Experimental rations, %			
		T1	T2	T3	T4
1	Yellow maize	47.71	47.71	47.71	47.71
2	De-oiled soybean meal	43.00	43.00	43.00	43.00
3	Vegetable oil	5.21	5.21	5.21	5.21
4	Dicalcium phosphate	2.35	2.35	2.35	2.35
5	Calcite	0.50	0.50	0.50	0.50
6	L-Lysine[1]	0.16	0.16	0.16	0.16
7	DL-Methionine[2]	0.20	0.20	0.20	0.20
8	Salt	0.50	0.50	0.50	0.50
		Feed Supplements, %			
1	Propionic acid[3]	0	0. 20	0	0.20
2	Formic acid[4]	0	0	0.20	0.20
3	Vitamin AB_2D_3K mix[5]	0.125	0.125	0.125	0.125
4	Choline chloride[6]	0.10	0.10	0.10	0.10
5	Trace mineral mix[7]	0.10	0.10	0.10	0.10
6	Vitamin B complex[8]	0.01	0.01	0.01	0.01
7	Toxin binder[9]	0.10	0.10	0.10	0.10
8	Anticoccidial[10]	0.02	0.02	0.02	0.02

1. L-Lysine Monohydrocloride 98.5 per cent (Promois)
2. DL-Methionine 99 per cent (Promois: Feedgrade)
3. Propionic acid 99 per cent (Varsha Group, Banglore)
4. Formic acid 85 per cent (Varsha Group, Banglore)
5. Rovimix AB_2D_3K (DSM Nutritional Products) containing Vitamin A – 82,500 IU, Vitamin B_2 – 50 mg, Vitamin D3 – 12,000 IU and Vitamin K – 10 mg per gram.
6. Choline chloride 60 per cent. (Corn cob N.B Group Company Ltd.)
7. Supplimin-TM, (Shree Pharma, Mehsana, India) contains- manganese sulphate equivalent to elemental manganese 54 g, zinc sulphate equivalent to elemental zinc 52 g, ferrous sulphate equivalent to elemental iron 30 g, copper sulphate equivalent to elemental copper 4 g, potassium iodide equivalent to elemental iodine 1 g, cobalt sulphate equivalent to elemental cobalt 0.1 g, chromium chloride equivalent to elemental chromium 0.2 g, Selenomethionine 100 ppm.
8. Meriplex (Vesper Pharmaceutical Group Pvt., Ltd. Bangalore) containing Vitamin B_1 – 8 mg, Vitamin B_6 – 16 mg, Vitamin B_{12} – 80mg, Niacin -120 mg, Calcium pantothenate – 80 mg, Vitamin E50 – 80 mg per gm, Folic acid – 8 mg and Calcium – 86 mg.
9. UTPP- 5 powder (Bio-Tech, Bangalore) containing treated Aluminosilicates, Propionates, Formates and Acetates.
10. Coxistac 120 (Vitec nutrition Ltd.) containing 120 g salinomycin sodium

Table 1b. Ingredient composition of experimental starter rations, %

Sl. No.	Ingredient	Experimental rations, %			
		T1	T2	T3	T4
1	Yellow maize	48.80	48.80	48.80	48.80
2	De-oiled soybean meal	40.60	40.60	40.60	40.60
3	Vegetable oil	6.55	6.55	6.55	6.55
4	Dicalcium phosphate	2.37	2.37	2.37	2.37
5	Calcite	0.52	0.52	0.52	0.52
6	L-Lysine[1]	0.11	0.11	0.11	0.11
7	DL-Methionine[2]	0.18	0.18	0.18	0.18
8	Salt	0.50	0.50	0.50	0.50
		Feed Supplements, %			
1	Propionic acid[3]	0	0.20	0	0.20
2	Formic acid[4]	0	0	0.20	0.20
3	Vitamin AB_2D_3K mix[5]	0.125	0.125	0.125	0.125
4	Choline chloride[6]	0.10	0.10	0.10	0.10
5	Trace mineral mix[7]	0.10	0.10	0.10	0.10
6	Vitamin B complex[8]	0.01	0.01	0.01	0.01
7	Toxin binder[9]	0.10	0.10	0.10	0.10
8	Anticoccidial[10]	0.02	0.02	0.02	0.02

1. L-Lysine Monohydrocloride 98.5 per cent (Promois)
2. DL-Methionine 99 per cent (Promois: Feedgrade)
3. Propionic acid 99 per cent (Varsha Group, Banglore)
4. Formic acid 85 per cent (Varsha Group, Banglore)
5. Rovimix AB_2D_3K (DSM Nutritional Products) containing Vitamin A – 82,500 IU, Vitamin B_2 – 50 mg, Vitamin D3 – 12,000 IU and Vitamin K – 10 mg per gram.
6. Choline chloride 60 per cent. (Corn cob N.B Group Company Ltd.)
7. Supplimin-TM, (Shree Pharma, Mehsana, India) contains- manganese sulphate equivalent to elemental manganese 54 g, zinc sulphate equivalent to elemental zinc 52 g, ferrous sulphate equivalent to elemental iron 30 g, copper sulphate equivalent to elemental copper 4 g, potassium iodide equivalent to elemental iodine 1 g, cobalt sulphate equivalent to elemental cobalt 0.1 g, chromium chloride equivalent to elemental chromium 0.2 g, Selenomethionine 100 ppm.
8. Meriplex (Vesper Pharmaceutical Group Pvt., Ltd. Bangalore) containing Vitamin B_1 – 8 mg, Vitamin B_6 – 16 mg, Vitamin B_{12} – 80mg, Niacin -120 mg, Calcium pantothenate – 80 mg, Vitamin E50 – 80 mg per gm, Folic acid – 8 mg and Calcium – 86 mg.
9. UTPP- 5 powder (Bio-Tech, Bangalore) containing treated Aluminosilicates, Propionates, Formates and Acetates.
10. Coxistac 120 (Vitec nutrition Ltd.) containing 120 g salinomycin sodium

Table 1c. Ingredient composition of experimental finisher rations, %

Sl. No.	Ingredient	Experimental rations, %			
		T1	**T2**	**T3**	**T4**
1	Yellow maize	53.50	53.50	53.50	53.50
2	De-oiled soybean meal	35.00	35.00	35.00	35.00
3	Vegetable oil	7.50	7.50	7.50	7.50
4	Dicalcium phosphate	2.35	2.35	2.35	2.35
5	Calcite	0.60	0.60	0.60	0.60
6	L-Lysine[1]	0.03	0.03	0.03	0.03
7	DL-Methionine[2]	0.16	0.16	0.16	0.16
8	Salt	0.50	0.50	0.50	0.50
		Feed Supplements, %			
1	Propionic acid[3]	0	0.20	0	0.20
2	Formic acid[4]	0	0	0.20	0.20
3	Vitamin AB_2D_3K mix[5]	0.125	0.125	0.125	0.125
4	Choline chloride[6]	0.10	0.10	0.10	0.10
5	Trace mineral mix[7]	0.10	0.10	0.10	0.10
6	Vitamin B complex[8]	0.01	0.01	0.01	0.01
7	Toxin binder[9]	0.10	0.10	0.10	0.10
8	Anticoccidial[10]	0.02	0.02	0.02	0.02

1. L-Lysine Monohydrocloride 98.5 per cent (Promois)
2. DL-Methionine 99 per cent (Promois: Feedgrade)
3. Propionic acid 99 per cent (Varsha Group, Banglore)
4. Formic acid 85 per cent (Varsha Group, Banglore)
5. Rovimix AB_2D_3K (DSM Nutritional Products) containing Vitamin A – 82,500 IU, Vitamin B_2 – 50 mg, Vitamin D3 – 12,000 IU and Vitamin K – 10 mg per gram.
6. Choline chloride 60 per cent. (Corn cob N.B Group Company Ltd.)
7. Supplimin-TM, (Shree Pharma, Mehsana, India) contains- manganese sulphate equivalent to elemental manganese 54 g, zinc sulphate equivalent to elemental zinc 52 g, ferrous sulphate equivalent to elemental iron 30 g, copper sulphate equivalent to elemental copper 4 g, potassium iodide equivalent to elemental iodine 1 g, cobalt sulphate equivalent to elemental cobalt 0.1 g, chromium chloride equivalent to elemental chromium 0.2 g, Selenomethionine 100 ppm.
8. Meriplex (Vesper Pharmaceutical Group Pvt., Ltd. Bangalore) containing Vitamin B_1 – 8 mg, Vitamin B_6 – 16 mg, Vitamin B_{12} – 80mg, Niacin -120 mg, Calcium pantothenate – 80 mg, Vitamin E50 – 80 mg per gm, Folic acid – 8 mg and Calcium – 86 mg.
9. UTPP- 5 powder (Bio-Tech, Bangalore) containing treated Aluminosilicates, Propionates, Formates and Acetates.
10. Coxistac 120 (Vitec nutrition Ltd.) containing 120 g salinomycin sodium.

Results

4. RESULTS

4.1 CHEMICAL COMPOSITION OF RATIONS

The per cent chemical composition of the broiler pre-starter, starter and finisher rations are presented in Tables 2, 3 and 4 respectively. The crude protein (CP) content of four pre-starter rations T1, T2, T3 and T4 ranged from 23.28 to 23.58 per cent while that for starter and finisher rations ranged from 22.22 to 22.49 and 20.65 to 20.91 per cent respectively. The gross energy (GE) value of the experimental rations T1, T2, T3 and T4 were 4128.81, 4030.10, 4143.36 and 4113.37 kcal per kg feed respectively in the pre-starter ration, 4306.64, 4311.06, 4340.82 and 4319.96 kcal per kg feed, in the starter ration and 4596.24, 4621.29, 4681.45 and 4528.25 kcal per kg feed, in the finisher ration, respectively.

The calcium (Ca) content of the experimental rations T1, T2, T3 and T4 were ranged from 1.00 to 1.12 per cent in the pre-starter ration, 1.02 to 1.23 per cent in the starter ration and 1.16 to 1.52 in the finisher rations respectively. The phosphorus (P) content of the four pre-starter rations T1, T2, T3 and T4 ranged from 0.46 to 0.49 per cent while that for starter and finisher rations ranged from 0.45 to 0.54 and 0.46 to 0.59 per cent respectively.

4.2 BODY WEIGHT, BODY WEIGHT GAIN AND AVERAGE DAILY GAIN

The mean body weight of birds under the four dietary treatments T1, T2, T3 and T4 recorded at weekly interval during the experimental period of 42 days are presented in Table 5 and graphically represented in Fig. 1. The average initial and final body weight of birds belongs to four groups T1, T2, T3 and T4 were 50.24, 50.24, 50.26 and 50.24 g and 2010.00, 2079.80, 1924.00 and 1988.00 g respectively.

The cumulative body weight gain and average daily gain of birds belonging to groups T1, T2, T3 and T4 are presented in Table 6 and is graphically depicted in Fig. 2 and 3, respectively. The cumulative body weight gain of birds belonging to four groups T1, T2, T3 and T4 was 1959.76, 2029.56, 1873.74 and 1937.76 g, respectively at sixth week of age. The average daily gain was 46.66, 48.32, 44.61 and 46.14 g respectively for the birds belonging to the groups T1, T2, T3 and T4.

4.3 FEED CONSUMPTION

Data on cumulative feed consumption of the birds as influenced by different dietary treatments are given in Table 7 and graphically depicted in Fig. 4. The mean cumulative feed intake recorded for four treatments T1, T2, T3 and T4 were 3575.30, 3409.40, 3739.20 and 3769.69 g, respectively at sixth weeks of age.

4.4 FEED CONVERSION RATIO

The cumulative feed conversion ratio (FCR) at weekly intervals of birds maintained on different treatments is presented in Table 8 and Fig. 5. The mean cumulative feed conversion ratio was 1.82, 1.68, 2.00 and 1.95 respectively for birds of T1, T2, T3 and T4 groups.

4.5 METABOLISM TRIAL

The chemical compositions of droppings voided by the experimental birds on four treatments T1, T2, T3 and T4 during the metabolism trial are given in Table 9. Data on percentage digestibility of nutrients are presented in Table 10 and Fig. 6. The per cent digestibility of four dietary rations T1, T2, T3 and T4 were 73.04, 78.61, 69.77 and 73.02 for dry matter, 49.14, 53.46, 42.31 and 46.61 for crude protein, 76.43, 84.13, 78.17 and 76.33 for ether extract, 34.67, 44.79, 38.45 and 40.88 for crude fibre and 82.36, 85.61, 80.12 and 80.90 for nitrogen free extract, respectively.

4.6 NITROGEN BALANCE

The data on nitrogen balance (g per day) of the experimental birds are shown in Table 10. The nitrogen balance for the experimental birds in T1, T2, T3 and T4 were 1.86, 2.31, 1.68 and 1.79 g per day, respectively.

4.7 AVAILABILITY OF MINERALS

The per cent availability of minerals is presented in Table 11 and is graphically represented in Fig. 7.

The per cent availability of minerals in the four dietary rations T1, T2, T3 and T4 were 48.24, 52.77, 50.49 and 44.81 for calcium and 56.98, 56.88, 53.87 and 55.60 for phosphorus, respectively.

4.8 SERUM BIOCHEMICAL PARAMETERS

4.8.1 Serum lipid profile

The serum lipid profile of birds maintained on different dietary treatments are presented in Table 12 and are graphically represented in Fig. 8.

The serum lipid profile (mg per dl) of experimental birds belonging to the groups T1, T2, T3 and T4 were 99.67, 96.89, 98.40 and 96.61 for total cholesterol, 47.18, 55.73, 49.73 and 49.65 for HDL cholesterol and 50.92, 56.43, 54.58 and 77.54 for triglycerides, respectively.

4.8.2 Serum mineral concentration

The serum mineral concentration of the experimental birds maintained on different dietary treatments are presented in Table 13 and are graphically represented in Fig. 9.

The serum mineral concentration (mg per dl) of birds maintained on different dietary treatments T1, T2, T3 and T4 were 10.08, 9.50, 9.92 and 10.02 for calcium and 4.61, 5.45, 4.91 and 5.02 for inorganic phosphorus, respectively.

4.8.3 Serum total protein

The serum total protein concentration of the experimental birds maintained on different dietary treatments are presented in Table 14 and graphically represented in Fig. 10.

The serum total protein concentration of birds maintained on different dietary treatments T1, T2, T3 and T4 were 7.70, 8.64, 8.90 and 7.05 g per dl, respectively.

4.9 CARCASS PARAMETERS

Data on live weight, carcass weight, giblet yield and dressing per cent of birds maintained on different dietary treatments at sixth weeks of age are presented in Table 15 and graphically represented in Fig. 11 and 12.

Average live weight of birds belonging to four dietary treatments T1, T2, T3 and T4 were 2242.40, 2283.40, 2168.60 and 2215.00 g, carcass weights were 1594.20, 1638.40, 1524.20 and 1556.80 g, giblet yield per cent were 4.03, 4.42, 4.65 and 4.43 per cent and dressing percentage were 71.04, 71.78, 70.20 and 70.21 per cent, respectively.

4.10 WEIGHT OF INTERNAL ORGANS

The weights of internal organs as percentage of carcass weight are presented in Table 16. The weight of internal organs as percentage of carcass weight for treatment groups belonging to T1, T2, T3 and T4 were 0.60, 0.66, 0.71 and 0.87 per cent for heart, 2.60, 2.88, 3.00 and 2.89 per cent for liver, 2.48, 2.62, 2.92 and 2.55 per cent for gizzard, 0.11, 0.15, 0.14 and 0.15 per cent for spleen and 6.27, 6.33, 7.63 and 6.73 per cent for intestine, respectively.

4.11 FAECAL MICROFLORA POPULATION

The data on faecal microbial count is presented in Table 17 and Fig. 13.. The total viable count in the droppings voided by birds maintained on four experimental treatments T1, T2, T3 and T4 was 7.32, 6.20, 6.28 and 5.33 $\log_{10}$ cfu per g, and coliform count was 5.56, 5.51, 5.62 and 5.65 $\log_{10}$ cfu per g, respectively.

4.12 PROTEIN EFFICIENCY RATIO, ENERGY EFFICIENCY RATIO AND PRODUCTION EFFICIENCY FACTOR

The protein efficiency ratio (PER), energy efficiency ratio (EER) and production efficiency factor (PEF) are presented in Table 18 and Fig. 14. The mean PER for birds belonging to T1, T2, T3 and T4 were 2.62, 2.85, 2.39 and 2.49, respectively. The PEF for T1, T2, T3 and T4 were 244.91, 271.54, 199.37 and 229.17, respectively. The mean EER was 16.05, 15.63, 14.76 and 15.86 for groups T1, T2, T3 and T4 respectively.

4.13 LIVABILITY PER CENT

The per cent livability was 100, 94, 96 and 100 for T1, T2, T3 and T4, respectively and is graphically represented in Fig. 15.

4.14 ECONOMICS OF PRODUCTION

Data on live weight gain, total feed intake, total feed cost and cost of feed per kg body weight gain of birds maintained on the four dietary treatments are presented in Table 19 and represented graphically in Fig. 16. The cost of ingredients used for the study was as per the rate contract fixed by the College of Veterinary and Animal Sciences, Mannuthy for the year 2013 – 2014. The total feed cost per bird for pre-starter ration was Rs.4.84, 4.89, 5.03 and 5.74, for starter ration was Rs. 31.56, 29.09, 34.57 and 38.79, and for finisher ration was Rs. 77.07, 80.14, 84.55 and 86.94 for groups T1, T2, T3 and T4, respectively. The cost of feed per kg live weight gain of birds was Rs.57.90, 56.23, 66.25 and 67.85 for groups T1, T2, T3 and T4, respectively

Table 2. Chemical and mineral composition of the pre-starter rations, %*

Parameter	Broiler pre-starter ration, %			
	T1	T2	T3	T4
Dry matter	89.43	88.76	88.81	90.45
Crude protein	23.43	23.29	23.58	23.28
Ether extract	2.43	2.28	2.35	2.22
Crude fibre	4.31	4.27	4.37	4.22
Nitrogen free extract	62.25	62.85	62.22	62.90
Total ash	7.58	7.32	7.48	7.38
Acid insoluble Ash	1.20	1.24	1.21	1.23
Gross energy kcal / kg	4128.8	4030.1	4143.4	4113.4
Calcium	1.00	1.02	1.04	1.12
Phosphorus	0.48	0.48	0.46	0.49

*On dry matter basis

Table 3. Chemical and mineral composition of the starter rations, %*

Parameter	Broiler starter ration, %			
	T1	T2	T3	T4
Dry matter	90.37	90.61	90.45	90.36
Crude protein	22.49	22.22	22.42	22.44
Ether extract	6.47	6.52	6.39	6.38
Crude fibre	4.82	4.56	4.49	4.84
Nitrogen free extract	58.82	59.30	59.16	58.98
Total ash	7.40	7.39	7.53	7.37
Acid insoluble Ash	1.01	1.12	1.24	1.33
Gross energy kcal / kg	4306.6	4311.1	4340.8	4320.0
Calcium	1.02	1.02	1.12	1.23
Phosphorus	0.46	0.45	0.53	0.54

*On dry matter basis

Table 4. Chemical and mineral composition of the finisher rations, %*

Parameter	Broiler finisher ration, %			
	T1	T2	T3	T4
Dry matter	92.01	91.86	91.77	91.97
Crude protein	20.91	20.81	20.87	20.65
Ether extract	7.48	7.56	7.33	7.66
Crude fibre	7.78	7.44	7.58	7.54
Nitrogen free extract	56.65	57.38	57.60	57.13
Total ash	7.18	6.81	6.61	7.00
Acid insoluble Ash	1.35	1.18	1.50	1.30
Gross energy kcal / kg	4596.2	4621.3	4681.5	4528.3
Calcium	1.16	1.31	1.52	1.28
Phosphorus	0.53	0.46	0.59	0.52

*On dry matter basis

Table 5. Weekly mean body weight of birds maintained on four dietary treatments, g

Week	Body weight, g†				p value
	T1	T2	T3	T4	
Initial	50.2 ± 0.10	50.2 ± 0.07	50.3 ± 0.11	50.2 ± 0.08	1.00
1	164.3 ± 3.01	159.1 ± 5.57	165.9 ± 1.84	164.2 ± 1.15	0.53
2	347.9 ± 8.68	344.4 ± 5.27	346.7 ± 8.01	353.1 ± 3.61	0.82
3	676.9 ± 11.64	658.8 ± 19.14	692.0 ± 14.43	671.7 ± 9.24	0.44
4	1072.0 ± 24.17	1068.0 ± 25.18	1058.0 ± 28.35	1076.0 ± 23.15	0.96
5	1500.0 ± 19.49	1552.0 ± 33.97	1490.0 ± 43.36	1508.0 ± 20.10	0.52
6	2010.0^{ab} ± 15.81	2079.8^{a} ± 24.00	1924.0^{b} ± 55.19	1988.0^{ab} ± 13.93	0.03*

*a, b – means with different superscripts within the same row differ significantly ($p < 0.05$)

† mean of five values with SE

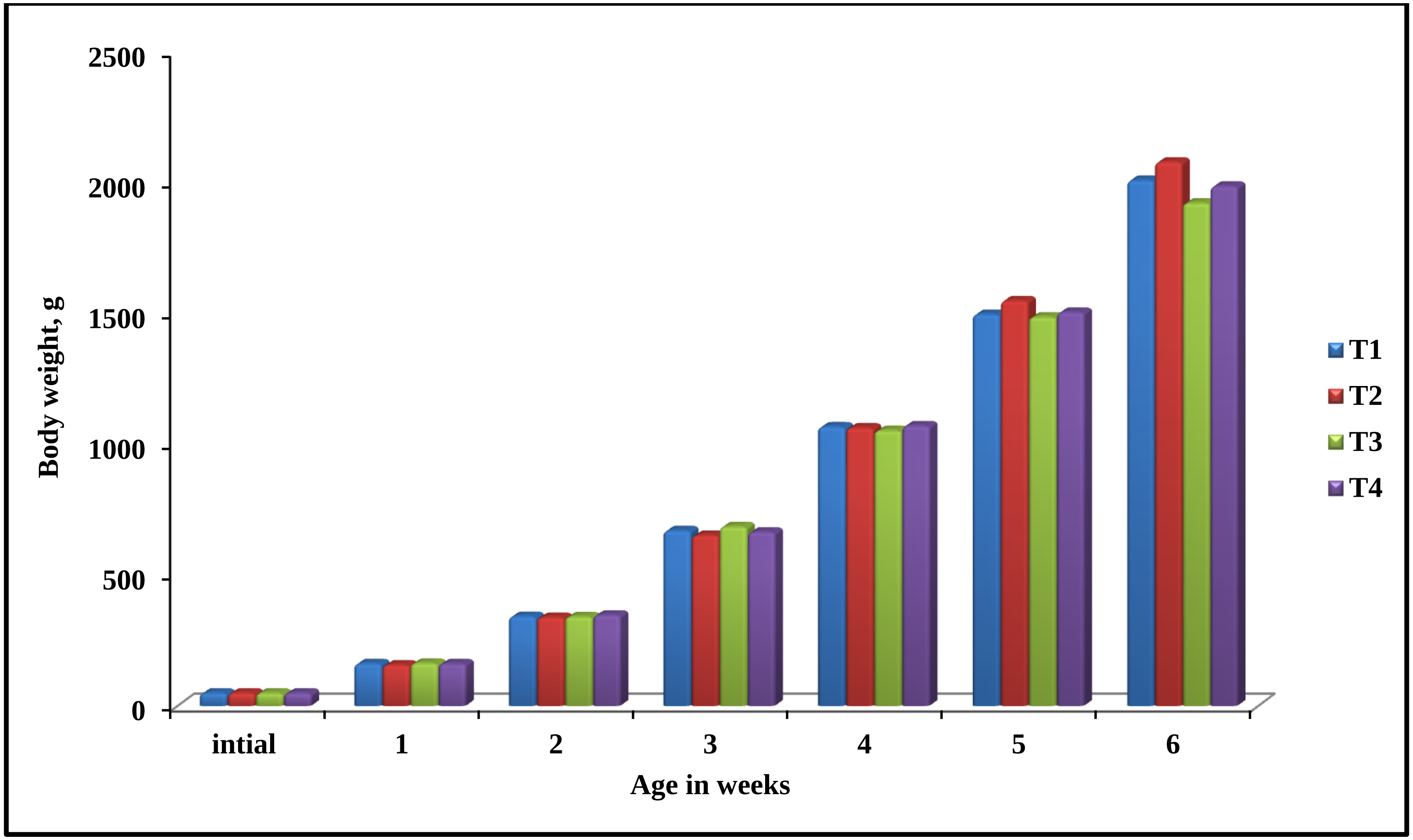

Fig. 1. Weekly mean body weight of birds maintained on four dietary treatments

Table 6. Cumulative body weight gain and average daily gain of birds maintained on four dietary treatments, g

Week	Cumulative body weight gain[†], g				p value
	T1	**T2**	**T3**	**T4**	
1	114.0 ± 3.10	105.5 ± 8.92	113.6 ± 2.85	114.0 ± 1.10	0.55
2	297.7 ± 8.75	294.2 ± 5.28	297.5 ± 7.58	302.9 ± 3.54	0.83
3	626.7 ± 11.69	608.6 ± 19.14	641.8 ± 14.33	621.5 ± 9.19	0.44
4	1021.8 ± 24.13	1017.8 ± 25.17	1007.7 ± 28.25	1025.8 ± 23.14	0.96
5	1449.8 ± 19.51	1501.8 ± 33.96	1439.7 ± 43.26	1457.8± 20.04	0.52
6	1959.8^{ab} ± 15.85	2029.6^{a} ± 23.99	1873.7^{b} ± 55.09	1937.8^{ab} ± 13.89	0.03*
Average daily gain, g	46.66^{ab} ± 0.38	48.32^{a} ± 0.57	44.61^{b} ± 1.31	46.14^{ab} ± 0.33	0.02*

a, b – means with different superscripts within the same row differ significantly ($p < 0.05$)

[†] mean of five values with SE

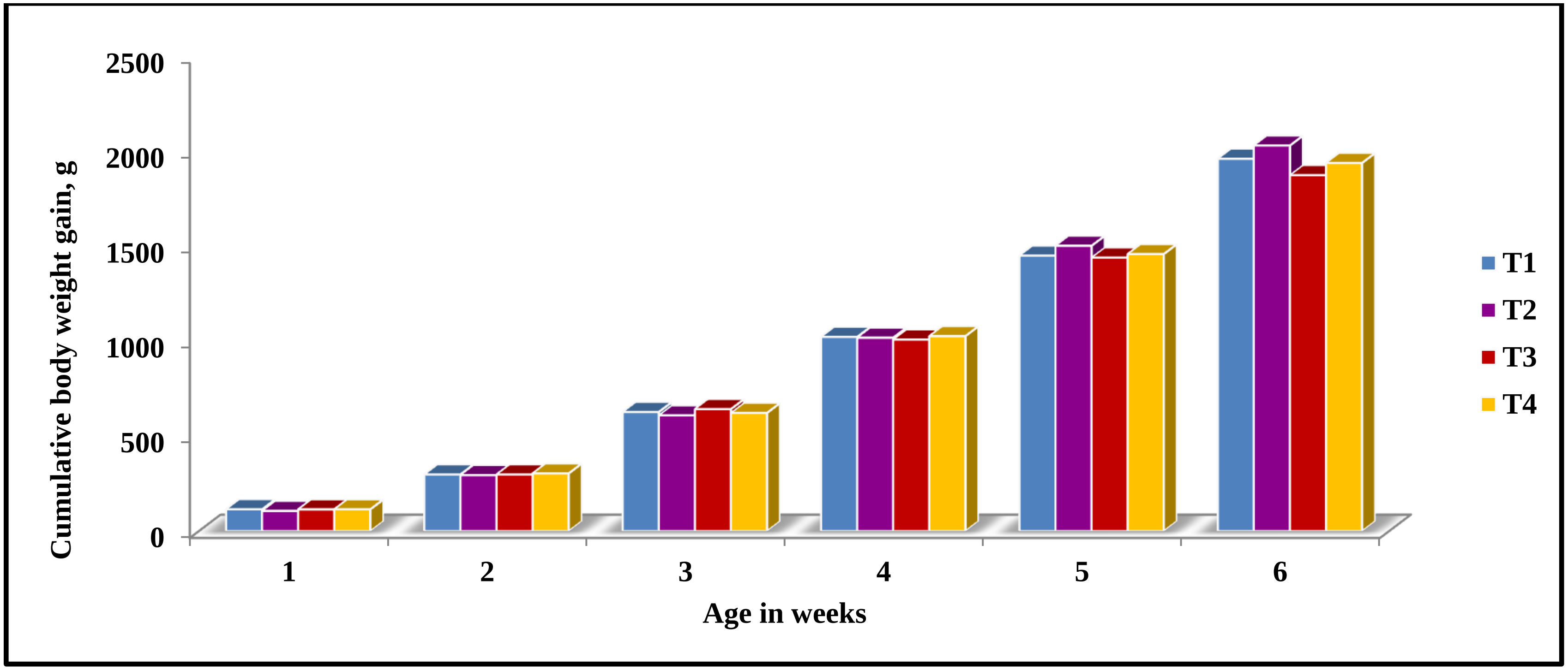

Fig. 2. Cumulative body weight gain of birds maintained on four dietary treatments

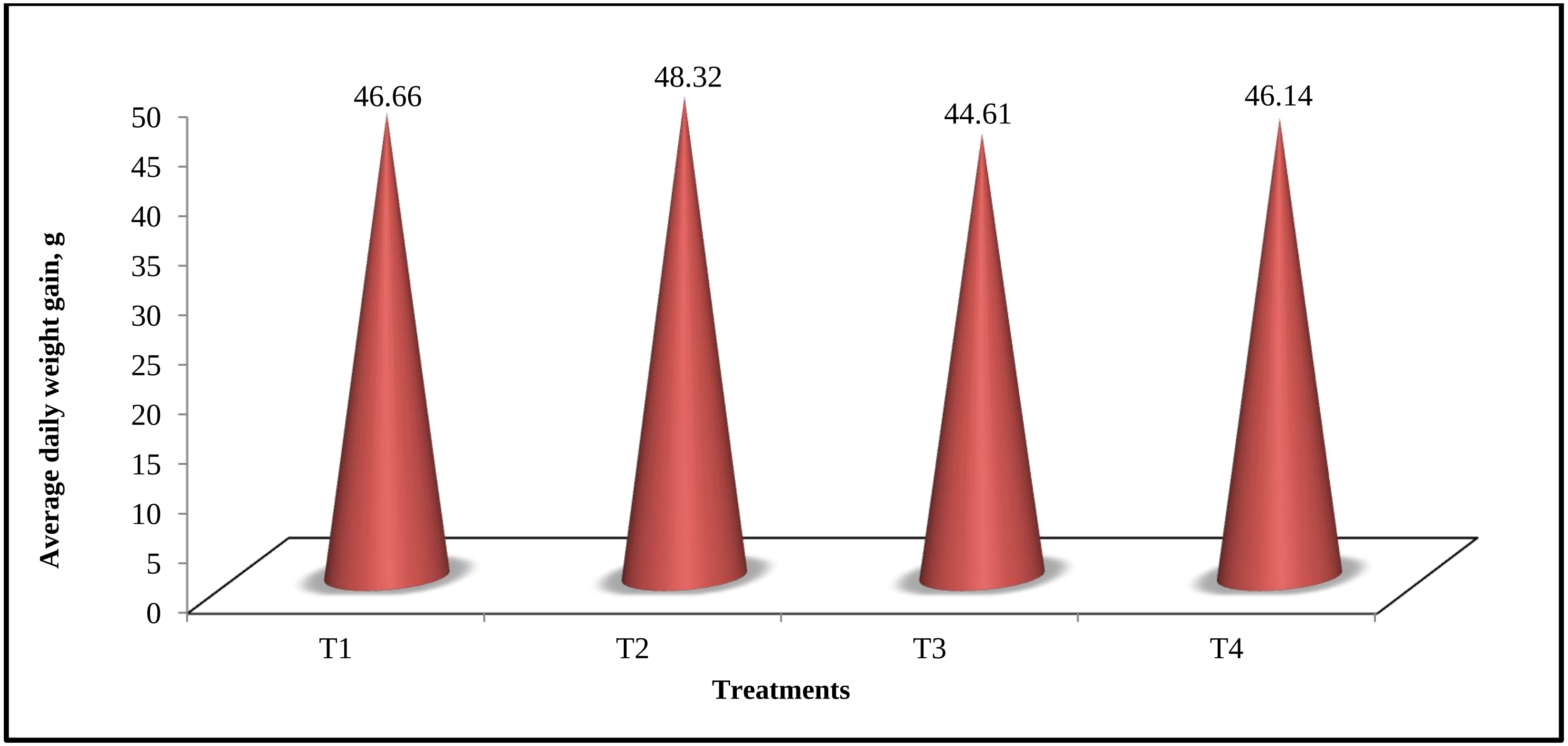

Fig. 3. Average daily weight gain of birds maintained on four dietary treatments

Table 7. Cumulative feed consumption of birds maintained on four dietary treatments, g

Week	Cumulative feed consumption†, g				p value
	T1	T2	T3	T4	
1	148.6 ± 4.93	142.5 ± 7.32	148.2 ± 3.69	160.9 ± 4.14	0.13
2	497.6^{a} ± 10.89	384.0^{b} ± 12.73	513.4^{a} ± 19.97	526.6^{a} ± 12.21	<0.001*
3	1120.9^{b} ± 27.45	992.5^{c} ± 34.97	1147.0^{ab} ± 36.95	1251.0^{a} ± 45.01	<0.001*
4	1763.9^{b} ± 24.57	1692.7^{b} ± 44.08	1922.5^{a} ± 40.85	1967.1^{a} ± 41.91	<0.001*
5	2606.1^{b} ± 20.70	2488.1^{b} ± 50.43	2782.8^{a} ± 46.65	2808.5^{a} ± 40.39	<0.001*
6	3575.3^{b} ± 21.60	3409.4^{c} ± 50.37	3739.2 ± 48.12	3769.7^{a} ± 48.02	<0.001*

*a, b, c – means with different superscripts within the same row differ significantly ($p < 0.05$)

† mean of five values with SE

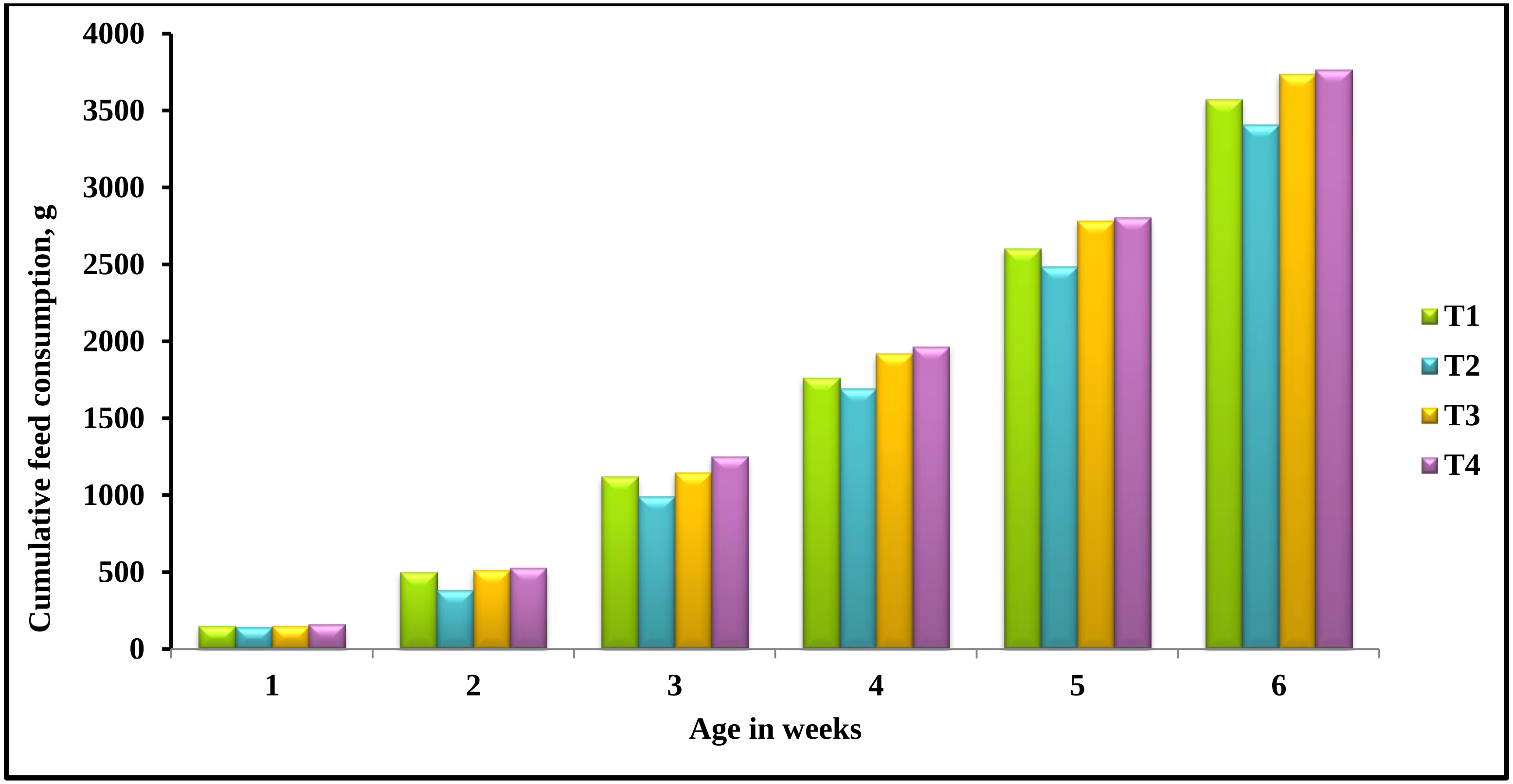

Fig. 4. Cumulative feed consumption of birds maintained on four dietary treatments

Table 8. Cumulative feed conversion ratios of birds maintained on four dietary treatments

Week	Cumulative feed conversion ratio†				p value
	T1	T2	T3	T4	
1	1.30 ± 0.03	1.38 ± 0.09	1.31 ± 0.04	1.41 ± 0.04	0.46
2	1.67^{a} ± 0.04	1.30^{b} ± 0.03	1.73^{a} ± 0.07	1.74^{a} ± 0.06	<0.001*
3	1.79^{b} ± 0.04	1.63^{b} ± 0.05	1.79^{b} ± 0.08	2.02^{a} ± 0.10	0.01*
4	1.73^{b} ± 0.04	1.67^{b}± 0.05	1.91^{a} ± 0.07	1.92^{a} ± 0.08	0.02*
5	1.80^{b} ± 0.02	1.66^{c} ± 0.03	1.94^{a} ± 0.06	1.93^{a} ± 0.05	<0.001*
6	1.82^{b} ± 0.01	1.68^{c} ± 0.03	2.00^{a} ± 0.06	1.95^{a} ± 0.02	<0.001*

*a, b, c – means with different superscripts within the same row differ significantly ($p < 0.05$)

† mean of five values with SE

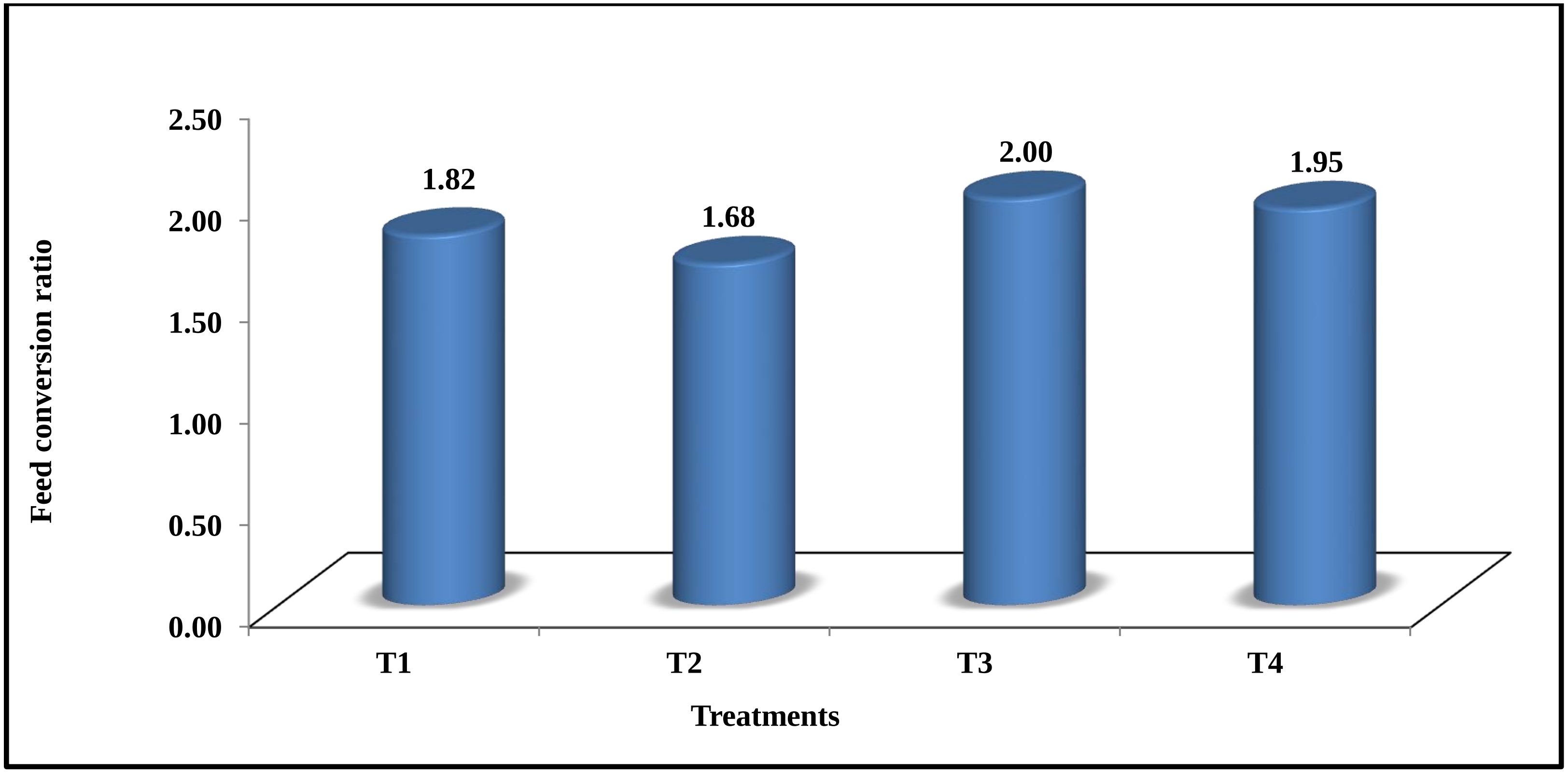

Fig. 5. Cumulative feed conversion ratio of birds maintained on four dietary treatments

Table 9. Chemical composition of the droppings voided by birds maintained on four dietary treatments, %

Parameter	Chemical composition of droppings†, %			
	T1	T2	T3	T4
Dry matter	20.99 ± 0.24	18.01 ± 0.30	23.42 ± 0.26	20.75 ± 0.40
Crude protein	8.25 ± 0.17	8.14 ± 0.14	9.31 ± 0.09	8.47 ± 0.11
Ether extract	6.55 ± 0.08	5.61 ± 0.15	5.29 ± 0.13	6.75 ± 0.10
Crude fibre	18.84 ± 0.11	19.19 ± 0.16	15.49 ± 0.20	16.62 ± 0.30
Nitrogen free extract	37.50 ± 1.27	38.69 ± 1.41	38.17 ± 1.47	40.12 ± 1.82
Total ash	17.05 ± 0.17	18.03 ± 0.24	17.40 ± 0.23	16.03 ± 0.21
Acid insoluble ash	2.35 ± 0.11	2.53 ± 0.06	2.09 ± 0.12	2.36 ± 0.07
Gross energy kcal/kg	4221.5 ± 37.81	4447.0 ± 60.86	4787.4 ± 53.04	4583.3 ± 54.89
Calcium	2.22 ± 0.10	2.89 ± 0.18	2.49 ± 0.08	2.60 ± 0.07
Phosphorus	0.85 ± 0.02	0.93 ± 0.01	0.90 ± 0.05	0.86 ± 0.03

**on dry matter basis*

† mean of five values with SE

Table 10. Nutrient digestibility, Energy efficiency (%) and Nitrogen balance (g/day) of four experimental rations

Parameters	Treatments†				p value
	T1	T2	T3	T4	
Dry matter	$73.04^{b} \pm 0.41$	$78.61^{a} \pm 0.74$	$69.77^{b} \pm 1.72$	$73.02^{b} \pm 1.66$	<0.001*
Crude protein	49.14 ± 2.27	53.46 ± 2.07	42.31 ± 3.87	46.61 ± 3.43	0.10
Ether extract	$76.43^{b} \pm 0.35$	$84.13^{a} \pm 0.74$	$78.17^{b} \pm 1.49$	$76.33^{b} \pm 1.32$	<0.001*
Crude fiber	$34.67^{b} \pm 1.36$	$44.79^{a} \pm 2.21$	$38.45^{ab} \pm 2.54$	$40.88^{ab} \pm 2.60$	0.04*
Nitrogen free extract	$82.36^{b} \pm 0.42$	$85.61^{a} \pm 0.67$	$80.12^{b} \pm 0.58$	$80.90^{b} \pm 1.86$	0.01*
Energy efficiency	$75.24^{b} \pm 0.37$	$79.42^{a} \pm 0.74$	$69.11^{c} \pm 1.70$	$72.77^{b} \pm 1.39$	<0.001*
Nitrogen balance g/day	1.86 ± 0.14	2.31 ± 0.15	1.68 ± 0.19	1.79 ± 0.16	0.06

b, c – means with different superscripts within the same row differ significantly ($p < 0.05$)

† mean of five values with SE

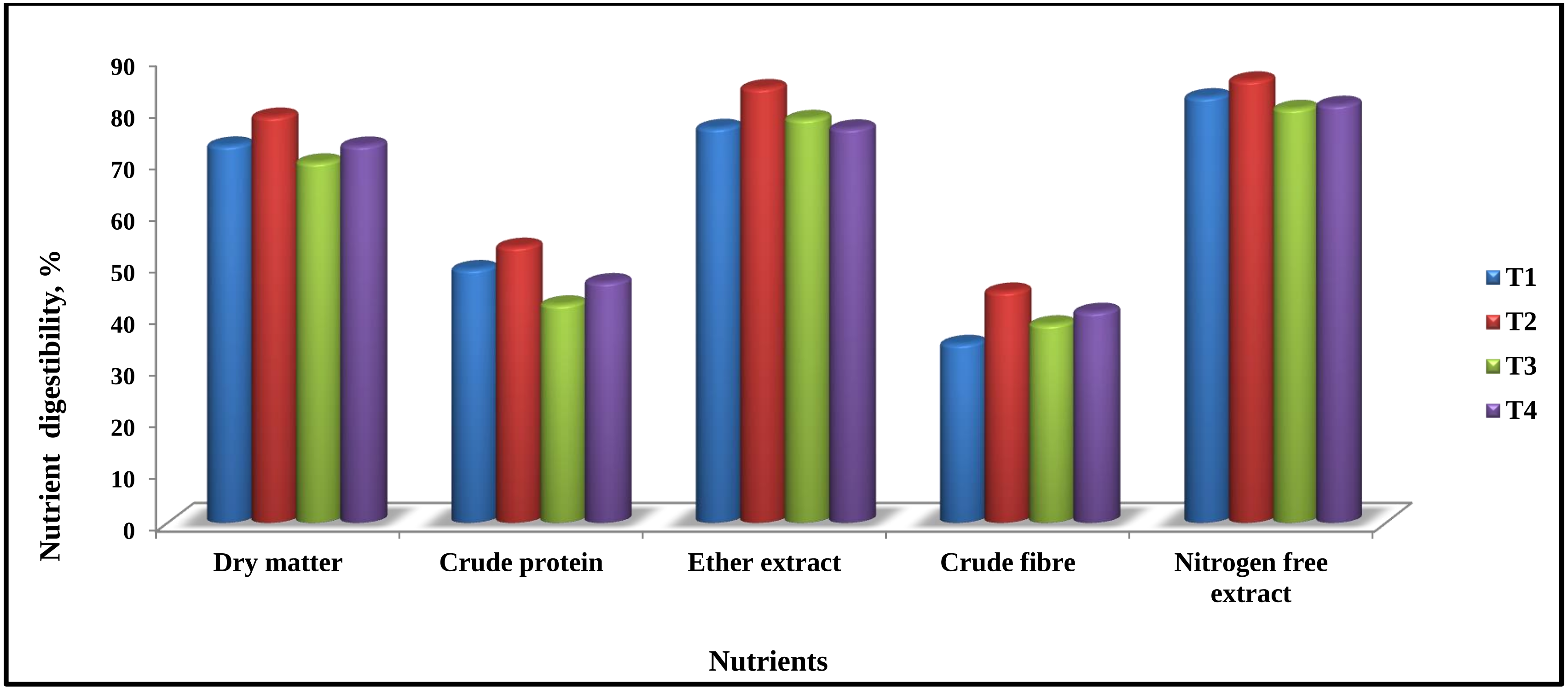

Fig. 6. Nutrient digestibility of four dietary treatments

Table 11. Mineral availability of birds maintained on four dietary treatments, %

Parameter	Treatment[†]				p value
	T1	T2	T3	T4	
Calcium	48.24 ± 3.87	52.77 ± 1.34	50.49 ± 2.21	44.81 ± 4.56	0.38
Phosphorus	56.98 ± 0.79	56.88 ± 1.82	53.87 ± 3.57	55.60 ± 1.52	0.73

[†] mean of five values with SE

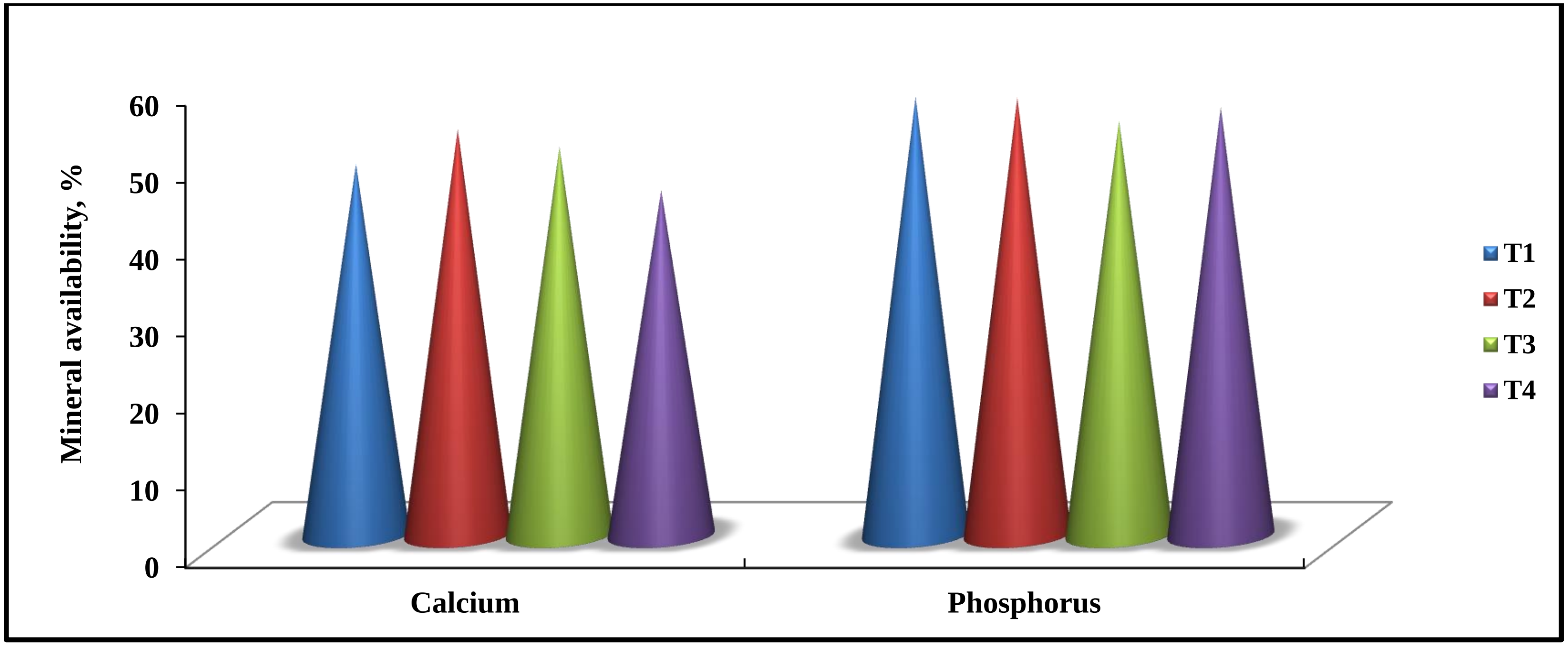

Fig. 7. Availability of calcium and phosphorus of four dietary treatments

Table 12. Serum lipid profile of birds maintained on four dietary treatments, mg per dl

Parameter	Serum lipid profile, mg per dl[†]				p value
	T1	T2	T3	T4	
Total Cholesterol	99.67 ± 0.34	96.89 ± 1.69	98.40 ± 1.45	96.61 ± 1.67	0.41
HDL Cholesterol	47.18[b] ± 2.32	55.73[a] ± 0.61	49.73[b] ± 1.83	49.65[b] ± 2.04	0.03*
Triglycerides	50.92[b] ± 3.66	56.43[b] ± 2.31	54.58[b] ± 4.43	77.54[a] ± 1.16	<0.001*

*a, b – means with different superscripts within the same row differ significantly (p<0.05)

[†] mean of five values with SE

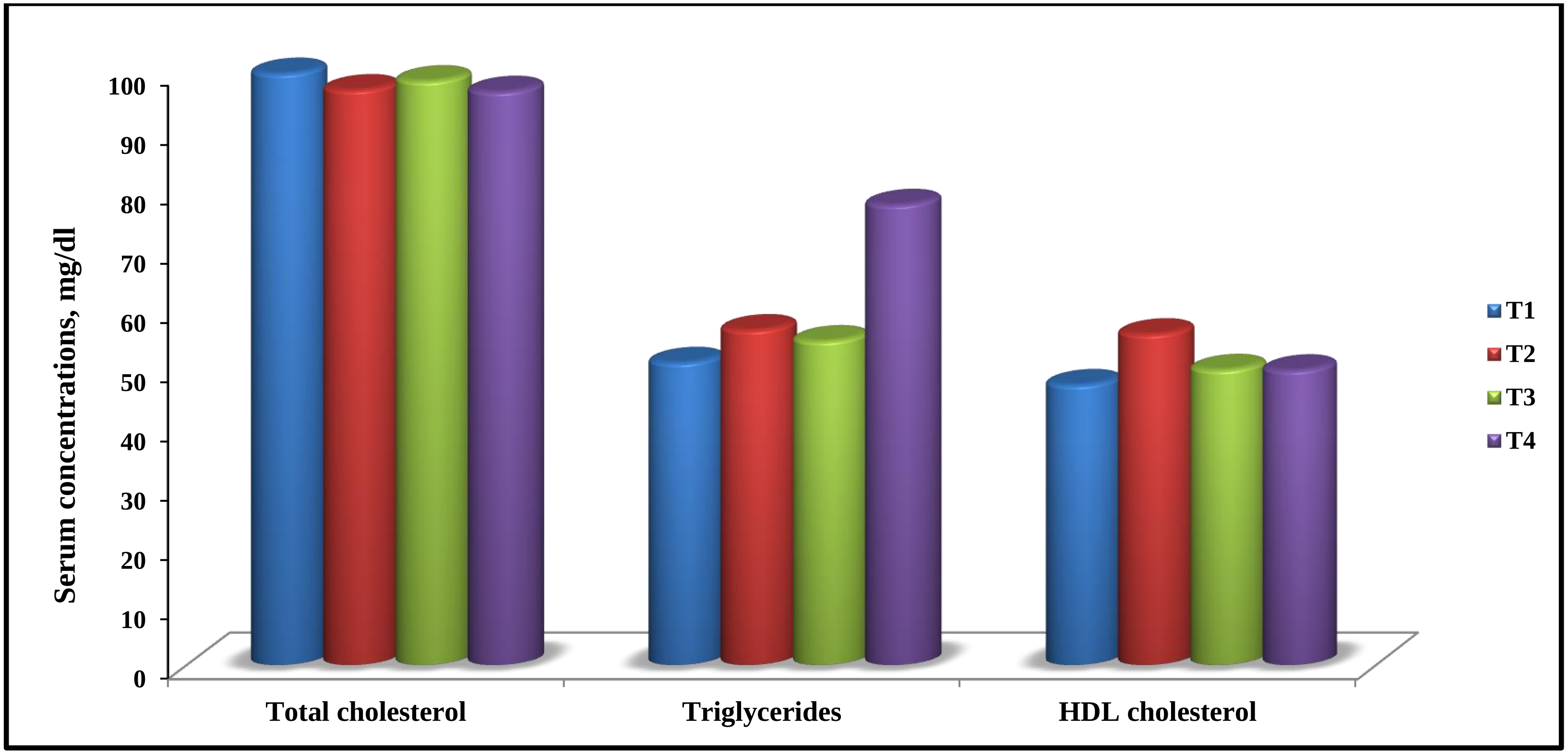

Fig. 8. Serum lipid profile of birds maintained on four dietary treatments

Table 13. Serum mineral concentration of birds maintained on four dietary treatments, mg per dl

Parameter	Serum mineral concentrations, mg per dl[†]				p value
	T1	T2	T3	T4	
Calcium	10.08 ± 0.90	9.50 ± 0.78	9.92 ± 0.83	10.02 ± 0.66	0.95
Inorganic phosphorus	4.61 ± 0.66	5.45 ± 0.58	4.91 ± 0.45	5.02 ± 0.24	0.71

[†] mean of five values with SE

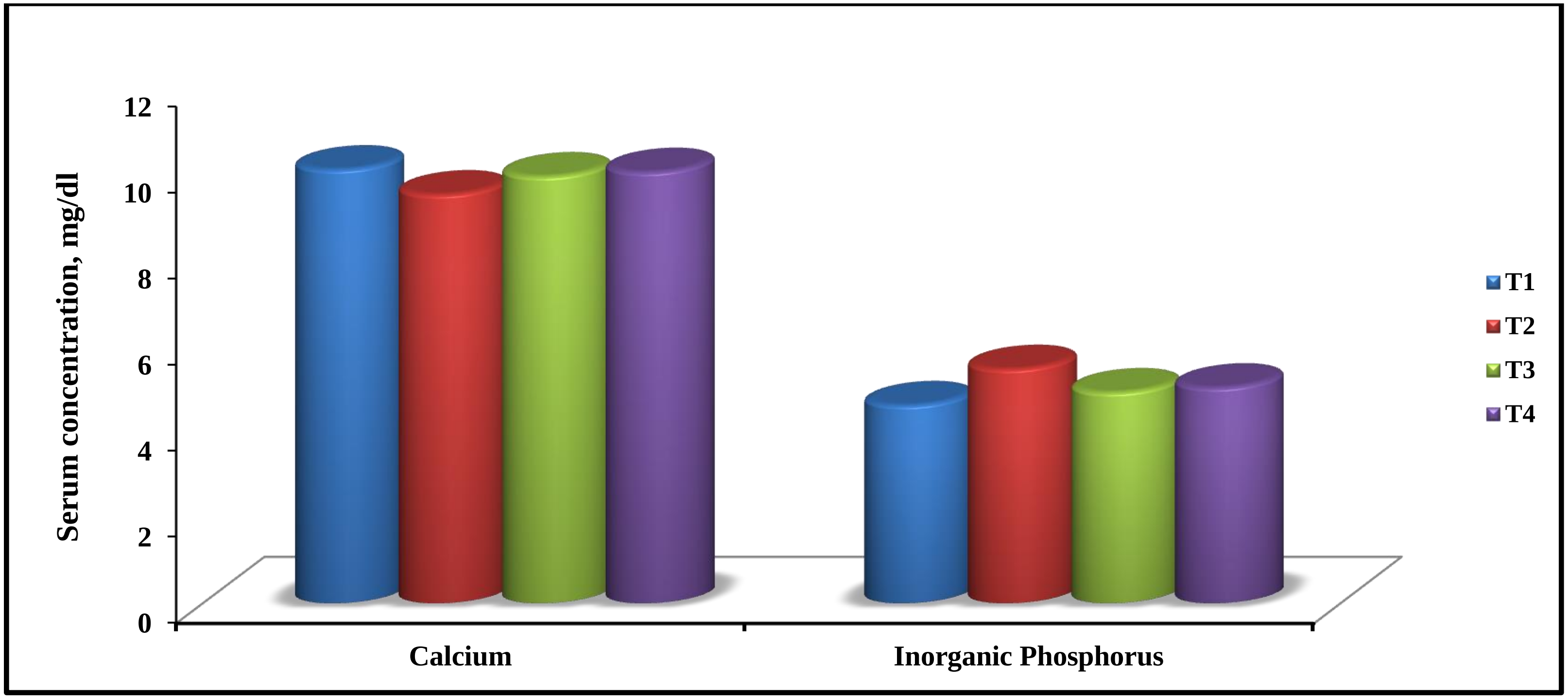

Fig. 9. Serum mineral concentrations of birds maintained on four dietary treatments

Table 14 . Serum total protein concentration of birds maintained on four dietary treatments, g per dl

Parameter	Treatment†				p value
	T1	T2	T3	T4	
Serum total protein g per dl	$7.70^{bc} \pm 0.24$	$8.64^{ab} \pm 0.50$	$8.90^{a} \pm 0.32$	$7.05^{c} \pm 0.24$	0.01*

*a, b, c – means with different superscripts within the same row differ significantly (p<0.05)

† mean of five values with SE

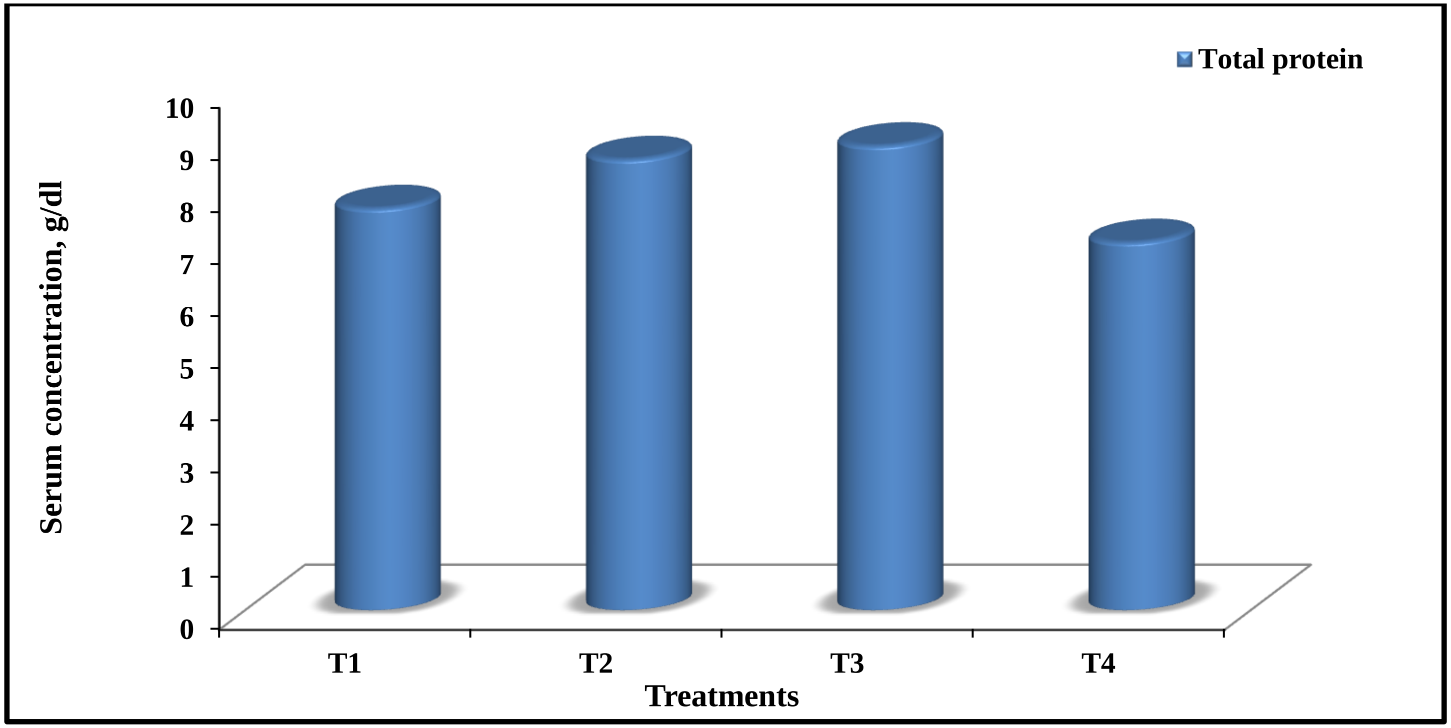

Fig. 10. Serum total protein concentration of birds maintained on four dietary treatments

Table 15. Slaughter data of birds maintained on four dietary treatments

Parameter	Carcass parameters[†]				p value
	T1	T2	T3	T4	
Live weight, g	2242.4 ± 64.08	2283.4 ± 68.13	2168.6 ± 96.21	2215.0 ± 81.76	0.77
Carcass weight, g	1594.2 ± 55.47	1638.4 ± 45.20	1524.2 ± 78.63	1556.8 ± 68.87	0.62
Giblet yield, %	4.03 ± 0.10	4.42 ± 0.13	4.65 ± 0.15	4.43 ± 0.29	0.16
Dressing percentage, %	71.04 ± 0.46	71.78 ± 0.46	70.20 ± 0.57	70.21 ± 0.69	0.18

[†] mean of five values with SE

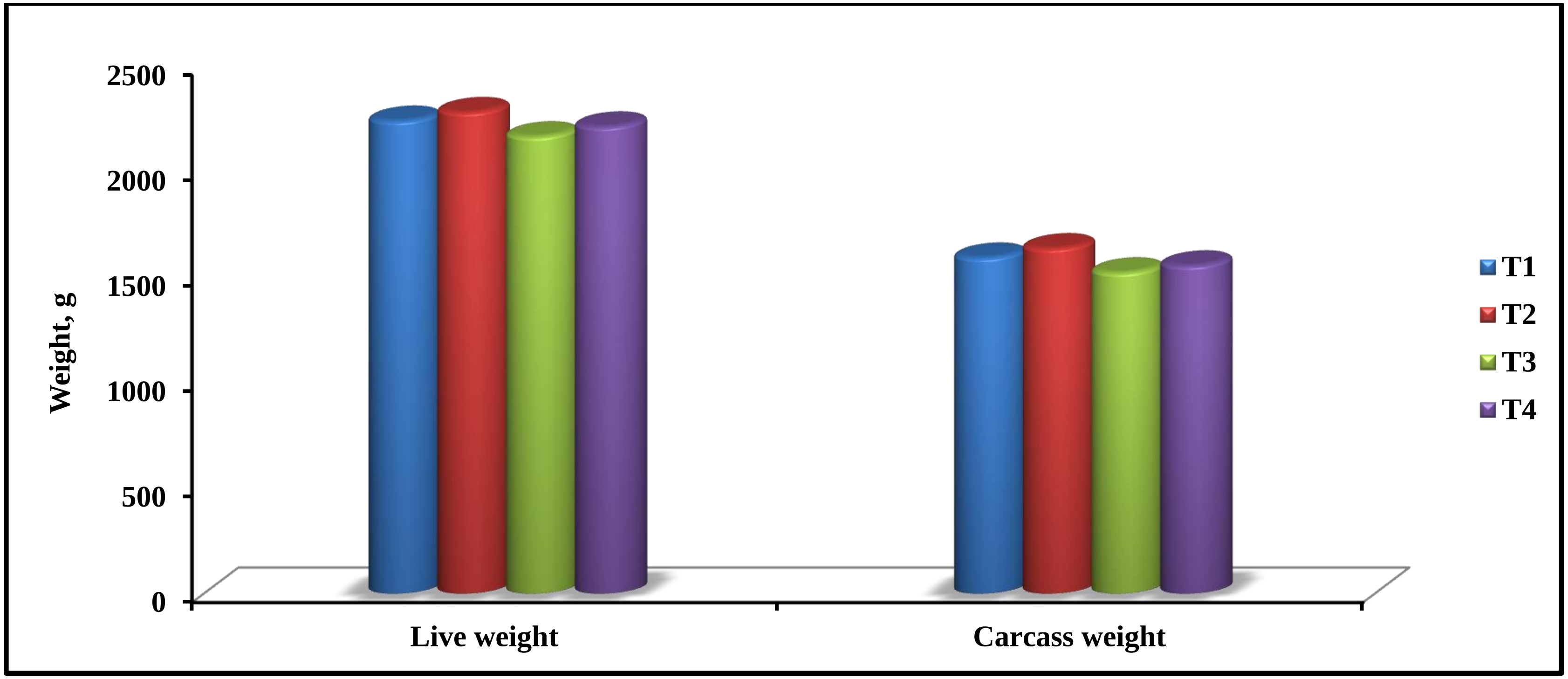

Fig. 11. Live weight and carcass weight of birds maintained on four dietary treatments

Table 16. Weight of internal organs of birds maintained on four different dietary treatments as percentage of carcass weight, %

Parameter	Weight of internal organs as percentage of carcass weight[†]				p value
	T1	T2	T3	T4	
Heart	0.60 ± 0.01	0.66 ± 0.01	0.71 ± 0.03	0.87 ± 0.13	0.07
Liver	2.60 ± 0.12	2.88 ± 0.16	3.00 ± 0.08	2.89 ± 0.18	0.26
Gizzard	2.48 ± 0.13	2.62 ± 0.19	2.92 ± 0.21	2.55 ± 0.16	0.32
Spleen	0.11 ± 0.01	0.15± 0.02	0.14 ± 0.02	0.15 ± 0.03	0.59
Intestine	6.27^{b}± 0.27	6.33^{b} ± 0.30	7.63^{a} ± 0.47	6.73^{ab}± 0.11	0.03*

* a, b – means with different superscripts within the same row differ significantly (P<0.05)

[†] mean of five values with SE

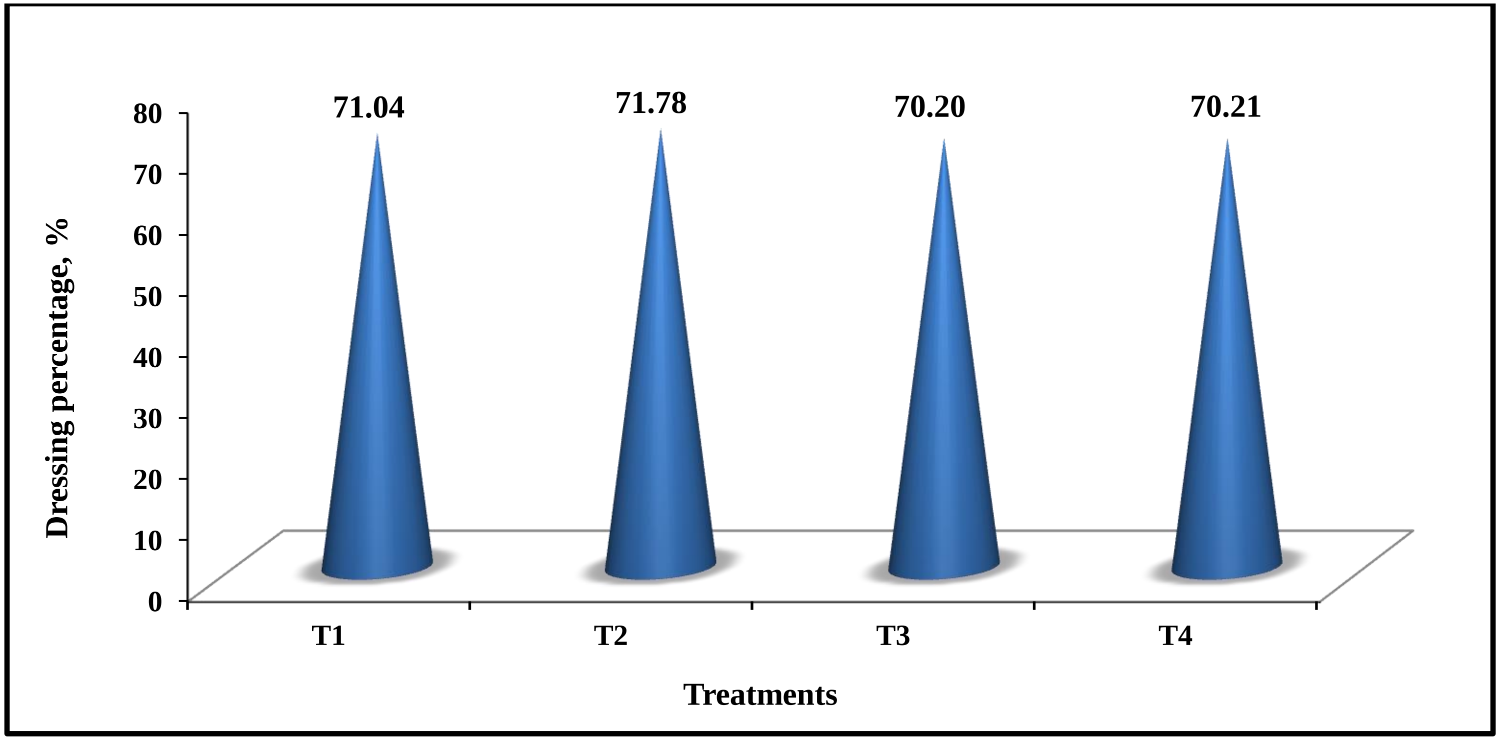

Fig. 12. Dressing percentage of birds maintained on four dietary treatments

Table 17. Faecal microbial count of birds maintained on four dietary treatments, log_{10} CFU per g

Parameter	Faecal microbial count, (log_{10} CFU per g)[†]				p value
	T1	T2	T3	T4	
Total viable count	$7.32^a \pm 0.01$	$6.20^c \pm 0.01$	$6.28^b \pm 0.01$	$5.33^d \pm 0.01$	<0.001*
Coliform count	$5.56^b \pm 0.03$	$5.51^b \pm 0.02$	$5.62^a \pm 0.02$	$5.65^a \pm 0.01$	<0.001*

*a, b, c, d – means with different superscripts within the same row differ significantly ($p<0.05$)

[†] mean of five values with SE

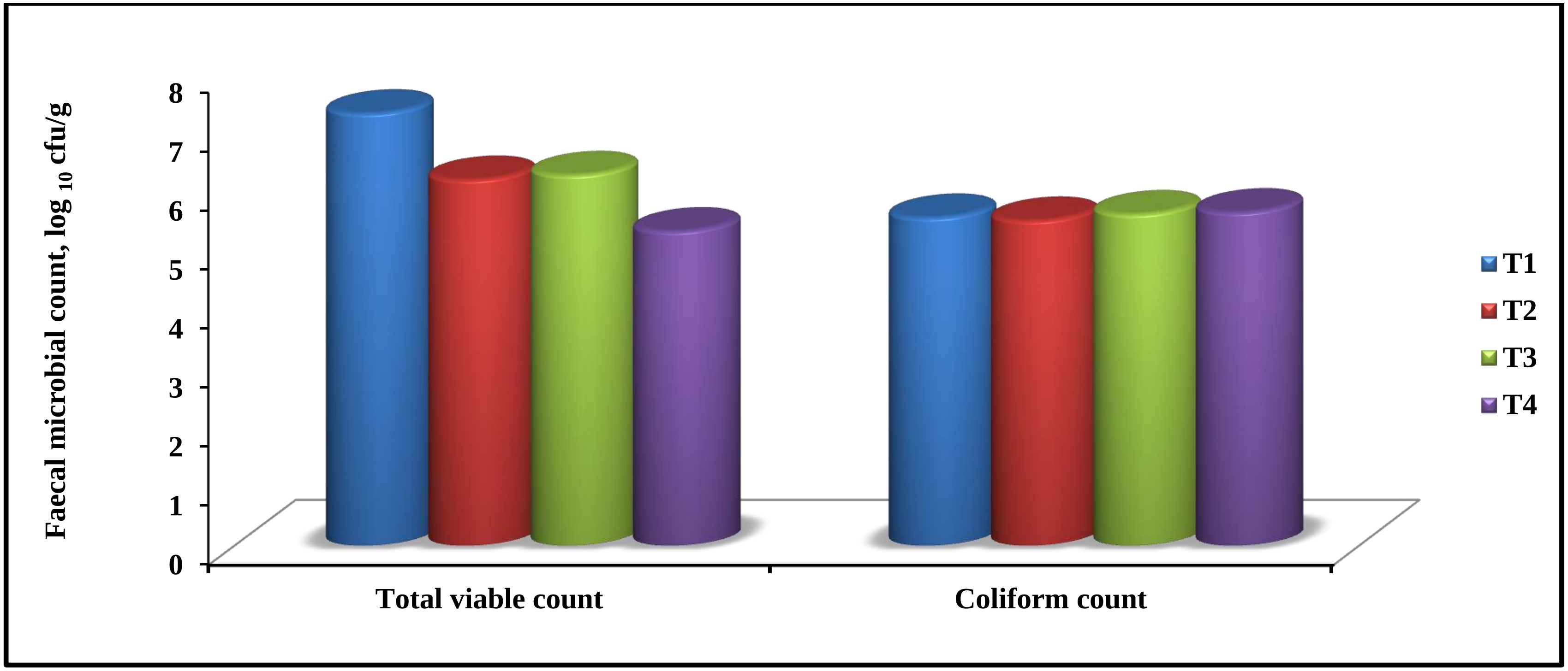

Fig. 13. Faecal microbial count of birds maintained on four dietary treatments

Table 18. Protein efficiency ratio, production efficiency factor and energy efficiency ratio of birds maintained on four dietary treatments

Parameter	Treatments				p value
	T1	T2	T3	T4	
Protein efficiency ratio	$2.62^{b} \pm 0.01$	$2.85^{a} \pm 0.07$	$2.39^{c} \pm 0.08$	$2.49^{bc} \pm 0.03$	<0.001*
Production efficiency factor	$244.9^{ab} \pm 11.22$	$271.5^{a} \pm 8.60$	$199.4^{c} \pm 13.48$	$229.2^{bc} \pm 4.55$	<0.001*
Energy efficiency ratio	16.05 ± 0.52	15.63 ± 0.58	14.76 ± 0.94	15.86 ± 0.27	0.54

*a, b, c – means with different superscripts within the same row differ significantly ($p < 0.05$)

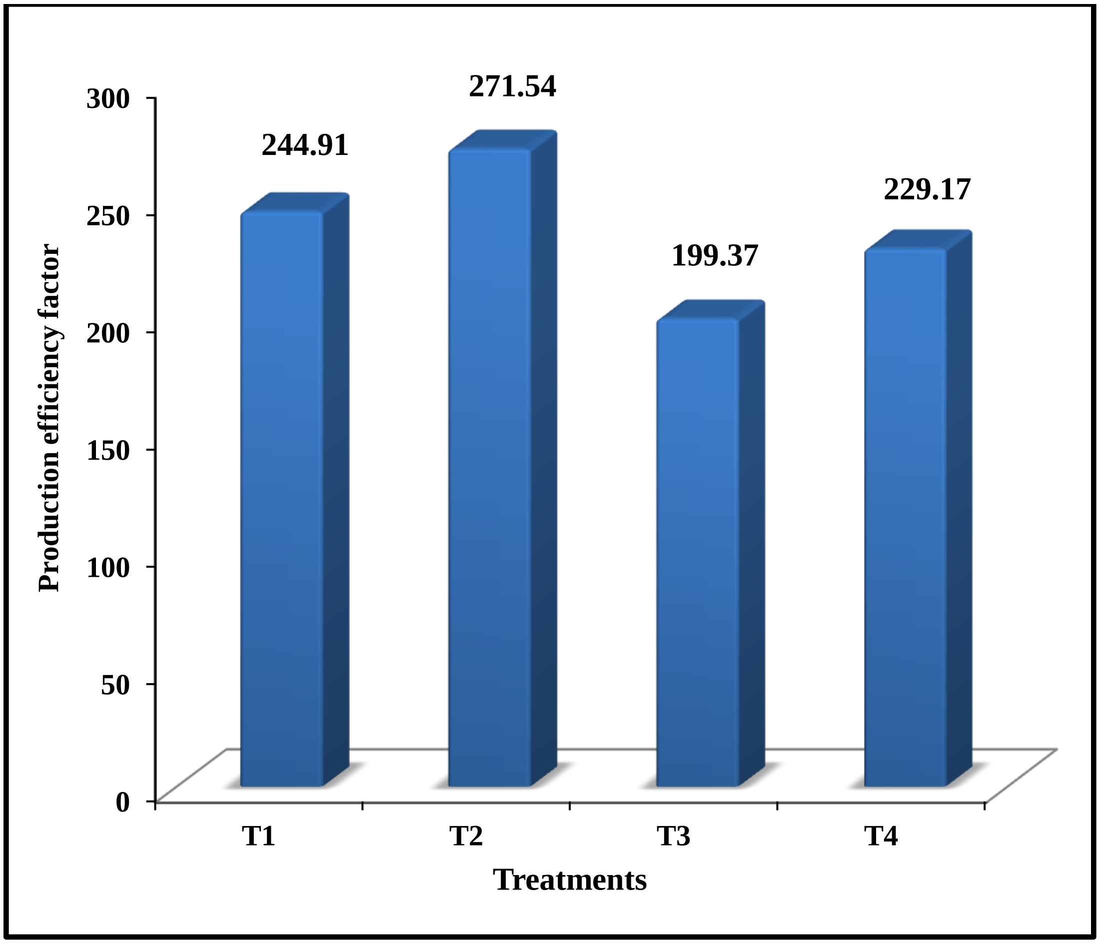

Fig. 14. Production efficiency factor of birds maintained on four dietary treatments

Table 19. Cost of feed per kg live weight gain of birds maintained on four dietary treatments, Rs.

Parameters		Treatments				
		T1	T2	T3	T4	P value
Total body weight gain, kg		1.96 ± 0.02	2.03 ± 0.04	1.87 ± 0.07	1.94 ± 0.01	0.10
Total feed intake, kg	**Pre-starter**	0.15 ± 0.00	0.14 ± 0.01	0.15 ± 0.00	0.16 ± 0.00	0.22
	Starter	0.97 ± 0.02[a]	0.85 ± 0.04[b]	1.02 ± 0.05[a]	1.09 ± 0.04[a]	<0.001*
	Finisher	2.45 ± 0.03[b]	2.42 ± 0.04[b]	2.58 ± 0.03[a]	2.52 ± 0.01[ab]	0.02*
Feed cost per kg, Rs.	**Pre-starter**	32.56	34.32	33.92	35.68	-
	Starter	32.46	34.22	33.82	35.58	-
	Finisher	31.40	33.16	32.76	34.52	-
Total feed cost, Rs.	**Pre-starter**	4.84 ± 0.16[b]	4.89 ± 0.44[b]	5.03 ± 0.13[ab]	5.74 ± 0.15[a]	0.05*
	Starter	31.56 ± 0.74[bc]	29.09 ± 1.49[c]	34.57 ± 1.60[b]	38.79 ± 1.48[a]	<0.001*
	Finisher	77.07 ± 0.81[c]	80.14 ± 1.43[b]	84.55 ± 1.02[a]	86.94 ± 0.32[a]	<0.001*
Cost of feed per kg body weight gain, Rs.		57.90 ± 0.22[b]	56.23 ± 0.92[b]	66.25 ± 2.71[a]	67.85 ± 0.82[a]	<0.001*

*a, b, c- means with different superscripts within the same row differ significantly ($p<0.05$)

**Cost calculated using the rate contract values fixed for feed ingredients by College of Veterinary and Animal Sciences, Mannuthy during period of conduct of experiment*

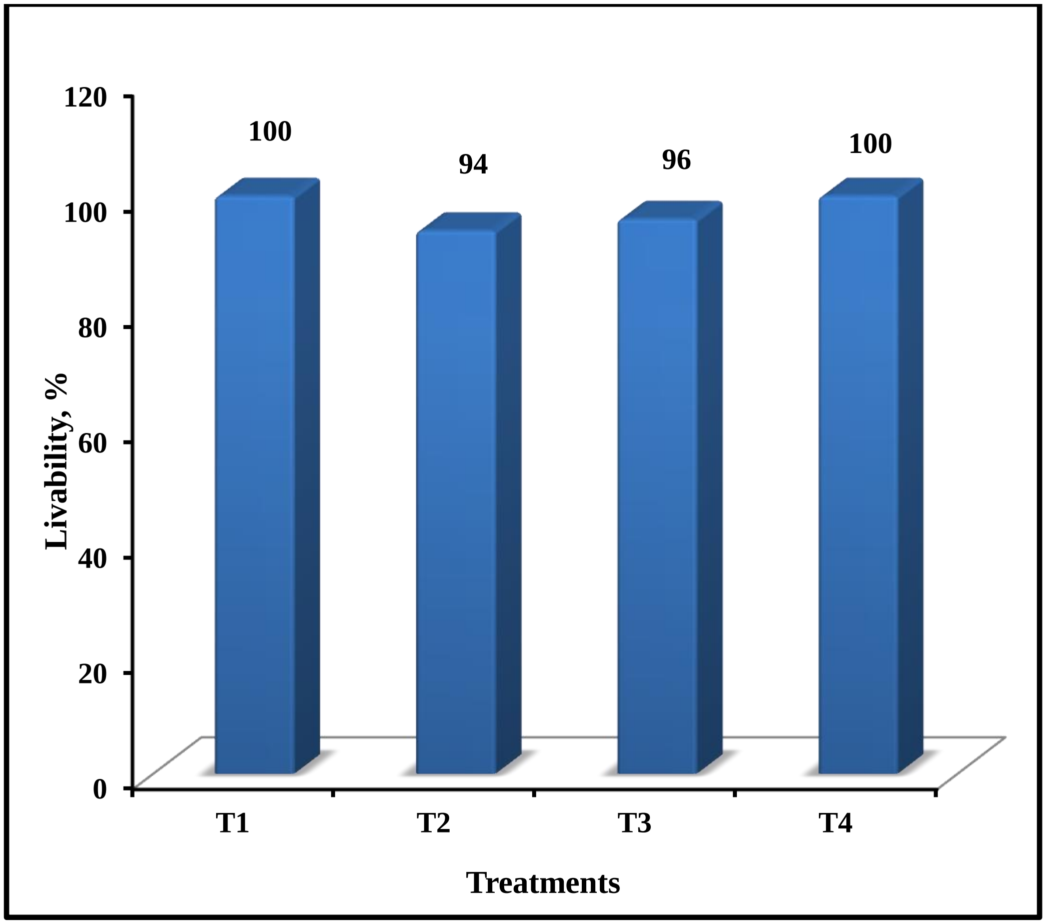

Fig. 15. Per cent livability of birds maintained on four dietary treatments

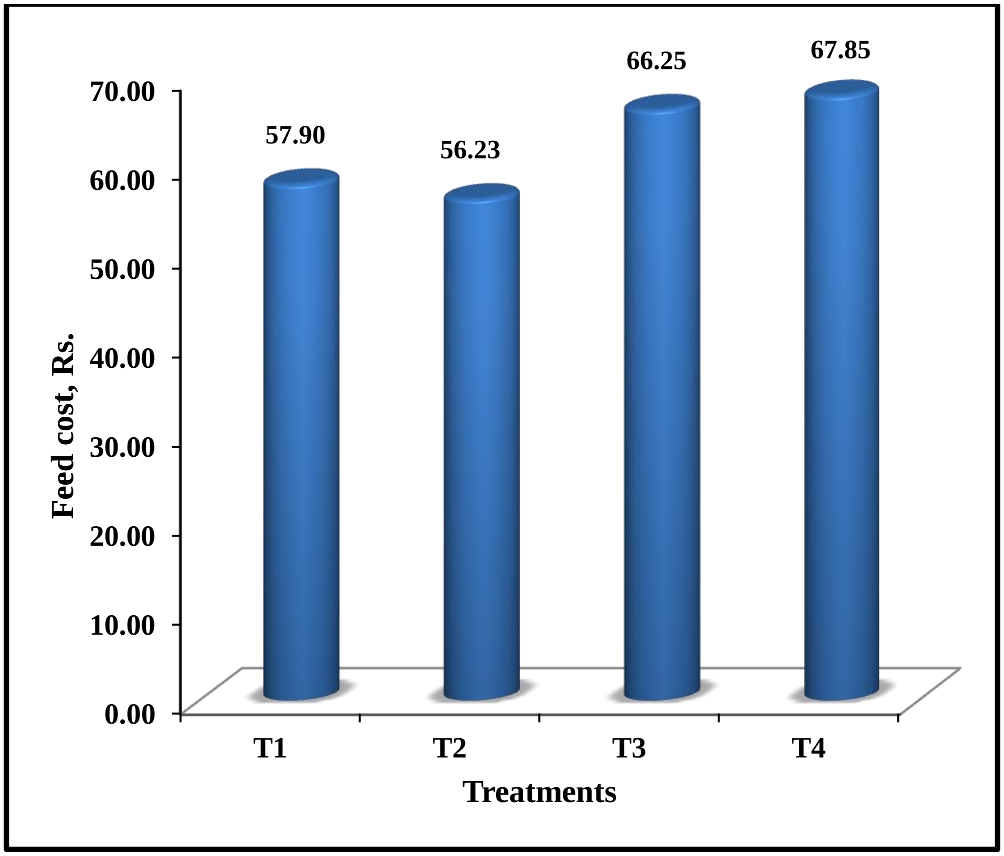

Fig. 16. Feed cost per kg live weight gain of birds maintained on four dietary treatments

Discussion

5. DISCUSSION

5.1 CHEMICAL COMPOSITION OF EXPERIMENTAL RATIONS

The data on chemical composition of the experimental broiler pre-starter, starter and finisher rations are given in Tables 2, 3 and 4. The percentage of dry matter (DM) in pre-starter ration varied from 88.76 to 90.45, ether extract (EE) from 2.22 to 2.43 and crude fibre (CF) from 4.22 to 4.37. The total ash (TA), acid insoluble ash (AIA) and nitrogen free extract (NFE) in the pre-starter ration varied from 7.32 to 7.58, 1.20 to 1.24 and 62.22 to 62.90 per cent, respectively. In starter ration, the percentage of DM varied from 90.36 to 90.61, EE from 6.38 to 6.52 and CF from 4.49 to 4.84. The TA, AIA and NFE varied from 7.37 to 7.53, 1.01 to 1.33 and 58.82 to 59.30 per cent, respectively in starter ration. In the finisher ration the per cent DM varied from 91.77 to 92.01, EE from 7.33 to 7.66 and CF from 7.44 to 7.78. The TA, AIA and NFE content varied from 6.61 to 7.18, 1.18 to 1.50 and 56.65 to 57.60 per cent, respectively in finisher ration.

The crude protein (CP) content of the broiler pre-starter ration ranged from 23.28 to 23.58 per cent while that for starter and finisher rations ranged from 22.22 to 22.49 and 20.65 to 20.91 per cent, respectively. As per BIS (2007), the broiler pre-starter, starter and finisher rations should contain 23.00, 22.00 and 20.00 per cent CP, thus the experimental rations used in the present study met the CP requirements as per the BIS (2007) specifications. The estimated gross energy (GE) of the experimental rations T1, T2, T3 and T4 were 4128.81, 4030.10, 4143.36 and 4113.37 kcal per kg feed, respectively in the pre-starter ration, 4306.64, 4311.06, 4340.82 and 4319.96 kcal per kg feed, respectively in the starter ration and 4596.24, 4621.29, 4681.45 and 4528.25 kcal per kg feed, in the finisher ration, respectively in the present study.

The mineral composition of the experimental pre-starter, starter and finisher rations are given in Tables 2, 3 and 4, respectively. The calcium (Ca) content of the experimental ration was ranged from 1.00 to 1.12 per cent in the pre-starter ration, from 1.02 to 1.23 per cent in the starter ration and from 1.16 to 1.52 per cent in the finisher rations, respectively. The phosphorus (P) content of the pre-starter ration ranged from 0.46 to 0.49 per cent while that for starter and finisher rations ranged from 0.45 to 0.54 and 0.46 to 0.59 per cent, respectively which meets the recommendation of calcium (1 per cent) and phosphorus (0.45 per cent) as per the BIS (2007) specifications.

5.2 BODY WEIGHT, BODY WEIGHT GAIN AND AVERAGE DAILY GAIN

The mean body weight of birds recorded at weekly intervals during the experimental period of 42 days under the four dietary treatments T1, T2, T3 and T4 are presented in Table 5 and Fig.1. The average final body weight of birds belonging to four groups T1, T2, T3 and T4 were 2010.00, 2079.80, 1924.00 and 1988.00 g, respectively and the corresponding cumulative body weight gain was 1959.76, 2029.56, 1873.74 and 1937.76 g, respectively at sixth week of age (Table 6 and Fig.2). The average daily gain of birds maintained on four dietary treatments T1, T2, T3 and T4 were 46.66, 48.32, 44.61 and 46.14 g, respectively (Table 6 and Fig.3). The final body weight, cumulative body weight gain and the average daily gain of birds belonging to propionic acid supplemented group (T2) was significantly higher ($p<0.05$) than that of formic acid supplemented group (T3), while it was similar to that of control diet fed group (T1) and blend of organic acid supplemented group (T4). Moreover, the final body weight, cumulative body weight gain and average daily gain of birds maintained on T1, T3 and T4 treatment groups were similar.

The results obtained in the present study shows that supplementation of propionic acid at 0.2 per cent level did not enhance the growth performance in birds compared to control diet fed group, which agrees with previous reports by Vogt and Matthews (1981) at 0.5, 1.0 and 2.0 per cent, Cave (1984) at 5 per cent and Paul *et al.* (2007) at 0.3 per cent levels. On contrary to the above observations, Khosravi *et al.*

(2008) and Al-Kassi and Mohssen (2009) reported a significant improvement in the growth performance of broiler chicken with dietary addition of propionic acid at 0.2 and 2.0 per cent levels, respectively. Similarly, Brzoska *et al.* (2013) also reported an increased growth rate in birds fed with varying levels (0.3, 0.6 and 0.9 per cent) of propionic acid in the diet than those fed basal diet.

The observations recorded on formic acid supplementation agrees with the earlier reports of Izat *et al.* (1990a) and Hernandez *et al.* (2006) who noted no significant effect on body weight gain in broiler chicken with the dietary addition of formic acid at 1.0 per cent and 0.5 and 1.0 per cent levels, respectively. However, an improvement in weight gain was observed when formic acid was supplemented at 1 per cent (Bozkurt *et al.*, 2009); at 0.25 and 0.50 per cent (Ghazalah *et al.*, 2011) and at 1.00, 1.50 and 2.00 per cent levels (Mishra *et al*., 2013) in the diet of broiler birds.

Visek (1978) and Kaniawati *et al.* (1992) recorded no improvement in live body weight and weight gain in birds supplemented with 1.0 per cent organic acid blend (propionic and formic acids). Moreover, Vale *et al.* (2004) reported a reduction in the body weight gain of broiler birds fed diet supplemented with 0.25 and 0.50 per cent levels of organic acid blend (70 per cent formic acid and 30 per cent propionic acid), as against control. These earlier results are in agreement with the present observations made in the blend of organic acids supplemented group. On the other hand, Thirumeignanam *et al.* (2006) found that blend of organic acids at 0.1 per cent level in the broiler diet increased the body weight gain. Similar results were also observed by Hernandez *et al.* (2006), Ghazalah *et al.* (2011) and Saki and Eftekhari (2012).

Variability observed in the above results might be due to factors like environmental conditions, heterogenicity of gut microflora that influence the effects of organic acids (Dibner *et al*., 2007) and differences in the dosage of organic acids (Venkatasubrahmani *et al*., 2014) used for various experiments.

5.3 FEED CONSUMPTION AND FEED CONVERSION RATIO

Data on cumulative feed consumption (Table 7 and Fig. 4) and cumulative feed conversion ratio (FCR) of birds (Table 8 and Fig. 5) revealed that there was no significant difference in the feed intake and FCR of birds maintained on four dietary treatments during the first week of age. However, during the second and third weeks of age, a significant reduction was observed ($p<0.05$) in the feed intake of birds in group T2 compared to other three treatment groups (T1, T3 and T4). Moreover, a significant reduction ($p<0.05$) in feed intake was also noted for T1 group, which was similar to group T2 at fourth and fifth weeks of age. The cumulative feed intake was lowest for T2 group and highest for birds belonging to T3 and T4 groups.

According to Cave (1984), a reduction in feed intake was observed in broiler birds fed diet supplemented with propionic acid which may be due to reduction in the palatability of diets and similar observations were also noted in the present study. The observations recorded in present study were similar to those earlier reports made by Pinchasov and Elmaliah (1994) and Paul *et al.* (2007) in broiler birds, in which, they reported a reduction in feed intake when propionic acid was supplemented in the diet. However, Venkatasubramani *et al.* (2014) observed no significant effect on feed intake when propionic acid was supplemented at 0.1 and 0.15 per cent levels in the diet.

Formic acid supplementation at 0.2 per cent level did not influence the feed intake and the recorded observations were similar to those birds fed control diet up to 21 days of age. But during the finisher period, significant increase ($p<0.05$) in feed intake was observed compared to control diet fed birds. These observations were in contrast with the earlier reports by Venkatasubramani *et al*. (2014) at 0.10 and 0.15 levels and Ologhobo *et al*. (2015) at 0.8 per cent level.

The cumulative feed intake of birds supplemented with blend of propionic and formic acid (group T4) were significantly higher than groups T1 and T2. Moreover, the feed intake of group T4 was statistically similar to group T3 during the entire experimental period. The increase in the feed intake due to supplementation of organic acid blend are in agreement with earlier reports by Vale *et al.* (2004) and Fallah and Rezaei (2013), but disagree with observations made by Venkatasubramani *et al.* (2014).

From the results presented in Table 8, it could be inferred that the birds maintained on dietary treatment T2 had a better ($p<0.05$) cumulative FCR compared to other three dietary treatments (T1, T3 and T4 groups) at second, fourth, fifth and sixth weeks of age. However, during third week of age the FCR in group T2 was similar to groups T1 and T3 and all the three treatment groups differed statistically from group T4. The results obtained in FCR with dietary supplementation of propionic acid were similar to earlier observations made by Khosaravi *et al.* (2008) at 0.2 per cent and Venkatasubramani *et al.* (2014) at 0.10 and 0.15 per cent levels in broiler birds.

From Table 8, it is clear that the supplementation of formic acid or blend of organic acids did not improve the cumulative FCR in broiler birds at six weeks of age compared to those fed control diet. Luckstadt and Theobald (2011); Mishra *et al.* (2013) and Ologhobo *et al*. (2015) reported a better FCR in formic acid supplemented group compared to control diet fed group and these findings are in contrast with the observations made in the present study. The poor FCR found in the formic acid supplemented group can be attributed to the lower digestibility of feed (Gracia *et al.,* 2007), as also evidenced from the results of present study.

Al-Kassi and Mohssen (2009) reported significantly higher value for FCR, when birds were fed diet containing 0.3 per cent of blend of propionic and formic acid than those fed control diet. The results obtained in the present study are also in agreement with the above observations. Variation observed among groups for FCR can be attributed to different factors like buffering capacity of the dietary ingredients, strength and dosage of the organic acids used, and the influence of one organic acid over the other (Reddy, 2011).

5.4 DIGESTIBILITY OF NUTRIENTS

The chemical compositions of droppings voided by the experimental birds maintained on four dietary treatments T1, T2, T3 and T4 during the metabolism trial are shown in Table 9. Data on percentage digestibility of nutrients are presented in Table 10 and is graphically depicted in Fig.6. The data on digestibility of nutrients reveal that there were statistical difference ($p<0.05$) among different treatment groups with respect to digestibility of DM, EE, CF and NFE. However, the digestibility of CP was statistically similar ($p>0.05$) among four treatment groups.

In the current study, supplementation of propionic acid (group T2) resulted in a significantly higher ($p<0.05$) digestibility of DM, EE, CF and NFE than other three treatment groups. Moreover, the nutrient digestibility in formic acid supplemented group (group T3) and blend of organic acid supplemented group (group T4) were similar to control diet fed group (group T1) for digestibility of all nutrients except CP. The energy efficiency observed for group T2 was significantly higher ($p<0.05$) compared to other three treatment groups. Moreover, the energy efficiency observed in group T3 was significantly lower compared to other three treatment groups.

On contrary to the results obtained in the present study, Izat *et al.* (1990b) reported no significant effect on nutrient digestibility by addition of propionic acid at 0.4 per cent level in the diet of broiler chicken. However, the present results corroborate with the findings of Venkatasubramani *et al*. (2014), who noted higher digestibility values for CF and EE, when birds were fed diet containing 0.15 per cent of propionic acid. The improvement in nutrient digestibility by propionic acid supplementation can be attributed to its antimicrobial activity, that enhance increased resistance to infections, thus promoting gut development and nutrient absorption, leading to better performance (Ganguly, 2013).

Hernandez *et al.* (2006) reported that supplementation of formic acid at 0.5 and 1.0 per cent levels did not affect the total tract digestibility of both DM and CP in broiler birds at 42 days of age. On a similar study, Gheisari *et al.* (2009) supplemented protected organic acid mixture (formic acid, propionic acid and their ammonium salts) at two dietary levels (0.2 and 0.4 per cent) and found no significant improvement in utilization of protein and digestibility of other nutrients (DM, CF and EE). However, Gracia *et al*. (2007) and Ghazalah *et al.* (2011) reported improvement in DM and CP digestibility when formic acid was supplemented in the diet. On contrary, Venkatasubramani *et al.* (2014) could not observe any significant improvement in nutrient digestibility, when birds were fed diets supplemented with either formic acid at 0.1 and 0.15 per cent levels or blend of propionic and formic acid at 0.1 per cent level compared to a control diet fed group and this observation corroborates with the results of present study.

5.5 NITROGEN BALANCE

The data on nitrogen balance (g per day) of experimental birds maintained on four experimental rations are shown in Table 10. The nitrogen balance for the groups T1, T2, T3 and T4 were 1.86, 2.31, 1.68 and 1.79 g per day, respectively. Positive nitrogen balance recorded in all the four groups indicates the adequate level of protein in the diet and the statistical analysis reveals that supplementation of organic acids had no significant ($p>0.05$) effect on nitrogen balance in broiler chicken. However, the results obtained in the present study for group T3 is in contrast with the earlier observations of Selle *et al.* (2004), who recorded an improvement in nitrogen retention when salts of formic acid were added at 0.3 and 1.2 per cent levels in the diet.

5.6 AVAILABILITY OF MINERALS

The per cent availability of minerals is presented in Table 11 and is graphically depicted in Fig. 7. The per cent availability of minerals in the four dietary rations T1, T2, T3 and T4 were 48.24, 52.77, 50.49 and 44.81 per cent for Ca and 56.98, 56.88, 53.87 and 55.60 per cent for P, respectively and the values were statistically similar ($p>0.05$) showing that supplementation of acidifiers did not influence the availability of calcium and phosphorus.

Reddy (2011) opined that organic acids improve the microbial phytase activity in animals resulting in better availability and retention of phosphorus. However, no such effects were observed during the present study. He also stated that the minerals present in the feed may have an alkaline effect in gut, that could reduce the growth promoting and nutrient utilizing effect of organic acids. Thus from the present study and from the available literature, there is lack of consistency in results regarding the effect of propionic acid, formic acid or their blends on mineral availability in poultry.

5.7 SERUM BIOCHEMICAL PARAMETERS

5.7.1 Serum lipid profile

The serum lipid profile of birds maintained on different dietary treatments are presented in Table 12 and are graphically represented in Fig. 8. The serum lipid profile (mg per dl) of experimental birds belonging to the treatment groups T1, T2, T3 and T4 were 99.67, 96.89, 98.40 and 96.61 for total cholesterol, 47.18, 55.73, 49.73 and 49.65 for HDL cholesterol and 50.92, 56.43, 54.58 and 77.54 for triglycerides, respectively.

The statistical analysis of data revealed that the serum total cholesterol concentration was unaffected by dietary treatments. However, there was significant difference ($p<0.05$) among different treatment group for HDL cholesterol concentrations with significantly higher values observed for propionic acid supplemented group. Moreover, significant difference was also observed for triglycerides with higher values for organic acid blend supplemented group.

Furthermore, the values recorded for lipid profile in the present study fall within the normal physiological range reported for the species

Hernandez *et al.* (2006) observed no change in serum total cholesterol concentration by addition of formic acid up to 1.0 per cent in the diet, and this observation is in agreement with the results of present study. Similar observations were also made by Brzoska *et al.* (2013) in broiler chicken fed diet supplemented with propionic acid at 0.3, 0.6 and 0.9 per cent levels. As against the results of the present study, Fallah and Rezaei (2013) reported a significant reduction in serum total cholesterol and triglycerides in broiler birds supplemented with blend of organic acids and their ammonium salts at 0.3 per cent level. Similarly, Venkatasubramani *et al.* (2014) also observed a reduction in the total serum cholesterol in broiler chicken when formic acid was supplemented at 0.1 and 0.15 per cent levels in the diet.

The results recorded for HDL cholesterol were in agreement with observations by Khosravi *et al*. (2008) who reported an increase in the HDL cholesterol when birds were fed diet supplemented with 0.2 per cent propionic acid. On contrary, Brzoska *et al.* (2013) reported no significant effect in serum HDL cholesterol and triglycerides in broiler chicken by dietary supplementation of propionic acid at 0.3, 0.6 and 0.9 per cent levels.

5.7.2 Serum mineral concentration

The serum mineral concentration of birds maintained on four dietary treatments T1, T2, T3 and T4 were 10.08, 9.50, 9.92 and 10.02 mg per dl for Ca and 4.61, 5.45, 4.91 and 5.02 mg per dl for inorganic P, respectively and did not show any significant difference among different treatment groups (Table 13 and Fig. 9.). Moreover, the values recorded in the present study falls within the normal range reported for poultry (Kaneko *et al*., 2008).

In contrast with the results of present study, Ghazalah *et al.* (2011) reported significantly higher serum Ca and inorganic P concentration in broiler birds when formic acid was supplemented at 0.25, 0.5 and 1.0 per cent levels. They hypothesized that the increase in the serum concentration of Ca and inorganic P could be due to lowering of gut pH that increases the absorption of minerals from gut into blood stream. However, no such effect was observed in the present study which could be due to lower dose level of formic acid used in current study.

5.7.3 Serum total protein

The serum total protein concentrations (g per dl) of the experimental birds maintained on various dietary treatments are shown in Table 14 and Fig.10. The serum total protein concentration of birds maintained on four dietary treatments T1, T2, T3 and T4 were 7.70, 8.64, 8.90 and 7.05 g per dl, respectively. On statistical analysis, it was found that the total protein concentration was higher ($p<0.05$) in group T3 than groups T1 or T4. However, total protein concentration was similar ($p>0.05$) among groups T1, T2 and T4. This result is in agreement with the earlier observation made by Ghazalah *et al.* (2011) who reported a significantly higher serum total protein concentration when formic acid was supplemented at 0.25, 0.5 and 1.0 per cent levels in the diet of broiler birds.

5.8 CARCASS PARAMETERS

Data on carcass weight, giblet yield and dressing percentage of birds maintained on different dietary treatments at sixth week of age are presented in Table 15 and are graphically represented in Fig.11 and 12.

5.8.1 Carcass weight

The birds achieved a mean carcass weight of 1594.20, 1638.40, 1524.20 and 1556.80 g for the treatment groups T1, T2, T3 and T4, respectively at the time of slaughter. There was no significant difference among the treatment groups for live weight and carcass weight. However, the slaughter characteristics of broiler birds were not negatively affected by any of the dietary supplementation.

Propionic acid supplementation in the current study was associated with better growth performance and nutrient utilization, but it did not resulted in better carcass yield. On contrary, Izat *et al.* (1990b), Hume *et al.* (1993) and Brzoska *et al.* (2013) reported significant increase in carcass weight of broiler birds when propionic acid was supplemented in the diet.

In agreement with results of the present study, Gracia *et al.* (2007) reported that formic acid supplementation at 0.5 or 1.0 per cent levels did not improve the carcass weight of broiler birds. Similarly, Bozkurt *et al.* (2009) also reported that the carcass weight of both male and female broiler birds were not affected by the addition of 0.1 per cent of formic acid in the diet.

Fallah and Rezaei (2013) reported that addition of 0.3 per cent blend of organic acids or their salts in the diet had no significant effect on the carcass weight in broiler birds. This observation is in agreement with results of the present study.

5.8.2 Dressing percentage

From the data presented in Table 15, it can be inferred that there was no significant difference ($p>0.05$) in the dressing percentage and giblet yield of birds maintained on different dietary treatments. However, a significant increase in the dressing percentage was observed in broiler birds fed diet supplemented with propionic acid at 0.2 per cent (Izat *et al.*, 1990b) and at 0.3 per cent levels (Brzoska *et al.*, 2013).

Gracia *et al.* (2007) recorded a significant reduction in the dressing percentage of birds when formic acid was supplemented at 1 per cent level. However, Mishra *et al.* (2013) reported a significant increase in the dressing percentage of birds when formic acid was supplemented at 1.5 and 2 per cent levels in the diet. Both these results were in contrast with the observations made in the present study.

Al-Kassi and Mohssen (2009) and Panda *et al.* (2011) reported significant increase in the dressing percentage of birds supplemented with 0.3 per cent blend of propionic and formic acid in the diet. These findings also disagree with the results of current study.

5.8.3 Weight of internal organs as percentage of carcass weight

The weight of internal organs such as heart, liver, gizzard, spleen and intestine as percentage of carcass weight are presented in Table 16. On statistical analysis, no significant differences was observed among birds of the four treatment groups except for the weight of intestine, with a significantly higher value observed for formic acid supplemented group (group T3) compared to control (group T1) and propionic acid supplemented group (group T2). Moreover, there was no significant difference between blend of organic acid supplemented group (group T4) and the other three treatment groups. The weight of the internal organs as percentage of carcass weight for treatment groups belonging to T1, T2, T3 and T4 were 0.60, 0.66, 0.71 and 0.87 per cent for heart, 2.60, 2.88, 3.00 and 2.89 per cent for liver, 2.48, 2.62, 2.92 and 2.55 per cent for gizzard, 0.11, 0.15, 0.14 and 0.15 per cent for spleen and 6.27, 6.33, 7.63 and 6.73 per cent for intestine, respectively.

Engberg *et al.* (2000) opined that dietary inclusion of acidifiers could result in reduced weight of intestine due to thinning of the intestine wall. However, no such effect was observed in present study by propionic acid supplementation. In agreement with the result of present study, Bozkurt *et al.* (2009) reported a significant increase in the weight of intestine as percentage of carcass weight in male broiler birds supplemented with 0.1 per cent formic acid in the diet. Similarly, Brzoska *et al.* (2013) and Venkatasubramani *et al.* (2014) recorded no significant effect ($p>0.05$) on weight of internal organs when birds were fed diet containing either propionic acid, formic acid or blends of organic acids and all these observations were in agreement with the results of the present study.

5.9 FAECAL MICROFLORA POPULATION

The data on faecal microbial count is presented in Table 17 and Fig.13. The total viable count in the droppings of birds maintained on four experimental treatments T1, T2, T3 and T4 were 7.32, 6.20, 6.28 and 5.33 $\log_{10}$ cfu per g, respectively and coliform count were 5.56, 5.51, 5.62 and 5.65 $\log_{10}$ cfu per g, respectively. Dietary supplementation of acidifiers resulted in significant reduction of total viable count in all the supplemented groups compared to control group and the lowest count was observed in T4 group. Furthermore, propionic acid supplementation resulted in lower coliform count compared to formic acid supplementation. From the Table 17, it is clear that supplementation of formic acid or blend of propionic acid and formic acid resulted in significant reduction in coliform count compared to propionic acid supplemented group or control diet fed group.

The observed result in the present study is in partial agreement with Al-Kassi and Mohssen (2009), who reported significant ($p<0.05$) reduction in the faecal total viable count and coliform count in broiler chicken, when fed diet supplemented with propionic acid at 0.2 per cent level compared to control diet fed group. On contrary, Ghazalah *et al.* (2011) observed that the caecal contents of birds supplemented with 0.5 per cent formic acid had higher counts of coliform and anaerobic bacteria than those fed un-supplemented diet. However, Ologhobo *et al.* (2015) observed a significant reduction in faecal total viable count and coliform count in broiler chicken when formic acid was added at 0.8 per cent along with 0.08 per cent DL- methionine in the diet.

The result of the present study is in agreement with the observations made by Gunal *et al.* (2006), who reported that blends of organic acids (0.2 per cent blend of formic and propionic acid) significantly reduced the faecal total bacteria count and gram negative bacteria count in broiler birds compared to those fed a control diet.

5.10 PROTEIN EFFICIENCY RATIO, PRODUCTION EFFICIENCY FACTOR AND ENERGY EFFICIENCY RATIO

The protein efficiency ratio (PER), production efficiency factor (PEF) and energy efficiency ratio (EER) of birds maintained on four dietary treatments are presented in Table 18. The PER of birds maintained on different treatments are depicted in Fig.14. The mean PER for birds belonging to treatment groups T1, T2, T3 and T4 were 2.62, 2.85, 2.39 and 2.49, respectively. The mean PEF were 244.91, 271.54, 199.37 and 229.17 and the mean EER were 16.05, 15.63, 14.76 and 15.86 for groups T1, T2, T3 and T4 respectively. The statistical analysis of the data revealed that there was significant difference among different treatment groups for PER and PEF. The EER of birds maintained on four different dietary treatments were statistically ($p>0.05$) similar. The results observed in PER for group T2 in the present study is in agreement with Khosravi *et al.* (2008). However, Ghazalah *et al*. (2011) reported that chicks fed 0.5 per cent formic acid had better ($p<0.01$) PEF compared to control group and this finding disagree with the result of present study.

5.11 LIVABILITY PER CENT

During the course of the experiment four birds from group T2 and two birds from group T3 i.e., a total of six birds died out of 200 birds (Fig. 15). The birds were dead during the first week of age and the per cent livability recorded was 100, 94, 96 and 100 for groups T1, T2, T3 and T4, respectively. The dead birds were subjected to necropsy procedures and the revealed necropsy lesions include enlarged spleen, fibrinous pericarditis and unabsorbed yolk sac, which are suggestive of neonatal *E. coli* septicaemia. The mortality observed in the present study might be due to the susceptibility of chicks in their early hatch life to infections due to various environment and management factors.

5.12 ECONOMICS OF PRODUCTION

Data on cost of feed per kg body weight gain of birds maintained on four dietary treatments are presented in Table 19. The costs of ingredients used for the present study were calculated as per the rate contract fixed by the College of Veterinary and Animal Sciences, Mannuthy for the year 2013–2014. The cost of feed per kg body weight gain of birds was Rs. 57.90, 56.23, 66.25 and 67.85 for groups T1, T2, T3 and T4, respectively. In the present study, supplementation of propionic acid reduced the cost of feed per kg body weight gain compared to other three treatment groups. However, the cost of feed per kg body weight gain recorded in control diet fed group was lower when compared to formic acid supplemented group and blend of organic acid supplemented group.

The results of the present study indicate that propionic acid supplementation at 0.2 per cent level improved the FCR, DM digestibility, PER and HDL cholesterol. Moreover, in comparison with the standard broiler ration, the cost of feed per kg body weight gain was reduced by Rs. 1.67 as a result of propionic acid supplementation. However, supplementation of formic acid at 0.2 per cent level or combination of acidifiers (propionic acid and formic acid each at 0.2 per cent level) did not improve the growth performance and carcass characteristics in broiler chicken. From the overall results, it can be concluded that propionic acid at 0.2 per cent level can be recommended as a feed additive in broiler chicken.

Summary

6. SUMMARY

An experiment was conducted for a period of 42 days using two hundred, day-old straight run commercial broiler chicks (Ven Cobb) to study the effect of dietary supplementation of feed acidifiers on growth performance, nutrient utilization, gut microbial load and carcass characteristics. The birds were divided into four treatment groups with five replicates of ten chicks in each replicate. The four dietary treatments consists of T1 (control ration as per BIS, 2007), T2 (control ration + propionic acid 2 g per kg of feed), T3 (control ration + formic acid 2 g per kg of feed) and T4 (control ration + propionic acid and formic acid each at 2 g per kg feed) using completely randomized design. The birds were reared under deep litter system of management. Feed and water were provided *ad libitum.* Birds were fed with standard broiler pre-starter ration up to one week of age, starter ration from two to three weeks of age and finisher ration fed till the end of experiment.

Body weight and feed intake of the birds were recorded at weekly intervals to study the growth performance, and the feed conversion ratio (FCR) was calculated using body weight gain and feed intake. Livability of birds was also recorded. At the end of the feeding trial, five birds from each treatment group were randomly selected and slaughtered to study the carcass traits such as live weight, carcass weight, giblet yield, weight of internal organs and dressing percentage. Blood samples were collected at the time of slaughter and analyzed for total protein, total cholesterol, HDL cholesterol, triglycerides, calcium and phosphorus by using standard kits. A metabolism trial of three days duration was conducted at the end of the feeding experiment using five birds from each treatment group to study the balance of nitrogen, calcium and phosphorus. The feed and droppings were analyzed for chemical composition to study the digestibility of nutrients. Data on total viable count and coliform count in the faecal sample were also recorded.

The average initial body weight of birds belonging to four dietary treatment groups T1, T2, T3 and T4 were 50.24, 50.24, 50.26 and 50.24 g, respectively. The average final body weight of birds belonging to four groups were 2010.0, 2079.8, 1924.0 and 1988.0 g, respectively and the corresponding cumulative body weight gain were 1959.8, 2029.6, 1873.7 and 1937.8 g, respectively at sixth week of age. The average daily gain was 46.66, 48.32, 44.61 and 46.14 g, respectively for the birds belonging to the groups T1, T2, T3 and T4. The results obtained in the present study showed significant difference among different treatment groups regarding the final body weight, cumulative body weight gain and average daily gain with a significantly higher ($p<0.05$) values observed for birds belonging to group T2 than group T3, while it was similar to that of groups T1 and T4.

The mean cumulative feed intake recorded for four treatments were 3575.3, 3409.4, 3739.2 and 3769.7 g, respectively at sixth week of age. The mean cumulative FCR was 1.82, 1.68, 2.00 and 1.95 respectively for birds of T1, T2, T3 and T4 groups at sixth week of age. There was no significant difference in the feed intake and FCR of birds maintained on four dietary treatments at first week of age. However, significant reduction ($p<0.05$) in the feed intake was observed for group T2 compared to other three treatment groups (T1, T3 and T4) during second, third and sixth weeks of age. Lowest feed intake was recorded for group T2, while it was highest for group T3 and T4 throughout the experiment period compared to control diet fed group. The birds maintained on dietary treatment T2 had a better ($p<0.05$) cumulative FCR at second, fourth, fifth and sixth weeks of age compared to other three dietary treatment groups (T1, T3 and T4). However, during the third week of age the FCR of group T2 differed from group T4, while it was similar to that of groups T1 and T3.

The data on per cent digestibility of four experimental rations T1, T2, T3 and T4 were 73.04, 78.61, 69.77 and 73.02 for dry matter (DM), 49.14, 53.46, 42.31 and 46.61 for crude protein (CP), 76.43, 84.13, 78.17 and 76.33 for ether extract (EE), 34.67, 44.79, 38.45 and 40.88 for crude fibre (CF) and 82.36, 85.61, 80.12 and 80.90

for nitrogen free extract (NFE), respectively. There existed significant difference ($p<0.05$) among the treatment groups with respect to digestibility of all the nutrients except CP. Supplementation of propionic acid (group T2) resulted in a significantly higher ($p<0.05$) digestibility of DM, EE, CF and NFE compared to other treatment groups. The energy efficiency observed for group T2 was significantly higher ($p<0.05$) compared to other three treatment groups. Moreover, the energy efficiency observed in group T3 was significantly lower compared to groups T1, T2 and T4.

The nitrogen balance for the experimental birds in groups T1, T2, T3 and T4 were 1.86, 2.31, 1.68 and 1.79 g per day, respectively and the statistical analysis of data revealed no significant ($p>0.05$) difference among different treatment groups.

The per cent availability of minerals in the four dietary rations T1, T2, T3 and T4 were 48.24, 52.77, 50.49 and 44.81 for calcium (Ca) and 56.98, 56.88, 53.87 and 55.60 for phosphorus (P), respectively and the values were statistically similar ($p>0.05$) indicating that supplementation of acidifiers did not influence the availability of Ca and P.

The serum lipid profile (mg per dl) of experimental birds belonging to the groups T1, T2, T3 and T4 were 99.67, 96.89, 98.40 and 96.61 for total cholesterol, 47.18, 55.73, 49.73 and 49.65 for HDL cholesterol and 50.92, 56.43, 54.58 and 77.54 for triglycerides, respectively. There was no significant difference among various treatment groups with regard to serum total cholesterol concentration. However, there was significant difference ($p<0.05$) among the four treatment groups for HDL cholesterol with significantly higher ($p<0.05$) values observed for propionic acid supplemented (T2) group. The serum concentration of triglycerides was significantly higher for organic acid blend (T4) supplemented group compared to other three treatment groups.

The serum mineral concentration (mg per dl) of birds maintained on different dietary treatments T1, T2, T3 and T4 were 10.08, 9.50, 9.92 and 10.02 for Ca and

4.61, 5.45, 4.91 and 5.02 for inorganic P, respectively and did not show any significant difference among the treatment groups. The serum total protein concentration of birds maintained on different dietary treatments T1, T2, T3 and T4 were 7.70, 8.64, 8.90 and 7.05 g per dl respectively. The statistical analysis revealed a significant difference among the four different treatment groups with a higher level of ($p<0.05$) total protein observed in group T3, which was similar to group T2. Furthermore, the group T4 had a significantly lower ($p<0.05$) total protein concentration compared to groups T2 and T3. However, a similarity was noted for group T1 compared to groups T2 and T4.

The slaughter data of birds belonging to four dietary treatments T1, T2, T3 and T4 were 2242.4, 2283.4, 2168.6 and 2215.0 g, respectively for live weight and 1594.2, 1638.4, 1524.2 and 1556.8 g, respectively for carcass weight. The giblet yield percentage and dressing percentage for birds maintained on dietary treatments T1, T2, T3 and T4 were 4.03, 4.42, 4.65 and 4.43 per cent, respectively for giblet yield and 71.04, 71.78, 70.20 and 70.21 per cent, respectively for dressing percentage. The weight of internal organs as percentage of carcass weight for treatment groups belonging to T1, T2, T3 and T4 were 0.60, 0.66, 0.71 and 0.87 for heart, 2.60, 2.88, 3.00 and 2.89 for liver, 2.48, 2.62, 2.92 and 2.55 for gizzard, 0.11, 0.15, 0.14 and 0.15 for spleen and 6.27, 6.33, 7.63 and 6.73 for intestine, respectively. There was no significant difference ($p>0.05$) among the birds in four different dietary treatments for slaughter data except for the weight of intestine, which was significantly higher in formic acid supplemented group (group T3) compared to control group (group T1) and propionic acid supplemented group (group T2).

The total viable count in the droppings of birds maintained on four dietary treatments T1, T2, T3 and T4 were 7.32, 6.20, 6.28 and 5.33 $\log_{10}$ cfu per g, and coliform count were 5.56, 5.51, 5.62 and 5.65 $\log_{10}$ cfu per g, respectively. Statistical analysis of total viable count and coliform count revealed that there was significant difference ($p<0.05$) among different treatment groups. The faecal total viable count lowered ($p<0.05$) with dietary supplementation of blend of organic acids (group T4). Moreover, supplementation of propionic acid at 0.2 per cent level (group T2) significantly reduced the faecal coliform count compared to groups T3 and T4 but similar to group T1.

The mean protein efficiency ratio (PER), energy efficiency ratio (EER) and production efficiency factor (PEF) for birds belonging to T1, T2, T3 and T4 were 2.62, 2.85, 2.39 and 2.49, respectively for PER, 16.05, 15.63, 14.76 and 15.86, respectively for EER and 244.91, 271.54, 199.37 and 229.17, respectively for PEF. There was significant difference ($p<0.05$) among different treatments for PER and PEF. A significantly higher PER ($p<0.05$) was observed in group T2 compared to other three groups. Furthermore, group T3 had significantly lower PER than groups T1 and T2, while it was similar to group T4. Propionic acid supplementation improved the PEF in broiler birds compared to formic acid supplementation. Moreover, group T4 had a similar PEF with respect to groups T1 and T3. The data on energy efficiency of birds maintained on different dietary treatments T1, T2, T3 and T4 were statistically similar.

During the course of the experiment three birds from group T2 and two birds from group T3 i.e., a total of five birds died out of 200 birds. The per cent livability recorded was 100, 94, 96 and 100 for groups T1, T2, T3 and T4, respectively. The cost of feed per kg live weight gain of birds was Rs. 57.90, 56.23, 66.25 and 67.85 for groups T1, T2, T3 and T4, respectively.

The results of this study indicated that dietary supplementation of 0.2 per cent propionic acid improved ($p<0.05$) the DM digestibility, FCR, PER and HDL cholesterol. Moreover, the cost of feed per kg body weight gain was reduced by Rs. 1.67 as a result of propionic acid supplementation. The supplementation of 0.2 per cent formic acid or 0.2 per cent blends of propionic acid and formic acid did not improve the growth performance and carcass characteristics in broiler chicken. From the overall results, it can be concluded that propionic acid at 0.2 per cent level can be recommended as a feed additive in broiler chicken.

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Abstract

DIETARY SUPPLEMENTATION OF FEED ACIDIFIERS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS IN BROILER CHICKEN

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(13-MVM-27)

ABSTRACT

Submitted in partial fulfillment of the requirement for the degree of

MASTER OF VETERINARY SCIENCE
(Animal Nutrition)
2015

Faculty of Veterinary and Animal Sciences
Kerala Veterinary and Animal Sciences University
Pookode, Wayanad

DEPARTMENT OF ANIMAL NUTRITION
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR – 680651
KERALA, INDIA

ABSTRACT

An experiment was conducted for a period of 42 days using two hundred, day-old straight run commercial broiler chicks (Ven Cobb) to study the effect of dietary supplementation of feed acidifiers on growth performance, nutrient utilization, gut microbial load and carcass characteristics. The birds were divided into four groups with five replicates of ten chicks in each replicate. The four dietary treatments consists of T1 (control ration as per BIS, 2007), T2 (control ration + propionic acid 2 g per kg of feed), T3 (control ration + formic acid 2 g per kg of feed) and T4 (control ration + propionic acid and formic acid each at 2 g per kg feed) using completely randomized design. Data on body weight, daily feed intake, feed conversion ratio, digestibility of nutrients, blood parameters (total protein, serum total cholesterol, HDL cholesterol, triglycerides, calcium and inorganic phosphorus) and carcass characteristics (live weight, carcass weight, giblet yield, weight of internal organs and dressing percentage) were used for the evaluation of work. The cost of production per kg body weight gain was also calculated.

The results obtained in the present study showed significant difference among different treatment groups regarding the final body weight, cumulative body weight gain and average daily gain with a significantly higher ($p<0.05$) values observed for birds belonging to group T2 than that of group T3, while it was similar to that of groups T1 and T4. Dietary supplementation with propionic acid significantly ($p<0.05$) improved the digestibility of all nutrients except crude protein. However, there was no significant difference among the different treatment groups for availability of Ca and P, nitrogen balance, energy efficiency ratio, serum total cholesterol and carcass characteristics. However, serum HDL cholesterol and triglycerides showed significant difference among the four treatment groups and better observations ($p<0.05$) were recorded in group T2. Dietary supplementation of acidifiers significantly reduced faecal total viable count and the lowest count was

observed in group T4. Moreover, propionic acid supplementation resulted in lower faecal coliform count compared to formic acid supplementation. Better protein efficiency ratio and production efficiency factor was also recorded with propionic acid supplementation. The feed cost per kg body weight gain for group T2 was lowest compared to other treatment groups. On summarizing the results, it can be concluded that propionic acid at 0.2 per cent level can be recommended as a feed additive in broiler chicken.

Academic form No.19

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY

Faculty of Veterinary and Animal Science

PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTER'S DEGREE

1. Title of thesis:

Dietary supplementation of feed acidifiers on growth performance and carcass characteristics in broiler chicken

2 (a) Title of the departmental / KVASU Research project of which this form a part:

Not applicable

(b) Code No. if any and order by which departmental/ KVASU Research project is approved:

Not applicable

3 (a). Name of the student:

Axsa P. Thomas

(b) Admission No. :

13-MVM-27

4(a) Name of the major advisor (Guide):

Dr. M.T.Dipu

(b) Designation:

Assistant Professor
Department of Animal Nutrition,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur -680651

5. Objective of the study:

Study the effect of dietary supplementation of propionic acid and formic acid as feed acidifiers on growth, nutrient utilization, gut microbial load, carcass traits and cost of production in broiler chicken

6. Practical /Scientific utility:

Poultry industry has emerged as the most dynamic and fast expanding segment of animal production in India. Antimicrobial feed additives were used as growth promoters in broiler ration for optimizing the production performance. However, the generalized use of antibiotics as feed additives has been severely restricted recently. This has lead to the use of feed acidifiers, probiotics and prebiotics as alternatives to maintain health and performance. Feed acidifiers are low molecular weight organic acids having specific antimicrobial activity which is pH dependent. Dietary acidifiers like propionic acid and formic acid inhibit growth of pathogenic bacteria, maintain beneficial microflora and helps in better nutrient digestibility. Moreover, the knowledge of synergistic effect of acidifiers in the bird is relatively few in literature. Hence, this research work is planned to evaluate the individual and synergistic effect of dietary supplementation of propionic and formic acids on growth performance and carcass characteristics in broiler chicken.

7. Important Publications on which the study is based:

Christian *et al*. (2004) observed that dietary inclusion of 0.3 per cent of organic acid mixture (70 per cent formic and 30 per cent propionic acids) increased live weight and average daily weight gain in broiler chicks compared to those fed with control diet.

Hernandez *et al*. (2007) observed a better feed conversion ratio and improved apparent ileal digestibility of dry matter in broiler chicken when formic acid was supplemented at 0.5 per cent level, compared to non supplemented group.

The supplementation of organic acid salts (ammonium formate and calcium propionate) each at 0.3 per cent level in the diet improved feed conversion ratio, increased intestinal villus height and better nutrient utilization in both treatment groups compared to control (Paul *et al*., 2007).

Al-Kassi and Mohssen (2009) reported that dietary supplementation of both 0.1 per cent formic acid and 0.2 per cent propionic acid increased average live weight, daily gain and feed consumption in Arbor-Acres broiler chicks compared to control.

Broiler chicks fed with a diet containing 0.2 per cent of propionic acid gained significantly more body weight than chicks fed a control diet (Khosravi *et al*., 2010).

Dietary inclusion of combination of 0.15 per cent ammonium formate and 0.15 per cent calcium propionate increased body weight in broiler chicken compared to control diet fed group (Roy *et al*., 2012).

8. Outline of technical programme:

Two hundred, day-old commercial broiler chicks will be used as the experimental birds. All the experimental birds will be wing banded and weighed individually before housing.

The birds will be randomly allotted to four treatment groups, with five replications of ten chicks each. The four dietary treatments are as follows:

Treatments	Ration
T1	Standard broiler ration (BIS, 2007)
T2	Standard broiler ration + Propionic acid 2g/ kg of feed
T3	Standard broiler ration + Formic acid 2g/kg of feed
T4	Standard broiler ration + propionic acid (2g/kg feed) + formic acid (2g/kg feed)`

All the birds will be fed as per standard broiler ration (BIS, 2007). Feed and water will be provided *ad libitum.* The birds will be reared under deep litter system of management. Body weight of the birds will be recorded at weekly intervals to study the growth rate. Feed intake of birds will be recorded replication wise at weekly intervals and feed conversion ratio will be calculated. Livability of birds will be recorded.

A metabolism trial of three days duration will be conducted after the feeding trial with four birds from each treatment group to study the balance of nitrogen, calcium and phosphorus. The feed and droppings will be analyzed for chemical composition (AOAC, 2012) to study the utilization of nutrients. Data on the total viable count and coliform count in the faecal sample will be recorded.

Four birds from each treatment will be randomly selected and slaughtered at six weeks of age to study the carcass traits.

Blood samples will be collected from four birds of each treatment groups at the time of slaughter and will be analyzed for total protein, triglycerides, total cholesterol and HDL cholesterol, phosphorus and calcium by using standard kits.

Cost of production will be worked out. Data will be analyzed statistically (Snedecor and Cochran, 1994).

9. Main Items of Observation to be made:

1. Body weight at weekly intervals
2. Feed consumption at weekly intervals
3. Blood parameters – total protein,

triglycerides, total cholesterol, HDL cholesterol, calcium and phosphorus.

4. Carcass traits
5. Chemical composition of feed and droppings
6. Faecal sample – total viable count and coliform count
7. Livability of birds

10. Facilities

a) Existing: Facilities available in the Department of Animal Nutrition and Center for Advanced Studies in Poultry Science, College of Veterinary and Animal Sciences, Mannuthy will be utilized for the study.

(b) Additional facilities required: Nil

-11. Duration of study:

Four semesters

12. Financial estimate:

Cost of feed / feed additives	= Rs. 10,000
Cost of birds and other Contingent expenditure	= Rs.10, 000
Total	= Rs. 20,000

Signature of the student:

Signature of the Major Advisor:

Place: Mannuthy

Date:

Name, designation and signature of Members of the Advisory Committee.

Chairperson

Dr. M. T. Dipu,
Assistant Professor,
Department of Animal Nutrition,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur - 680651.

Members

Dr. P. Gangadevi,
Professor and Head,
Department of Animal Nutrition,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur - 680651.

Dr. K. M. Syam Mohan,
Associate Professor,
Department of Animal Nutrition,
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Mannuthy, Thrissur - 680651.

Dr. S. Sankaralingam,
Assistant Professor and Head,
University Poultry and Duck Farm,
Mannuthy, Thrissur – 680651.

APPENDIX-I

References:

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APPENDIX-II

Time frame of work

Semester I

1. Collection of literature.
2. Planning of programme for research.
3. Preparation for synopsis.

Semester II

1. Literature collection.
2. Formulation of experimental feeds.
3. Conducting the experiment.

Semester III

1. Conducting of the experiment.
2. Collection of data.
3. Feed and faecal analysis.

Semester IV

1. Statistical analysis of data.
2. Preparation of thesis.
3. Submission of thesis

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to Animals Act (Amendment, 1998).

Place: Mannuthy Dr. M. T. Dipu

Date: Major Advisor

CURRICULAM VITAE

1. Name of candidate : Dr. Axsa P. Thomas
2. Date of birth : 12 – 03 – 1990
3. Place of birth : Kottayam
4. Marital status : Married
5. Permanent address : Puthenpurackal house
S H Mount P. O
Kottayam- 686006
6. Major field of specialization : Animal Nutrition
7. Educational status : Completed BVSc & AH in the year 2013 from
CV&AS, Mannuthy.
8. Professional experience : -
9. Publications made :

1.“Assessment of feeding practices and nutritional status of captive lion and tigers in Thrissur zoo.” in the proceedings of Kerala Veterinary Science Congress, 2014

2. “Lameness in dairy cattle: Nutritional approaches for prevention and management.” in the Journal of Indian Veterinary Association (JIVA).

3. “Nutritional strategies for environment friendly swine production.” In the compendium of 5th International Congress on Kerala Studies, 2015.

10. Membership of professional Societies : Kerala State Veterinary Council
Indian Veterinary Association
Indian Dairy Association