STUDY ON EVALUATION OF EXOGENOUS EMULSIFIERS IN ENERGY-RESTRICTED DIET FED BROILER CHICKEN

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By

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CERTIFICATE - I

This is to certify that the thesis entitled **"Study on Evaluation Of Exogenous Emulsifiers in Energy-Restricted Fed Broiler Chicken"** submitted in partial fulfillment of the requirement for the award of the degree of **Master of Veterinary Sciences** in the discipline of **Animal Nutrition** of the faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is a bonafide research work carried out by **Dr. AJIT SHEKHAR, Admission No.: VM0030/2019-20** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been fully acknowledge

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CERTIFICATE – II

This is to certify that the thesis entitled **" Study on Evaluation of Exogenous Emulsifiers in Energy-Restricted Fed Broiler Chicken"** submitted by **Dr. AJIT SHEKHAR,** Registration No**.: VM0030/2019-20,** to the Bihar Animal Sciences University, Patna in partial fulfillment of the requirements for the degree of **Master of Veterinary Sciences** in the discipline of **Animal Nutrition** has been approval by Student's Advisory Committee after an oral examination of the student in collaboration with External Examiner

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ABBREVIATIONS

ADF	-	Acid Detergent Fibre
ADG	-	Average Daily Gain
ALA	-	Alpha- Linoleic Acid
ALP	-	Alkaline Phosphatase
ALT	-	Alanine Transaminase
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemist
AST	-	Aspartate Transaminase
BIS	-	Bureau of Indian Standard
BUN	-	Blood Urea Nitrogen
BW	-	Body weigh
Ca	-	Calcium
CF	-	Crude Fibre
CP	-	Crude Protein
DM	-	Dry Matter
DMI	-	Dry Matter Intake
DTNB	-	5,5-dithiobis-(2-Nitrobenzoic Acid
EDTA	-	Ethylene Diamine Tetra Acetate
EE	-	Ether Extract
FAO	-	Food and Agricultural Organization
FCR	-	Feed Conversion Ratio
FI	-	Feed Intake
g	-	Gram
GSH	-	Glutathione
HA	-	Haemagglutination
Hb	-	Haemoglobin

HDL	-	High Density Lipoprotein
HI	-	Haemagglutination Inhibition
HSP	-	Heat Shock Protein
IU	-	International Unit
Kcal	-	Kilocalorie
Kg	-	Kilogram
LDH	-	Lactate dehydrogenase
LDL	-	Low Density Lipoprotein
LPO	-	Lipid peroxidation
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration
MCV	-	Mean Corpuscular Volume
ME	-	Metabolizable Energy
ml	-	Millilitre
Na	-	Sodium
ND	-	Newcastle Disease
NDF	-	Neutral Detergent Fibre
NDV	-	Newcastle Disease Vaccine
NFE	-	Nitrogen Free Extract
OM	-	Organic Matter
P	-	Phosphorus
PBS	-	Phosphate-Buffered Saline
PCV	-	Packed Cell Volume
PI	-	Performance index
RBC	-	Red Blood Corpuscles
ROS	-	Reactive Oxygen Species
SBM	-	Soybean Meal
SEM	-	Standard Error of Mean
SGOT	-	Serum Glutamic Oxaloacetic Transaminase

SGPT	-	Serum Glutamic Pyruvic Transaminase
SOD	-	Superoxide dismutase
SPSS	-	Statistical Packages for Social Science
TA	-	Total Ash
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TEC	-	Total Erythrocyte Count
TLC	-	Total Leucocytes Count
U/L	-	Unit PerLiter
VLDL	-	Very Low Density Lipoprotein
WBC	-	White Blood Cell
WHO	-	World Health Organization

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ABSTRACT

Present study was conducted to evaluate the effect of synthetic and herbal exogenous emulsifiers on growth performance, nutrients utilization, haemato-biochemical profile, antioxidant capacity and carcass quality in broiler chickens fed energy restricted diet during a 35-day feeding trial. A total of 180, day-old Cobb 400Y strain broiler chicks were locally procured, weighed individually and divided into four treatment groups (T_1: Standard basal diet without emulsifier (control), T_2: Basal diet with 3% less metabolisable energy, Treatment T_3:Basal diet with 3% less metabolisable energy + synthetic emulsifier @250 g/tonne of feed and T_4: Basal diet with 3% less metabolisable energy + herbal emulsifier@250 g/tonne of feed) each with three replicates of 15 chicks following completely randomised design. Growth performance of broilers in terms of weekly body weight gain, feed conversion ratio and performance index and economic return decreased ($P<0.05$) due to reduction in dietary energy content. Supplementation of either synthetic or herbal exogenous emulsifier enhanced($P<0.05$)growth performance and economics of broiler chicken.There was no significant ($P>0.05$) effect of exogenous emulsifiers on dry matter, crude protein, calcium and phosphorus retention in broiler chickens fed energy restricted diet, however, retention of ether extract retention improved significantly ($P<0.05$) due to supplementation of the emulsifiers. Haematological parameters viz. haemoglobin, packed cell volume, total erythrocyte count, mean corpuscular volume and mean corpuscular haemoglobin values in broiler chicks fed diets supplemented with emulsifiers in energy restricted diets didn`t differ significantly. Serum glucose, total protein, albumin and globulin, total cholesterol, triglycerides, HDL and LDL concentration (mg/dl) levels and serum enzymes *viz.*, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in broiler chickens did not differ significantly ($P>0.05$) due to supplementation of synthetic and herbal emulsifier in energy restricted fed broiler chicken. There was no significant ($P>0.05$) change in antioxidant status of serum in terms of activity of glutathione peroxidise (GSH), superoxide dismutase (SOD) and catalase (CAT) among different dietary treatments.The carcass characters (dressing yield, eviscerated yield, blood loss, feather loss, meat to bone ratio), relative organ weights (giblet, heart, liver and gizzard, spleen, bursa of fabricious), cut-up parts (thigh, drumstick, breast, back, wing and neck) among different dietary treatments were statistically similar ($P>0.05$). Birds fed diet having optimum energy had highest abdominal fat value, whereas, birds fed energy restricted diet had lowest abdominal fat value. Dietary treatments didn`t affect ($P>0.05$) appearance, flavour, tenderness, juiciness and overall acceptability of meat of broiler chicken. Similar response in all observed parameters indicated that herbal emulsifier was equally effective in utilization of fat in broiler chickens. Fromthe results obtained from present study, it can be concluded that decrease in 3% metabolisable energy of broiler chicken diet depressed growth performance, ether extract retention and economics of broiler chicken whereas, dietary supplementation of synthetic or herbal emulsifier @ 250 gm/tonne of energy restricted soyabean oil based broiler feed improved the growth performance, ether extract utilization and economics without affecting haemato-biochemical constituents, antioxidant activity and carcass quality of broiler chickens.

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1 INTRODUCTION

Poultry production is one of the most dynamic and fastest segments in animal husbandry sector in India. Rearing of poultry has showed tremendous change over past few decades in terms of nutrition, genetics and management. Nutrition plays vital role in supporting the desired growth and production performance of birds.

Current intensive poultry farming has necessitated dense, highly nutritive rations and the dwindling supply of conventional feedstuff has forced mankind to search an alternative source of feed and fodder (Rameshwari *et al*., 2005). Energy is a major cost component factor in diets for high performance animals, such as broilers. The global demand for high energy source feed stuff for use in animal nutrition is increasing but the energy source like cereal sand grains has limited resource. To meet the energy requirement of poultry, commercial poultry feeds are commonly supplemented with fats and oils to provide high energy density to support growth performance (Blanch *et al.*, 1996) and enhanced productivity in layers and breeders. Dietary fats and oils provide 2.25 times more energy than carbohydrate and they are also supplier of essential fatty acids and fat soluble vitamins. Fats are also added because of their effect on physical properties of feed like the reduction of dustiness and reduced particle separation in mash diet. However, the addition of high levels of saturated fat to broiler rations may result in excessive visceral and carcass fat, inadequate vitamin A and E and poorer meat quality (Chae *et al.*, 2006; Zulkifli *et al.*, 2007). In recent years, because of the ever-increasing energy costs, there is greater interest in maximising the use of supplemental fats as nutritionists strive to increase the dietary energy density to meet the requirements of high-performing birds.

Fat digestion in poultry occurs mainly in the small intestine. Fats and oils are broken down into diglycerides, monoglycerides and fatty acids with the help of bile salts, lipases, deoxycholates, phospholopids, etc. For birds to utilize fat, they have to digest and absorb fats and oils from gastro intestinal tract. It has been reported that fat absorption increases with bird age, as young broilers have a physiological limitation to absorb that nutrient (Jeason and Kellog, 1992; Nir *et al.*, 1993; Lima *et al.*, 2003). The inability of young birds to absorb fat efficiently has been attributed to its poor

emulsification ability and poor lipase activity; however, these biological processes improve with the age and adapt to cope with higher unsaturated fatty acids (Meng *et al.*, 2004).

Fats are insoluble in water and cannot be solubilised in the gastrointestinal tract, and have to be emulsified before lipolytic enzymes can digest them. Emulsification is the process of breaking down of the large fat globules into smaller globules and makes them water soluble. An emulsion is a mixture of two products such as oil and water that do not mix together i.e. that are immiscible. An emulsifier is a substance that stabilises an emulsion and prevents the coalescence of the globules of the dispersed phase. An emulsifier is a blend of hydrophobic tail and hydrophilic head. Adding an emulsifier to the mixture causes the oil to be broken down into smaller pieces that can then be dispersed throughout the water. An emulsifier is able to bridge between water soluble and fat soluble material. Emulsifiers facilitate the formation of emulsion droplets, which lowers the surface tension (Ashraf, 2007), stimulates the formation of micelles, causes high levels of monoglycerides in the intestine and facilitates the nutrient transport through the membrane (Melegy *et al.*, 2010). Emulsification depends on the characteristics of the fat such as chain length, position of fatty acids on the triglycerides and fat saturation (Gu and Li, 2003). The emulsifier also helps to increase the digestibility of long chain of fatty acids and hence may be economic value because both animal fat and some vegetable fat contain these long chain fatty acid.

Bile salts are natural emulsifiers. Poultry produces emulsifier in the form of bile salts however it is sometimes insufficient in view of added fats and oils at higher levels. The utilization of dietary fats in young birds is poor because they have a limited capacity to produce and secrete bile salts and lipase until their gastrointestinal tract matures at 10-14 days of age (Noy and Sklan, 1998).When levels of emulsifier are less than optimum in relation to the diet, fats are not fully digested and energy is wasted. Emulsifiers help to improve the utilisation of lipids, particularly animal fats, and play a role in performing the insufficiencies of naturally low bile production and recirculation in young birds (Siyal *et al.*, 2017).The use of emulsifiers in poultry diets have increased feed efficiency, lipids absorption, growth performance and modified the lipids present in the blood (Udomprasert and Rukkwamsuk, 2006).It was reported that addition of fat emulsifier in vegetable oil based broiler diets resulted in an

improved weight gain (Luc *et al.*, 2013; Gaiotto *et al.*, 2001; Wongsuthavas *et al.*, 2007; GuerreiroNeto *et al.*, 2011). Hence, addition of emulsifier from external source becomes obligatory in poultry feed.

Commercial emulsifiers which are usually used in the feed industry can be categorized into two groups *viz.* natural emulsifier and synthetic emulsifiers. Natural emulsifier ones are those produced in the animal body such bile and phospholids, and those from food materials such as soylecithin (Soares and Lopez-Bote, 2002), whereas synthetic emulsifiers are modified emulsifiers such as lysolecithin or lysophosphatidylcholine (Zhang *et. al.*, 2011).Bile salts are natural emulsifiers. Young birds have inadequate portions of bile salt production so fat digestibility is poor in the early stage of life (Siyal *et al.*, 2017). Exogenous supply of bile salts in broiler chicken diet improved growth performance, enzymes activity of intestinal tract and metabolisable energy, and decreased plasma cholesterol (Kussaibati *et al.*, 1982). Supplementation of bile salts in poultry diets increased fat digestion but this may not be economically viable (Al-Marzooqi and Leeson, 1999). Natural emulsifiers like soylecithin obtained from soyabean cause absorption of fatty acids into micelles and improve fat digestion in chicks (Polin, 1980), resulting in improved growth performance and decrease in LDL level in broiler chicken (Huang et. al., 2007; Siyal *et al.*, 2017). Lecithin structure contains choline which is also effective in preventing perosis in birds (Schaible, 1970). Similarly, exogenous supply of synthetic emulsifiers like polyethylene glycol mono and dioleates and sodium stearoyl-2-lactylate in poultry diets improves body weight, feed intake and utilization efficiency of fat, protein and metabolisable energy (Jones *et al.*, 1992; Roy *et al.*, 2010; Yordan *et al.*, 2013).

Thus, exogenous emulsifiers can be used to improve fat digestibility and energy efficiency. The addition of an effective emulsifier to a diet can compensate for a reduction in dietary energy. As a result, lower energy diets can be formulated for birds whilst maintaining the same performance, leading to lower feed cost and more economical and sustainable production (Siyal *et al.*, 2017). There are many reports available on evaluation of natural and synthetic exogenous emulsifiers in poultry and swine diets. However, there is lack of research data on use of comparative efficacy of natural and synthetic emulsifiers in broiler chickens. Therefore, the present study is planned for efficacy evaluation of exogenous natural and synthetic feed emulsifier in

energy-restricted feed of broiler chicken for economic poultry production with the following objectives:

Objectives of Investigation:

- To study the effect of exogenous emulsifiers on growth and performance parameters in energy-restricted fed broiler chicken
- To study the effect of exogenous emulsifiers on retention of nutrients in energy-restricted fed broiler chicken
- To study the effect of exogenous emulsifiers on hemato-biochemical parameters in energy-restricted fed broiler chicken
- To study the effect of exogenous emulsifiers on carcass characteristic in energy-restricted fed broiler chicken

2 REVIEW OF LITERATURE

Feed accounts for 65- 70% of broiler production cost, thus, feed cost deserves befitting attention. Energy is a major cost component factor in diets of broiler chickens. Due to its high energy density, fats and oils are important energy sources in feed formulation. Fat provides more than twice energy than either protein or carbohydrates on equal weight basis. Fat also provides essential fatty acids which have crucial importance in making prostaglandins which help in combating infections. Fat is not just a source of energy but much more than energy, it helps in digestion and absorption of fat soluble vitamins.

Fat digestion in poultry occurs mainly in the small intestine. Fats and oils are broken down into diglycerides, monoglycerides and fatty acids with the help of bile salts, lipases, deoxycholates, phospholopids etc. Fatty acids passes through the liquid phase of the small intestine and, after aggregating to form micelles, are absorbed as hydrophobic components. This process is naturally mediated by endogenous emulsifiers, such as bile salts. Bile salts are natural emulsifiers. During the first week post-hatch, young chicks have limited bile and lipase secretion and therefore are not able to break down lipids effectively (Upadhaya *et al.*, 2017). The physiological inability of the GIT to utilize dietary lipids effectively can be aided in the use of dietary emulsifiers. Emulsifiers are used to act as a catalyst to break down dietary fats and to enhance the action of lipase during lipid hydrolysis (Upadhaya *et al.*, 2018). The use of emulsifiers also encourages the chick's lipase enzymatic activity in lipid digestion thereby making lipid absorption more efficient.

2.1 Emulsifier:

Emulsifiers by definition are surfactive substance that acts on the surface between two media that are considered immiscible (e.g. water and oil) (Tan *et al.*, 2016). The dietary lipids consumed by animals are insoluble in the aqueous environment of their gastrointestinal tract and require the action of bile and lipase for lipid digestion (Siyal *et al.*, 2017). The mode of action of emulsifiers is to increase the active surface of fats, allowing the action of lipase, which hydrolyze triglyceride molecules into fatty acids and monoglycerides. Emulsifiers facilitate the formation of

emulsion droplets, which lowers the surface tension (Ashraf, 2007), stimulates the formation of micelles, causes high levels of monoglycerides in the intestine and facilitates the nutrient transport through the membrane (Melegy *et al.*, 2010). An emulsifier breaks the fat globules into small micelles, which are easily digested, absorbed and assimilate into the system, resulting in availability of extra metabolizable energy to the birds.

Benefits of use of an emulsifier –

- Improves digestibility of a fat thereby optimising utilization of energy of fat/ oil
- Improves the bioavailability of both water soluble and fat soluble nutrients
- Prevents wastage of fat/oil through indigestion
- Prevents fat build up and minimizes abdominal fat
- Improves feed conversion ratio and overall performances
- Helps extract maximum nutritional value from high density rations

Thus, emulsifiers can be used to improve fat digestibility and energy efficiency. As a result, lower energy diets can be formulated for birds whilst maintaining the same performance, leading to lower feed cost and more economical and sustainable production.

2.2 Types of Emulsifier:

Different types of emulsifiers have been used in animal feed and include 1,3 diacylglycerol (Upadhaya *et al.*, 2017), polyethylene glycol riconoleate (Tan *et al.*, 2016), lysophospholipids (Zampiga *et al.*, 2016), Liprex (Aguilar *et al.*, 2013) and Lysoforte booster (Melegy*et al.*, 2010). There are two types of emulsifier.

i. Natural emulsifier
ii. Synthetic emulsifier

2.2.1 Natural emulsifiers:

Bile salts are natural endogenous emulsifiers responsible for the emulsification of fats into triglycerides and phospholipids in the duodenum tract of broilers (Doreau and Chilliard, 1997) and also play an important role in improving the action of lipase for the hydrolysis of lipids into triglycerides and monoglycerides needed for micelle

production (Upadhaya *et al.*, 2018). When supplemented in the diet of broilers, bile salts improved the average daily gain and improved the final weight of broilers when compared to the control (without bile salts) (Lai *et al.*, 2018). Another common natural emulsifier used to improve broiler performance is lecithin. Lecithin are naturally occurring emulsifiers consisting of phosphatidylcholine with different fatty acids that can include oleic, stearic and palmitic acids and are commercially produced from plant oils seeds (sunflower and soybean oil) or can be of animal origin (milk, brain tissue and egg yolk) (Oke *et al.*, 2010). When provided in the diet of broilers, the supplementation of lecithin improved lipid digestion (Woodgate and Van der Veen, 2014), improved feed intake, improved daily gain and growth (Siyal *et al.*, 2017) and have also shown to regulate fat metabolism in broilers (Huang *et al.*, 2008). Proteins such as caseins and whey proteins have been used for many decades as emulsifiers in the emulsion of food products such as milk, ice cream and various dairy products (Kralova and Sjöblom, 2009). Casein is another naturally occurring polymeric emulsifier (Dickinson, 1993) and is commonly found in bovine milk (constitutes about 80% of milk protein) (Kralova and Sjöblom, 2009). When supplemented as a feed additive in broiler production, it has shown to improve weight gain, FCR and improved pancreatic lipase activity (GuerreiroNeto *et al.*, 2011). Globin is another naturally occurring protein emulsifier used as a feed additive in broiler production. It is a protein-based emulsifier that contains active hydrophilic protein and is made from porcine blood during red cell fractionation and has similar properties to that of soy lecithin (Dabbou *et al.*, 2019). When used as an emulsifier in the diet of broilers, improvements in fat digestibility, protein metabolism, FCR and in the net energy production were found.

2.2.2 Synthetic emulsifiers:

There are many synthetic emulsifiers which are used in poultry feed. Sodium stearoyl-2-lactylate (SSL) is a synthetic emulsifier and is a sodium salt consisting of a long chain carbolylic acid with two esters linkages with a very high hydrophilic-lipophilic balance and is a good fat-in water emulsifier (Cho *et al.*, 2012). Sodium stearoyl-2-lactylate is formed by the esterification of stearic acid with lactic acid which is then neutralized to form sodium salt (Gheisar *et al.*, 2015). When added to the diet of broilers, improvement in feed conversion ratio and in the digestibility of

energy and nitrogen where found (Gheisar *et al.*, 2015). Furthermore, when added to a diet of low energy improvements in the average daily gain were also found to the same level as that of diets with a high energy level (Cho *et al.*, 2012).

Diacylglycerol (DAG) is another synthetic emulsifier consisting of 70% medium chain fatty acids and 30% fatty acids (Upadhaya *et al.*, 2017). The amphiphilic ability of DAG is responsible for its ability to take-up free fatty acids that are not efficiently broken down by bile salts (Dierick and Decuypere, 2004). When added to the diet of broilers, improvement in FCR, ADG and dry matter digestibility were found (Upadhaya *et al.*, 2017). Lysophosphatidylcholine (LPC), also known as lysolecithins, is derived by enzymatic conversion of lecithin (Jansen *et al.*, 2015). Lysolecithins are considered better emulsifier agents when compared to bile due to its emulsification capacity (Zhang *et al.*, 2011). There are various types of lysolecitihins depending on the lecithin source and can include soybean and rapeseed lecithin (Jansen *et al.*, 2015), from sunflower seeds and from animal sources of which can include milk, eggs and brain tissue (Oke *et al.*, 2010). When used in the diet of broilers, lysolecithins were able to improve the digestibility and the energy of broiler feeds containing saturated fat sources (Jansen). Furthermore, improvements in the FCR and in fat absorption were found in broilers when supplemented in their diets irrespective of fat type (Khonyoung *et al.*, 2015).

EFFECTS OF USE OF EMULSIFIERS IN POULTRY:

2.3 Growth Performance

2.3.1 Body weight gain

Jones *et al.* (1992) determined whether emulsifiers improve utilization of fat from diets for early-weaned pigs. In Exp. one, 96 weanling pigs (17 days old) were used in metabolism cages, with main effects of fat source (soybean oil, tallow, lard and coconut oil) and emulsifier treatment (no emulsifier, lecithin, and lysolecithin as 10% of the added fat). They observed that pigs fed soybean oil gained weight faster than pigs fed the other treatments ($P < 0.06$), and pigs fed tallow without emulsifiers had the lowest average daily gain (ADG). Considering all experiments, addition of emulsifiers increased digestibility of nutrients but had minimal effect on growth performance.

Azman *et al.* (2004) investigated the effects of soybean lecithin (SL) as substitute for soybean oil (SO) or beef tallow (BT) added in broiler diets on growth performance. Four hundred 5-day-old chicks were divided into 4 equal groups receiving eventually SL-supplemented starter diets for 17 days and grower diets for 14 days. SO was gradually replaced by SL in diets with a rate of 0% (control group, n = 100), 25% (SL1 group, n = 100), 50% (SL2 group, n = 100). Another group (TL group, n = 100) received a dietary fat mixture containing 50% BT and 50% SL. Although birds presented comparable body weights at the end of experiment, a slight increase of daily weight gain was observed in SL1 group during the growing period while this parameter was reduced in TL group in the same period. Even if decreases of food intake were noticed during starter and grower periods and for all the treatment duration in SL2 group, no significant improvement of food conversion ratio was obtained. On the contrary, during growing period and during all the treatment period, food intake was significantly enhanced in TL group, leading to significant increases of food conversion ratio.

Ferreira *et al.* (2005) conducted experiment to determine the energy values of soyabean oil, beef tallow and their blends (0:100; 25:75; 50:50; 75:25 and 100:0), and to evaluate the effect of inclusion of 6.0% of these blends on performance parameters and carcass characteristics of broilers. During the performance experiment, the fat sources did not influence the performance and carcass characteristics evaluated.

Arnouts *et al.* (2006) studied the effects of Globin (produced by hydrolysis of porcine haemoglobin into haem and globin, is a water-soluble protein with a strong emulsifying capacity) on broiler chickens. Goblin was included @ 0.05% in broiler diets containing a combination of soya oil (S) and palm fat (P) on average daily growth (ADG) and feed conversion ratio(FCR). Three hundred male broiler chickens (Ross 308) were equally divided in 10 pens of 30 birds (5 pens per treatment; 2 treatments: Control (C) and Globin (G). They concluded that addition of 0,05% of globin from starter to finisher did not affect average daily growth but clearly reduced feed conversion ratio with 2,9%.

Haldar *et al.* (2010) reported that reduction in dietary energy content may result in significant improvement in feed conversion by lowering the feed intake under the influence of nutritional emulsifiers. These improvements in FCR indicate

the nutritional emulsifiers compensate for an energy reduction in broiler diets without reducing growth parameters.

Roy *et al.* (2010) studied the effects of an exogenous emulsifier, glyceryl polyethylene glycol ricinoleate, on performance and carcass traits of broiler chickens were assessed. The emulsifier was added to the diet at dose rates of 0 (control), 1 (E1) and 2 (E2) % of added fat (saturated palm oil). Live weight gain and feed conversion ratio in 39 days were higher in the E1 dietary group. Gain: ME intake and gain:protein intake during the grower phase improved quadratically ($P < 0.05$). Gross carcass traits were not affected. Body fat content and fat accretion increased ($P < 0.05$) and liver fat content decreased ($P < 0.05$) linearly with the level of emulsifier in diet. Fat excretion decreased ($P < .001$) leading to increased ileal fat digestibility ($P < .06$) in the E1 group (quadratic response). Metabolizable intake of N and fat increased quadratically due to supplementation of emulsifier in diet. Metabolism of trace elements and serum lipid profiles were not affected. The study revealed that supplementation of exogenous emulsifiers in diets containing moderate quantities of added vegetable fats may substantially improve broiler performance

Gaiotto *et al.* (2001) evaluated less expensive fat sources as alternatives to soybean oil in broiler diets. A total of 1,440 day-old male Ross chicks were raised to 42 days of age in a randomized block design of six treatments and six replicates, fed diets containing 4% supplemental fat from the sources: soybean oil (SOY4), beef tallow (TAL4), acidulated soapstock (SOAP4), mixtures 2%:2% (SOAP2/TAL2), (SOAP2/SOY2) and (SOY2/TAL2). Live weight, weight gain and feed:gain of soybean oil were better ($p<.05$) than those devoid of soybean oil in the diet, but feed intake, and viability did not differ. The mixtures containing 2% soybean oil resulted in performance similar to soybean oil in all variables ($P>.05$) and soybean oil in the mixture equally improved the results of the alternative sources. The performance of birds fed acidulated soapstock was inferior to those fed mixtures of acidulated soapstock, soybean oil (25%) ($P<.06$) but was similar to those fed with mixture of beef tallow acidulated soapstock, soybean oil. The abdominal fat did not differ among the treatments, but abdominal fats reflected the composition of the different fats. These results confirmed the superiority of soybean oil relative to the other fat sources fed to broiler and demonstrated that the quality of acidulated soapstock and beef tallow may be improved when used in 1:1 mixture with soybean oil.

GuerreiroNeto *et al.* (2011) studied the effect of the addition of an emulsifier to diets containing soybean oil, poultry fat or their blend, on the performance, carcass traits, serum lipid levels, pancreatic lipase concentration and nutrient digestibility of broilers in randomized block design with three fat sources (soybean oil, poultry fat, and a blend of 50% soybean oil and 50% poultry fat) and the addition or not of an emulsifier. Broilers fed the diet containing soybean oil and emulsifier presented higher body weight, weight gain and better feed conversion ratio. When birds were fed poultry fat and the fat blend (soybean oil and poultry fat) and the emulsifier was added to the diets, pancreatic lipase concentration increased. Soybean oil, poultry fat and their blend does no in the diet does not influence the performance, carcass traits, or serum cholesterol, HDL and triglyceride levels of 42-day-old broilers. The addition of emulsifiers to diets containing poultry fat improves ether extract digestibility and increases the production and secretion of pancreatic lipase.

Patra *et al.* (2011) conducted an experiment to assess the effects of different sources of fats added with an external emulsifier (lecithin) on the performances of Khaki Campbell Ducks in an eight-week trial. The ducks were fed with a basal diet supplemented with 3% soybean oil and without emulsifier (C1), 3% palm oil without emulsifier (C2), 3% soybean oil with emulsifier (T1), 3% palm oil with emulsifier (T2) and 3% lard with emulsifier (T3). Feed intakes by ducks were also similar ($P > 0.1$) among treatments within the periods. Similarly, feed intakes to gain ratios were not affected by any dietary treatments. They concluded that supplementation of lecithin as an emulsifier to the diets containing different sources of fats (3%) appears to have no major impact on the overall performances of Khaki Campbell ducks in their grower phase

Cho *et al.* (2012) conducted a trial to determine the effects of emulsifier and multi-enzyme in different energy density diet on growth performance. A total of five hundred and forty two day old male Ross broilers were used in a 35-d experiment and randomly divided into 5 treatment groups: 1) NC [low energy diet, 3% tallow, ME = 3000 (1 to 21 d) and 3100 kcal/kg (22 to 35 d)], 2) PC [high energy diet, 5.5% soybean oil, ME = 3150 (1 to 21 d) and 3250 kcal/kg (22 to 35 d))], 3) P2 (NC + 0.05% emulsifier), 5) P3 (NC + 0.1% multi-enzyme + 0.05% emulsifier). Multi-enzymecontained α-galactosidase, galactomannase, xylanase, and β-glucanase. They concluded that emulsifier andmulti-enzymein low-density diets can partially improve

growth performance, triglyceride, and relative organ weight in broiler chickens, which can counterpart the negative effects caused by the decreased nutrient concentration.

Kaczmarek *et al.* (2015) conducted a trial in completely randomised design study with a 2 × 2 factorial arrangements was conducted to observe effects of an emulsifier (glyceryl polyethylene glycol ricinoleate (GPR)) on nutrient utilisation and performance of broiler chickens. A total of 384 male broiler chickens were used to determine the influence of GPR (without addition or added at 0.04% of diet) and two levels of apparent metabolisable energy (AMEN) (according to standard requirements (Diets SE) or energy reduced by 0.4 MJ/kg diet (Diets LE)) on birds' performance and apparent total tract digestibility (ATTD). During the grower period and the whole trial, birds fed diets supplemented with GPR were characterized by higher body weight gain (BWG) and lower feed conversion ratio (FCR) compared to chicken receiving diets without GPR ($P< 0.05$). At the end of experiment, birds fed Diet LE without GPR were characterized by lower BWG and higher FCR.

Zampiga *et al.* (2016) conducted an experiment to evaluate productive performance, nutrient digestibility and carcass quality traits of broiler chickens fed diets supplemented with an exogenous emulsifier based on lysophospholipids prepared by enzymatic conversion of soy lecithin. One thousand seven hundred and fifty-five one-day-old male Ross 308 chicks were randomly divided into three experimental groups of nine replications each: control group (CON) fed a corn–soybean basal diet, and two groups fed CON diet supplemented with constant (1 kg/ton) or variable (1–1.5 kg/ton) level of emulsifier (CONST and VARI, respectively). At the end of the trial (42 d), birds receiving the emulsifier had a statistically significant ($p < 0.05$) lower feed conversion rate compared to the control. Body weight and daily weight gain were only slightly influenced by lysophospholipids supplementation, while mortality and feed intake resulted similar among the groups. No statistically significant effect of the emulsifier was observed on nutrient digestibility as well as slaughtering yields, skin pigmentation and incidence of foot pad dermatitis. The results obtained in this study suggested that the use of an emulsifier based on lysophospholipids improves feed efficiency while showed limited effect on carcass quality traits.

Bontempo *et al.* (2018) studied the effect of different doses of emulsifier on 600 one-day-old ROSS 308 broiler chicks for 44 days. Control diet (CTR) or diet

supplemented with emulsifier (AVI-MUL TOP) @ 1 g/kg from day 0 to 12, 0.75 g/kg from day 12 to 22 and 0.5 g/kg from day 22 to 44. They observed that emulsifier supplementation to broiler chicks had beneficial effect on growth performances and carcass dressing yield.

Dabbou *et al.* (2018) evaluated the effect of dietary Globin on the energy efficiency and digestibility of starter feeds and on the production performance of broilers throughout the whole rearing cycle. A total of 224-day-old ROSS 708 chickens (14 birds/pen, 8 replicates/treatment) were fed ad libitum with either a basal diet (C) or a basal diet with the addition of 0.05% Globin during the starter (d1–10), growing (d10–25) and finisher (d25–35) periods. Globin significantly decreased FCR and increased nutrient digestibility of fat and net energy for production during the starter period. The overall performance was similar between groups, although Globin tended to increase PER overall.

Yun *et al.* (2018) conducted a research trial to evaluate the effect of supplementation of sodium stearoyl-2-lactylate as fat emulsifier in low-density diet on the growth performance and meat quality of finishing pigs. A total of 84 mixed-sex finishing pigs [(Landrace × Yorkshire) × Duroc] at 112 d of age with an average body weight (BW) of 60 ± 0.75 kg were used in a 56 d experiment. The following three treatments were used (1) control basal diet (T1), (2) low-energy diet (T2), and (3) T2 + 0.1% sodium stearoyl-2-lactylate emulsifier (T3). The supplementation of sodium stearoyl-2-lactylate as fat emulsifier in energy-reduced diet did not have significant effects on growth performance compared with energy-reduced diet without emulsifier, although it slightly increased final BW by 1.45%, average daily gain by 3.3%, gain to feed ratio by 3.77%, and reduced average daily feed intake by 0.64%.

Dabbou *et al.* (2019) conducted a trial on 224-day-old ROSS 708 chickens divided into either a basal diet (C) or a basal diet with the addition of 0.05% Globin during the starter (1–10 days), growing (10–25 days) and finisher (25–35 days) periods. The average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and protein efficiency ratio (PER) were measured of each growth period. Globin significantly decreased FCR and increased overall PER.

Hu *et al.* (2019) conducted a 2-factor test design was used to investigate the effect of an emulsifier (Aldo®, Lonza, America) (200g/t) in the diet of Cherry Valley meat ducks to replace some of 2 different oils (animal fat and vegetable oil) on meat

production performance, slaughter traits, and fat metabolism. The 900 healthy 18-day-old ducks were grouped into 6 treatments, each with 5 replicates and 30 meat ducks per replicate. The 2 fat sources were established as a positive control group, a negative control group (positive control group-some oil (equivalent to metabolic energy of 50kcal/ton)), and an emulsifier group (negative control group + 200g/ton Aldo). The results showed that addition of different fat sources in feed had no significant effect on growth performance, carcass properties, and fat metabolism of 18- to 42-day-old meat ducks. Reducing the amount of oil used in the feed lowered the growth performance, carcass properties, and affected fat metabolism of meat ducks.

Kulkarni *et al.* (2019) evaluated two emulsifiers Orffa energizer-01 (OE1) and Orffa energizer-02 (OE2) s for diets with two different oil sources, soybean oil and rice bran oil on the performance on 240, Cobb-430 broiler chicks for six weeks. T1 received control diet (without emulsifier) prepared with soybean oil and T2 received control diet (without emulsifier) prepared with rice bran oil. The chicks from group T3 and T5 received feed containing soybean oil with addition of OE1 and OE2, respectively @ 350 g/Ton in each of the diets. Whereas, the chicks from T4 and T6 received feed containing rice bran oil with addition of OE1 and OE2, respectively @ 350 g/Ton in each of the diets. Both emulsifiers showed on both vegetable oils a significant positive effect on body weight gain over the whole period and both emulsifiers improved both the energy metabolizability and feed conversion ratio. Hence, it is concluded that the effect of emulsifier EO2 on rice bran oil is higher than the effect on soybean oil. Rice bran oil with OE2 resulted in the highest body weight gain, feed intake and feed conversion ratio in broiler diet. The combination of rice bran oil with OE2 emulsifier was found the best supplement for the growth performance followed by soybean oil with OE2.

An *et al.* (2020) conducted an experiment to study the effects of exogenous emulsifier supplementation @0.1% and 0.2% on growth performance, energy digestibility, and meat quality in broiler chicks. They concluded that the addition of 0.1% exogenous emulsifier to broiler feed lowered the FCR and improved the growth performance. Also, the addition of 0.2% exogenous emulsifier improved meat quality by increasing the water holding capacity and decreasing the shearing force of broiler breast meat.

Saleh *et al.* (2020) conducted a trial to investigate the influence of emulsifiers on broilers fed low-energy diets, the birds were distributed into three sets: the control was fed the basal diet, the second group was fed diets 50 kcal/kg less than control, and the third group was fed diets 50 kcal/kg less than control and supplemented with 500 g/ton of emulsifiers. The used mixture of exogenous emulsifiers contains phosphatidyl choline, lysophosphatidyl choline, and polyethylene glycol ricinoleate. Although the feed intake was not meaningfully affected by dietary low-energy level with emulsifier inclusion, the weight gain and FCR were clearly enhanced. Protein and lipids utilization were decreased by reducing energy level, but they were increased by emulsifier supplementation. The mixture of emulsifier supplementation to low-energy diets enhanced fat utilization and resulted in positive effects on the growth performance, nutrient utilization, lipid peroxidation, and modified plasma lipid profiles in broilers

Srinivasan *et al.* (2020) studied about the efficacy of crude soya oil and emulsifier in ration on production performance of broilers. A 90 day- old Vencobb 320 broiler chicks were randomly divided into 3 treatment groups. Treatment groups were fed with basal diet contains crude soya oil as energy source (T1), basal diet supplemented with fat emulsifier at the rate of 250 g per metric tonne of feed (T2) and 80 kcal energy reduced from basal diet supplemented with emulsifier at the rate of 250 g per metric tonne of feed (T3).They concluded that inclusion of emulsifier at the rate of 250 g / MT of feed containing crude soya oil with 80 kcal reduced metabolic energy improved body weight and feed conversion ratio at market age

2.3.2 Feed intake

Kussaibati *et al.* (1982) conducted an experiment to evaluate the effect of fat and emulsifier on broiler chicken for 11 days and concluded that there was no effect of dietary fat and exogenous fat emulsifier on feed intake during all phases of broiler life.

Melegy *et al.* (2010) investigated responses to dietary fortification of natural bio surfactant using broiler performance, serum chemistry and carcass traits. A total of 996 day old broiler chicks (Cobb 500 strain) were randomly assigned into four groups and three replicates per group with 83 birds per replicate. Group I, (negative control) was fed a lower nutrient density test diet without Lysoforte Booster®

(lysolecithin). While, group II, (positive control) was fed the basal diet (corn-soybean meal based), recommended by breed catalogue without Lysoforte Booster®. However, groups III and I were fed the negative control diet with Lysoforte Booster at a rate of 250 and 500 g/ton of feed on top, respectively. Results showed significantly ($P < 0.05$) lowest final weight, lowest weight gain, poorest FCR and highest feed intake in group I in comparison to the positive control and the Lysoforte Booster® supplemented groups. The negative control also had a significant ($P < 0.05$) higher mortality rate compared to the Lysoforte Booster supplemented groups.

Udomprasert *et al.* (2006) conducted a trial involving 1,885 weanling pigs (24 d of age) were conducted to determine the effect of exogenous emulsifier, glyceryl polyethyleneglycolricinoleate (Bredol®). These results demonstrated that addition of Bredol® to diets for weanling pigs improved growth performance and reduced deterioration of fat in the diets

Rahman *et al.* (2010) investigated the effect of dietary inclusion of 2, 3, 4 and 5% of palm oil on feed consumption, growth performance and profitability of broiler. Addition of 4% palm oil in diet found to attribute additive effect on the growth of the bird from 2nd to 4th week of the trial. Broilers of 2 and 3% oil added group attained 1791g and 1777.67g live weight, respectively which was 4% and 3% higher than that of the control. Similar effect of different levels of palm oil was also observed in case of live weight gain and dietary inclusion of palm oil improved feed consumption and significantly ($p<0.05$) higher feed consumption was recorded in 4% palm oil group than 5% oil group.

Aguilar *et al.* (2013) studied the growth performance of broiler chicks fed with an exogenous emulsifier and increasing levels of energy provided by palm oil on broiler chicks. The treatments consisted of three increasing levels of metabolizable energy (ME) of 12.13 MJ/kg (starter) and 12.80 MJ/kg (finisher) (T1); 12.38 MJ/kg (starter) and 13.05 MJ/kg (finisher) (T2), 12.64 MJ/kg (starter) and 13.51 MJ/kg (finisher) (T3); and the supplementation of an exogenous emulsifier to liquid dose of 0.5 ut on diets (T4) with the same ingredients and contributions that the T1. Mortality and feed intake were not affected ($P>0.05$) by the experimental diets in any experimental period. However, the T3 increased the body weight (2477.37g) and decreased feed conversion ratio (1.845) with significant differences ($P>0.05$) between treatments.

Mandalwi *et al.* (2015) studied the effect of the inclusion of raw glycerine (GLYC) and lecithin in the diet on egg production. Egg quality and total tract apparent retention (TTAR) of dietary components was studied in brown egg laying hens from 23 to 51 wk of age. The experimental design was completely randomized with six diets combined as a 2x2 factorial with two levels of GLYC(0-70g/kg) and three animal fat lecithin rations (40:0, 20:20 and 0:40g/kg).each treatment was replicated eight times and the experimental unit was a cage with ten hens. Production was recorded by replicate every 28- day period and cumulatively. The replacement of animal fat by lecithin (40:0, 20:20 and 0:40 g/kg) increased ($P<0.05$) weight and egg mass production and feed conversion ratio per kilogram of eggs, however, feed intake, egg production and body weight gain were not affected.

Abbas *et al*. (2016) conducted an experiment to evaluate the effect of fat emulsifier on fat utilization in broiler chickens using 240 days old chicks for 35 days. Experimental diets were formulated using 1, 2 or 3% fat with or without fat emulsifier (Lecithin) at 350 mg/kg. The result revealed that feed intake was not affected ($p>0.05$) by fat and fat emulsifier. Also, increasing level of dietary fat supplementation did not affect ($p<0.05$) starter, finisher and overall feed intake.

Kulkarni *et al.* (2019) evaluated two emulsifiers Orffa energizer-01 (OE1) and Orffa energizer-02 (OE2) for diets with two different oil sources, soybean oil and rice bran oil on the performance of broilers chicks for six weeks. T1 received control diet (Without emulsifier) prepared with soybean oil and T2 received control diet (Without emulsifier) prepared with rice bran oil. The chicks from group T3 and T5 received feed containing soybean oil with addition of OE1 and OE2, respectively @ 350 g/ton in each of the diets. Whereas, the chicks from T4 and T6 received feed containing rice bran oil with addition of OE1 and OE2, respectively @ 350 g/Ton in each of the diets. They reported that supplementing emulsifier in the diet prepared with different oil sources like soya bean oil and rice bran oil as fat source, the feed intake was comparable among different treatment groups during staring phase (0-3 week) but significant increase in feed intake was observed during finishing (3-6 weeks).

Srinivasan *et al. (*2020) studied the efficacy of crude soya oil and emulsifier in ration on production performance of broilers. A 90 day- old Vencobb 320 broiler chicks were randomly divided into 3 treatment groups. Treatment groups were fed with basal diet contains crude soya oil as energy source (T1), basal diet supplemented

with fat emulsifier at the rate of 250 g per metric tonne of feed (T2) and 80 kcal energy reduced from basal diet supplemented with emulsifier at the rate of 250 g per metric tonne of feed (T3) The cumulative feed intake of birds fed with or without emulsifier revealed that no significant difference in feed intake noticed between treatment groups at 6^{th} week of age, but numerically lowered feed intake was observed in T3 and T2 groups.

2.3.3 Feed Conversion ratio (FCR)

Panja *et al*. (1995) observed the effects of varying palm oil levels (0 to 8%) in the diets on performance of broilers under the warm humid tropical environment. Increasing palm oil levels resulted in corresponding rise in the dietary energy concentrations, and broilers fed on higher energy diets improved feed and energy consumption, daily weight gain and feed conversion ratio.

Zollitsch *et al*. (1997) reported that there was a significant effect of fat and fat emulsifier on FCR in broilers fed diets containing a blend of different fats supplemented with choline.

Azman and Ciftci (2004) investigated the effect of replacing dietary fat with lecithin on broiler chicken zoo technical performance. Broiler birds were divided in 4 equal groups and fed with starter diets containing 4% soybean oil (control) and soybean lecithin mixtures (in proportion of 75/25 for SL1 group and 50/50 for SL2 group) or 4% of beef tallow and soybean lecithin mixtures. It was observed that body weights in groups supplemented with lecithin at 21 days were not significantly different from those of control group, but during grower period (from 22nd to 35th day) a moderate and significant increase of daily weight gain was obtained in group supplemented with soybean oil and soya lecithin mixture in proportion 75/25. It was also observed highest body weight after 35 days in same group.

Kim *et al*. (2008) evaluated the effect of dietary lecithin with or without chitooligosaccharide (COS) on the performance, blood metabolites, pork cholesterol, fatty acid composition and quality of finishing pigs. In exp. 1, 36 pigs (Landrace Yorkshire Duroc, 84.590.60 kg initial body weight) were fed lecithin at 0, 2.5 or 5.0% of the diet. It was observed that, lecithin improved average daily gain and FCR, however, addition of 2.5 and 5% lecithin resulted in 15.6 and 16.2% greater body

weight gain of pigs and decreased the FCR by 7.2 and 14.3%, respectively, when compared with those fed diets without lecithin.

GuerreiroNeto *et al.* (2011) studied the effect of the addition of an emulsifier to diets containing soybean oil, poultry fat or their blend, on the performance, carcass traits, serum lipid levels, pancreatic lipase concentration and nutrient digestibility of broilers. A randomized block design was applied using a 3 x 2 factorial arrangement, with three fat sources (soybean oil, poultry fat, and a blend of 50% soybean oil and 50% poultry fat) and the addition or not of an emulsifier reported that there was no effect of fat and fat emulsifier interaction on overall FCR in broiler however, starter FCR was affected. Increasing FCR with increasing fat level in starter might be attributed to no lipase activity in starter phase resulting in lower absorption of fats and as a result lower energy absorption from iso-caloric.

Mandalwi *et al*. (2014) studied the effect of the inclusion of raw glycerine (GLYC) and lecithin in the diet on egg production, egg quality and total tract apparent retention (TTAR) of dietary components was studied in brown egg laying hens from 23 to 51 wk of age. The experimental design was completely randomized with six diets combined as a 2x2 factorial with two levels of GLYC(0-70g/kg) and three animal fat to lecithin rations (40:0,20:20 and 0:40g/kg). The replacement of animal fat by lecithin (40:0, 20:20 and 0:40 g/kg) didn`t affect feed intake.

Abbas *et al*. (2016) conducted an experiment for 35 days to evaluate the effect of fat emulsifier on fat utilization in broiler chickens using 240 days old chicks which were divided into 24 replicates in a 3×2 factorial arrangements under Completely Randomized Design (CRD). Experimental diets were formulated using 1, 2 or 3% fat with or without fat emulsifier (Lecithin) at 350 mg kgG1. Starter and overall FCRs were found unaltered ($p>0.05$) however, finisher FCR was significantly affected ($p<0.05$) with combination of fat and fat emulsifier in diets. Fat emulsifier did not affect ($p>0.05$) starter and overall FCR but finisher FCR was affected ($p<0.05$). Increasing dietary fat levels had no significant effect ($p>0.05$) on finisher and overall FCR however, starter FCR increased with increasing fat levels ($p<0.05$)

Bontempo *et al*. (2018) studied effect of emulsifier AVI-MUL TOP (AMT) at 1 g/kg from day 0 to 12, 0.75 g/kg from day 12 to 22 and 0.5 g/kg from day 22 to 44 in broiler chicks and reported that FCR was lower when fed AMT emulsifier from 22nd day to 44th day.

2.4 Nutient Utilization:

Jones *et al*. (1992) carried out series of experiments to study effect of exogenous emulsifiers and fat sources on nutrient digestibility in weanling pigs. It was observed that when lecithin was added as 10% of added fat (soybean oil) nitrogen digestibility was increased from 87.4% to 90.4% likewise when lysolecithin was added as 10% of added fat (soybean oil) nitrogen digestibility was increased from 87.4% to 88.4%

Huang *et al*. (2007) examined the effects of different soy-oil and soy-lecithin levels nutrient utilization in broiler chickens. Two hundred and forty 1-day-old Arbor Acres chicks were randomly divided into 4 groups and treated as follows: basal diet with 2% soy-oil (SO); soy-oil and soy- lecithin mixture in proportion of 75/25 (SOL1), 50/50 (SOL2) and 2% lecithin (SL). At the end of the trial (42 d),it was observed that the utilization of ether Extract was improved in SOL1 group ($p<0.05$) but apparent metabolizable energy (AME) and utilization of other nutrients decreased in SOL2 and SL group from 19 to 21 days. No significant effects were observed in apparent metabolizable energy, dry matter, crude protein and ether extract but the utilization of calcium and phosphorus was significantly improved (70.42%, 65.09%), respectively, in SL group ($p<0.05$) during 39 to 42 d.

Price (2007) studied effect of diet physical form, fatty acid chain length and emulsification on improving fat utilization in weaned pigs. He stated that digestibility of tallow as fat was increased from 80.9% to 88.4% when lecithin was included in diet while digestibility was increased slightly, by 3 units when lyso-lecithin was included in tallow containing diet.

Kim *et al.* (2008) observed the effect of lecithin with or without chitooligosaccharide on the nutrient digestibility in pigs. Two experiments were conducted to evaluate the effect of dietary lecithin with or without chitooligosaccharide (COS) on the performance, fatty acid composition and quality of finishing pigs. There was no effect on apparent nutrient digestibility in pigs

Zampiga *et al.* (2016) evaluated nutrient digestibility of broiler chickens fed diets supplemented with an exogenous emulsifier based on lysophospholipids prepared by enzymatic conversion of soy lecithin. birds treated with the emulsifier showed no significant effect on digestibility of dry matter (62.9 and 62.5% vs. 59.7%,

respectively for CONST, VARI and CON), crude fat (61.5 and 62.1 vs. 59.4%, respectively) and crude protein (61.3 and 60.6 vs. 60.5%, respectively

Siyal *et al.* (2017) studied the effect of SL (soya lecithin) on nutrient utilization in broilers. Broiler birds were divided in three groups as follow: the first group was fed a basal diet (BD) without emulsifier; the second and third groups were led basal diet supplemented with 0.05 (SL0.05) and 0.1% (SLO. 10) of SL, respectively. On day 21, digestibility of dry matter (86.609 %) and ether extract (78.559 %) and protein (77.048 %) in chicken fed diet with SLO. 10 was significantly improved in comparison with those fed SL0.05 and control.

2.5 Haemato-biochemical Parameters:

Jones *et al.* (1992) conducted a series of experiments to study effect of exogenous emulsifiers and fat sources on serum lipids in weanling pigs. In one experiment fat sources like soybean oil, coconut oil, tallow and lard were added as 10% of the diet, lecithin and lysolecithin were added as 10% of added fat. It was observed that pigs fed lecithin had lower serum triglycerides of (50.0 mg/dl, 70.3 mg/dl, 66.4 mg/dl and 63.7 mg/dl respectively, and cholesterol of (93.0 mg/dl, 109.1 mg/dl, 99.8 mg/dl and 94.0 mg/dl respectively than pigs fed lysolecithin.

Lechowski *et al.* (1999) carried out an experiment to observe the effect of lecithin supplementation on the biochemical profile of rats fed different animal fats. Levels of supplementation were not specified in article It was observed that total serum cholesterol concentration decreased significantly ($P<0.05$) from 1.79 to 1.5 nmol/L after lecithin supplementation in rats fed with control diet.

Spilburg *et al.* (2003) studied the effect of fat free foods supplemented with soy stanol-lecithin powder on cholesterol absorption and LDL cholesterol in 16 females and 8 males. Throughout the study subjects consumed daily a beverage containing formulated soy stanols (1.9 g). It was observed that soy stanol lecithin reduced total serum cholesterol by 10.1%, HDL and LDL cholesterol by 10.2 % and 14.3% respectively. Concluded that powdered soy stanol-lecithin when consumed in fat free diets lowers cholesterol absorption.

Huang *et al.* (2007) conducted a study to examine the effects of different soy-oil and soy-lecithin levels on growth performance, nutrient utilization and serum parameters in broiler chickens. Two hundred and forty 1-day-old Arbor Acres chicks

were randomly divided into 4 groups and treated as follows: basal diet with 2% soy-oil (SO); soy-oil and soy-lecithin mixture in proportion of 75/25 (SOL1), 50/50 (SOL2) and 2% lecithin (SL) The birds fed with lecithin had lower serum total cholesterol and triglyceride than the control group (SO). Broilers fed with 2% lecithin (SL) had the highest insulin level ($p<0.05$). The results implied that soy-lecithin and soy-oil in a proportion of 25:75 had the highest growth performance and that soy-lecithin had cholesterol lowering capacity.

Melegy *et al.* (2010) investigated the responses to dietary fortification of natural biosurfactant using broiler serum chemistry. A total of 996day old broiler chicks (Cobb 500 strain) were weighed individually and randomly assigned into four groups and three replicates per group with 83 birds per replicate. Group 1, (negative control) was fed a lower nutrient density test diet without Lysoforte Booster (lysolecithin). While, group II, (positive control) was fed the basal diet (corn-soyabean meal based), recommended by breed catalogue without Lysoforte Booster. However, groups III and IV were fed the negative control diet with Lysoforte Booster@ at a rate of 250 and 500 g/ton of feed on top. It was observed that serum metabolic profile was not significantly affected by dietary fortification with lysoforte booster.

GuerreiroNeto *et al.* (2011) studied the effect of the addition of an emulsifier to diets containing soybean oil, poultry fat or their blend, on the serum lipid levels, pancreatic lipase concentration of broilers in randomized block design with three fat sources (soybean oil, poultry fat, and a blend of 50% soybean oil and 50% poultry fat) and the addition or not of an emulsifier. When birds were fed poultry fat and the fat blend (soybean oil and poultry fat) and the emulsifier was added to the diets, pancreatic lipase concentration increased. Soybean oil, poultry fat and their blend does not in the diet does not influence the serum cholesterol, HDL and triglyceride levels of 42-day-old broilers. The addition of emulsifiers to diets containing poultry fat improved ether extract digestibility and increases the production and secretion of pancreatic lipase.

Cho *et al.* (2012) studied the effect of emulsifier (Prosol® containing sodium stearoyl-2-lactylate. and multienzyme (alpha-galactosidase, galactomannase, xylanase, and beta-glucanase) in different energy density diet on blood profiles in broiler chickens. A total of five hundred and forty two-days-old male Ross broilers

were used in a 35-days experiment and randomly divided into 5 treatment groups: 1) NC [low energy diet, 3% tallow, ME =15 3000 (1 to 21 days) and 3100kcal/kg (22 to 35 days)], 2) PC [high energy diet, 5.5% soybean oil, ME 3150 (1 to 21 days) and 3250 kcal/kg (22 to 35 days)], 3) P1 (NC + 0.1% multi-enzyme). 4) P2 (NC + 0.05% emulsifier), 5) P3 (NC + 0.1% multienzyme + 0.05% emulsifier). No differences were observed on white blood cell (WBC), red blood cell (RBC) and glucose concentration.

Elkhair *et al.* (2015) evaluated dried vegetable fat blend with emulsifier and yeast culture or their combination on some biochemical parameters of broiler chickens. A total of 150 (Cobb) day old chicks were divided into five groups of 30 birds each. The treatments were: control group received no supplement (CON), basal diet with dried fat (T1), dried fat with 250 g ton - 1 of emulsifier (T2), dried fat with 3 g kg -1 of yeast culture (T3), dried fat with emulsifier and yeast culture (T4) they observed that T1 recorded higher serum cholesterol (235.6 mg/dl), triglycerides (69.51 mg/dl). LDL (145.58 mg/dl) and VLDL (31.60 mg/dl) was comparable to T3 and T4.

Wang *et al.* (2016) evaluated the effects of dietary supplementation of emulsifier and carbohydrate on the breast meat fatty acids profile of broiler chickens a for 35-days experiment and were randomly divided into five treatments (1) NC(low energy); (2) (high energy diet); (3) P1 (NC+0.1%carbohydrase); (4) P2 (NC+0.05%emulsifier); and (5) P3 (NC+0.1% carbohydrases + 0.05% emulsifier). It was observed on days 35, serum total cholesterol (115.2 mg/dl, 128.9‘mg/dl) and low density lipoprotein cholesterol concentration (46.2 mg/dl, 62.3 mg/dl) were higher ($p<0.05$) and high density lipoprotein cholesterol was lower (67.96 mg/dl, 66.4"mg/dl) ($p< 0.05$) respectively in NC and P2 treatments than in PC, P2 and P3 treatments.

Gheisari *et al.* (2017) investigated the effect of supplementing saturated and unsaturated fat sources on serum metabolites in the diets of broiler chickens. A total of 360 day-old male broiler chicks (Ross 308) were used in a completely randomized design with five treatment and six replicates of 14 chicks. The diets were prepared by applying basal diet with no supplemented fat and the addition of soybean oil (SO), Lecithinized palm oil (LPO), a 50 : 50 mix of SO and LPO (ESL), and 75 : 25 mix of SO and LPO (HSL) ratios to the basal diet. The inclusion levels of experimental fats were 2% and 4% in the starter and growing periods, respectively. On day 41 Chickens

led LPO added diets had substantially greater serum triacylglycerol_(85.55 mg/dl) and very low density lipoprotein concentrations (10.13 mg/dl) compared with those that received other dietary treatments (P <0.05).

Siyal *et al.* (2017) conducted feeding trial to study the effectof SL on improvement of poultry performance and nutrient utilization. Broiler birds were divided in three groups as the first group was fed a Basal Diet (BD) without emulsifier; the second and third groups were fed basal diet supplemented with 0.05 (SL0.05) and 0.1% (SLO.10) of SL, respectively. It was observed at 21 and 42 days old broiler, cholesterol (2.309 mmol LG), triglyceride (0.45 mmol LG) and low density lipoprotein concentration (0.75mmol LG) were decreased in SL.0.10 group in comparison with control.

Zhao and Kim (2017) observed the effect of diets with different energy and emulsifier (Lipidol, active ingredient: lysophospholipids; LPL) levels on lipid profile in broilers. A total of 864 one- day-old male Ross 308 broilers (45.3 + 0.6 g) were used in a 28-day experiment. Broilers were allotted to a 2 x 3 factorial arrangement design with 2 levels of energy (starter: ME = 2,950 kcal/kg for energy reduced diet and 3,050 kcal/kg for basal diet; finisher: ME - 3,100 kcal/kg for energy reduced diet and 3,200 kcal/kg for basal diet) and 3 levels of emulsifier supplementation (zero, 0.05, and 0.10%) according to their initial BW. It was observed on d 14, the concentration of HDL, LDL total cholesterol, and triglycerides was not influenced by energy. However, the (LDL cholesterol - 26.6. 24.8 and 22.6 mg/dl), (total cholesterol 118.6, 115.4 and 112.4mg/dl) and (triglyceriodes=87.0, 84.0 and 84.6 mg/dl) concentration were decreased (p<0.05) by LPL supplementation.

Bontempo *et al.* (2018) studied on total of 600 one-day-old ROSS 308 broiler chicks were assigned to 2 experimental groups consisting of 15 pens with 20 birds/ per pen each, to compare the different dietary treatments: control diet (CTR) or diet supplemented with emulsifier AVI-MUL TOP (AMT) at 1 g/kg from day 0 to 12, 0.75 g/kg from day 12 to 22 and 0.5 g/kg from day 22 to 44, AMT dietary supplementation increased total cholesterol (P = 0.02) and HDL cholesterol (P = 0.02) plasma concentrations.

2.6 Carcass characteristics

Roy *et al.* (2010) conducted research study explaining nutritional emulsifier as an innovative approach to enhance productivity in broilers. The diet was supplemented with exogenous synthetic emulsifier at a dose rate of 2% of added fat. Carcass weight was found to be increased by almost 6% and breast meat yield was increased by more than 8% in emulsifier supplemented group

Collins *et al.* (2011) conducted a dose-response study by the Pork CRC during 2009 to assess the impact of lecithin concentration on pork tenderness (0, 4, 20 or 80 g lecithin/kg diet). Diets were fed to individually housed finisher gilts for a period of six weeks prior to slaughter. The results showed an increase in carcass weight (76.8kg) and dressing percentage (78.5%) associated the lecithin supplementation. Following slaughter, meat quality assessment found differences in the colour of the loin due to lecithin supplementation. It was concluded that dietary lecithin increased carcass weight and dressing percentage of pigs housed in groups

GuerreiroNeto *et al.* (2011) studied the effect of the addition of an emulsifier to diets containing soybean oil, poultry fat or their blend, on the performance, carcass traits, serum lipid levels, pancreatic lipase concentration and nutrient digestibility of broilers in randomized block design with three fat sources (soybean oil, poultry fat, and a blend of 50% soybean oil and 50% poultry fat) and the addition or not of an emulsifier. Soybean oil, poultry fat and their blend in the diet did not influence the performance, carcass traits, or serum cholesterol, HDL and triglyceride levels of 42-day-old broilers.

Patra *et al.* (2011) conducted an experiment to assess the effects of different sources of fats added with an external emulsifier (lecithin) on the performances of Khaki Campbell Ducks in an eight-week trial. The ducks were fed with a basal diet supplemented with 3% soybean oil and without emulsifier (C1), 3% palm oil without emulsifier (C2), 3% soybean oil with emulsifier (T1), 3% palm oil with emulsifier (T2) and 3% lard with emulsifier (T3). Various carcass traits such as percentages of hot carcass, breast, legs, lungs, hearts, gizzard, giblets weight relative to bodyweights did not vary ($P > 0.1$) among the groups. The carcass yield tended ($P = 0.06$) to be greater in the T1 than in the C1 group. The moisture, fats, protein and ash composition of meat (percent on fresh basis) was similar ($P > 0.1$) among treatments.

Cho *et al.*(2012) conducted an study to determine the effects of emulsifier and multi-enzyme in different energy density diet on five hundred and forty2-d-oldmale Ross broilers in a 35-d experiment and randomly divided into 5 treatment groups: 1) NC [low energy diet, 3% tallow, ME = 3000 (1 to 21 d) and 3100 kcal/kg (22 to 35 d)], 2) PC [high energy diet, 5.5% soybean oil, ME = 3150 (1 to 21 d) and 3250 kcal/kg (22 to 35 d))], 3) P1 (NC+0.1% multi-enzyme), 4) P2 (NC + 0.05% emulsifier), 5) P3 (NC + 0.1% multi-enzyme + 0.05% emulsifier). Multi-enzyme contained α-galactosidase, galactomannase, xylanase, and β-glucanase. Emulsifier was a commercial product named Prosol® which was sodium stearoyl-2-lactylate.The relative weight of the spleen was significantly decreased ($P < 0.05$) in P3 treatment compared with PC treatment. Furthermore, the relative weight of the bursa of Fabricius in P3 treatment was higher ($P < 0.05$) than PC, P1 and P2 treatments.

Aguilar *et al.* (2013) determined the carcass traits of broiler chicks fed with an increasing levels of energy provided by palm oil (12.13, 12.80, 12.38, 13.05, 12.64 and 13.51MJ/kg) and the supplementation of an exogenous emulsifier to liquid dose of 0.5 lt on640 one-day-old male Ross 308® broilers. The treatments did not influenced on the carcass, breast and abdominal fat weight and yield, breast meat colour ($P>0.05$). Thus, the exogenous emulsifier had no effect on productive indicators and lipid profile in broiler and showed similar results to T1 and T2; the T3 with more ME and oil palm showed the results best.

Ayed *et al.* (2015) examined the effect of fatty acid compositions of oils (soybean and palm oils) on the performance of broilers. At the end of trial, the carcass fat content was higher in all treated groups (Group2 = 8.362 %, Group3 = 7.79 %) compared to the control (Group1=5.43%).

Abbas *et al.* (2016) conducted to evaluate the effect of fat emulsifier on fat utilization in broiler chickens using 240 days old chicks which were divided into 24 replicates in a 3×2 factorial arrangements under Completely Randomized Design (CRD). Experimental diets were formulated using 1, 2 or 3% fat with or without fat emulsifier (Lecithin) at 350 mg/kg. The trial lasted for 35 days and was divided into starter and finisher phases. Fat emulsifier supplementation did not affect ($p>0.05$) liver, spleen and gizzard weight however, heart weight was affected ($p<0.05$) and gizzard weight decreased ($p<0.05$) linearly with increasing fat level.

Nagargoje *et al.* (2016) conducted on four hundred commercial day old (Vencobb) broiler chicks for 42 days to evaluate the effect of crude soy lecithin with or without lipase on carcass traits and keeping quality of meat of broiler chickens. The birds were divided into four dietary treatments with four replicates of 25 birds each. The edible carcass yield percent was found significantly ($P<0.05$) higher in lecithin alone or lipase enzyme supplemented groups (Group C=76.006 % and Group D=77.642 %) respectively.

Wang *et al.* (2016) studied the effects of dietary supplementation of emulsifier and carbohydrate on the breast meat fatty acids profile of broiler chickens a total of 540 2-day old male Ross 308 broilers were used in 35-days experiment and were randomly divided into five treatment: (1) NC (low energy); (2) PC (high energy diet) ; (3) P1 (NC+0.1% carbohydrase); (4) P2 (NC+0.05% emulsifier); and (5) P3 (NC+0.1% carbohydrates + 0.05% emulsifier). From days 0 to 35, abdominal fat weight was heavier ($p<0.05$) in PC, P2 and P3 treatments than in NC and P1 treatments.

Zampiga *et al.* (2016) evaluated carcass traits of broiler chickens fed diets supplemented with an exogenous emulsifier based on lysophospholipids prepared by enzymatic conversion of soy lecithin. One thousand seven hundred and fifty-five one-day-old male Ross 308 chicks were randomly divided into three experimental groups of nine replications each: control group (CON) fed a corn-soybean basal diet, and two groups fed CON diet supplemented with constant (1 kg/ton) or variable (1-1.5 kg/ton) level of emulsifier (CONST and VARI, respectively). At the end of the trial (42 d), birds, treated with the emulsifier showed no differences among the experimental groups regarding the eviscerated yield (68.4, 68.7 and 68.3% respectively for CON, CONST and VARI), as well as for the percentage of breast (30.1, 30.2, 30.1%), legs (43.1, 43.1, 43.1%) and un separated wings (19.1, 19.2 and 19.3%).

Bontempo *et al.*(2018) studied on total of 600 one-day-old ROSS 308 broiler chicks were assigned to 2 experimental groups consisting of 15 pens with 20 birds/ per pen each, to compare the different dietary treatments: control diet (CTR) or diet supplemented with emulsifier AVI-MUL TOP (AMT) at 1 g/kg from day 0 to 12, 0.75 g/kg from day 12 to 22 and 0.5 g/kg from day 22 to 44. AMT supplementation modified carcass and meat characteristics, increasing dressing percentage and yellowness compared to control group.

Yun *et al.* (2018) conducted a research trial to evaluate the effect of supplementation of sodium stearoyl-2-lactylate as fat emulsifier in low-density diet on the growth performance and meat quality of finishing pigs. A total of 84 mixed-sex finishing pigs [(Landrace × Yorkshire) × Duroc] at 112 d of age with an average body weight (BW) of 60 ± 0.75 kg were used in a 56 d experiment. The following three treatments were used (1) control basal diet (T1), (2) low-energy diet (T2), and (3) T2 + 0.1% sodium stearoyl-2-lactylate emulsifier (T3). The supplementation of sodium stearoyl-2-lactylate as fat emulsifier in energy-reduced diet did not have significant effects on growth performance compared with energy-reduced diet without emulsifier, although it slightly increased final BW by 1.45%, average daily gain by 3.3%, gain to feed ratio by 3.77%, and reduced average daily feed intake by 0.64%. The supplementation of emulsifier in energy-reduced diet did not have any adverse effect ($P > 0.05$) on meat quality attributes, as well as back fat thickness and lean muscle percentage (LMP), compared with energy-reduced diet without emulsifier or basal diet.

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3 MATERIALS AND METHODS

An experiment on broiler chicken was carried out to discern the effect of study on evaluation of exogenous emulsifiers in energy-restricted fed broiler chicken on production performance, nutrient utilization, haemato-biochemical, antioxidant status, and carcass characteristics in broiler chicken. The experiment was conducted at Instructional Livestock Farm Complex, Bihar Veterinary College, Patna, Bihar.

In this chapter, a brief description of experimental design, selection and management of experimental birds and analytical techniques followed during the study have been presented here under:

3.1 Experimental birds

For the experimental feeding trial one hundred and eighty, day- old Cobb 400Y strain broiler chicks were procured locally. All the chicks were individually weighed and wing banded. Thereafter, chicks were randomly allotted to four treatment groups each with three replicates of 15 chicks following completely randomised design (CRD) in such a way that average body weight was approximately similar for all the treatment groups.

3.2 Housing and feeding management

All the broiler chicks were housed in a deep litter system and provided *ad libitum* feed and water throughout the feeding trial. All the chicks were provided proper lighting throughout the experimental period. The experiment was conducted at Livestock Farm Complex of Bihar Animal Sciences University, Patna, Bihar, India during the month of February- March 2021. A layer of 4-5 cm rice husk was spread as litter material. The standard vaccination schedule was followed during the feeding trial. Standard managemental practices were adapted identically to all treatments during the entire experimental period of 35 days. Proper ventilation and free from dust condition was maintained throughout the experimental feeding trial. The experiment was conducted strictly in accordance with the guidelines of 'Institutional Animal Ethics Committee (IAEC)', Bihar Veterinary College, Patna, India. Weighed amount of the experimental feed was offered daily at 8.00 A.M to ensure *ad libitum* feeding at

all the time, but taking care to avoid spillage and wastage of feed. Fresh and clean water was always made available in suitable water troughs to all the birds during the study period.

3.3 Experimental feeds

Feed ingredients, supplements and feed additives in required quantities for formulation of experimental diets were procured from the local market nearby Patna, Bihar, India. Standard basal diets for pre-starter (0-7 days) starter (8-21 days) and finisher (21-35 days) phases of growth of broiler chickens were prepared by mixing the different ingredients (Table 1) to meet the nutrient requirements of broiler chicken as per recommendation of (BIS 2007). Standard basal ration T1 (control) contained all nutrients as per specification of BIS (2007) whereas T2 (Negative control) had 3% less metabolisable energy (kcal/kg feed) as compared to T_1. Treatment group T1 and T2 didn't contain exogenous feed emulsifier. Treatment group T3 and T4 were supplemented with synthetic feed emulsifier (Volamel) of Nukamel,Industriekade, the Netherlands, and herbal emulsifier (AV/PEE/15) of Ayurvet Limited, Katha, Baddi, Solan, India @ 250 g per ton of feed, respectively. Proximate composition, phosphorus (AOAC, 2007) and calcium (Talapatra *et al.* 1940) contents of the feed ingredients used in the experiments were determined following standard techniques. Calculated value of metabolisable energy, available phosphorus, lysine, methionine was used to balance the ration to meet the nutrients by the broiler chickens. Feed costs were calculated on the basis of local price of individual feed ingredients used in the ration.

There were 4 dietary treatment groups as follows:

Group	Description of treatments	No. of birds/ replicate	No. of replicates	Total No. of birds
T1	Standard basal diet(Table 1)	15	3	45
T2	Basal diet with 3% less energy	15	3	45
T3	Basal diet with 3% less energy + synthetic emulsifier @250 g/tonne of feed	15	3	45
T4	Basal diet with 3% less energy + herbal emulsifier@250 g/tonne of feed	15	3	45

Table-1: Composition of experimental ration

Ingredients (kg/tonne of feed)	Pre-starter (0-7 days)		Starter (8-21 days)		Finisher 22-35 days)	
	T1	T2	T1	T2	T1	T2
Maize	587	535	645	607	687	647
Soya meal	310	300	240	230	205	195
Mustard Deoiled Cake	15	15	20	20	18	20
Maize Gluten Meal	20	20	20	20	25	25
Rice Polish	14.75	17.75	18.8	20.8	14.3	51.3
Deoiled Rice Bran	0	58	0	53	0	18
Soya oil	7	7	15	7	15	8
Common salt	2.2	3.1	1.8	2.6	1.5	2.4
Monocalcium Phosphate	10.2	10.2	0.5	0.5	6.3	6.3
Limestone Phosphate	16.4	16.4	0.5	0.5	12.6	12.6
Sodium Bicarbonate	2.4	2.4	0.1	0.1	2.2	2.2
Vitamin Premix	0.5	0.5	0.7	0.7	0.5	0.5
Trace mineral Premix	0.5	0.5	7.8	7.8	0.5	0.5
Vitamin E	0.2	0.2	15.2	15.2	0.1	0.1
Lysine Sulphate	5.7	5.8	2.4	2.4	0.8	0.8
DL Methionine	3.2	3.2	5.6	5.8	0.5	0.5
L Threonine	1.4	1.4	2.7	2.7	1	1
Liver tonic	0.5	0.5	1.35	1.35	0.4	0.4
Choline chloride	0.9	0.9	0.4	0.4	0.15	0.15
Toxin binder	1.0	1.0	1.0	1.0	0	0
Firbre degrading enzyme	0.5	0.5	0.5	0.5	5.7	5.8
Coccidiostat	0.5	0.5	0.5	0.5	2.3	2.3
Phytase enzyme	0.15	0.15	0.15	0.15	1.15	1.15
Nutrients composition						
Dry matter (%)	90.18	90.14	90.18	90.22	90.26	90.28
Crude protein (%)	23.02	22.96	21.68	21.72	20.41	20.57
Metabolizable energy (kcal/kg)*	3002	2908	3103	2992	3165	3073
Ether extract (%)	3.47	3.41	4.78	4.03	4.84	4.41
Crude fibre (%)	4.54	5.24	4.38	5.03	4.32	4.79
Total ash (%)	5.05	5.94	4.59	5.40	4.42	4.14
Calcium (%)	1.11	1.11	1.01	1.02	1.01	0.99
Total phosphorus (%)	0.79	0.80	0.72	0.73	0.68	0.70
Available phosphorus (%)*	0.55	0.56	0.49	0.49	0.45	0.45
Lysine (%)*	1.61	1.60	1.43	1.44	1.34	1.34
Methionine (%)*	0.66	0.67	0.59	0.59	0.54	0.54
Feed cost per Kg (Rs.)*	33.52	32.78	32.00	29.77	30.38	28.91

*Calculated values

Ration T2 contained 3% less metabolisable energy as compared to T1; Ration T3 and T4 was prepared supplementing synthetic and herbal emulsifier@ 250 gram /tonne of feed in T2, respectively.

[1]Trace mineral premix supplied (per kg diet): Magnesium- 300 mg, Manganese- 55 mg, Iodine-0.4 mg, Iron- 56 mg; Zinc- 30 mg and Copper 4 mg.

[2] Vitamin premix supplied (per kg diet): vitamin A-8250 IU, vitamin D_3- 1200 ICU; vitamin K-1 mg; vitamin B_1- 2 mg, vitamin B_2- 4 mg; niacin- 60 mg, pantothenic acid-10 mg, cyanocobalamin-10 µg and choline-500 mg.

3.3 Production performance

The following parameters were recorded and calculated during the experimental period for growth studies.

3.3.1 Feed intake

Daily record of feed given to various groups was maintained. Left over of feed was weighed weekly. The feed intake in different groups was calculated by subtracting the weight of left over feed from the weight of total feed offered in a week.

3.3.2 Body weight gain

The body weight of individual birds was recorded at the start of the experiment and also at every week till the end of experiment. On the basis of these weights, weekly and overall body weight gains in different groups of broiler chicks were calculated

3.3.3 Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was calculated by dividing the feed consumed from the body weight gain as per the formula given below:

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

3.3.4 Performance index (PI)

The performance index of broilers during different periods of growth was calculated by using the formula given by Bird (1995).

$$\text{Performance index} = \text{Body weight gain (g)} \div \text{FCR}$$

3.3.5 Feed Cost of body weight gain

The cost of different rations used in the present study was calculated based on the actual market price of feed ingredients which prevailed at the time of experiment. The cost was calculated for a period of 5 weeks as follows:

$$\text{Feed cost (Rs./kg body weight gain)} = \frac{\text{Feed consumption (kg)} \times \text{Feed cost (Rs./kg)}}{\text{Body weight gain (kg)}}$$

3.4 Metabolism trial

A metabolism trial of three days was conducted to investigate the apparent total tract nutrient retention at the end of 5th week of feeding trial. Six broiler chicks (2 birds per replicates) representing the average body weight of the group were randomly selected and kept in metabolic cages. The experimental feed and fresh drinking water were provided *ad lib*. Initially, 3 days adaptation period was observed followed by 5 days excreta collection period. During the collection period, weighed amount of feed was offered to all broilers in the morning at 9 A.M. and the residue left was weighed next morning at the same time. Simultaneously, faecal trays coveredwith polythene sheets were placed for the collection of excreta. Care was taken to collect the excreta free from feed, feathers and scales. Excreta was pooled and dried in hot air oven at 70ºC for 48 hrs for dry matter estimation and thereafter stored for further analysis. Representative samples of feed and excreta were drawn for chemical analysis. For nitrogen estimation, fresh samples of excreta were preserved in 5% sulphuric acid (v/v). The samples of the experimental diets and the dropping were analysed for proximate principles, phosphorus (AOAC, 2007),calcium (Talapatra *et al.*, 1940). The retention of a nutrient was calculated by applying the following formula:

$$\text{Retention of nutrient (\%)} = \frac{(\text{Nutrient intake} - \text{Nutrient outgo})}{\text{Nutrient intake}} \times 100$$

3.5 Analysis of feed, excreta and meat samples

The representative samples of broiler starter feed, broiler finisher feed, laying hen feed, excreta obtained during metabolism trial and representative meat samples from breast and thigh were collected and analyzed for proximate principles, phosphorus (AOAC, 2007),calcium (Talapatra *et al.*, 1940)

3.5.1 Determination of dry matter (DM)

A known quantity of ground sample (about 10-50 g) was taken in a pre-weighed moisture cup. The cup was placed in hot air oven at 100 ± 2^0C for 24 h. The loss in moisture content after drying was estimated and DM was calculated as follows:

$$\text{Dry matter (\%)} = \frac{(\text{Wt. of moisture cup + sample after drying} -}{} \times 100$$

Wt. of moisture cup)

Wt. of fresh sample

3.5.2 Determination of nitrogen and crude protein

The crude protein content was determined by micro–Kjeldahl method. For this purpose 2 g of sample was taken in a digestion flask followed by addition of 5g of digestion mixture (K_2SO_4:$CuSO_4$ in 9:1) and 25 ml of concentrated sulphuric acid. The contents were digested till blue/ green transparent liquid was obtained. The volume of digested mixture was made up to 100 ml with distilled water. A 25 ml aliquot of digested mixture was distilled in Kjeldahl distillation apparatus (KELPLUS Nitrogen Analyzer) with excess of 40% NaOH solution and liberation of ammonia was collected in 20 ml of 2% boric acid solution containing 2 to 3 drops of mixed indicator (10 ml of 0.1% bromocresol green + 0.1% methyl red). A reagent blank was similarly digested and distilled which was titrated against N/10 H_2SO_4. Nitrogen content (%) in sample was calculated as follows

$$\text{Nitrogen (\%)} = \frac{(\text{Sample Titre} - \text{Blank Titre}) \times \text{Normality of } H_2SO_4 \times 0.014 \times \text{Volume made up}}{\text{Aliquot} \times \text{Weight of sample taken}}$$

Nitrogen per cent was converted to crude protein per cent by multiplying with factor 6.25. This was based on the principle that all the protein contains 16% nitrogen

3.5.3 Determination of ether extract

For estimation of ether extract Soxhlet extraction method was used. A known quantity of ground sample (about 2-3 g) was taken in a cellulose thimble and extracted for 6-8 hours with petroleum ether (Boiling point 40-60^{0}C) in Soxhlet extraction apparatus attached to a preweighed oil flask. The oil flask was removed and after evaporating the excess of ether, it was dried overnight in a hot air oven (60±2°C). The flask was cooled in a desiccator and weighed to a constant weight. The difference in weights gave the amount of ether extract in the sample as follows:

$$\text{Ether extract (\%)} = \frac{[A-B]}{C} \quad X\ 100$$

Where, A= Weight of oil flask with ether extract (g)

B= Weight of empty oil flask (g),

C= Weight of sample (g)

3.5.4 Determination of crude fibre

Moisture and fat free samples were transferred from thimbles to spoutless tall beaker of one litre capacity and in each beaker, 200 ml 1.25% H_2SO_4 was poured. It was refluxed for 30 minutes on hot plate after the boiling started and thereafter filtered through muslin cloth. The residue was washed 5-6 times with hot water until it became free from acid. The residual material on muslin cloth was again transferred to the respective spoutless tall beakers and in each beaker 200 ml of 1.25% sodium hydroxide (NaOH) solution was added. It was again refluxed for 30 minutes after the boiling started and thereafter filtered through muslin cloth and washed with hot water for 5-6 times until it became free from alkali. Thereafter, total residue was transferred in a clean, dry silica crucible and dried in hot air oven at 100^0C for 24 hours. Then, it was cooled in dessicators and weighed. The residue was then ignited in Muffle furnace at 600^0C for 2 hours. After 12 hrs silica crucibles containing ash were removed from the furnace and transferred into desiccators, cooled and weighed again. Weight loss during ignition was recorded as the weight of crude fibre:

$$\text{Crude fibre (\%)} = \frac{(B-A)}{A} \quad X100$$

Where, A = weight of samples on DM basis (g)

B= weight of silica crucible plus residue before ignition (g)

C = weight of silica crucible containing ash after ignition (g)

3.5.5 Determination of total ash

A known quantity of sample (5 g) was taken in pre-weighed silica crucible. After charring the sample on heater (till the smoke disappeared), the crucible was kept in Muffle furnace for ignition at 600°C for 2 h. The crucible was removed on cooling and kept in a desiccator and weighed again to find out weight of ash. The ash content was calculated as given below:

(Wt. of crucible + ash - Wt. of crucible)

$$\text{Total ash (\%)} = \frac{\text{Wt. of ash (g)}}{\text{Wt. of sample (g)}} \times 100$$

3.5.6 Estimation of acid insoluble ash (AIA)

About 50 ml of 50 per cent hydrochloric acid was added to total ash in the above mentioned crucible and contents were boiled in the water bath for 10 minutes, filtered through Whatman's filter paper no. 42 after giving washings with the distilled water to washout the crucible completely. Filter paper along with the residue was transferred to the same crucible again and it was burnt on the heater and ignited in the Muffle furnace at 600°C for 2 hours. After cooling of Muffle furnace, the crucible containing acid insoluble ash were removed and kept in desiccators, cooled and weighed. The acid insoluble ash content was calculated by the formula given below:

$$\text{Acid insoluble ash (\%)} = \frac{(W_2 - W_1)}{W} \times 100$$

Where, W_1 = Weight of crucible (g)

W_2 = Weight of crucible with acid insoluble ash (g)

W = Weight of original sample (g)

3.5.7 Calcium estimation

Calcium was estimated in the acid mineral extract prepared from ashing of sample and then dissolving in dilute hydrochloric acid extraction (Talapatra*et al.* 1940). An aliquot of 10 ml mineral extract was transferred into a 250 ml beaker and to it 10 ml saturated ammonium oxalate solution was added with constant stirring. Then, two drops of methyl red indicator were added. Dilute ammonia solution was added drop wise to develop a faint yellow colour which was again adjusted by adding dilute HCl drop wise with constant stirring so as to get faint pink colour. The contents were heated on a hot plate for about 5 minutes and kept overnight for proper precipitation of calcium oxalate. On the next day, contents were filtered through Whatman filter paper no. 40 with minimum five washings with hot distilled water. After that, filter papers containing whitish calcium oxalate precipitate was quantitatively transferred into a beaker and dissolved by adding dilute sulphuric acid, followed by gentle heating and titrated against standard N/10 or N/100 potassium permanganate solution ($KMnO_4$) solution.

Calcium (%) in the sample was calculated as follows:

$$\text{Ca (\%)} = \frac{\text{N/10 } KMnO_4 \text{ used (ml) x 0.002 x dilution factor}}{\text{Wt of sample}} \times 100$$

3.5.8 Estimation of phosphorus

The phosphorus content of samples was estimated as per method of (AOAC 2007) using UV visible spectrophotometer as described below. In a large beaker 20 g ammonium molybdatetetrahydrate was taken and dissolved in about 300 ml hot distilled water and cooled by keeping in cold water trough. In a separate beaker two grams of ammonium metavandate was dissolved in 250 ml hot distilled water, cooled and 125 ml of 70% perchloric acid ($HClO_4$) was added to it slowly. Molybdate solution was added to vandate solution with constant stirring and transferred to a volumetric flask. The volume was made up to one liter mark with distilled water.

Different volumes of working standard solution were taken in the test tubes so as to have 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg P and volume was made to 6 ml by adding distilled water. Then, 4 ml of molybdo-vandate reagent was added to all the test tubes, mixed and OD was taken at 400 nm after 10 minutes of incubation in UV visible spectrophotometer. The amount of phosphorus present in the samples was determined by using regression equation obtained from the standard curve

Collection of blood and separation of serum

Blood samples were collected from six experimental birds of each group i.e. two broiler chicks from each replicate on 35^{th} days of experimental feeding. Blood samples (about 3.0 ml) were collected aseptically from their wing vein, using sterilized syringes and needles. Collected blood samples were divided into two parts. One part (1.5ml) was transferred to the vials containing anticoagulant (EDTA) for analysis of haematological parameters. The second part (1.5 ml) of blood was used for separation of serum. For separation of serum, remaining 1.5 ml blood sample was allowed to stand at room temperature in slanting position for clot formation for threeto four hours. After clotting of blood, plunger was removed from the syringes and serum was stored at -20^0C with date and sample number for further analysis.

3.6 Haematological parameters

3.6.1 Haemoglobin (Hb)

Haemoglobin concentration was estimated following the method described by (Sharma and Singh, 2000) using Sahli's haemoglobinometer with acid haematin method. The brown colour was matched with glass standard and haemoglobin concentration (g/ dl) was recorded

3.6.2 Packed cell volume (PCV)

Packed cell volume was estimated by using micro haematocrit method as described by (Sharma and Singh 2000). Fresh anticoagulant added blood was drawn into micro capillaries and sealed with wax at one end. Capillaries were centrifuged at 10,000 rpm for 30 minutes. Packed cell volume was directly measured by using Citro Cap Microhaematocrit tube reader and expressed in per cent

3.6.3 Total Erythrocyte Count (TEC)

The blood specimen is diluted with 1:200 with the RBC diluting fluid and cells are counted under high [40s objective] by using Neubauers chamber. The number of cell in undiluted blood are calculated and reported as the total number of cell per cubic mm of whole blood. Total count is equal to total number of RBC in all the five squares (upper left, upper right,, lower right, lower left andcentral) multiplied by 10,000

3.6.4 Mean Corpuscular Volume (MCV)

Mean Corpuscular Volume (MCV) is measure of the average volume of the red blood corpuscules. The measure is attained by multiplying a volume of blood by the proportion of blood i.e cellular and dividing that product by the number of erythrocyte in that volume. It is expressed infemto litre (fL). It was calculated by using the formula:

$$MCV = (PCV \times 10) / TEC$$

3.6.5 Mean Corpuscular Haemoglobin (MCH)

Mean Corpuscular Haemoglobin (MCH) is the average mass of haemoglobin per blood cell (RBC) in a sample of blood It is expressed in picograms per cells (pg). It was calculated by using the formula:

MCH= (Hb × 10) / TEC

3.7 Serum biochemical parameters

3.7.1 Serum glucose

Estimation of serum glucose was done by enzymatic GOD-POD method with the help of Span Diagnostic Kit at 505 nm wavelength against blank reagent (Sacks, 1998). In this procedure, glucose oxidase (GOD) oxidises glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidase, released hydrogen peroxide is coupled with phenol and 4-aminoantipyrine (4-AAP) to form coloured Quinoneimine dye. Absorbance of coloured dye measured at 505 nm was directly proportional to glucose concentration in the sample. Concentration of serum glucose was expressed in mg/dl.

3.7.2 Serum protein profile

3.7.2 (a). Serum total protein

Total protein concentration in serum was estimated by biuret method with the help of Erba diagnostic kit at 540 nm wavelength (Johnson *et al.* 1999). The peptide bonds of proteins react with cupric ion in alkaline solution to form a colored chelate (Protein + Cu^{2+} → Cu-protein complex), which is measured at 578 nm. Concentration of plasma total protein was expressed in g/dl.

3.7.2 (b). Serum albumin

Albumin concentration in the serum was estimated by bromocresol green end point assay method (Albumin + bromocresol green → Green coloured complex) with the help of Autopak diagnostic kit at 630 nm wavelength (Johnson *et al.* 1999). Concentration of serum albumin was expressed in g/dl.

3.7.3 (c) Serum globulin

The serum albumin content was subtracted from serum total protein content to calculate globulin content and expressed in g/dl.

Globulin (g/dl) = Total protein (g/dl) – Albumin (g/dl)

3.7.3 (d) Albumin: Globulin Ratio (A: G ratio)

The albumin-globulin ratio was calculated by using following formula:

$$\text{Albumin- globulin ratio} = \frac{\text{Serum albumin (g/dl)}}{\text{Serum globulin (g/dl)}}$$

3.7.4 Serum lipid profile

3.7.4 (a). Estimation of serum total cholesterol and HDL cholesterol

The total cholesterol and HDL (high density lipoprotein) cholesterol concentration in serum was estimated spectrophotometrically using Span Diagnostic kit with enzymatic cholesterol oxidase/peroxidase (CHOD-POD) method. The enzyme, cholesterol esterase catalyzes hydrolysis of cholesterol esters to free cholesterol and fatty acid molecules. Then free cholesterol gets oxidized in the presence of cholesterol to form cholest-4en- 3-one and H_2O_2. Liberated H_2O_2 reacts with phenol and 4- Aminoantigpyrine (4-AAP) in presence of peroxidase to form red coloredquinoneinine complex, the intensity of which was measured at 505 nm. The concentration of standard cholesterol used was 200mg/dl. Concentration of serum cholesterol was expresses in mg/dl.

3.7.4(b) Serum triglycerides

Serum triglyceride was estimated using Span diagnostic kit based on the method at 505 nm wavelength. The enzyme, lipoprotein lipase catalyzes hydrolysis of triglycerides to glycerol and fatty acid. Glycerol then is phosphorylated in an ATP - requiring reaction catalyzed by glycerophosphate. The formed glycerophosphate is oxidized to dihydroxyacetone and H_2O_2 in a glycerophosphate oxidase catalyzed reaction. H_2O_2 then reacts with 4- Aminoantigpyrine (4-AAP) and 4 - chlorophenol under the catalytic influence of peroxidase to form coloredquinoneimine complex, the

intensity of which was measured at 505 nm. Concentration of serum triglycerides was expressed in mg/dl.

3.7.4(c) Serum LDL cholesterol:

Serum LDL (low density lipoprotein) cholesterol was calculated by using the following formula

LDL cholesterol=Total cholesterol – HDL cholesterol – triglyceride

LDL cholesterol level in plasma was expressed as mg/dl.

3.7.5 Serum enzyme profile

Following serum enzymes were estimated using the serum obtained from the blood samples.

3.7.5 (a). Serum alkaline phosphatase (ALP)

The alkaline phosphatase activity in serum was assayed using Span diagnostic Kit at 510 nm wavelength (Bergmeyer *et al.,* 1986). Concentration of serum ALP was expressed in IU/L.

3.7.5 (b). Estimation of alanine aminotransferase (ALT)

Alanine amiontransferase (ALT) is also called as serum glutamate pyruvate transaminase (SGPT). For the estimation of ALT, 4 - DNPH method of (Reitman and Frankel, 1957) was used. The principle in this reaction is that ALT catalyses the following reaction

α- Ketoglutarate + L- Alanine ↔ L- Glutamate + Pyruvate

Pyruvate so formed is coupled with 2, 4-Dinitrophenyl hydrazine (2, 4 - DNPH) to give the corresponding hydrazone which gives brown colour in alkaline medium and this can be measured calorimetrically at 505 nm. ALT activity was expressed as IU/L.

3.7.5 (c) Serum aspartate aminotransferase (AST)

The activity of serum glutamate oxaloacetate transaminase (SGOT) or aspartate aminotransferase (AST) was measured following 2, 4 - DNPH method of Reitman and Frankel (1957) using a diagnostic kit (Span Diagnostic Ltd.). The principle in this reaction is that aspartate aminotransferase (AST) catalyses the

transamination of L- aspartate and α- ketoglutarate to form oxaloacetate and L-glutamate.

α- Ketoglutarate + L- Aspartate ↔ L- Glutamate + Oxaloacetate

Oxaloacetate so formed is coupled with 2, 4 – Dinitrophenyl Hydrazine (2, 4 - DNPH) to give the corresponding hydrazone, a brown colour complex in alkaline medium and this can be measured colourimetrically at 505 nm. AST activity was expressed as IU/L.

3.7.6 Serum Antioxidant status

3.7.6 (a) Catalase activity

Serum catalase activity was estimated spectrophotometrically as per the method described by (Cohen *et al.* 1970). The reaction commences with the addition of 50µL of serum to 2.95ml of phosphate buffer- H_2O_2 solution. In the blank, sample was substituted by same amount of PBS. Phosphate buffer- H_2O_2 gives an absorbance of 0.5-0.6 at 240 nm. The decrease in absorbance was measured for every 20 seconds up to 1 minute. Since a decrease in absorbance of 0.05 at 240 nm corresponds to the disappearance of 3.45 µmoles of H_2O_2.

The units of catalase activity per ml serum was calculated as below.

0.05 change in absorbance = 3.45 µmoles of H_2O_2 disappeared

'A' change in absorbance in 50µl sample = 3.45 X A/ 0.05

So, 'A' change in absorbance in 1ml sample = 3.45 X A/ (0.05 X 0.05)

= 1380 A

3.7.6 (b) Lipid peroxidation

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) as per method given by (Buege and Aust1978) modified by (Suleiman *et al.* 1996). First of all 0.2 ml serum sample was diluted to 1 ml with normal saline solution. Lipid peroxide levels were measured in the serum after addition of 2 ml of TBA-TCA reagent to 1ml of diluted serum. The mixture was heated in a boiling water bath for 15 minutes. After cooling, the suspension was centrifuged at 3000rpm for 10 minutes. The supernatant was separated and absorbance was measured at 535 nm. The MDA concentration was determined by specific molar extinction coefficient of 1.56 $X10^5 mol^{-1} cm^{-3}$.

MDA value (µmoles/ml) = Absorbance X dilution factor/ (1.56 X 10^5).

3.7.6 (c) Reduced glutathione (GSH)

Reduced glutathione (GSH) level in serum was estimated using the method described by (Lin *et al.* 1988) with some modifications. An aliquot of serum (400µL) was mixed with 400µl of tris-EDTA buffer followed by addition of 40µl of 10mM 5,5'-dithiobis (2-nitro benzoic acid) [DTNB] and 3.16ml of absolute methanol. Colour was developed after incubation at 37^0C for 30 minutes. Now, the suspension was centrifuged at 3500 rpm for 10 minutes. The absorbance of the supernatant was measured at 412nm (A) and subtracted from a DTNB blank (B) and a blank containing the sample without DTNB. In agreement with (Sedlak and Lindsay, 1968), a value of 0.03 at 412nm for the sample blank was consistently obtained. Consequently, individual sample blanks were not critical and were taken as 0.03. GSH levels were conveniently calculated using an absorptivity of 13600$cm^{-1}M^{-1}$ as follows:

(A-B-0.03) X (4.0/0.4)/ 13.6 = (A-B-0.03) X 0.735 mM.

3.7.6 (d) Superoxide dismutase (SOD) activity

Serum superoxide dismutase (SOD) activity was measured using the method as given by (Madesh and Balasubramanian, 1998) with some modifications. In the micro-titre plate method, the assay mixture in a total volume of 300µl per well consisted of 120µl PBS, 10µl serum sample, 5µl of 1.25 mM MTT and 15µl of freshly prepared 1mM pyragallol solution to be added at the end. Sample was replaced with PBS in the blank. After an incubation period of 15 minutes, 150µl DMSO was added and absorbance was taken in ELISA reader at 570 nmThe percent inhibition by the presence of SOD was calculated from the reduction of the MTT colour formation as compared to the MTT formazan formed in the absence of SOD which was taken as 100%. One unit of SOD was defined as the amount of protein required to inhibit the MTT reduction by 50%.

SOD activity (units/ml) = 2 X 100 X A_T/ A_B

Where, A_T= Absorbance for test.

And A_B= Absorbance for blank

3.8 Carcass quality traits

At the end of 42 days of experimental period, two representative broiler chicks from each replicate (six birds per treatments) of all treatment groups were sacrificed for evaluation of carcass characteristics, organ weight, cut-off parts and sensory evaluation.

3.8.1 Dressing yield and organs weight

The broiler chicks were off fed for 12 hours before slaughter. However, water was provided *ad libitum.* Before slaughter, each broiler chicks was weighed. The chicks were sacrificed by cervical dislocation and allowed to bleed completely by cutting the jugular vein. After complete bleeding, weight of the bled carcass was recorded. The weight was again recorded after defeathering manually. Head and shank were removed by giving cuts at atlanto-occipital and hock joints, respectively and their respective weights were taken. Thereafter, a horizontal cut was applied posterior to keel bone. Breast was pushed forward to expose the viscera, which was then pulled out. Weight of the carcass was then recorded as dressed yield by the following formula:

Dressing yield = live weight –(weight loss as blood, feathers, head, shank and viscera)

The weight of different cut up parts viz., thigh, breast, drumstick, back, neck and wings were recorded by separating them from carcass and expressed as a percentage of live body weight. Internal organs (heart, liver, gizzard and spleen) and small intestine were detached from rest of the viscera, weighed individually and expressed as percentage of live weight. Gall bladder was removed from liver. Gizzard was opened, its contents were removed and epithelial linings were detached. Around 100 g of meat sample from both breast and thigh muscles were collected for the nutritional analysis of meat (AOAC, 2007).

3.8.2 Sensory evaluation of meat

The sensory quality of cooked meat samples was evaluated by the standard sensory evaluation method (Keeton and Feedings, 1984). The broiler meat sample of

breast muscle of broiler chickens from each group collected and was boiled by addition of 1.5% salt solution and served warm to panelist for sensory evaluation. A sensory panel (semi trained) of eight judges drawn from post graduate students and teaching staff were requested to evaluate the meat sample for different sensory attributes *viz*., appearance, flavor, texture, juiciness and overall acceptability. All the food products were presented in small plates labelled with three digit random codes.Panelists were provided with drinking water to rinse their mouth between samples. The samples were coded and presented in random order and panelists were asked to rate their unbiased assessment of colour, taste, texture and overall acceptability on a 9-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely). A score of 5 or below was considered a limit of acceptability for all sensory attributes tested.

3.9 Statistical analysis

The experimental data obtained were analysed statistically (Snedecor and Cochran, 1994) as a completely randomized design by analysis of variance (ANOVA) by using general linear model (GLM) procedure of SPSS. Difference between treatments means were compared using Duncan's Multiple Range Test (Kramer 1957). Statistical significance was declared at $P \leq 0.05$.

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4 RESULTS AND DISCUSSION

The present investigation was conducted to study the effect of exogenous emulsifier in energy restricted fed broiler chicken on growth performance, haemato-biochemical constituents, oxidative status, nutrient utilization and carcass characteristics in Cobb 400Y broiler chicks. The results obtained have been presented as follows:

4.1 Production performance

The data on growth performance of broilers of different treatment groups during 5 weeks experimental period are presented in Table 2 to 5.

4.1.1 Pre-starter phase (0-7 days)

The cumulative growth performance of broilers in terms of average weight gain, feed intake, FCR and performance index as influenced by supplementation of exogenous emulsifier in energy restricted fed broiler chicken during pre-starter phase (0-7 days) is presented in Table 2 and depicted in Figure 1 and 2.

During the pre-starter phase (0-7 days) feed consumption, body weight gain, and performance index in broilers of various treatment groups didn`t differ-significantly ($P>0.05$), however, there was significantly ($P<0.05$) change in feed intake and feed conversion ratio. In body weight gain there was numerically ($P>0.05$) highest body weight gain(114.83 g) in the group supplemented with herbal emulsifier (T_4), that was followed by the groups supplemented with basal diet with 3% less energy (T_2) (113.49g), synthetic emulsifier (T_3) (104.61 g) and the control group (T_1) (109.86 g). Maximum feed intake was recorded in T_2 group (227.00 g) which was followed by the groups T1, T3 and T4 that is 190.96 g, 180.85 g and 174.53 g, respectively. There were significant ($P<0.05$) difference in FCR among different treatments. The best FCR was obtained in T4 (1.52) group whereas the worst FCR was recorded in T2 treatment group (2.00). Performance index was numerically ($P>0.05$) highest in the synthetic emulsifier supplemented group (T4) (76.12) followed by T1, T3 and T2 that is 63.49, 60.73 and 57.19, respectively.

Table-2: Effect of exogenous emulsifier on growth performance in energy restricted fed broiler chicks during pre-starter phase (0-7days)

Treatments	BWG(g)	FI (g)	FCR	PI
T_1	109.86±4.56	190.96^{a}±9.79	1.74^{ab}±0.080	63.49±4.79
T_2	113.49±2.78	227.00^{b}±8.88	2.00^{b}±0.121	57.19±5.11
T_3	104.61±3.97	180.85^{a}±4.71	1.73^{ab}±0.075	60.73±3.55
T_4	114.83±5.35	174.53^{a}±4.39	1.52^{a}±0.089	76.12±8.25
SEm	2.17	6.88	0.064	3.24
P value	0.389	0.004	0.042	0.180

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05)

Table-3:Effect of exogenous emulsifier on growth performance in energy restricted fed broiler chicken during Starter phase (8-21days)

Treatments	BWG(g)	FI (g)	FCR	PI
T_1	832.88^{c}±18.20	1075.68^{b}±21.95	1.29^{a}±0.020	645.20^{b}±20.51
T_2	736.55^{a}±13.19	1159.50^{c}±16.91	1.57^{b}±0.045	468.64^{a}±20.93
T_3	772.95^{ab}±17.07	1012.95^{a}±23.11	1.31^{a}±0.066	591.62^{b}±16.10
T_4	797.62^{bc}±19.84	1078.86^{b}±12.53	1.35^{a}±0.018	589.90^{b}±22.41
SEm	12.90	17.67	0.038	21.30
P value	0.024	0.005	0.006	0.002

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05)

Figure 1: Effect of exogenous emulsifiers on growth performance in restricted fed broiler chicken in pre-starter phase (0-7 days)

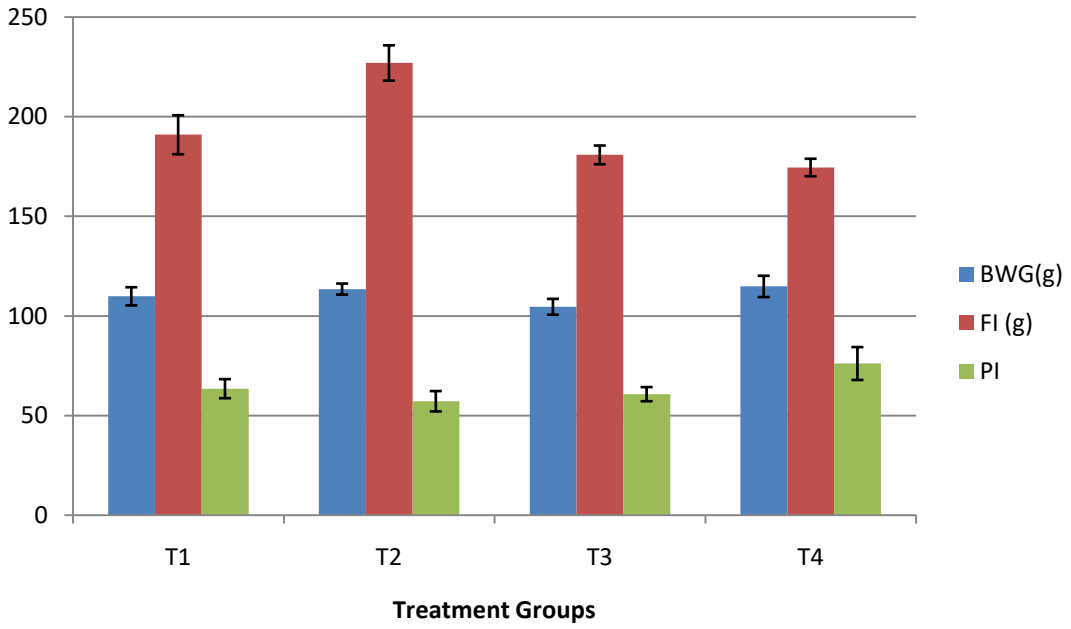
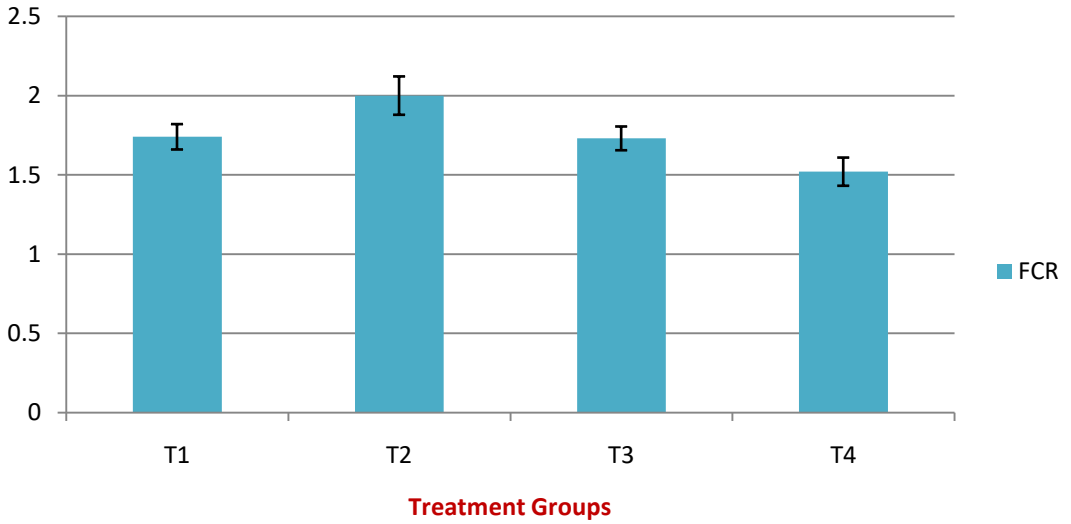


Figure 2: Effect of exogenous emulsifiers on FCR in restricted fed broiler chicken in Pre starter phase (0-7 days)



The study of our result in agreement with An *et al.,* (2020) who found that at the end of the experiment, the final body weight of the broilers was significantly higher (P< 0.05) in broilers fed the diet supplemented with 0.2% exogenous emulsifier than in other treatment group. Findings of Bontempo *et al.* (2018) showed a significant improvement in average daily gain with the supplementation of synthetic emulsifier to the feed for one to 12 days. GuerreiroNeto *et al.* (2011) did not find any difference in broiler growth performance on addition of emulsifier with fat source at 42 days of age. In a similar study, Dabbou *et al.* (2019) reported that there were no significant differences in body weight gain or feed intake by supplementation with natural emulsifiers for one to 10 days Kulkarni *et al.* (2019) reported that supplementing emulsifier in the diet prepared with different oil sources like soya bean oil and rice bran oil as fat source, the feed intake was comparable among different treatment groups during staring phase (0-3 week) but significant increase in feed intake was observed during finishing (3-6 weeks). The results of this study indicated that the low energy density can reduce body weight gain from d 1 to 21 and increase feed intake from d 21 to 35. In general, the lower energy (ME) level can result in a depression of growth performance. Broilers had the ability to regulate feed intake based on the energy levels of the diet, however this effect was limited during the first week of age, especially if feed the mash feed (Jones and Wiseman, 1985). The reason may be mainly attributed to the capacity limitation of the gastro-intestine of chicks. GuerreiroNeto *et al.* (2011) also reported improvement in FCR in broiler fed diet containing soya bean oil with emulsifier.

4.1.2 Starter phase (8-21 days)

The cumulative growth performance of broilers in terms of average weight gain, feed intake, FCR and performance index as influenced by supplementation of exogenous emulsifiers in energy restricted fed broiler chicken during starter phase (8-21 days) is presented in Table 3 and depicted in Figure 3 and 4.

During the starter phase (8-21 days) feed consumption, body weight gain, FCR and performance index in broilers of various treatment groups differed significantly (P<0.05). There was significantly (P<0.05) highest body weight gain, (832.88 g) in the control group (T1), that was followed by the groups supplemented

Fig. 3: Effect of exogenous emulsifier on growth performance in energy restriced fed broiler chicken during starter phase

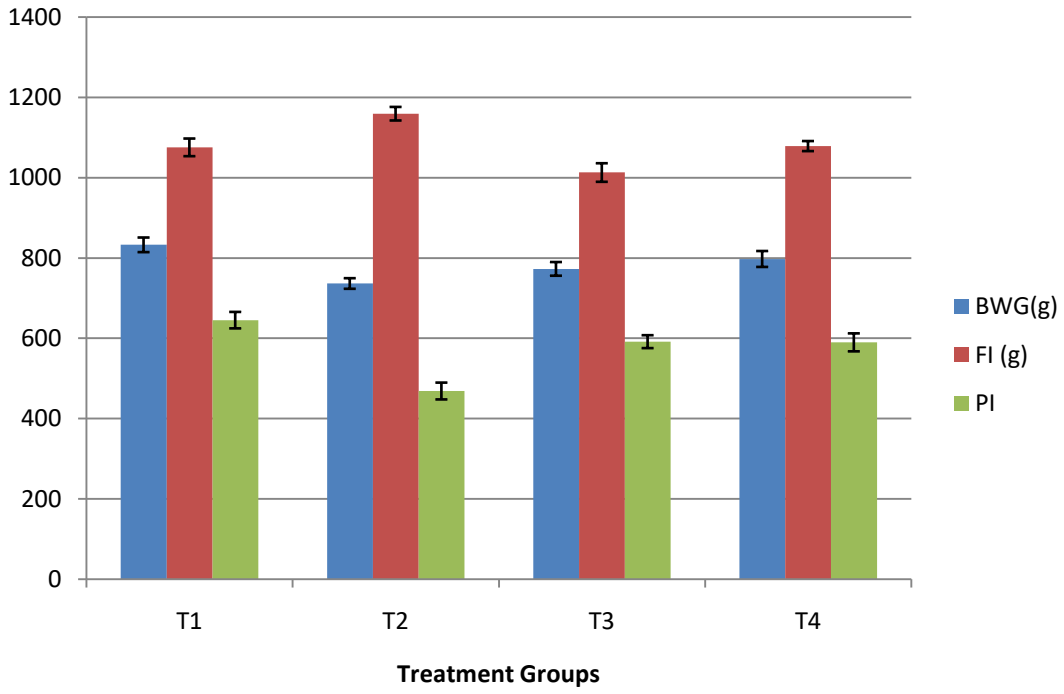
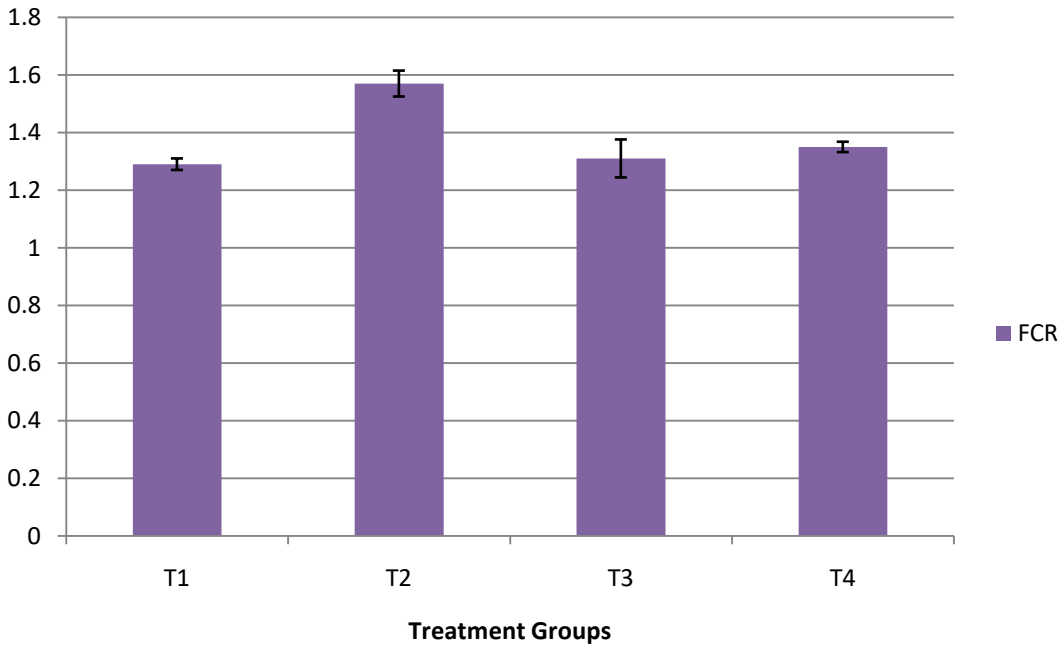


Fig. 4 Effect of exogenous emulsifiers on FCR in energy restricted fed broiler during starter phase (8-21 days)



with herbal emulsifier (T4) (797.62 g), synthetic emulsifier (T3) (772.95 g) and the control group with 3 % less energy (T2) (736.55g). Maximum feed intake ($P<0.05$) was recorded in T2 group (1159.50 g) followed by the groups T4, T1 and T3. Increase in feed intake in the experimental animals fed on low energy ration was also observed by Sayed, (2009) and Nideou *et al*. (2017). The highest feed intake in T2 might be due to an attempt to compensate the energy demand by broiler chicken fed low energy based feed as birds eat to satisfy their energy requirement (Griffith *et al.*,1977). There was significantly ($P<0.05$) better FCR (1.29) obtained in T1 group (1.70) as compared to T2,T3,T4 group but it was comparable to T4 group (1.35) and T3(1.31). The best FCR (1.29) was recorded in control group fed standard diet. Performance index increased significantly ($P<0.05$) in the control group (T1) (645.20) followed by T3, T4 and T2 that is 591.62, 589.90 and 468.64, respectively.

4.1.3 Finisher phase (22-35 days)

The cumulative growth performance of broilers as influenced by synthetic emulsifier, herbal emulsifier in energy restricted feed broiler chicken during finisher phase (22-35 days) is presented in Table 4 and depicted in Fig. 5 and 6.

During the finisher phase, the average weight gain of broilers was significantly ($P<0.05$) influenced by the dietary treatments. The body weight gain in broiler chickens fed standard basal diet (T_1) group was significantly ($P<0.05$) higher (982.78 g) than synthetic emulsifier (T_3) (923.86 g) and those supplemented with herbal emulsifier (T4) (911.48 g) and basal diet with 3% less energy (T_2) (830.95 g). Feed intake didn`t differ significantly ($P>0.05$) among different treatment. No change in feed intake in broiler chicks fed exogenous emulsifiers during finished phase was also reported by Ashraf (2007); Roy *et al.* (2010) and Guerreiro Neto *et al.* (2011). Lack of pronounced effect of emulsifier during finished stage as compared to younger age may be potentiated by the fact that young birds are unable to absorb fat efficiently due to its poor emulsification ability and poor lipase activity; however, these biological processes improve with the age and adapt to cope with higher unsaturated fatty acids (Meng *et al*., 2004). FCR was significantly lowest in the T_1 (1.85) group and that was succeeded by T_4 (1.80), T_3 (1.81) and T2 (1.82). Performance index was significantly ($P<0.05$) higher in T1 (531.64) group followed by T_3 (474.73), T_4 (465.91) and T_2

(373.11) groups, respectively. Birds fed diets containing synthetic emulsifier (T_3) and herbal emulsifier (T_4) had comparable performance index.

Table-4:Effect of exogenous emulsifier on growth performance in energy restricted fed broiler chicken during finisher phase (22-35days)

Treatments	BWG(g)	FI (g)	FCR	PI
T_1	982.78^{c}±17.31	1817.39±16.08	1.85^{a}±0.024	531.64^{c}±7.72
T_2	830.95^{a}±15.13	1862.83±22.42	2.23^{b}±0.055	373.11^{a}±5.09
T_3	923.86^{b}±10.35	1799.00±23.02	1.94^{a}±0.033	474.73^{b}±6.28
T_4	911.48b±13.90	1783.70±19.38	1.95^{a}±0.024	465.91^{b}±6.62
SEm	17.43	12.48	0.045	17.36
P value	0.001	0.107	0.000	0.000

^{a,b,c}Mean values with different superscripts within a column differ significantly (P<0.05)

Table-5:Effect of Exogenous emulsifier on growth performance in energy restricted fed broiler chicken during overall period (0-35days)

Treatments	BWG(g)	FI (g)	FCR	PI
T_1	1925.53^{c}±16.32	3084.04^{b}±17.27	1.60^{a}±0.017	1202.51^{c}±22.76
T_2	1681.00^{a}±14.04	3239.33^{c}±20.64	1.92^{b}±0.031	872.81^{a}±20.69
T_3	1801.43^{b}±12.25	2995.13^{a}±25.84	1.66^{a}±0.043	1084.95^{b}±16.50
T_4	1823.93^{b}±13.22	3037.10^{ab}±11.77	1.66^{a}±0.027	1095.94^{b}±31.05
SEm	26.89	29.07	0.040	37.40
P value	0.001	0.001	0.001	0.001

^{a,b,c}Mean values with different superscripts within a column differ significantly (P<0.05)

Fig.5 Effect of exogenous emulsifier on growth performance in energy restricted fed broiler chicken during finisher phase(22-35 days)

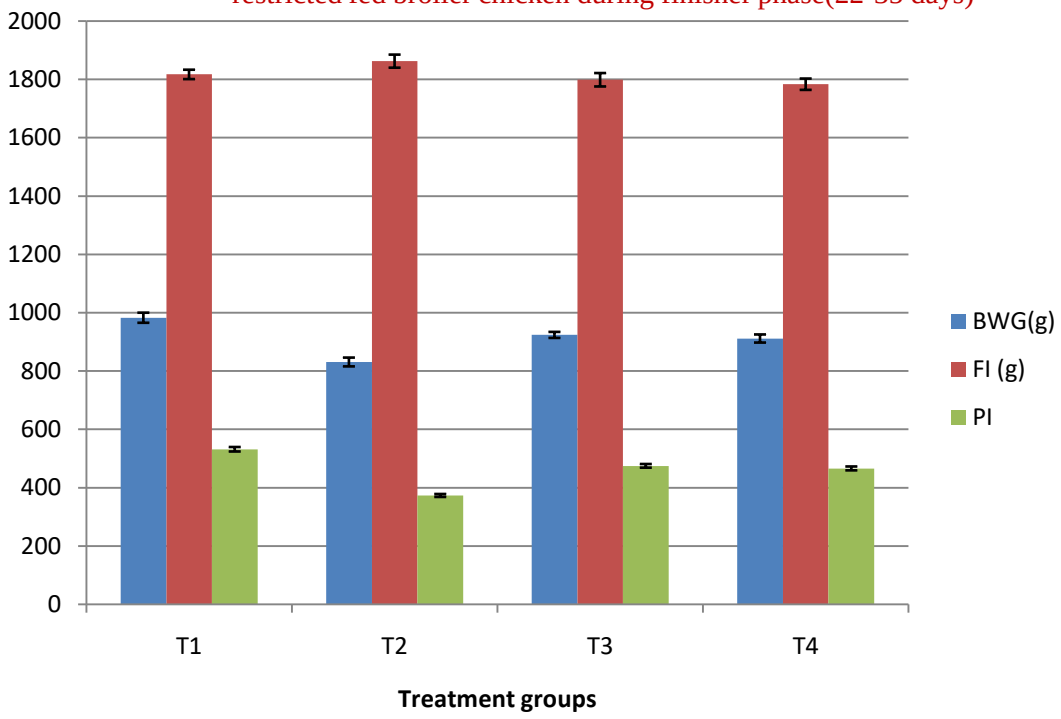
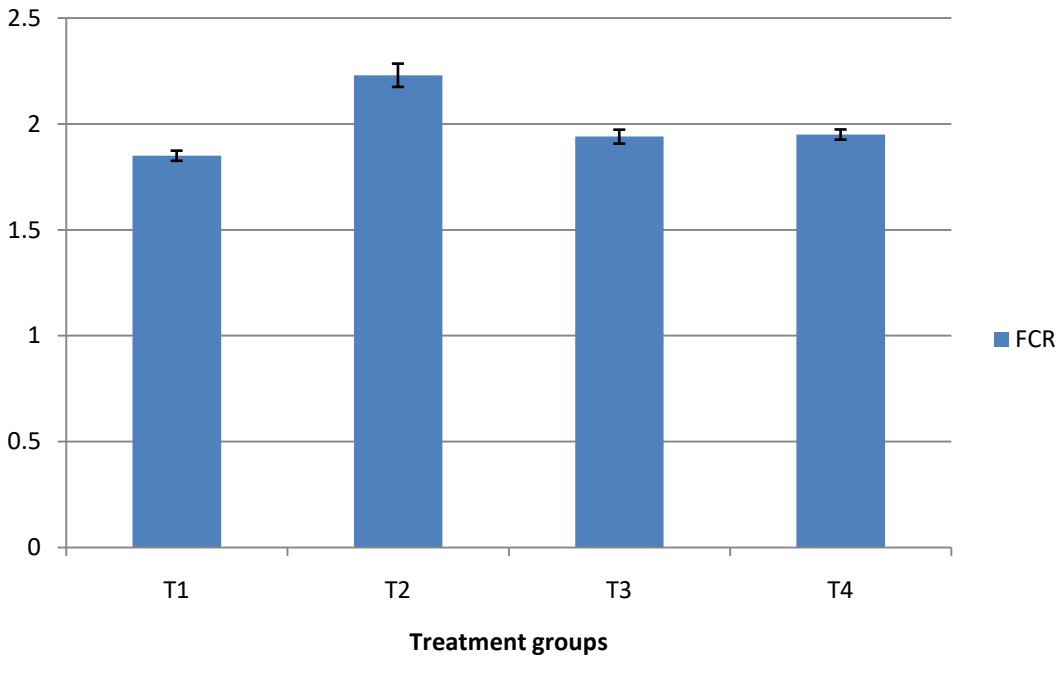


Fig. 6 Effect of exogenous emulsifier on FCR in energy restricted fed broiler chicken during finisher phase (22-35 days)



4.1.4 Overall period (0-35 days)

The cumulative growth performance of broilers in terms of average weight gain, feed intake, FCR and performance index as influenced by supplementation of exogenous emulsifier in energy restricted fed broiler chicken for overall period of 35 days is presented in Table 5 and depicted in Fig. 7 and 8.

There was significant difference ($P<0.05$) observed in body weight gain among different groups. Maximum weight gain (1925.53 g) was recorded in broilers fed with basal diet (T_1) followed by T_4 (1823.93 g), T_3 (1801g) and T_2 (1681 g) groups, T3 and T4 are non-significant ($P>0.05$) and comparable. There was significant difference ($P<0.05$) observed in feed intake among different groups. Maximum feed intake (3239.33g)(T2) was recorded in broilers fed with basal diet with restricted energy (T_2) followed by T_1 (3084.04 g), T_4 (3037.10 g)and T_3 (2995.13 g) groups. However, feed intake in T3 and T4 were comparable. There was significant difference ($P<0.05$) observed in FCR results among different treatment groups, T3 and T4 are comparable. Lowest FCR (1.60) recorded in birds fed with basal diet i.e control group (T_1) which was significantly higher as compared to birds supplemented with restricted energy basal diet(T2) (1.92) and synthetic emulsifier (T3) and herbal emulsifier (T4) group FCR (1.66) is comparable. Overall best FCR was recorded in control group i.e birds supplemented with standard basal diet as compared with other treatment groups. Synthetic and herbal emulsifier supplemented groups had comparable FCR. Overall lowest FCR was noted in bird supplemented with basal diet having 3% less energy Similarly, performance index was significantly ($P<0.05$) highest in T_1 (1202.51) followed by T_4 (1095.94), T_3 (1084.95) and T_2 (872.81) groups.

Results of the experiment revealed that decrease in dietary fat content decreased growth performance in terms of body weight gain, feed conversion ratio and performance index as compared to control group in broiler chicken during the experiment period of 35 days. After adding the emulsifier (synthetic or herbal), the performance index of the broiler chicken improved but didn't differ from the positive control. This indicates that some fats (3% less metabolisable energy content in this experiment) could be replaced by adding either herbal or synthetic emulsifier. The result of the experiment is in agreement with Luc *et al*. (2013); Gaiotto *et al*. (2001);

Fig. 7 Effect of exogenous emulsifiers on growth performance in energy restricted fed broiler chicken during overall period (0-35 days)

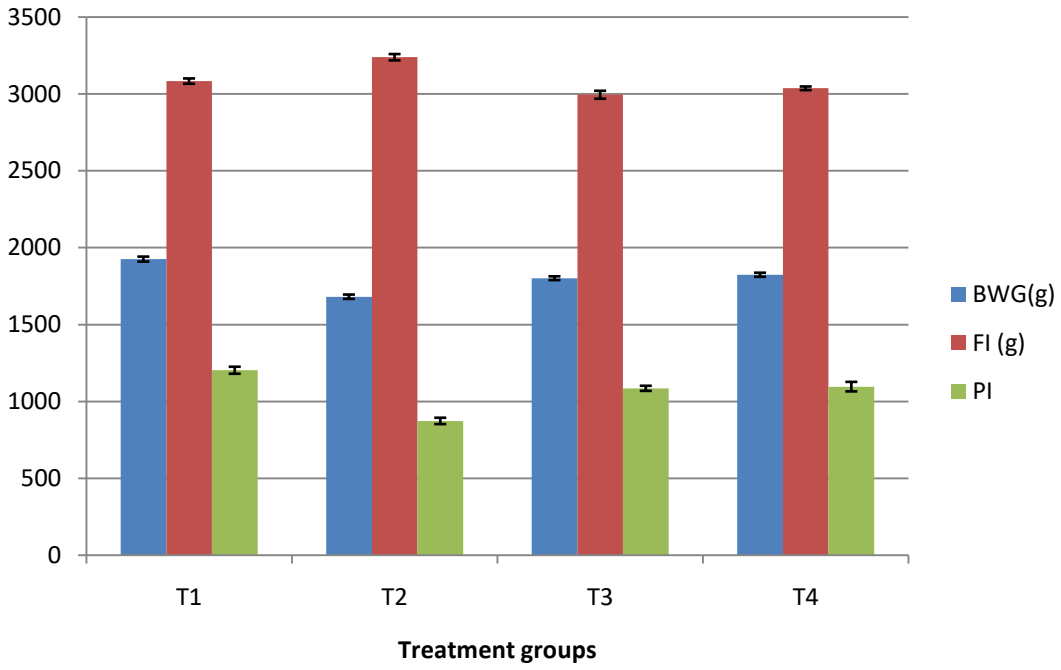
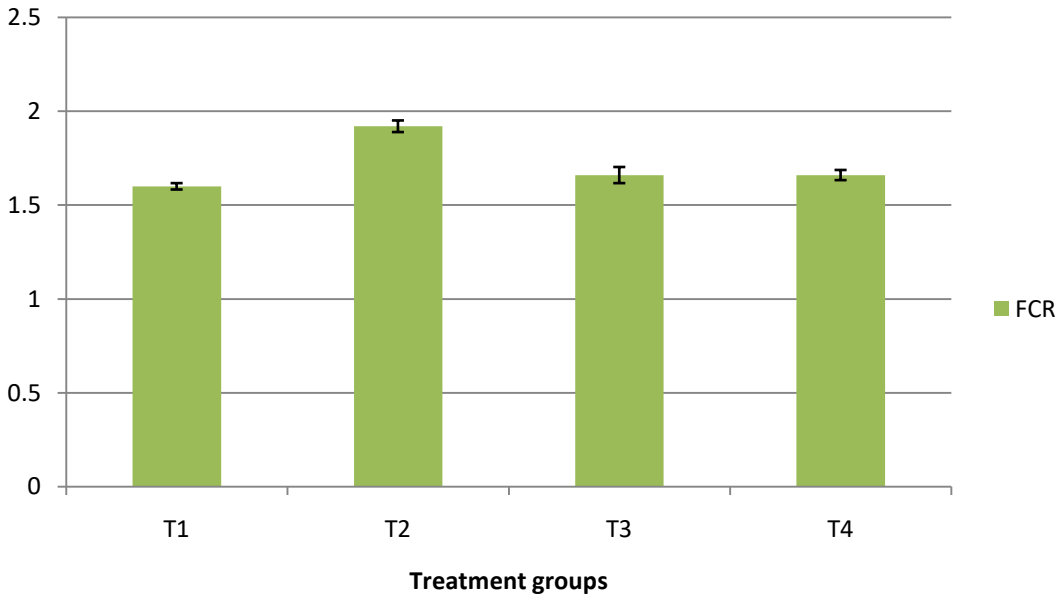


Fig. 8 Effect of exogenous emulsifier on FCR in energy restricted fed broiler chicken during overall period (0-35 days)



Wongsuthavas *et al*. (2007); GuerreiroNeto *et al*. (2011) who reported that addition of fat emulsifier in vegetable oil based broiler diets resulted in an improved weight gain during starter and finisher phase. Kaczmarek *et al*. (2015) found that glyceryl polyethyleneglycol ricinoleate (GPR) addition was characterized by higher body weight gain and lower feed conversion ratio in chickens compared to receiving diets without GPR. Haldhar *et al*. (2010) reported that reduction in dietary energy content may result in significant improvement in feed conversion by lowering the feed intake under the influence of nutritional emulsifiers. Zosangpuii *et al*. (2015) reported that soybean oil and palm oil with glycerol polyethylene glycol ricinoleate as emulsifier could be added in duck diets containing high amounts of rice bran without affecting performance. Bontempo *et al*. (2016) observed that a synthetic emulsifier consisting of bidistillated oleicacid andglyceryl polyethyleneglycol ricinoleate (GPR) had beneficial effects on the growth performance of chickens for fattening. Hu *et al*. (2019) evaluated supplementation of emulsifier in soyabean and poultry fat based diet in Cherry Valley ducks and reported that emulsifier improved growth performance in negative control diets. The main function of adding emulsifier in feed is to promote emulsification of fat, make up for the deficiency of bile acid and lipase in the digestive tract of poultry, promote the utilization of oil by poultry, and reduce the waste of oil and the damage caused by excessive oil to the animal body (Allahyari-Bake and Jahanian, 2017; Zhao and Kim, 2017; Bontempo *et al*., 2018). Exogenous emulsifiers are capable of improving fat digestibility and subsequently sustaining or enhancing the growth performance of broiler chickens fed a low-density energy diet (Wickramasuriya *et al*., 2020). Thus, these improvements in overall growth performance indicate that the nutritional emulsifiers compensate for an energy reduction in broiler diets without reducing growth parameters.

Results indicated that reduction or restriction in dietary energy (metabolisable energy) level by 3% resulted in significant ($P<0.05$) decrease in growth performance of broiler chickens. Addition of emulsifiers (synthetic or herbal) @250 g per tonne of feed in energy restricted (3% less metabolisable energy) broiler diet improved the growth performance of the birds. After addition of the emulsifiers, growth performance was similar to the control group having optimum dietary energy level. Effect of herbal and synthetic feed emulsifier on improvement in growth performance in broiler chicken was similar ($P>0.05$).

4.2 Feed Economics

Calculation of feed cost per kg body weight gain is presented in Table. 6.

Feed cost per kg body weight gain achieved during 35 days of the experiment was Rs. 49.85 in treatment group T1 (birds fed basal diet having optimum feed energy). Decrease in energy content of feed reduced price (Rs.per kg feed procured) of feed but due to comparatively less body weight gain and poor feed conversion ratio, feed cost per kg body weight was highest (Rs. 56.84 per kg body weight gain) in the birds of experimental group fed 3% less metabolisable energy (T2). Although addition of synthetic or herbal emulsifier in energy restricted diet slightly increased price of feed but due to improvement in growth rate and feed conversion ratio, feed cost per kg body weight gain was reduced to Rs. 49.13 and Rs. 49.19 in T3 and T4, respectively. This clearly indicates that feed emulsifier (either synthetic or herbal) was useful in improving feed efficiency by utilizing dietary fat efficiently in broiler chicken. This also indicates that addition of synthetic or herbal emulsifier @ 250 g per tonne of feed with soyabean oil based diet can save the cost of production by decreasing the source of energy (fat) in broiler chicken diet. Slightly higher feed cost per kg body weight gain in treatment group supplemented with herbal emulsifier may be due to lower dose of herbal emulsifier. Higher dose of herbal emulsifier may result in comparable feed economics with synthetic feed emulsifier. Dose response of the herbal or natural emulsifier in energy restricted broiler diet may be tested in future.

4.3 Nutrients utilization

The data representing to the average total tract nutrient utilization in broilers during finisher phase are summarized in Table 7 and depicted in Fig. 9

Data pertaining to the average total tract nutrient utilization revealed that dry matter retention did not change significantly ($P>0.05$) but numerically higher in T1 (74.28 %) followed by T4 (73.60%), T3 (72.72%), T2 (70.62%), respectively. Difference in crude protein retention among different treatments was non-significant ($P>0.05$) but numerically highest in herbal emulsifier group T4 (65.60 %) followed by T3 (64.78%), T1 (64.77 %) and lowest in basal diet fed with restricted energy in broiler chicken T2 (63.55 %).

There was significant difference in retention of ether extract among different treatments. Ether extract retention was significantly ($P<0.05$) highest in T1 (76.66%)

Blank

i.e birds fed basal diet optimum energy. Ether extract retention reduced significantly (P<0.05) (73.34%) in birds fed energy restricted diet (T2) as compared to birds fed diet having optimum energy (T1) (76.66%). The calorie: protein ratio of diets had been found to play a prominent role in the performance of broiler chicken (NRC, 1994; Lesson *et al*., 1996; Aftab et al., 2006). The calorie: protein ratio was low and below the recommended level of BIS (2007), which might have reduced ether extract digestibility in treatment group T2. There was significant improvement in ether retention due to supplementation of synthetic emulsifier (T3) and herbal emulsifier (T4). Emulsifier helps in digestion and utilization of dietary fat in broiler chicken. Improved utilization of ether extract in the emulsifier supplemented birds indicated positive effects of the emulsifier on digestion and absorption of fat in broiler chicken. Similar response in ether extract retention indicated that herbal emulsifier was equally effective in utilization of fat in broiler chickens.

There was no significant (P>0.05) change in the calcium retention although numerically higher retention observed in T_4 (18.03) group followed by T_1 (17.06), T3 (16.76) and T2 (16.74). There was no significant (P>0.05) change in the phosphorous retention among the groups supplemented with emulsifiers or control group although numerically higher retention observed in T_1 (14.40) group followed by T_4 (13.53), T_3 (11.12) and T_2 (14.40).

Results of this study are in agreement with Jones *et al*. (1992), Soares and Lopez-Bote (2002), Huang *et al.* (2007), Kim *et al*. (2008) and Zampiga *et al* (2016). Polin & Hussein (1982) observed an increase in lipid retention in 7-day old broilers when bile salts (sodium taurocholate at 0.4%) in the diets, whereas the absence of bile salt supplementation reduced fat utilization in 25% as seven days of age as compared to 14 and 21 days of age. Kussaibati *et al*. (1982) showed that the supplementation of bile salts in broiler diets increased the digestibility of less saturated fats, such as the blends of animal and vegetable fats. Jones *et al*. (1992) reported an increase in fat digestibility when lecithin or lysolecithin were added to nursery diets containing soybean oil or tallow. Soares and Lopez-Bote (2002) reported that supplementation of lecithin or dietary fat had no effect on the dry matter, crude protein, or total mineral digestibility. Huang *et al.* (2007) observed significant effect of soy-lecithin as emulsifier on utilization of ether extract in broiler chicken during 42 days' research trial. Kim *et al.* (2008) also observed the effect of lecithin with or without

Table-7: Effect of exogenous emulsifiers on total tract nutrient retention (%) in energy restricted fed broiler chicken

Treat-ments	DM retention (%)	Crude protein retention (%)	EE retention (%)	Calcium retention (%)	Phosphorous retention (%)
T_1	74.28±2.40	64.77±3.19	76.66^{b} ±0.93	17.06±0.58	14.40±0.80
T_2	70.62±1.15	63.55±2.72	73.34^{a}±0.68	16.74±0.14	10.63±1.65
T_3	72.72±1.60	64.78±3.00	75.93^{b}±0.90	16.76±0.17	11.12±0.60
T_4	73.60±1.32	65.60±1.52	75.44^{ab} ±0.69	18.03±0.48	13.53±1.30
SEM	0.83	2.41	0.47	0.23	0.69
P value	0.489	0.828	0.041	0.147	0.139

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05)

Table-8: Effect of exogenous emulsifiers on hematological parameters in energy fed broiler chicken

Treatments	Hb	PCV	TEC	MCV	MCH
T1	8.44±0.12	25.35±0.24	2.25±0.029	112.30±1.13	37.42±0.542
T2	8.33±0.16	25.03±0.37	2.24±0.018	111.79±2.03	37.20±0.000
T3	8.46±0.05	25.10± 0.38	2.27±0.026	110.68±2.79	37.29±0.396
T4	8.46±0.14	25.08±0.30	2.28±0.015	109.99±1.49	37.09±0.000
SEM	0.13	0.15	0.011	0.93	0.158
P value	0.280	0.905	0.641	0.836	0.910

Table-9: Effect of exogenous emulsifier on serum bio-chemical parameters in energy restricted fed broiler chickens

Treatments	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
T_1	249.15±1.19	4.10±0.046	3.13±0.021	1.83±0.025
T_2	245.20±0.816	4.01±0.053	3.17±0.069	1.77±0.022
T_3	244.16±1.89	4.01±0.068	3.22±0.025	1.80±0.025
T_4	246.91±2.34	4.08±0.027	3.28±0.067	1.82±0.026
SEm	0.87	0.025	0.026	0.012
P- value	0.197	0.491	0.238	0.322

chitooligosaccharide on the nutrient digestibility in pigs. However, Zampiga *et a.l* (2016) observed that nutrient digestibility of broiler chickens fed diets supplemented with an exogenous emulsifier based on lysophospholipids prepared by enzymatic conversion of soy lecithin showed no significant effect on digestibility of dry matter, crude fat and crude protein.

4.4 Haematological Parameters

The data representing to the haematological parameters in broilers of different treatment groups are summarized in Table 8 and depicted in Fig. 10

Haemoglobin (Hb) and packed cell volume (PCV) didn`t differ significantly (P>0.05) among different treatment groups. Numerically haemoglobin level was highest in birds fed with synthetic and herbal emulsifier T3 (8.46),T4 (8.46), which was followed by birds fed with basal diet T1 (8.44) birds fed with 3% less energy basal diet T2 (8.33) whereas in PCV was numerically was highest in T1 (25.35) followed by T3 (25.10),T4 (25.08) and T2 (25.03) Total erythrocyte count (TEC) was found to be non-significant (P>0.05) but numerically higher in T4 (2.28million /mm^3)followed by T3 (2.27million/$mm^{3)}$, T1(2.25 million /mm^3) and T2 (2.24 million /mm^3). MCV was found to be non-significant (P>0.05) but numerically higher in T1 (112.30) followed by T2 (111.79), T3 (110.68), T4 (109.99). MCH was found to be non-significant (P>0.05) but numerically higher in T1 (37.42), T3 (37.29), T2(37.20), T4 (37.09). No significant change in haematological parameters indicated that emulsifiers didn't exert any harmful effects on broiler chicken. Results of haematological parameters are in agreement with the result of Cho *et al*. (2012). They observed on significant difference in white blood cell (WBC) and red blood cell (RBC) due to emulsifier supplementation in broiler chickens.

4.5 Serum biochemical parameters

The data on serum biochemical parameters in broiler chickens fed diet supplemented with herbal and synthetic emulsifier on 35^{th} day of feeding trial are summarized in Table 9 and depicted in figure 9 and depicted in figure 11 and 12.

The values of mean serum glucose concentration in broiler chickens did not differ significantly (P>0.05) among different treatments although there was numerically lower serum glucose concentrations found in the T2 as compared to

control group. Numerically it was higher in bird fed with standard basal diet T1 (249.15 mg/dl) followed by T4 (246.91 mg/dl), T2 (245.20) and T3 (244.16) All values are within the normal range (130 to 270 mg/dl) as mentioned by Reece (2004). In the present study no significant difference (P>0.05) was recorded in the total protein, albumin and globulin among all the treatment groups. In total protein numerically higher group is T1 (4.10 g/dl) which was followed by T4 (4.08 g/dl) and T2 and T3 have same value (4.01 g/dl). Albumin level in T4 was 3.28 g/dl, which followed T3 (322 g/dl), T2 (3.17 g/dl), T1 (3.13 g/dl). Similarly, globulin level was numerically highest in T1 (1.83 g/dl) followed by T4 (1.82 g/dl), T3(1.80 g/dl) and T2 (1.77 g/dl). No significant change in serum glucose, total protein, albumin and globulin indicated that supplementation of emulsifiers didn't pose any harmful effects on broiler chicken.

In corroboration of the result of the present study, GuerreiroNeto *et al.* (2011), Upadhaya *et al*. (2018) and Wickramasuriya *et al*. (2020) reported that emulsifier supplementation did not affect the blood metabolites of broiler chickens. However, Roy et al. (2010) observed that supplemental emulsifier had variable effects on serum metabolites. Serum glucose increased linearly with the dose of emulsifier supplementation in the diet. However, total protein and albumin concentrations were similar across the treatments. These changes may be due to interaction of emulsifiers and fat used in the experiments.

4.6Serum lipid profile

The data on serum lipid profile in terms total cholesterol, triglycerides, HDL and LDL concentration (mg/dl) in broiler chickens fed diet supplemented with herbal and synthetic emulsifier on 35th day of feeding trial are summarized in Table 10 and depicted in figure 13 and 14.

There was no significant change in the serum cholesterol, triglycerides, HDL and LDL concentration level due to supplementation of exogenous emulsifiers. Serum cholesterol in T1 (149.44 mg/dl) was numerically higher followed by T3 (147.33 mg/dl), T2(146.18 mg/dl) and T4(146.00 mg/dl) Similarly, triglyceride level in serum was numerically highest in T3 (90.89 mg/dl) followed by T4 (90.55 mg/dl),T1 (89.59 mg/dl) and T2 (89.59 mg/dl). Numerically highest In HDL was observed in T2 (99.68 mg/dl) followed by T3 (99.61 mg/dl), T4 (99.38 mg/dl) and T1 (99.25 mg/dl).

Fig. 9 Effect of exogenous emulsifier on total tract nutrient retention (%) in energy restricted fed broiler chicken

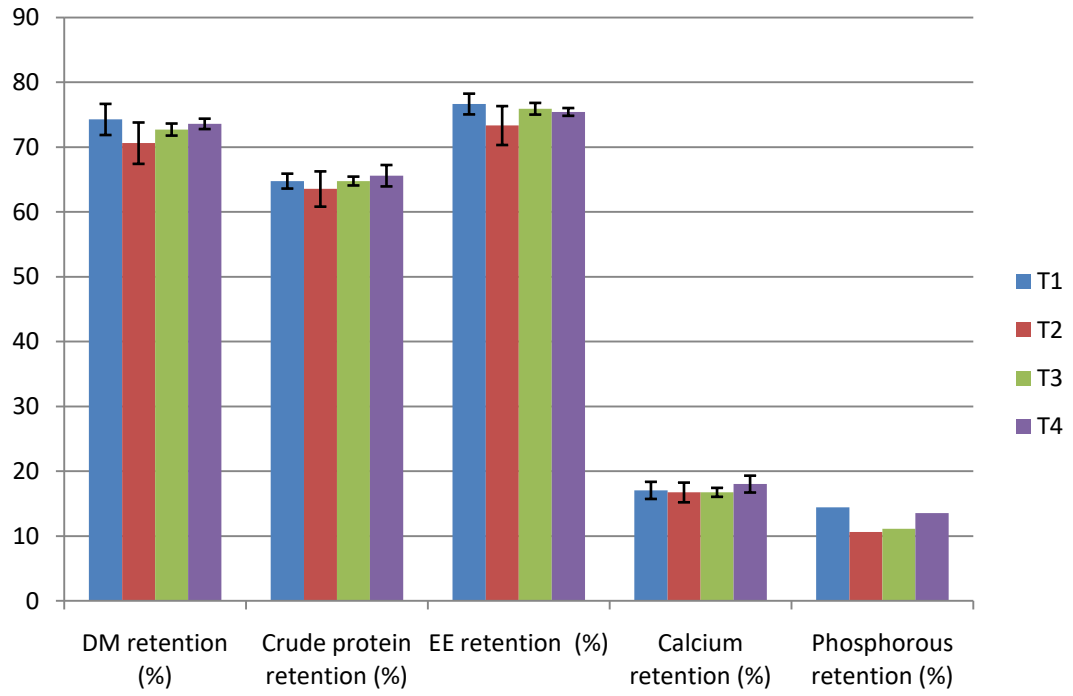


Fig. 10 Effect of exogenous emulsifier on Hb,PCV,MCV in energy restricted fed broiler chicken

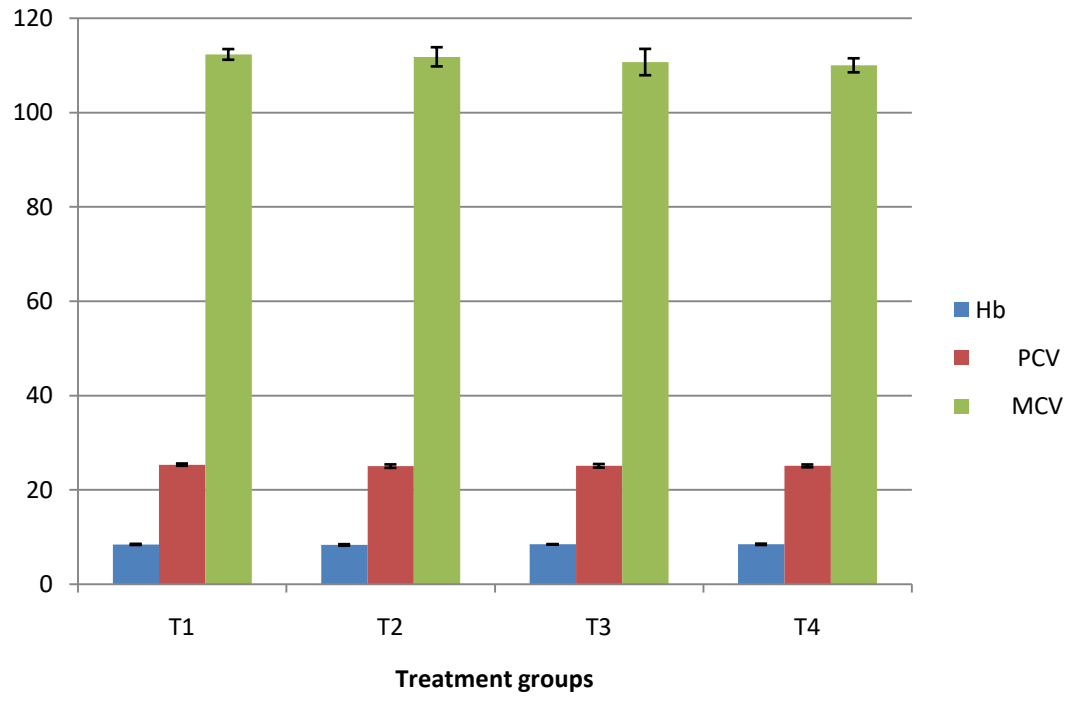


Fig. 11: Effect of exogenous emulsifier on serum glucose in energy restricted fed broiler chicken

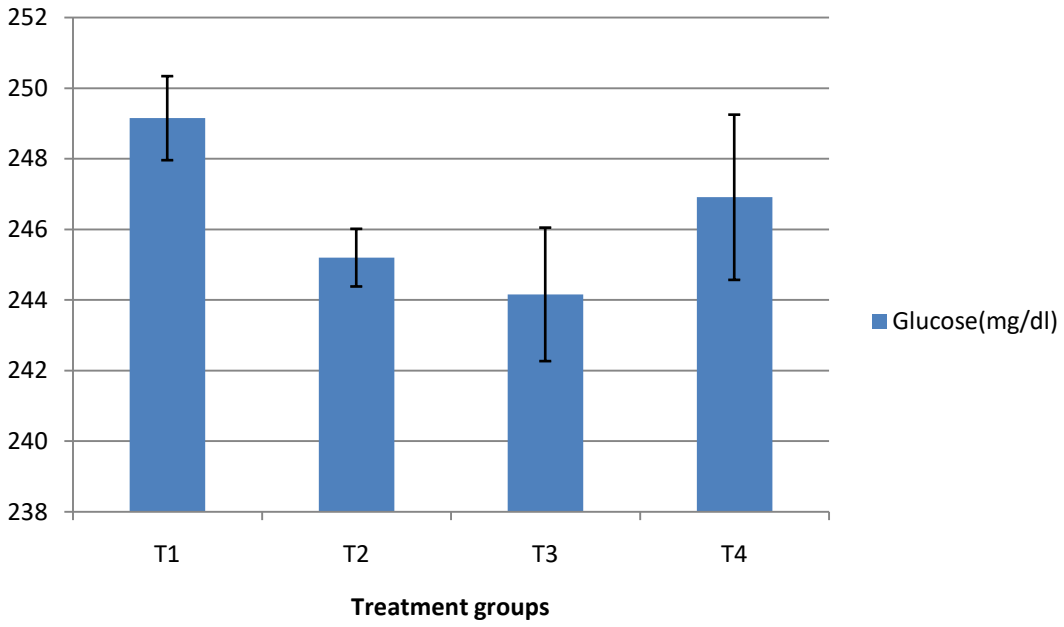
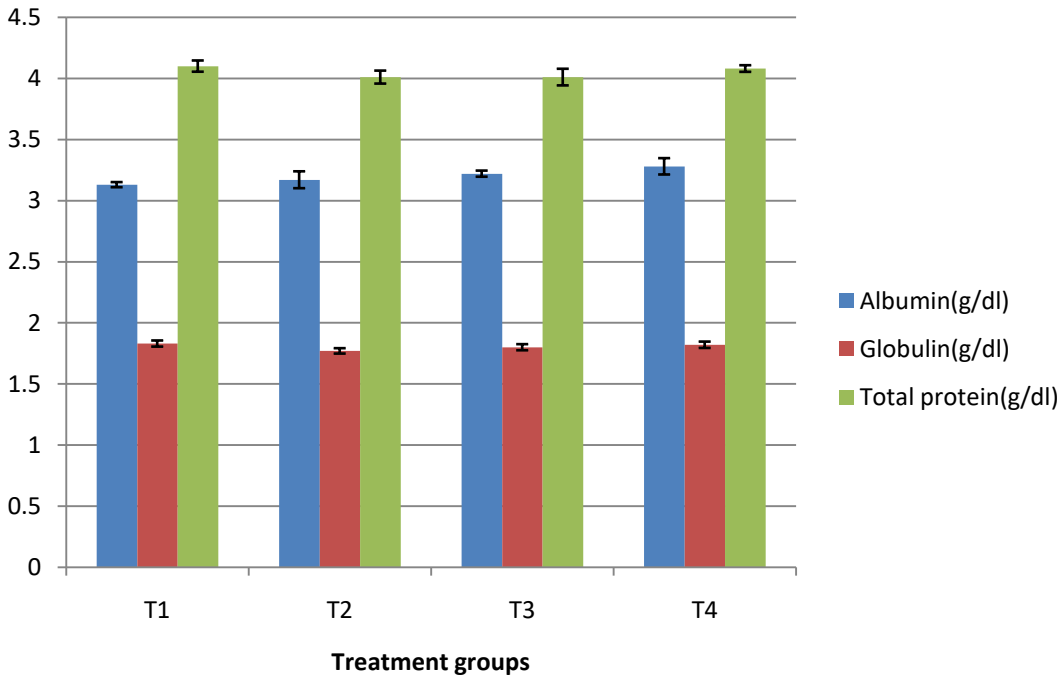


Fig. 12 Effect of exogenous emulsifier on serum albumin, globulin and total protein in broiler chicken



Similar trend was observed in serum LDL concentration in birds of different treatment groups.

The result corroborated with other research findings of Melegy *et al*. (2010), Roy et al. (2010), GuerreiroNeto *et al*. (2011) and Updhyaya *et al*. (2018). Roy et al. (2010) observed that total cholesterol and LDL cholesterol decreased linearly with the level of emulsifier (glyceryl polyethylene glycol ricinoleate) in diet on day 20 although the difference disappeared on day 39. However, HDL cholesterol fraction remained unaffected by the level of emulsifier in diet. Added emulsifier exerted non significant effect in serum tryacylglycerol concentration. It was observed further that irrespective of the level of emulsifier in diet serum tryacylglycerol concentration decreased with age. Similarly, GuerreiroNeto *et al*. (2011) reported that soybean oil, poultry fat and their blend in the diet did not not influence the serum cholesterol, HDL and triglyceride levels of broilers. Melegy *et al*. (2010)observed that serum metabolic profile was not affect by dietary fortification with lysoforte booster. Updhyaya *et al*. (2018) reported that total cholesterol, triglycerides and HDL concentration didn't affected, however, there was reduction in LDL concentration due to supplementation of emulsifier in broiler diet. Hu *et al*. (2019) observed that after reducing the use of oil, the serum triglycerides content of meat ducks was significantly higher than that of the positive control group. However, after adding emulsifier, the triglycerides level in serum was significantly lower than for the negative control group, but did not significantly differ from the positive control group. There were no significant differences in serum total cholesterol, LDL and HDL levels among all groups. However, Zhao and Kim (2017) reported that lysophospholipid supplementation reduced total cholesterol, LDL, and triglycerides in broiler chickens fed tallow incorporated into corn soy-bean diets. Similarly, another study observed reduced total cholesterol, LDL, and triglyceride profiles in broiler chickens fed emulsifier-supplemented broiler diets (Roy *et al*., 2010; Hu *et al*., 2018). Elevated serum lipids, such as cholesterol and triglycerides, due to the inclusion of animal fat in broiler diets can be efficiently reduced by the inclusion of emulsifiers (Upadhaya *et al.*¸ 2018). Observed differences in results of different workers may be ascribed to the consequences of the interactions between metabolism and absorption mechanisms.

Table-10: Effect of exogenous emulsifier on serum lipid profile (mg/dl) in energy restricted fed broiler chickens determined at 35 days of age

Treatments	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
T_1	149.44±1.55	89.59±0.609	99.25±0.599	35.64±0.944
T_2	146.18±0.57	88.69±0.545	99.68±0.724	35.15±0.738
T_3	147.33±1.73	90.89±0.773	99.61±0.168	36.26±1.244
T_4	146.00±0.67	90.55±0.503	99.38±0.635	35.69±0.689
SEm	0.63	0.339	0.280	0.435
P- value	0.291	0.081	0.606	0.987

Table-11: Effect of exogenous emulsifier on serum enzymes in energy restricted fed broiler chickens determined at 35 days of age

Treatments	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
T_1	28.46±1.30	167.87±1.00	216.82±0.87
T_2	28.37±1.15	168.00±1.31	213.37±3.42
T_3	26.87±1.10	171.51±1.69	217.94±1.53
T_4	27.42±1.50	170.50±1.53	214.87±0.92
SEm	1.37	0.73	0.99
P value	0.126	0.205	0.392

Table-12: Effect of exogenous emulsifier on serum anti-oxidant enzyme activity of energy restricted fed broiler chickens at 35 days of age

Treatments	GSH (U/ml)	CAT (U/ml)	SOD (U/ml)
T_1	251.00±11.78	2.39±.075	421.33±1.76
T_2	249.66±4.25	2.19±.058	415.33±3.17
T_3	255.00±14.43	2.31±.057	415.66±1.45
T_4	257.00±5.77	2.31±.037	412.33±5.92
SEm	4.34	0.032	1.80
P-value	0.948	0.197	0.397

Fig. 13 Effect of exogenous emulsifier on serum lipid profile (mg/dl) in experimental broiler chicken

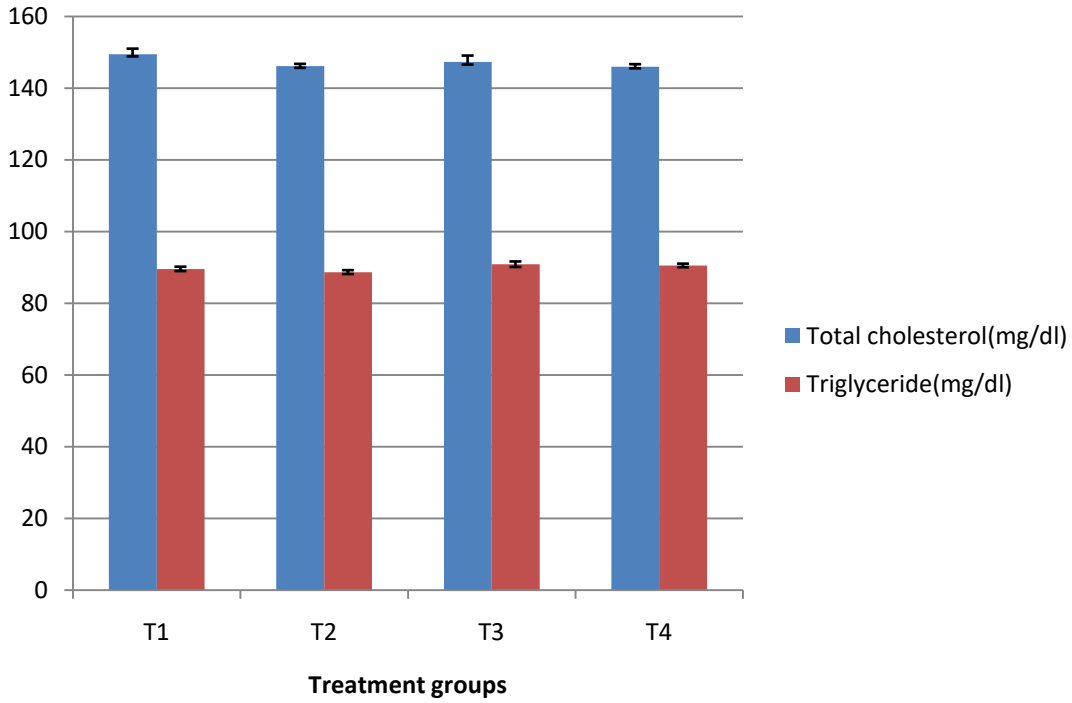


Fig. 14 Effect of exogenous emulsifier on serum lipid profile (mg/dl) in energy restricted fed broiler chicken

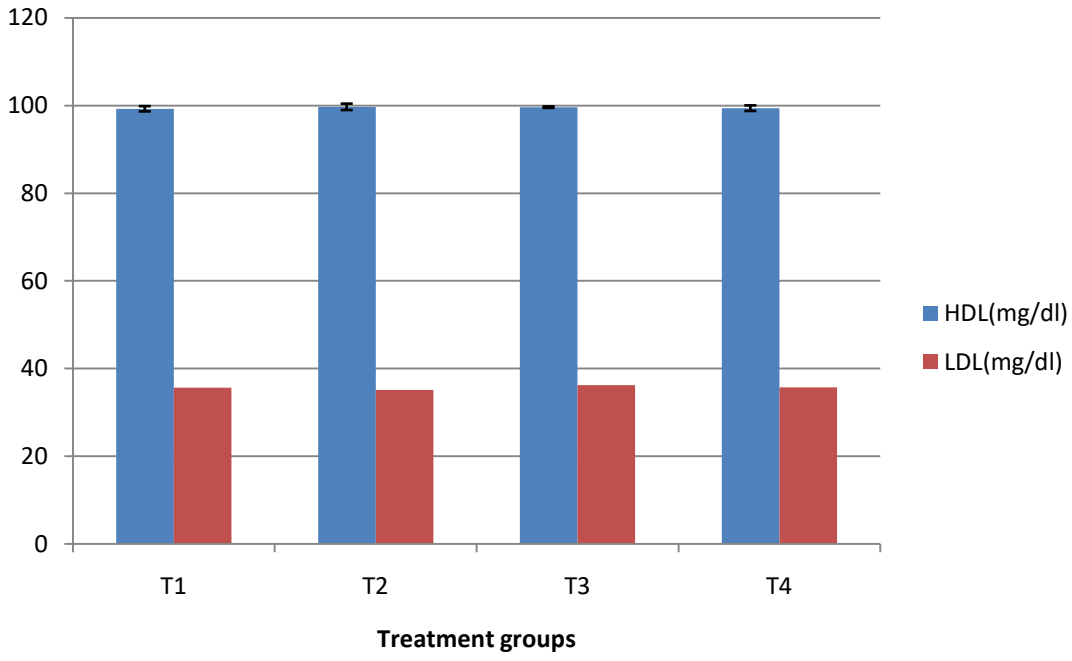


Fig. 15: Effect of exogenous emulsifier on serum enzymes in energy restricted fed broilers

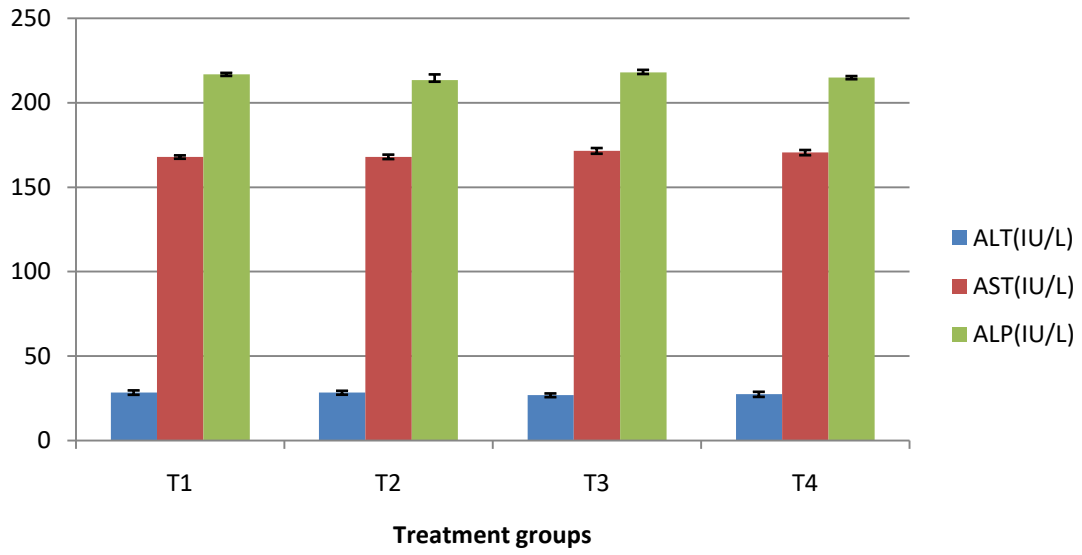
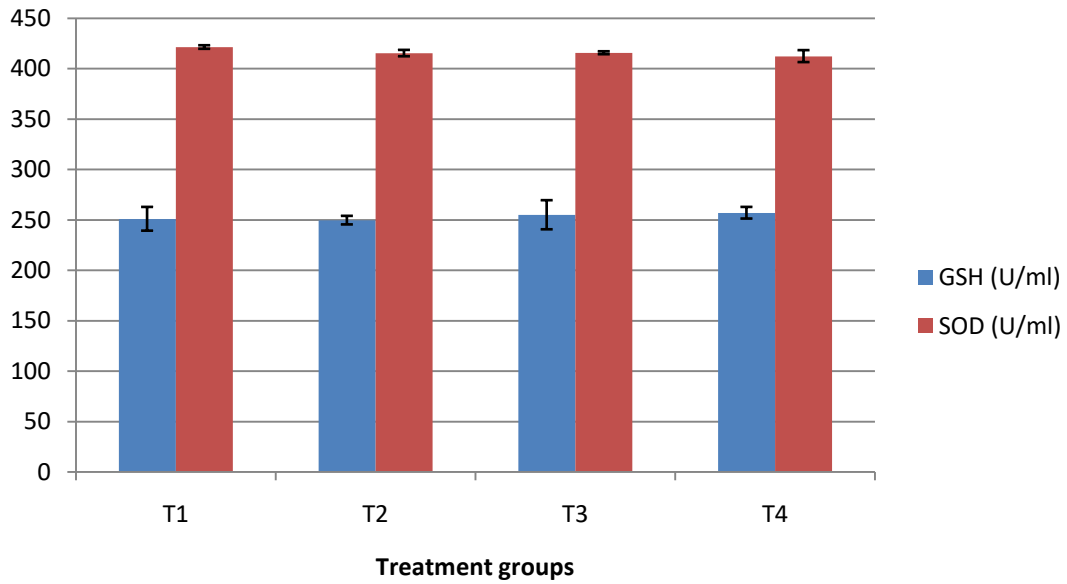


Fig. 16 Effect of exogenous emulsifier on serum anti-oxidant enzyme in energy restricted fed broiler chicken



4.7 Serum enzymes

The data representing the activity of enzymes *viz.* alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) in broilers fed diet supplemented exogenous emulsifier in energy Table 11 and depicted in figure 15.

In the present study there was no significant (P>0.05) change observed in the AST, ALT and ALP of broiler fed energy restricted and emulsifier supplemented diet. Serum alanine aminotransferase (ALT) was numerically (P>0.05) highest in treatment group T1(28.46) followed by T2 (28.37), T4 (27.42) and T3 (26.87). Similarly,aspartate aminotransferase (AST) level was not affected by dietary treatments, however, numerically higher AST values observed in T3 (171.51IU/L) which was followed by T4 (170.50 IU/L),T2 (168.00 IU/L) and T1 (167.87 IU/L). Serum alkaline phosphatase (ALP) level was also not affected by dietary treatment levels. Serum ALP was highest in T3 (217.94 IU/L) which was followed by T1 (216.82 IU/L) ,T3 (217.94 IU/L) and T2 (213.37 IU/L).

Supporting finding of the present study, the responses of all blood metabolites at 35 days of age did not differ between the dietary treatments. Similarly, GuerreiroNeto *et al*. (2011), Upadhaya *et al*. (2018) and Wickramasuriya *et al*. (2020) reported that emulsifier supplementation did not affect the blood metabolites of broiler chickens at 35 days.

4.8 Antioxidant status of serum

The data representing the antioxidant status of serum in terms of glutathione peroxidise (GSH), superoxide dismutase (SOD) and catalase (CAT) in broilers fed diet supplemented exogenous emulsifier in energy Table 12 and depicted in figure 16.

In the present study, there was no significant (P>0.05) change in antioxidant status of serum in terms of activity of glutathione peroxidise (GSH), superoxide dismutase (SOD) and catalase (CAT) among different dietary treatments. Glutathione is the ubiquitous thiol found in cells, at high concentrations of 1 to 10 mmol/L. Its primary biological function is to act as a non enzymatic reducing agent to keep the protein thiol groups in a reduced state, which is essential for the functional activity of proteins, particularly their antioxidant activity. Superoxide dismutase and catalase are

antioxidant enzymes that, by dismutation reaction, are capable of eliminating peroxides from the environment, thereby preventing the formation of other free radicals and their derivatives. Superoxide dismutase is a major antioxidant enzyme that protects cells and organisms from the damaging effects of superoxide anion. Superoxide dismutase works in conjunction with catalase and peroxidases to diminish the harmful effects of free radicals. No significant change in antioxidant status of broilers indicated that inclusion of both herbal or synthetic emulsifiers in the diets of birds did not produce any detectable changes in the activity dynamics of the antioxidative enzymes studied (i.e., glutathione peroxidise, superoxide dismutase and catalase). According to sadeghi *et al. (2016)* studied the effects of purslane (*Portulaca oleracea L*.) powder supplementation in broiler chicken diet on antioxidant status There were no significant differences among dietary treatments in liver SOD and GSH-Px activities,

4.9 Carcass traits

Data pertaining to the average dressing yield, processing losses, organ weight, cut- up parts and abdominal fat of broilers of different treatment groups due to exogenous emulsifier are presented in Table 13, 14 and 15

The carcass characters *viz*., dressing yield, eviscerated yield, blood loss, feather loss, meat to bone ratio (Table 13) and relative weights of giblet, heart, liver, spleen, bursa of fabricious (Table 14) were found to be statistically similar ($P>0.05$) among the broilers of different treatment groups. Similarly, different cut-up parts (% of live weight) *viz.* thigh, drumstick, breast, back, wing and neck did not differ among the different dietary treatments. There was significant ($P<0.05$) change in abdominal fat among different dietary treatments (Table 15). Birds fed diet having optimum energy (T1) had highest abdominal fat value, whereas birds fed energy restricted diet (T2) had lowest value and, T3 and T4 were comparable.

These results are in agreement with the findings of Andreotti *et al.* (2004), Ferreira *et al.* (2005), GuerreiroNeto *et al*. (2011); Cho *et al.* (2012) and Luc *et al.* (2013). Aguilar *et al.* (2013) determined the carcass traits of broiler chicks fed with an exogenous emulsifier and concluded that it did not influenced on the carcass, breast and abdominal fat weight, breast meat colour ($P>0.05$). Abbas *et al.* (2016) found that fat emulsifier supplementation did not affect ($P>0.05$) liver, spleen and gizzard weight

however, heart weight was affected (P<0.05) and gizzard weight decreased (p<0.05) linearly with increasing fat level. Roy *et al.* (2010) concluded that carcass weight was found to be increased by almost 6% and breast meat yield was increased by more than 8% in emulsifier supplemented group. GuerreiroNeto *et al.* (2011) reported that the use of soybean oil, poultry fat and their blend in the diet did not influence the performance and carcass traits. Collins *et al.* (2011) reported that dietary lecithin increased carcass weight and dressing percentage of pigs housed in groups. Huang *et al.* (2007) observed that lecithin improved relative weight of liver which can be correlated with the enhanced lipid metabolism in liver. Ayed *et al.* (2015) examined the effect of fatty acid compositions of oils (soybean and palm oils) on the performance of broilers and concluded that the carcass fat content was generally higher in all treated groups compared to the control. Nagargoje *et al.* (2016) conducted that the supplementation of soy lecithin alone or in combination with lipase enzyme through feed in broilers was beneficial for bird's carcass traits.

The spleen and the bursa of fabricius (BF) are known as the lymphoid organs and play an important role in the immune response of poultry (Khoso *et al.,* 2017). It was reported by Cho *et al*. (2012) that a reduction in relative spleen weight in broilers supplemented with an emulsifier could lead to an immunosuppressive effect and thus health problems. In the current study, no differences (P > 0.05) were found in the weight and in the portion of the spleen and bursa of fabricious among different treatments indicating that none on the emulsifiers had an immunosuppressive effect on broilers.In contrary to the result of the present study, Allahyari-Bake &Jahanian (2017) and Cho *et al*. (2012) and reported an increase in the bursa of fabricious weight when an emulsifer was provided in the diet. Difference in bursa of fabricious weight could be as a result of emulsifier and fat type interaction.

Table-13: Effect of exogenous emulsifier on carcass characteristics (% of live weight) of energy restricted fed broiler chickens

Treatments	Blood loss	Feather loss	Dressing Yield	Eviscerated yield	Giblet	Meat:Bone
T_1	3.19±0.095	5.04±0.031	70.41±0.50	65.56±0.61	5.35±0.09	2.21±0.028
T_2	3.11±0.019	5.06±0.063	68.40±0.44	63.38±0.54	5.25±0.08	2.16±0.023
T_3	3.18±0.025	5.12±0.035	69.04±1.13	63.53±1.22	5.20±0.14	2.25±0.040
T_4	3.22±0.10	5.18±0.091	70.42±0.43	65.11±0.63	5.28±0.03	2.28±0.046
SEm	0.034	0.030	0.37	0.42	0.04	0.015
P value	0.758	0.409	0.131	0.166	0.775	0.479

Table-14: Effect of exogenous emulsifier on different organs (% of live weight) of energy restricted fed broiler chickens

Treatments	Heart	Liver	Gizzard	Spleen	Bursa of fabricius
T_1	0.68±0.013	2.35±0.042	2.24±0.030	0.090±0.005	0.220±0.0085
T_2	0.63±0.011	2.19±0.051	2.22±0.020	0.095±0.007	0.203±0.0084
T_3	0.64±0.027	2.20±0.075	2.22±0.047	0.093±0.007	0.210±0.0068
T_4	0.66±0.008	2.28±0.024	2.28±0.009	0.106±0.004	0.200±0.0025
SEm	0.008	0.027	0.015	0.003	0.0036
P value	0.190	0.146	0.479	0.161	0.227

Table 15

Table-16: Effect of exogenous emulsifiers on sensory evaluation of meat in energy restricted fed broiler chickens

Treatments	Appearance	Flavour	Juiciness	Tenderness	Overall Acceptability
T_1	6.58±0.13	6.43±0.23	6.33±0.063	6.19±0.03	6.20±0.10
T_2	6.33±0.063	6.00±0.05	6.11±0.060	6.08±0.05	6.07±0.065
T_3	6.33±0.067	6.19±0.03	6.52±0.21	6.45±0.22	6.10±0.05
T_4	6.50±0.057	6.22±0.06	6.51±0.20	6.50±0.057	6.16±0.05
SEm	0.049	0.071	0.083	0.73	0.09
P-value	0.080	0.206	0.126	0.245	0.063

Table-17: Effect of exogenous emulsifier on mortality in energy restricted fed broiler chickens

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5
T_1	0	0	1	1	0
T_2	0	0	0	0	0
T_3	0	0	0	1	0
T_4	0	0	0	0	1

4.10 Organoleptic test (Hedonic test) of meat

Data pertaining to the organoleptic test of broiler meat of different treatment groups due to exogenous emulsi fier are presented in Table 16

Dietary treatments didn`t affect appearance, flavour, tenderness, juiciness and overall acceptability of meat of broiler chicken fed diets supplemented with synthetic or herbal exogenous emulsifier.

4.11 Mortality

Data on mortality of broiler chicks during the experimental period are presented in Table 17.

During the trial of exogenous emulsifiers in energy restricted fed broiler chicken, it was found that there was no effect of dietary treatments on mortality and mortality was within the permissible limit.

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5 SUMMARY AND CONCLUSIONS

Poultry production is one of the most dynamic and fastest segments in animal husbandry sector in India. The high growth rate and feed efficiency are the two main targets in broiler production. Therefore, the most important key in the broiler industry is to provide feed that contains all the necessary nutrients needed for broilers to grow to their full genetic potential. Feed accounts for 65- 70% of broiler production cost, thus, feed cost deserves befitting attention. Energy is a major cost component factor in diets of broilers. Fats (animal or vegetable) are used in the diet in order to meet the energy requirements of birds due to fats have a higher energy value and can provide about 2.25 times more energy than carbohydrates

Fat digestion in poultry occurs mainly in the small intestine. Fats are insoluble in water and cannot be solubilised in the gastrointestinal tract, and have to be emulsified before lipolytic enzymes can digest them. Emulsification is the process of breaking down of the large fat globules into smaller globules and makes them water soluble and an emulsifier is able to bridge between water soluble and fat soluble material. Bile salts are natural emulsifiers. The utilization of dietary fats in young birds is poor because they have a limited capacity to produce and secrete bile salts and lipase until their gastrointestinal tract matures at 10-14 days of age (Noy and Sklan, 1998). Emulsifiers help to improve the utilisation of lipids, particularly animal fats, and play a role in performing the insufficiencies of naturally low bile production and recirculation in young birds (Siyal *et al.*, 2017). Therefore, broiler ration is supplemented with feed emulsifier to improve fat digestibility and energy efficiency. Commercial emulsifiers which are commonly used in the feed industry can be categorized into two groups *viz.* natural emulsifier and synthetic emulsifiers. Natural emulsifier ones are those produced in the animal body such bile and phospholids, and those from food materials such as soylecithin (Soares and Lopez-Bote, 2002), whereas synthetic emulsifiers are modified emulsifiers such as lysolecithin or lysophosphatidylcholine (Zhang *et. al.*,2011).Natural emulsifiers like soylecithin obtained from soyabean and synthetic emulsifiers like polyethylene glycol mono and dioleates and sodium stearoyl-2-lactylate in poultry diets have been used to improve fat digestion in chicks, resulting in improved growth performance. Thus, the addition

of an effective emulsifier to a poultry diet can compensate for a reduction in dietary energy by improving fat digestibility and energy efficiency. There are many reports available on evaluation of natural and synthetic exogenous emulsifiers in poultry and swine diets. However, there is lack of research data on use of comparative efficacy of herbal and synthetic emulsifiers in broiler chickens. Therefore, the present study was planned for comparative efficacy evaluation of herbal and synthetic feed emulsifier in energy-restricted feed of broiler chicken for economic poultry production with the following objectives:

- To study the effect of exogenous emulsifiers on growth and performance parameters in energy-restricted fed broiler chicken
- To study the effect of exogenous emulsifiers on retention of nutrients in energy-restricted fed broiler chicken
- To study the effect of exogenous emulsifiers on hemato-biochemical parameters in energy-restricted fed broiler chicken
- To study the effect of exogenous emulsifiers on carcass characteristic in energy-restricted fed broiler chicken

A feeding trial of 35 days was conducted to to compare the effects of synthetic and herbal emulsifier on the growth performance, nutrients utilization, haemato-biochemical profile meat quality, antioxidant capacity and carcass quality of broiler chickens.

There were 4 dietary treatment groups as follows:

Treatment T_1: Standard basal diet without emulsifier (control)

Treatment T_2: Basal diet with 3% less metabolisable energy

Treatment T_3:Basal diet with 3% less metabolisable energy + synthetic emulsifier @250 g/tonne of feed

Treatment T_4:Basal diet with 3% less metabolisable energy + herbal emulsifier@250 g/tonne of feed

A total of one hundred and eighty, day-old Cobb 400Y strain broiler chicks were procured, weighed individually and randomly allotted to four treatment groups each with three replicates of 15 chicks in such a way that average body weight was approximately similar for all the treatment groups. Standard basal diets for pre-starter

(0-7 days) starter (8-21 days) and finisher (21-35 days) phases of growth of broiler chickens were prepared by mixing the different ingredients to meet the nutrient requirements of broiler chicken as per recommendation of BIS (2007).Standard basal ration T1 (control) contained all nutrients as per specification of BIS (2007) whereas T2 (Negative control) had 3% less metabolisable energy (kcal/kg feed) as compared to T_1. Treatment group T1 and T2 didn't contain exogenous feed emulsifier. Treatment group T3 and T4 were supplemented with synthetic feed emulsifier (Volamel) of Nukamel, Industriekade, the Netherlands, and herbal emulsifier (AV/PEE/15) of Ayurvet Limited, Katha, Baddi, Solan, India @ 250 g per ton of feed, respectively. The chicks were provided the experimental feeds and water *ad libitum.* Body weight of individual chick and feed consumption of each replicate in different group was recorded weekly upto 5 weeks of age, and feed conversion ratio (FCR) and performance index was calculated. Blood samples were collected from wing vein of two birds from each replicates on 35^{th} day for analyses of haemato-biochemical parameters and anti-oxidant status. A metabolism trial was also conducted on two birds from each replicate during the 5^{th} week of feeding trial to know the nutrient utilization. At the end of feeding trial, two chicks per replicate in each treatment group were sacrificed for the study of carcass yield, cut up parts, organ weights, processing losses and antioxidant status in breast meat.

The results of the present study are summarized as under:

I. Effect of synthetic and herbal feed emulsifiers on growth performance of broiler chicken:

Result on cumulative growth performance of broilers in terms of average weight gain, feed intake, FCR and performance index as influenced by supplementation of synthetic and herbal emulsifiers were divided into different phases *viz.* pre-starter phase (0-7 days), starter phase (8-21 days), finisher phase (22-35 days) and overall period (0-35 days).

During the pre-starter phase (0-7 days) there was significantly ($P<0.05$) change in feed intake and feed conversion ratio. In body weight gain there was numerically ($P>0.05$) highest body weight gain(114.83 g) in the group supplemented with herbal emulsifier (T_4), that was followed by the groups supplemented with basal diet with 3% less energy (T_2) (113.49g), synthetic emulsifier (T_3) (104.61 g) and the control group (T_1) (109.86 g). Maximum feed intake was recorded in T_2 group

(227.00 g) which was followed by the groups T1, T3 and T4, respectively. The best FCR obtained in T4 (1.52) group whereas the worst FCR was recorded in T2 treatment group (2.00). Performance index was numerically (P>0.05) highest in the synthetic emulsifier supplemented group (T4) (76.12) followed by T1, T3 and T2 that is 63.49, 60.73 and 57.19, respectively.

During the starter phase (8-21 days) feed consumption, body weight gain, FCR and performance index in broilers of various treatment groups differed significantly (P<0.05). There was significantly (P<0.05) highest body weight gain, (832.88 g) in the control group (T1) and lowest in T2 (736.55g). Maximum feed intake (P<0.05) was recorded in T2 group (1159.50 g) followed by the groups T4 , T1 and T3 that is 1078.86 g, 1075.68 g and 1012.95 g, respectively. The best FCR (1.29) was recorded in control group fed standard diet. Performance index increased significantly (P<0.05) in the control group (T1) (645.20) followed by T3, T4 and T2 that is 591.62, 589.90 and 468.64, respectively.

During the finisher phase, body weight gain in broiler chickens fed standard basal diet (T_1) group was significantly (P<0.05) higher (982.78 g) than synthetic emulsifier (T_3) (923.86 g) and those supplemented with herbal emulsifier (T4) (911.48 g) and basal diet with 3% less energy (T_2) (830.95 g). Feed intake didn`t differ significantly (P>0.05) among different treatment groups. FCR result was significantly lowest in the T_1 (1.85) group and that was succeeded by T_4 (1.80), T_3 (1.81) and T2 (1.82). Performance index was highest (531.64) in broilers fed diet standard basal diet followed by T_3 (474.73), T_4 (465.91) and T_2 (373.11) groups, respectively. Birds fed diets containing synthetic emulsifier (T_3) and herbal emulsifier (T_4) had comparable performance index. Lowest PI was seen in T2 (373.11) which was fed with restricted energy basal diet.

During overall experimental period (0-35 days) of experiment, there was significant difference (P<0.05) observed in body weight gain among different groups. Maximum weight gain (1925.53 g) was recorded in broilers fed with basal diet (T_1) followed by T_4 (1823.93 g), T_3 (1801g) and T_2 (1681 g) groups, whereas, T3 and T4 were non-significant (P>0.05) and comparable. Significantly (P<0.05) highest feed intake (3239.33g) (T2) was recorded in broilers fed with basal diet with restricted energy (T_2) followed by T_1 (3084.04 g), T_4 (3037.10 g) and T_3 (2995.13 g) groups. However, feed intake in T3 and T4 were comparable. There was significant difference

(P<0.05) observed in FCR results among different treatment groups, T3 and T4 are comparable. Lowest FCR (1.60) recorded in birds fed with basal diet i.e control group (T_1) which was significantly higher as compared to birds supplemented with restricted energy basal diet(T2) (1.92) and synthetic emulsifier (T3) and herbal emulsifier (T4) group FCR (1.66) was comparable. Overall best FCR was recorded in control group i.e birds fed standard basal diet as compared with other treatment groups. Synthetic and herbal emulsifier supplemented groups had comparable FCR. Overall lowest FCR was noted in bird supplemented with basal diet having 3% less energy. Similarly, performance index was significantly (P<0.05) highest in T_1 (1202.51) followed by T_4 (1095.94), T_3 (1084.95) and T_2 (872.81) groups.

Results indicated that reduction or restriction in dietary energy (metabolisable energy) level by 3% resulted in significant (P<0.05) decrease in growth performance of broiler chickens. Addition of emulsifiers (synthetic or herbal) @250 g per tonne of feed in energy restricted (3% less metabolisable energy) broiler diet improved the growth performance of the birds. After addition of the emulsifiers, growth performance was similar to the control group having optimum dietary energy level. Effect of herbal and synthetic feed emulsifier on improvement in growth performance in broiler chicken was similar (P>0.05), however, feed cost per kg body weight gain was almost for synthetic and herbal emulsifier.

II. Effect of supplementation of synthetic and herbal emulsifier on nutrients utilization in energy restricted fed broiler chicken:

There was no significant (P>0.05) effect of exogenous emulsifiers on dry matter, crude protein, calcium and phosphorus retention in broiler chickens fed energy restricted diet. There was significant (P<0.05) difference in retention of ether extract among different treatments. Ether extract retention was significantly (P<0.05) highest in T1 (76.66%) i.e birds fed basal diet optimum energy, whereas ether extract retention was significantly (P<0.05) lowest (73.34%) in birds fed energy restricted diet (T2). There was significant improvement in ether retention due to use of emulsifier @250 g per tonne of feed. Emulsifier helps in digestion and utilization of dietary fat in broiler chicken. Improved utilization of ether extract in the emulsifier supplemented birds indicated positive effects of the emulsifier on digestion and

absorption of fat. Similar response in ether extract retention indicated that herbal emulsifier was equally effective in utilization of fat in broiler chickens.

III. Effect of supplementation of synthetic and herbal emulsifier on haematological parameters in energy restricted fed broiler chicken:

Haematological parameters viz. haemoglobin, packed cell volume (PCV), Total erythrocyte count (TEC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) values in broiler chicks fed diets supplemented with emulsifiers in energy restricted diets didn`t differ significantly. No significant change in haematological parameters indicated that emulsifiers did not exert any harmful effects on broiler chicken.

IV. Effect of supplementation of synthetic and herbal emulsifier on serum biochemical parameters in energy restricted fed broiler chicken:

Serum glucose, total protein, albumin and globulin, total cholesterol, triglycerides, HDL and LDL concentration (mg/dl) levels and serum enzymes *viz.*, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in broiler chickens did not differ significantly ($P>0.05$) due to supplementation of synthetic and herbal emulsifier in energy restricted fed broiler chicken. There was no significant variation in serum biochemical constituents due to type of emulsifier (herbal or synthetic).

V. Effect of supplementation of synthetic and herbal emulsifier on antioxidant status of broiler chicken:

There was no significant ($P>0.05$) change in antioxidant status of serum in terms of activity of glutathione peroxidise (GSH), superoxide dismutase (SOD) and catalase (CAT) among different dietary treatments due to supplementation of emulsifiers. No significant change in antioxidant status of broilers indicated that inclusion of either herbal or synthetic emulsifiers @250 g per tonne of feed in the diets of birds did not produce any detectable changes in the activity dynamics of the antioxidative enzymes studied.

VI. Effect of supplementation of synthetic and herbal emulsifier on carcass characteristics of broiler chicken:

The carcass characters *viz*., dressing yield, eviscerated yield, blood loss, feather loss, meat to bone ratio and relative weights of giblet, heart, liver and gizzard among different dietary treatments didn`t differ significantly (P>0.05) among different treatment groups. Similarly, different cut-up parts (% of live weight) *viz.* thigh, drumstick, breast, back, wing and neck did not differ among the different dietary treatments. Relative weights of giblet, heart, liver, spleen, bursa of fabricious were found statistically similar (P>0.05). Birds fed diet having optimum energy (T1) had highest abdominal fat value, whereas, birds fed energy restricted diet (T2) had lowest value. Addition of emulsifier @250 g per tonne of energy restricted fed broiler diet (T3 and T4) had comparable abdominal fat. Dietary treatments didn`t affect appearance, flavour, tenderness, juiciness and overall acceptability of meat of broiler chicken fed diets supplemented with synthetic or herbal exogenous emulsifier.

Conclusion:

Based on results obtained from present study, it can be concluded that:

I. Decrease in 3% metabolisable energy of broiler chicken diet depressed growth performance, ether extract retention and economics of broiler chicken.

II. Dietary supplementation of synthetic or herbal emulsifier @ 250 gm/tonne of energy restricted soyabean oil based broiler feed improved the growth performance, ether extract utilization and economics without affecting haemato-biochemical constituents, antioxidant activity and carcass quality of broiler chickens.

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Table-15: Effect of exogenous emulsifiers on carcass characteristics (% of live weight) in energy restricted fed broiler chickens

Treatments	Thigh	Drumstick	Breast	Back	Wing	Neck	Abdominal Fat
T_1	10.55±0.029	9.38± 0.085	18.26±0.040	17.44±0.231	8.60±0.106	4.69±0.055	1.62^{c}±0.017
T_2	10.33±0.168	9.20±0.027	18.33±0.216	16.91±0.252	8.13±0.317	4.49±0.103	1.34^{a}±0.053
T_3	10.37± 0.107	9.22±0.045	18.31±0.193	17.01±0.052	8.51±0.189	4.48±0.083	1.53^{bc}±0.017
T_4	10.39± 0.036	9.22±0.070	18.26±0.089	17.00±0.024	8.65±0.152	4.69±0.067	1.48^{b}±0.015
SEm	0.050	0.032	0.071	0.091	0.105	0.042	0.025
P value	0.481	0.162	0.983	0.170	0.314	0.111	0.001

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

Table-6 Economics of broiler production in terms of feed cost per kg of body weight gain (0-35 days)

Treatments	Feed Intake (gram)			Feed cost (Rs./ kg)			Feed cost for body weight gain			Total feed cost for 0-35 days	Body weight gain (0-35 days)	Feed cost (Rs./Kg weight gain)
	Pre-starter (0-7 day)	Starter (8-21 days)	Finisher (22-35days)	Pre-starter feed (0-7 day)	Starter feed (8-21 days)	Finisher feed (22-35days)	Pre-starter (0-7 day)	Starter (8-21 days)	Finisher (22-35days)			
T1	191	1076	1817	33.52	32.00	30.38	6.40	34.43	55.20	96.03	1926	49.85
T2	227	1150	1863	32.78	29.77	28.91	7.44	34.24	53.86	95.54	1681	56.84
T3	180	1013	1799	32.93	29.92	29.06	5.93	30.31	52.29	88.53	1802	49.13
T4	175	1078	1784	32.88	29.87	29.17	5.76	32.20	51.76	89.72	1824	49.19

LIST OF PLATES

(1) Day old chicks

(2) Brooding of day old chicks

(3) Wing banding of day old chicks

(4) Synthetic emulsifier

(5) Herbal emulsifier

(6) Premixing of emulsifier

(7) Weight at 3 weeks of age

(8) Weight at 5 weeks of age

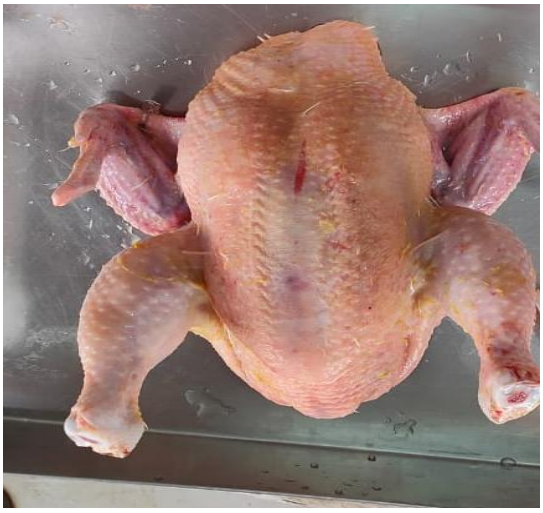
(9) Blood collection from wing vien

(10) Metabolic trial cage

(11) Feed intake calculation during metabolic trial

(12) Faecal sample collection

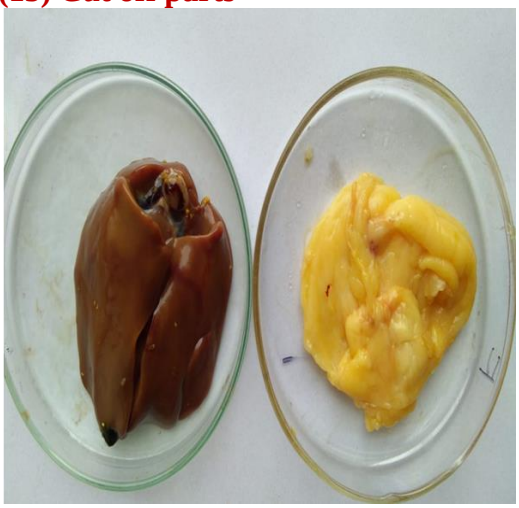
(13) Faecal sample weight



(14) Dressed weight

(15) Cut off parts

(16) Giblet



(17) Liver and Abdominal Fat

(18) Biochemical test

APPENDICES

Table 1: Effect of Exogenous emulsifier on body weight gain in energy restricted fed broiler chicken(0-35 days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	109.86±4.56	314.56±9.53	518.31^{c}±8.66	499.11^{b}±8.44	483.66^{c}±9.13
T_2	113.49±2.78	278.92±10.17	457.63^{a}±9.58	443.95^{a}±6.73	387.00^{a}±9.07
T_3	104.61±1.80	309.26±19.88	463.68^{ab}±13.97	487.16^{b}±4.08	436.70^{b}±6.82
T_4	114.83±5.35	300.78±9.34	496.83^{bc}±11.39	471.76^{ab}±15.33	439.71^{b}±11.21
SEm	2.04	6.89	8.83	7.44	11.03
P value	0.318	0.298	0.015	0.018	0.001

Table-2: Effect of Exogenous emulsifier on Body weight in energy restricted fed broiler chicks during Overall Period (0-35days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	157.66±4.76	472.23±10.81	990.55^{c}±18.99	1489.66^{c}±17.06	1973.33^{c}±16.44
T_2	160.56±2.67	439.48±9.15	897.11^{a}±14.19	1341.06^{a}±14.47	1728.06^{a}±14.51
T_3	151.96±2.36	461.23±8.56	924.91^{ab}±11.53	1412.08^{b}±12.43	1845.45^{b}±19.02
T_4	161.15±4.67	461.93±10.26	958.77^{bc}±21.15	1430.53^{b}±15.04	1870.25^{b}±22.27
SEm	1.95	5.49	12.81	17.19	27.43
P value	0.361	0.193	0.021	0.001	0.000

^{a,b,c}Mean values with different superscripts within a column differ significantly ($P<0.05$)

Table-3: Effect of Exogenous emulsifier on feed intake in energy restricted fed broiler chicks during overall period (0-35 days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	190.96^{a}±9.79	377.06^{ab}±3.53	698.61^{ab}±19.16	838.69±6.57	978.70±22.29
T_2	227.00^{b}±8.88	397.33^{b}±6.22	762.16^{c}±11.70	860.43±13.67	992.40±12.42
T_3	180.85^{a}±4.79	358.25^{a}±11.92	657.03^{a}±14.95	837.80±20.99	961.20±18.65
T_4	174.53^{a}±4.39	369.26^{a}±6.61	709.60^{b}±7.43	835.06±14.21	948.63±20.60
SEm	6.88	5.41	12.77	7.00	9.48
P value	0.004	0.038	0.005	0.616	0.426

Table-4 Effect of Exogenous emulsifier on FCR in energy restricted fed broiler chicks during (0-35days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	1.74^{ab}±0.08	1.20±0.03	1.34^{a}±0.02	1.68^{a}±0.03	2.02^{a}±0.03
T_2	2.00^{b}±0.12	1.42±0.04	1.66^{b}±0.06	1.93^{b}±0.05	2.56^{b}±0.06
T_3	1.73^{ab}±0.07	1.17±0.10	1.42^{a}±0.07	1.71^{a}±0.04	2.20^{a}±0.03
T_4	1.52^{a}±0.08	1.23±0.08	1.43^{a}±0.07	1.77^{a}±0.03	2.16^{a}±0.09
SEm	0.06	0.04	0.04	0.03	0.06
P value	0.042	0.137	0.028	0.011	0.002

^{a,b} Mean values with different superscripts within a column differ significantly ($P<0.05$)

Table-5 Effect of Exogenous emulsifier on Performance Index in energy restricted fed broiler chicks during (0-35days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	63.53±4.88	263.12±15.98	735.77^{b}±13.83	297.49^{b}±10.66	239.20^{c}±6.55
T_2	57.28±5.16	196.22±12.82	540.19^{a}±26.71	229.32^{a}±9.27	151.14^{a}±7.07
T_3	60.78±3.56	271.77±44.66	654.41^{b}±32.02	283.62^{b}±7.89	198.34^{b}±5.07
T_4	76.16±8.22	246.17±22.34	670.84^{b}±49.27	267.03^{b}±13.39	203.57^{b}±3.73
SEm	3.25	14.50	25.48	8.90	9.75
P value	0.183	0.272	0.018	0.009	0.000

^{a,b,c} Mean values with different superscripts within a column differ significantly ($P<0.05$)

List of ANOVA tables

Table 6 : Analysis of varience (ANOVA) of weekly Body weight in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Week1	Between Groups	158.642	3	52.881	1.230	.361
	Within Groups	343.949	8	42.994		
	Total	502.590	11			
Week2	Between Groups	1707.970	3	569.323	1.999	.193
	Within Groups	2278.849	8	284.856		
	Total	3986.819	11			
Week3	Between Groups	14825.634	3	4941.878	5.765	.021
	Within Groups	6857.179	8	857.147		
	Total	21682.813	11			
Week 4	Between Groups	33739.698	3	11246.566	17.010	.001
	Within Groups	5289.411	8	661.176		
	Total	39029.109	11			
Week5	Between Groups	91309.854	3	30436.618	30.297	.000
	Within Groups	8036.738	8	1004.592		
	Total	99346.592	11			

Table 7: Analysis of varience (ANOVA) of weekly Body weight gain in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Week1	Between Groups	187.766	3	62.589	1.377	.318
	Within Groups	363.670	8	45.459		
	Total	551.436	11			
Week2	Between Groups	2219.294	3	739.765	1.456	.298
	Within Groups	4064.535	8	508.067		
	Total	6283.829	11			
Week3	Between Groups	7350.725	3	2450.242	6.637	.015
	Within Groups	2953.342	8	369.168		
	Total	10304.067	11			
Week 4	Between Groups	5109.595	3	1703.198	6.160	.018
	Within Groups	2211.770	8	276.471		
	Total	7321.365	11			
Week5	Between Groups	14055.114	3	4685.038	18.474	.001
	Within Groups	2028.828	8	253.604		
	Total	16083.942	11			

Table 8: Analysis of varience (ANOVA) of weekly Feed Intake in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Week1	Between Groups	4944.957	3	1648.319	10.112	.004
	Within Groups	1304.088	8	163.011		
	Total	6249.046	11			
Week2	Between Groups	2446.692	3	815.564	4.586	.038
	Within Groups	1422.655	8	177.832		
	Total	3869.347	11			
Week3	Between Groups	16850.952	3	5616.984	9.563	.005
	Within Groups	4698.995	8	587.374		
	Total	21549.947	11			
Week 4	Between Groups	1237.253	3	412.418	.630	.616
	Within Groups	5239.598	8	654.950		
	Total	6476.850	11			
Week5	Between Groups	3333.620	3	1111.207	1.041	.426
	Within Groups	8543.207	8	1067.901		
	Total	11876.827	11			

Table 9: Analysis of varience (ANOVA) of weekly FCR in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Week1	Between Groups	.346	3	.115	4.393	.042
	Within Groups	.210	8	.026		
	Total	.556	11			
Week2	Between Groups	.119	3	.040	2.460	.137
	Within Groups	.129	8	.016		
	Total	.248	11			
Week3	Between Groups	.173	3	.058	5.201	.028
	Within Groups	.089	8	.011		
	Total	.262	11			
Week 4	Between Groups	.116	3	.039	7.349	.011
	Within Groups	.042	8	.005		
	Total	.159	11			
Week5	Between Groups	.483	3	.161	13.430	.002
	Within Groups	.096	8	.012		
	Total	.346	3	.115	4.393	.042

Table 10: Analysis of varience (ANOVA) of weekly Performance index in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Week1	Between Groups	608.847	3	202.949	2.067	.183
	Within Groups	785.564	8	98.196		
	Total	1394.411	11			
Week2	Between Groups	10272.939	3	3424.313	1.567	.272
	Within Groups	17486.763	8	2185.845		
	Total	27759.701	11			
Week3	Between Groups	59607.829	3	19869.276	6.079	.018
	Within Groups	26148.809	8	3268.601		
	Total	85756.639	11			
Week 4	Between Groups	7809.842	3	2603.281	7.861	.009
	Within Groups	2649.227	8	331.153		
	Total	10459.069	11			
Week5	Between Groups	11773.601	3	3924.534	39.442	.000
	Within Groups	796.009	8	99.501		
	Total	12569.610	11			

Table 11: Analysis of varience (ANOVA) of growth performance in energy restricted fed broiler chicks during Pre-starter phase (0-7days)

Anova						
Week	**Source of varience**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
BWG	Between Groups	187.766	3	62.589	1.141	.389
	Within Groups	438.670	8	54.834		
	Total	626.436	11			
FI	Between Groups	4944.957	3	1648.319	10.112	.004
	Within Groups	1304.088	8	163.011		
	Total	6249.046	11			
FCR	Between Groups	.346	3	.115	4.393	.042
	Within Groups	.210	8	.026		
	Total	.556	11			
PI	Between Groups	610.765	3	203.588	2.090	.180
	Within Groups	779.255	8	97.407		
	Total	1390.020	11			

Table 12: Analysis of varience (ANOVA) of growth performance in energy restricted fed broiler chicks during starter phase (8-21days)

Anova						
Week	**Source of varience**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
BWG	Between Groups	14832.111	3	4944.037	5.536	.024
	Within Groups	7144.410	8	893.051		
	Total	21976.521	11			
FI	Between Groups	32470.862	3	10823.621	9.886	.005
	Within Groups	8758.723	8	1094.840		
	Total	41229.585	11			
FCR	Between Groups	.151	3	.050	9.203	.006
	Within Groups	.044	8	.005		
	Total	.195	11			
PI	Between Groups	50197.321	3	16732.440	13.763	.002
	Within Groups	9726.339	8	1215.792		
	Total	59923.660	11			

Table 13: Analysis of varience (ANOVA) of growth performance in energy restricted fed broiler chicks during finisher period (22-35 days)

Anova						
Week	**Source of varience**	Sum of Squares	df	Mean Square	F	Sig.
BWG	Between Groups	35160.522	3	11720.174	18.841	.001
	Within Groups	4976.395	8	622.049		
	Total	40136.917	11			
FI	Between Groups	10581.947	3	3527.316	2.820	.107
	Within Groups	10005.533	8	1250.692		
	Total	20587.480	11			
FCR	Between Groups	.242	3	.081	19.788	.000
	Within Groups	.033	8	.004		
	Total	.275	11			
PI	Between Groups	38779.750	3	12926.583	101.928	.000
	Within Groups	1014.562	8	126.820		
	Total	39794.312	11			

Table 14: Analysis of varience (ANOVA) of growth performance in energy restricted fed broiler chicks during overall phase (0-35days)

Anova						
Week	**Source of varience**	Sum of Squares	df	Mean Square	F	Sig.
BWG	Between Groups	90720.542	3	30240.181	51.114	.000
	Within Groups	4732.990	8	591.624		
	Total	95453.532	11			
FI	Between Groups	102387.511	3	34129.170	29.718	.000
	Within Groups	9187.530	8	1148.441		
	Total	111575.041	11			
FCR	Between Groups	.189	3	.063	20.972	.000
	Within Groups	.024	8	.003		
	Total	.213	11			
PI	Between Groups	171592.613	3	57197.538	34.936	.000
	Within Groups	13097.719	8	1637.215		
	Total	184690.332	11			

Table 15: Analysis of variance (Anova) for Effects of treatments on total nutrients retention performance in energy restricted fed broiler chicken during overall phase

Anova						
Week	**Source of varience**	Sum of Squares	df	Mean Square	F	Sig.
DM	Between Groups	22.823	3	7.608	.884	.489
	Within Groups	68.872	8	8.609		
	Total	91.695	11			
Crude protien	Between Groups	6.405	3	2.135	.295	.828
	Within Groups	57.934	8	7.242		
	Total	64.340	11			
EE	Between Groups	18.274	3	6.091	4.444	.041
	Within Groups	10.965	8	1.371		
	Total	29.239	11			
Ca	Between Groups	3.307	3	1.102	2.366	.147
	Within Groups	3.727	8	.466		
	Total	7.034	11			
P	Between Groups	30.126	3	10.042	2.443	.139
	Within Groups	32.882	8	4.110		
	Total	63.008	11			

Table 16: Analysis of variance (Anova) for Effects of treatments on total haematological parameters in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	Sum of Squares	df	Mean Square	F	Sig.
Hb	Between Groups	.069	3	.023	1.373	.280
	Within Groups	.335	20	.017		
	Total	.404	23			
PCV	Between Groups	.368	3	.123	.185	.905
	Within Groups	13.274	20	.664		
	Total	13.642	23			
TEC	Between Groups	.006	3	.002	.570	.641
	Within Groups	.065	20	.003		
	Total	.070	23			
MCV	Between Groups	19.781	3	6.594	.284	.836
	Within Groups	463.714	20	23.186		
	Total	483.495	23			
MCH	Between Groups	.361	3	.120	.178	.910
	Within Groups	13.529	20	.676		
	Total	13.890	23			

Table 17: Analysis of variance (Anova) for Effects of treatments on total Serum parameters in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	Sum of Squares	df	Mean Square	F	Sig.
Glucose	Between Groups	85.748	3	28.583	1.709	.197
	Within Groups	334.423	20	16.721		
	Total	420.171	23			
Total protien	Between Groups	.040	3	.013	.835	.491
	Within Groups	.316	20	.016		
	Total	.356	23			
Albumin	Between Groups	.072	3	.024	1.529	.238
	Within Groups	.314	20	.016		
	Total	.386	23			
Globulin	Between Groups	.014	3	.005	1.240	.322
	Within Groups	.075	20	.004		
	Total	.088	23			
Total cholestrol	Between Groups	37.342	3	12.447	1.334	.291
	Within Groups	186.624	20	9.331		
	Total	223.966	23			
ALT	Between Groups	10.561	3	3.520	2.152	.126
	Within Groups	32.722	20	1.636		
	Total	43.284	23			
AST	Between Groups	59.845	3	19.948	1.672	.205
	Within Groups	238.585	20	11.929		
	Total	298.429	23			
ALP	Between Groups	74.213	3	24.738	1.051	.392
	Within Groups	470.649	20	23.532		
	Total	544.862	23			

Table 18: Analysis of variance (Anova) for Effects of treatments on carcass and cut off parts in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	Sum of Squares	df	Mean Square	F	Sig.
Blood loss	Between Groups	.038	3	.013	.395	.758
	Within Groups	.634	20	.032		
	Total	.672	23			
Feather loss	Between Groups	.067	3	.022	1.010	.409
	Within Groups	.439	20	.022		
	Total	.506	23			
Dressing yield	Between Groups	18.448	3	6.149	2.114	.131
	Within Groups	58.186	20	2.909		
	Total	76.634	23			
Eviscerated yield	Between Groups	21.769	3	7.256	1.875	.166
	Within Groups	77.412	20	3.871		
	Total	99.181	23			
Giblet	Between Groups	.065	3	.022	.371	.775
	Within Groups	1.168	20	.058		
	Total	1.233	23			
Heart	Between Groups	.009	3	.003	1.747	.190
	Within Groups	.034	20	.002		
	Total	.043	23			
Liver	Between Groups	.097	3	.032	2.004	.146
	Within Groups	.321	20	.016		
	Total	.418	23			
Gizzard	Between Groups	.014	3	.005	.859	.479
	Within Groups	.112	20	.006		
	Total	.126	23			
Thigh	Between Groups	.163	3	.054	.854	.481
	Within Groups	1.269	20	.063		

	Total	1.432	23			
Drumstick	Between Groups	.128	3	.043	1.899	.162
	Within Groups	.448	20	.022		
	Total	.576	23			
Breast	Between Groups	.023	3	.008	.055	.983
	Within Groups	2.827	20	.141		
	Total	2.850	23			
Back	Between Groups	1.004	3	.335	1.851	.170
	Within Groups	3.616	20	.181		
	Total	4.620	23			
Wing	Between Groups	.971	3	.324	1.262	.314
	Within Groups	5.130	20	.257		
	Total	6.101	23			
Neck	Between Groups	.259	3	.086	2.272	.111
	Within Groups	.759	20	.038		
	Total	1.017	23			
adominalfat	Between Groups	.236	3	.079	14.198	.000
	Within Groups	.111	20	.006		
	Total	.346	23			

Table 19: Analysis of variance (Anova) for Effects of treatments on sensory evaluation of meat in energy restricted fed broiler chicken

Anova						
Week	**Source of varience**	Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	.148	3	.049	2.275	0.080
	Within Groups	.173	8	.022		
	Total	.321	11			
Flavour	Between Groups	.279	3	.093	1.916	0.206
	Within Groups	.388	8	.049		
	Total	.667	11			
Juciness	Between Groups	.324	3	.108	1.465	0.126
	Within Groups	.589	8	.074		
	Total	.913	11			
Tenderness	Between Groups	.366	3	.122	2.772	0.245
	Within Groups	.352	8	.044		
	Total	.717	11			
Overall Acceptability	Between Groups	.034	3	.011	.709	0.063
	Within Groups	.126	8	.016		
	Total	.160	11			

APPENDIES

Table-1: Effect of exogenous emulsifier on weekly body weight in energy restricted fed broiler chicks during overall period (0-35days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	157.66±4.76	472.23±10.81	990.55^{c}±18.99	1489.66^{c}±17.06	1973.33^{c}±16.44
T_2	160.56±2.67	439.48±9.15	897.11^{a}±14.19	1341.06^{a}±14.47	1728.06^{a}±14.51
T_3	151.96±2.36	461.23±8.56	924.91^{ab}±11.53	1412.08^{b}±12.43	1845.45^{b}±19.02
T_4	161.15±4.67	461.93±10.26	958.77^{bc}±21.15	1430.53^{b}±15.04	1870.25^{b}±22.27
SEm	1.95	5.49	12.81	17.19	27.43
P value	0.361	0.193	0.021	0.001	0.000

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05)

Table 2: Effect of exogenous emulsifier on weekly body weight gain in energy restricted fed broiler chicken (0-35 days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	109.86±4.56	314.56±9.53	518.31^{c}±8.66	499.11^{b}±8.44	483.66^{c}±9.13
T_2	113.49±2.78	278.92±10.17	457.63^{a}±9.58	443.95^{a}±6.73	387.00^{a}±9.07
T_3	104.61±1.80	309.26±19.88	463.68^{ab}±13.97	487.16^{b}±4.08	436.70^{b}±6.82
T_4	114.83±5.35	300.78±9.34	496.83^{bc}±11.39	471.76^{ab}±15.33	439.71^{b}±11.21
SEm	2.04	6.89	8.83	7.44	11.03
P value	0.318	0.298	0.015	0.018	0.001

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05)

Table-3: Effect of exogenous emulsifier on feed intake in energy restricted fed broiler chicks during overall period (0-35 days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	190.96^{a}±9.79	377.06^{ab}±3.53	698.61^{ab}±19.16	838.69±6.57	978.70±22.29
T_2	227.00^{b}±8.88	397.33^{b}±6.22	762.16^{c}±11.70	860.43±13.67	992.40±12.42
T_3	180.85^{a}±4.79	358.25^{a}±11.92	657.03^{a}±14.95	837.80±20.99	961.20±18.65
T_4	174.53^{a}±4.39	369.26^{a}±6.61	709.60^{b}±7.43	835.06±14.21	948.63±20.60
SEm	6.88	5.41	12.77	7.00	9.48
P value	0.004	0.038	0.005	0.616	0.426

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05)

Table-4 Effect of exogenous emulsifier on weekly FCR in energy restricted fed Broiler chicks during (0-35 days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	1.74^{ab}±0.08	1.20±0.03	1.34^{a}±0.02	1.68^{a}±0.03	2.02^{a}±0.03
T_2	2.00^{b}±0.12	1.42±0.04	1.66^{b}±0.06	1.93^{b}±0.05	2.56^{b}±0.06
T_3	1.73^{ab}±0.07	1.17±0.10	1.42^{a}±0.07	1.71^{a}±0.04	2.20^{a}±0.03
T_4	1.52^{a}±0.08	1.23±0.08	1.43^{a}±0.07	1.77^{a}±0.03	2.16^{a}±0.09
SEm	0.06	0.04	0.04	0.03	0.06
P value	0.042	0.137	0.028	0.011	0.002

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05)

Table-5 Effect of Exogenous emulsifier on weekly performance index (PI) in energy restricted fed broiler chicks during (0-35days)

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5
T_1	63.53±4.88	263.12±15.98	735.77^{b}±13.83	297.49^{b}±10.66	239.20^{c}±6.55
T_2	57.28±5.16	196.22±12.82	540.19^{a}±26.71	229.32^{a}±9.27	151.14^{a}±7.07
T_3	60.78±3.56	271.77±44.66	654.41^{b}±32.02	283.62^{b}±7.89	198.34^{b}±5.07
T_4	76.16±8.22	246.17±22.34	670.84^{b}±49.27	267.03^{b}±13.39	203.57^{b}±3.73
SEm	3.25	14.50	25.48	8.90	9.75
P value	0.183	0.272	0.018	0.009	0.000

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05)

Table 6: Analysis of Variance (ANOVA) of weekly body weight

Week	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
Week1	Between Groups	158.642	3	52.881	1.230	.361
	Within Groups	343.949	8	42.994		
	Total	502.590	11			
Week2	Between Groups	1707.970	3	569.323	1.999	.193
	Within Groups	2278.849	8	284.856		
	Total	3986.819	11			
Week3	Between Groups	14825.634	3	4941.878	5.765	.021
	Within Groups	6857.179	8	857.147		
	Total	21682.813	11			
Week 4	Between Groups	33739.698	3	11246.566	17.010	.001
	Within Groups	5289.411	8	661.176		
	Total	39029.109	11			
Week5	Between Groups	91309.854	3	30436.618	30.297	.000
	Within Groups	8036.738	8	1004.592		
	Total	99346.592	11			

Table 7: Analysis of variance (ANOVA) of weekly body weight gain

Week	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
Week1	Between Groups	187.766	3	62.589	1.377	.318
	Within Groups	363.670	8	45.459		
	Total	551.436	11			
Week2	Between Groups	2219.294	3	739.765	1.456	.298
	Within Groups	4064.535	8	508.067		
	Total	6283.829	11			
Week3	Between Groups	7350.725	3	2450.242	6.637	.015
	Within Groups	2953.342	8	369.168		
	Total	10304.067	11			
Week 4	Between Groups	5109.595	3	1703.198	6.160	.018
	Within Groups	2211.770	8	276.471		
	Total	7321.365	11			
Week5	Between Groups	14055.114	3	4685.038	18.474	.001
	Within Groups	2028.828	8	253.604		
	Total	16083.942	11			

Table 8: Analysis of variance (ANOVA) of weekly feed intake

Week	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
Week1	Between Groups	4944.957	3	1648.319	10.112	.004
	Within Groups	1304.088	8	163.011		
	Total	6249.046	11			
Week2	Between Groups	2446.692	3	815.564	4.586	.038
	Within Groups	1422.655	8	177.832		
	Total	3869.347	11			
Week3	Between Groups	16850.952	3	5616.984	9.563	.005
	Within Groups	4698.995	8	587.374		
	Total	21549.947	11			
Week 4	Between Groups	1237.253	3	412.418	.630	.616
	Within Groups	5239.598	8	654.950		
	Total	6476.850	11			
Week5	Between Groups	3333.620	3	1111.207	1.041	.426
	Within Groups	8543.207	8	1067.901		
	Total	11876.827	11			

Table 9: Analysis of variance (ANOVA) of growth performance during pre-starter phase

Parameters	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
BWG	Between Groups	187.766	3	62.589	1.141	.389
	Within Groups	438.670	8	54.834		
	Total	626.436	11			
FI	Between Groups	4944.957	3	1648.319	10.112	.004
	Within Groups	1304.088	8	163.011		
	Total	6249.046	11			
FCR	Between Groups	.346	3	.115	4.393	.042
	Within Groups	.210	8	.026		
	Total	.556	11			
PI	Between Groups	610.765	3	203.588	2.090	.180
	Within Groups	779.255	8	97.407		
	Total	1390.020	11			

Table 10: Analysis of variance (ANOVA) of growth performance during starter phase

Parameters	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
BWG	Between Groups	14832.111	3	4944.037	5.536	.024
	Within Groups	7144.410	8	893.051		
	Total	21976.521	11			
FI	Between Groups	32470.862	3	10823.621	9.886	.005
	Within Groups	8758.723	8	1094.840		
	Total	41229.585	11			
FCR	Between Groups	.151	3	.050	9.203	.006
	Within Groups	.044	8	.005		
	Total	.195	11			
PI	Between Groups	50197.321	3	16732.440	13.763	.002
	Within Groups	9726.339	8	1215.792		
	Total	59923.660	11			

Table 11: Analysis of variance (ANOVA) of growth performance during finisher phase

	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
BWG	Between Groups	35160.522	3	11720.174	18.841	.001
	Within Groups	4976.395	8	622.049		
	Total	40136.917	11			
FI	Between Groups	10581.947	3	3527.316	2.820	.107
	Within Groups	10005.533	8	1250.692		
	Total	20587.480	11			
FCR	Between Groups	.242	3	.081	19.788	.000
	Within Groups	.033	8	.004		
	Total	.275	11			
PI	Between Groups	38779.750	3	12926.583	101.92	.000
	Within Groups	1014.562	8	126.820		
	Total	39794.312	11			

Table 12: Analysis of variance (ANOVA) of growth performance during overall phase (0-35days)

	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
BWG	Between Groups	90720.542	3	30240.181	51.114	.000
	Within Groups	4732.990	8	591.624		
	Total	95453.532	11			
FI	Between Groups	102387.511	3	34129.170	29.718	.000
	Within Groups	9187.530	8	1148.441		
	Total	111575.041	11			
FCR	Between Groups	.189	3	.063	20.972	.000
	Within Groups	.024	8	.003		
	Total	.213	11			
PI	Between Groups	171592.613	3	57197.538	34.936	.000
	Within Groups	13097.719	8	1637.215		
	Total	184690.332	11			

Table 13: Analysis of variance (ANOVA) for effects of treatments on total nutrients retention

	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
DM	Between Groups	22.823	3	7.608	.884	.489
	Within Groups	68.872	8	8.609		
	Total	91.695	11			
Nitrogen	Between Groups	6.405	3	2.135	.295	.828
	Within Groups	57.934	8	7.242		
	Total	64.340	11			
EE	Between Groups	18.274	3	6.091	4.444	.041
	Within Groups	10.965	8	1.371		
	Total	29.239	11			
Ca	Between Groups	3.307	3	1.102	2.366	.147
	Within Groups	3.727	8	.466		
	Total	7.034	11			
P	Between Groups	30.126	3	10.042	2.443	.139
	Within Groups	32.882	8	4.110		
	Total	63.008	11			

Table 14: Analysis of variance (ANOVA) of haematological parameters

	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
Hb	Between Groups	.069	3	.023	1.373	.280
	Within Groups	.335	20	.017		
	Total	.404	23			
PCV	Between Groups	.368	3	.123	.185	.905
	Within Groups	13.274	20	.664		
	Total	13.642	23			
TEC	Between Groups	.006	3	.002	.570	.641
	Within Groups	.065	20	.003		
	Total	.070	23			
MCV	Between Groups	19.781	3	6.594	.284	.836
	Within Groups	463.714	20	23.186		
	Total	483.495	23			
MCH	Between Groups	.361	3	.120	.178	.910
	Within Groups	13.529	20	.676		
	Total	13.890	23			

Table 15: Analysis of variance (ANOVA) on effects of treatments on serum parameters

Parameters	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
Glucose	Between Groups	85.748	3	28.583	1.709	.197
	Within Groups	334.423	20	16.721		
	Total	420.171	23			
Total protein	Between Groups	.040	3	.013	.835	.491
	Within Groups	.316	20	.016		
	Total	.356	23			
Albumin	Between Groups	.072	3	.024	1.529	.238
	Within Groups	.314	20	.016		
	Total	.386	23			
Globulin	Between Groups	.014	3	.005	1.240	.322
	Within Groups	.075	20	.004		
	Total	.088	23			
Total cholesterol	Between Groups	37.342	3	12.447	1.334	.291
	Within Groups	186.624	20	9.331		
	Total	223.966	23			
ALT	Between Groups	10.561	3	3.520	2.152	.126
	Within Groups	32.722	20	1.636		
	Total	43.284	23			
AST	Between Groups	59.845	3	19.948	1.672	.205
	Within Groups	238.585	20	11.929		
	Total	298.429	23			
ALP	Between Groups	74.213	3	24.738	1.051	.392
	Within Groups	470.649	20	23.532		
	Total	544.862	23			

Table 16: Analysis of variance (ANOVA) of carcass and cut off parts

Parameters	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
Blood loss	Between Groups	.038	3	.013	.395	.758
	Within Groups	.634	20	.032		
	Total	.672	23			
Feather loss	Between Groups	.067	3	.022	1.010	.409
	Within Groups	.439	20	.022		
	Total	.506	23			
Dressing yield	Between Groups	18.448	3	6.149	2.114	.131
	Within Groups	58.186	20	2.909		
	Total	76.634	23			
Eviscerated yield	Between Groups	21.769	3	7.256	1.875	.166
	Within Groups	77.412	20	3.871		
	Total	99.181	23			
Giblet	Between Groups	.065	3	.022	.371	.775
	Within Groups	1.168	20	.058		
	Total	1.233	23			
Heart	Between Groups	.009	3	.003	1.747	.190
	Within Groups	.034	20	.002		
	Total	.043	23			
Liver	Between Groups	.097	3	.032	2.004	.146
	Within Groups	.321	20	.016		
	Total	.418	23			
Gizzard	Between Groups	.014	3	.005	.859	.479
	Within Groups	.112	20	.006		
	Total	.126	23			
Thigh	Between Groups	.163	3	.054	.854	.481
	Within Groups	1.269	20	.063		
	Total	1.432	23			
Drumstick	Between Groups	.128	3	.043	1.899	.162
	Within Groups	.448	20	.022		
	Total	.576	23			
Breast	Between Groups	.023	3	.008	.055	.983
	Within Groups	2.827	20	.141		
	Total	2.850	23			
Back	Between Groups	1.004	3	.335	1.851	.170
	Within Groups	3.616	20	.181		
	Total	4.620	23			
Wing	Between Groups	.971	3	.324	1.262	.314
	Within Groups	5.130	20	.257		
	Total	6.101	23			
Neck	Between Groups	.259	3	.086	2.272	.111
	Within Groups	.759	20	.038		
	Total	1.017	23			
Abdominal fat	Between Groups	.236	3	.079	14.19	.000
	Within Groups	.111	20	.006		
	Total	.346	23			

Table 17: Analysis of variance (ANOVA) of effects of treatments on sensory evaluation of meat of broiler chickens

Parameters	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	.148	3	.049	2.275	0.080
	Within Groups	.173	8	.022		
	Total	.321	11			
Flavour	Between Groups	.279	3	.093	1.916	0.206
	Within Groups	.388	8	.049		
	Total	.667	11			
Juciness	Between Groups	.324	3	.108	1.465	0.126
	Within Groups	.589	8	.074		
	Total	.913	11			
Tenderness	Between Groups	.366	3	.122	2.772	0.245
	Within Groups	.352	8	.044		
	Total	.717	11			
Overall Acceptability	Between Groups	.034	3	.011	.709	0.063
	Within Groups	.126	8	.016		
	Total	.160	11			

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RESUME

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