QUALITY CHARACTERISTICS OF FRESH & STORED MEAT AS INFLUENCED BY SUPPLEMENTATION OF SOY ACID OIL AND CRUDE SOY LECITHIN ALONE OR IN COMBINATION IN VEN COBB BROILER DIET

M. V. Sc. THESIS

By

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This is to certify that the thesis entitled **"Influence of chromium and zinc on growth performance, blood biochemical constituents and mineral profile in goats"** submitted in partial fulfillment of the requirements for the degree of **"Master of Veterinary Science"** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), is a record of bonafide research work carried out by Hemant Kumar under my guidance and supervision. The Student's Advisory Committee and the Director of Instruction have approved the subject of the thesis.

No part of the thesis has been submitted for any other degree or diploma or has been published / published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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Date: October, 2008 *Dr. Hemant Kumar*
Place: Anjora, Durg

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LIST OF ABBREVIATIONS/SYMBOLS

%	**Percent**
/	**Per**
ADG	**Average Daily Gain**
AIA	**Acid Insoluble Ash**
ALP	**Alkaline phosphatase**
Avg.	**Average**
b. wt	**Body weight**
CF	**Crude fiber**
CP	**Crude protein**
CRD	**Completely randomized design**
DCP	**Digestible crude protein**
df	**Degree of freedom**
DM	**Dry matter**
Edn.	**Edition**
EE	**Ether Extract**
et al	**And other (Latin *et alii)***
FCR	**Feed Conversion Ratio**
Fig.	**Figure**
g	**gram**
g/dday	**gram/day**
Hb	**Haemoglobin**
i.e.	**That is to say**
IU	**International unit**
kcal	**Kilo calorie**
kg	**Kilogram**
mg	**Milligram**
N	**Nitrogen**
NFE	**Nitrogen Free Extract**
No.	**Number**
NRC	**National Research Council**
NS	**Not significant**
P<0.05	**Significant at 5% level**
P>0.05	**Non Significant at 5% level**
P	**Phosphorus**
PCV	**Packed cell volume**
ppm	**Parts Per Million**
Ref.	**Reference**
Rs.	**Rupees**
Tab	**Table**
TDN	**Total Digestible Nutrients**

TEC	**Total erythrocyte count**
VFA	**Volatile fatty acid**
viz	**Namely**

INFLUENCE OF CHROMIUM AND ZINC ON GROWTH PERFORMANCE, BLOOD BIOCHEMICAL CONSTITUENTS AND MINERAL PROFILE IN GOATS

MVSc THESIS

By

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DEPARTMENT OF ANIMAL NUTRITION

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INDIRA GANDHI KRISHI VISHWAVIDYALAYA RAIPUR (C.G.)

2008

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Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

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No part of the thesis has been submitted for any other degree or diploma or has been published / published part has been fully acknowledged. All the assistance and help received during the course of the investigations has been duly acknowledged by her.

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This is to certify that the thesis entitled **"Quality characteristics of fresh & stored meat as influenced by supplementation of soy acid oil and crude soy lecithin alone or in combination in vencobb broiler diet"** submitted by **S.A.Dani Preetha** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) in partial fulfillment of the requirements for the degree of **M.V.Sc**. in the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Anjora, Durg has been approved by the student's Advisory Committee after oral examination in collaboration with the external examiner.

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Dr. S. A. Dani Preetha

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LIST OF ABBREVIATIONS

%	Per cent
&	And
/	Per
˚C	Degree celcius
A.H.,	Animal Husbandry
CFU	Colony Forming Unit
cm	Centimeter
CP	Crude protein
CSL	Crude Soy oil
d	Day
DCP	Dicalcium Phosphate
DHC	Docosa hexenoic acid
DM	Dry matter
Edn.	Edition
EE	Ether extract
EE	Ether Extract
EPA	Eicosa pentenoic acid
ERV	Extract Release volume
et al	And other (Latin *et alii)*
Fig.	Figure
g	Gram
h	Hour
i.e.	That is to say
ISF	Isoflavone
IU	International unit
Kcal	Kilo calorie
Kg	Kilo gram
LS	Limestone
Ltd	Limited
M	Mole
MA	Malanoldehyde
ME	Metabolizable Energy
mg	Milligram
Min	Minute
ml	Milliliter
n	Number

N	Normality
nm	Nanometer
no.	Number
NRC	National Research Council
NS	Not significant
P<0.05	Significant at 5% level
ppm	Parts per million
PUFA	Poly unsaturated fatty Acid
Rs.	Rupees
TBA	Thio barbituric acid
TCA	Trichloro Acetic acid
TP	Total protein
Viz	Namely
WHC	Water holding capacity
δ	Delta

CHAPTER-I

INTRODUCTION

Poultry has emerged as one of the fastest growing segments of agriculture domain in India with an average annual growth rate of 8% in egg production and 12% in broiler production (Kumar and Chandra, 2006) with the production level of 44 billion eggs and 1.6 billion broilers per annum. India is now the world's 4^{th} largest egg producer and the 5^{th} major producer of broilers. Consistent with the increase in the production and productivity, the per capita annual availability has also increased to 44 eggs and 1.76 kg poultry meat, which is still lower than the recommended levels of 180 eggs and 11 kg of meat.

Foods derived from animal products are an important source of nutrients in the human diet and will definitely play an increasing role in the human nutrition in future (Givens, 2005). Poultry products are universally popular and in recent years the consumption of poultry meat has risen dramatically. However, more and more concerns have been focused on poultry meat quality and its food safety (Le Behan-Dval, 2004). The meat quality can be expressed by several quality characteristics such as flavor, colour, water holding capacity, extract release volume and nutritional value. Hoffman (1973) defined four groups of quality classes – eating quality, nutritional quality, technological quality and hygienic quality - to define meat quality objectively. Meat quality generally is described as the sum of all meat quality characteristics (Hoffmann, 1986).

The use of nutritional strategies to improve the quality of meat is a relatively new approach that has emerged at the interface of animal science and food science. It often represents the only technology available to alter the quality

of intact muscle, where utilization of exogenous compounds is difficult if not impossible. Nutritional approaches are often more effective than direct addition of the additive to meat since the compound is preferably deposited where it is most needed (Govaris *et al.*, 2004).

Nutrition may affect meat quality by means of feeding level and feed composition. Higher feeding level has positive effects on tenderness and juiciness of the meat (Wood *et al*., 1994). Also the ingredients making up the feed have crucial effects on the quality. For instance, effects of dietary fat composition on the fatty acid profile in both the intramuscular fat and other fat depots (Warnants *et al*., 1999).

Among the various energy rich ingredients used in poultry diets, fats and oils are the most concentrated source of energy. They are usually added to broiler diet to improve productivity (Huang *et al*., 2007). The inclusion of fats in the diet improves the broiler performance (Vieira *et al*., 2002) and enhances the palatability of feed (NRC, 1994). The dietary fatty acids affects the composition of body fat in broilers (Waldroup & Waldroup, 2005), and therefore body fat composition can be modified by dietary fat (Crespo & Esteve-Garcia, 2002). However use of refined oils in feed formulation as energy source increases the cost of feed, hence poultry producers always look for some alternative source of energy which can reduce the cost of production.

Soy acid oil (acidulated soybean soapstock) and crude soy lecithin are the two by products of soybean oil refinery. Acid oil is composed of 75 to 95% of the total fatty acids from the original oil and they are present primarily as free fatty acids with a variable amount of triacylglycerols. These acid oils are not fit for human

consumption and mostly used for soap production. The acid oil may be utilized as an alternative to conventional oil as the energy source (Inal *et al*., 1994).The acid oils were also used to produce healthy lean meat since it had high level of omega-3 fatty acids (Balevi *et al.,* 2001). In addition the market price of soy acid oil is just half (Rs 30/kg) as compared to soy oil.

Crude soybean lecithin is another by-product of soybean oil refinery, which is obtained during the degumming operation, *i.e*. treatment of the crude oil with steam or hot water. Lecithin is a multi-functional surface-active agent. It is a lipid that consists mostly of choline, but also includes inositol, phosphorus, and linoleic acid. Lecithin helps to prevent arteriosclerosis, protects against cardiovascular disease, improves brain function, helps to keep the liver and kidneys healthy, aids in thiamine and vitamin A absorption, and can even help to repair liver damage caused by alcoholism. This nutrient is essential to every living cell in the human body. The choline and inositol in lecithin protect against hardening of the arteries and heart disease by promoting normal processing of fat and cholesterol. Lecithin itself helps to bind fats and cholesterol to water so that they can pass through the body rather than cause a potentially harmful build up in the heart or liver. The addition of lecithin to the diet causes an increase in the linolenic acid concentration in the serum and an increase δ-6- desaturase activity (Biagi *et al*., 1993).

In India, consumers purchase meat in fresh or frozen form mostly. The quality of meat and meat products is defined by the criteria like palatability (typical texture and consistency, juiciness, good flavour), proportion of lean meat to fat, freshness and adequate conservability of the products, absence of harmful

micro-organisms or substances and appropriate (preferably minimal) use of additives and meat extenders. The different criteria need different methods of quality control, such as organoleptic evaluation, physical test methods, chemical analysis and microbiological examination.

Different oils due to their varying degree of unsaturation affect the fatty acid composition of neutral lipids and to some extent the fatty acid composition of phospholipids which in turn influence the oxidative stability of meat during storage. Fatty acid composition of the phospholipids fraction of the intramuscular fat could have an effect on membrane stability, oxidation processes, flavour development and possibly water holding capacity (Monahan *et al*., 1992; Van Laack and Spencer, 1999). There is lack of information for the quality characteristics of meat when supplemented with soy acid oil and crude soy lecithin. With all this in view the present study was designed to study the quality characteristics of fresh & stored meat as influenced by soy acid oil and crude soy lecithin alone or in combination in vencobb broiler diet with the following objectives.

OBJECTIVES

- To study the effect of soy acid oil and crude soy lecithin alone or in combination on the nutritive quality (proximate principles) of meat at different storage period
- To study the effect of soy acid oil and crude soy lecithin alone or in combination on the physico-chemical changes in meat at different storage period

- To make a comparative study on the microbial quality of meat at different storage period by feeding soy acid oil, crude soy lecithin and their combination
- To determine the total lipid content in meat at different storage period when feeding soy acid oil and crude soy lecithin alone or in combination

CHAPTER-II

REVIEW OF LITERATURE

Zinc is no doubt dietary essential for all animals, and should be fed in quantities sufficient enough to ensure proper growth and productivity. Problems can easily arise when Zn is either deficient or in excessive quantities. When dietary Zn is deficient, growth, feed consumption, and feed efficiency are poor. Furthermore, Zn-deficient animals can exhibit an impaired immune system. When the concentration of dietary Zn is in excess, feed consumption is decreased and consequently, animal growth is decreased.

Trivalent Cr is an essential trace mineral that is involved in carbohydrate, lipid, and protein metabolism. Mertz *et al*. (1974) showed that Cr potentiates the effects of insulin and, therefore, improves carbohydrate metabolism, and they concluded that protein synthesis may be affected positively as well.

2.1 Zinc

Zinc participates in the structure, catalytic, and regulatory actions of metalloenzymes (Brandao-Neto *et al*., 1995). Some of the more important Zn-dependent enzymes include carbonic anhydrase alcohol dehydrogenase, glutamic dehydrogenase, lactic dehydrogenase, alkaline phosphatase, and carboxypeptidase A and B (McDowell, 1992). In addition to its role in these enzymes, Zn has also been associated with DNA, RNA, and protein; cellular division, growth, and repair (Miller *et al*., 1968); and immune function (Keen and Gershwin, 1990).

With such a vast biological significance, ensuring that animals consume adequate quantities of Zn is vital to maintaining a healthy, productive animal. In

many instances, it is necessary to provide some form of supplemental Zn to animals in order to meet production goals.

2.1.1 Sources of Dietary Zinc

Chelated minerals seem to be the most beneficial in the diets of animals that are subjected to nutritional and (or) environmental stressors (Spears, 1988; Hutcheson, 1989). Research also has shown that these chelated sources of minerals are advantageous in enhancing reproductive efficiency and immune responsiveness (Spears, 1988; Kropp, 1990; Chirase *et al*., 1991). The question of the overall bioavailability of these chelated minerals compared with inorganic forms, however, has yet to be fully answered.

The NRC (1996) set the Zn requirement for growing goat at 30 mg/kg of the dietary dry matter (DM). Traditionally, the major forms of supplemental Zn used to meet these requirements have been the inorganic form, including oxides, sulfates, chlorides, and carbonates. However in animal tissues, micro minerals are often chelated to specific binding proteins, which serve as transport proteins, storage proteins, active enzymes, and a host of other functions (Greene, 1995).

The amine and carboxyl groups of peptides and amino acids readily bind to metal ions via coordinate-covalent bonds. The strength of these bonds varies as a result of the specific nature of the metal and the organic constituent (Kratzer and Vohra, 1986). The stability of chelates (e.g., the relative strength or weakness of the bonds formed) determines the true value of the chelated mineral as a supplement. The strength of the bonds formed depends largely on the binding affinity between the organic molecule and the metal, and the ability of the dietary chelates to stay bound within the gastrointestinal tract depends on pH and other

Table 2.1: Chemical Characteristics of soy oil

Characteristics	Wiseman *et al.*, (1986)	Gunstons *et al.*,(1994)	Blanch *et al.*,(1995)	Vila and Esteve-Garci *et al.*, (1996)	Karleskind and Wolff(1996)	Pesti *et al.*,(2002)
Moisture(%)	<10	-	0.11	-	-	0.01
Hexane insoluble/Insoluble impurities (%)	-	-	0.18	-	-	0.11
Unsaponifiable matter (%)	<10	<15	0.5	1.58	-	0.43
Free fatty acids (%)	13.0	-	0.86	33.2	-	1.5
Iodine Number	75.99	120-143	-	-	125-128	128.9
Peroxide Value (mEq/kg)	-	-	-	-	-	3.8
Saponification Value	-	189-195	-	-	188-195	-

digestive components (Kratzer and Vohra, 1986). Therefore, chelates used by the feed industry must be strong enough to survive the digestive environment of the animal, yet not so strong that they are rendered unavailable after absorption.

2.1.2 Zinc Absorption

The small intestine is generally regarded as the main site of Zn absorption (Miller, 1970; Hampton *et al*., 1976a). For many years, the duodenum, or proximal portion of the small intestine was considered to be a much more active site of absorption than more distal portions; however, later research showed that true Zn absorption per unit of small intestinal length was fairly uniform, and that relative Zn absorption is not affected by Zn status (Hampton *et al*., 1976a, 1976b). Although the small intestine is the primary site of Zn absorption, an appreciable quantity of Zn also is absorbed from the rumen and abomasum of both cattle (Miller and Cragle, 1965) and sheep (Arora *et al*., 1969).

2.1.3 Excretion of zinc

It has been well established that under normal dietary circumstances, the feces is the major route of Zn excretion in ruminants, as well as other animals (Miller, 1969).

Zinc found in the feces is not limited to excretion of unabsorbed dietary Zn (normally a large proportion) but also consists of excretion of endogenous Zn (Hambidge *et al*., 1986). Endogenous Zn can be found in almost all segments of the digestive tract including the rumen and reticulum. The proximal portion of the small intestine, however, is probably the most important site of endogenous excretion (Stake *et al*., 1974; Miller *et al*., 1991). The quantity of Zn reabsorbed from the small intestine depends, in part, on the Zn status of the animal. Miller *et*

al. (1991) studied Zn absorption, metabolism, and endogenous excretion in both Zn-deficient and normal calves, and found that Zn-deficient animals had a higher endogenous Zn excretion into the upper portions of the intestinal tract.

Urinary excretion of Zn by calves and lambs is generally low (< 1 mg/d), with little effect as a result of dietary Zn supply (Hambidge *et al*., 1986).

2.1.4 Factors Affecting Zinc Absorption

There are a number of factors that affect absorption of Zn from the gastrointestinal tract. The single most important of these factors is the Zn content of the diet itself (Miller, 1970). It has been shown that Zn-deficient calves can absorb as much as 80% of an oral Zn dose (Miller, 1970). Although low Zn or Zn deficient diets increase Zn absorption.

Animal maturity is another factor that seems to have an effect on Zn absorption. Younger calves absorb a greater percentage of dietary Zn than older animals (Miller, 1970). However, in the same study, it also was discovered that when a Zn-deficient diet was fed, absorption was not affected by age of the animal (Miller *et al*., 1968).

Other factors that can greatly affect Zn absorption are related to components of the diet itself. Mineral interactions often occur and can be either advantageous or deleterious to absorption. Metal ion interactions are positive when one ion enhances the bioavailability of another, and negative interactions occur when one ion decreases the bioavailability of another (O'Dell, 1989).

2.1.5 Zinc Deficiency

As noted previously, Zn has been well established as an essential nutrient for the health and normal functioning of all animals, including ruminants

Miller (1970) described clinical signs of Zn deficiency in ruminants to include: (a) inflammation of the nose and mouth with submucous hemorrhages; (b) unthrifty appearance; (c) rough hair coat; (d) stiffness of the joints, with soft edematous swelling of the feet in front of the fetlocks; (e) cracks in the skin of the coronary bands around the hooves that later become deep fissures; (f) dry, scaly skin on the ears; (g) thickening and cracking of the skin around the nostrils; (h) development of horny overgrowths of the mucosa of the lips and dental pads; (i) gnashing of the teeth; (j) excessive salivation; (k) alopecia; (l) red, scabby, and wrinkled scrotal skin; and (m) bowing of the hind legs. Decreased feed intake, growth rate, and feed efficiency are often early signs of Zn deficiency (McDowell, 1992). The signs of Zn deficiency, however, are not always overt.

A borderline Zn deficiency is difficult to diagnose clinically, but it might be of more economic importance than a severe Zn deficiency (McDowell, 1992).

2.1.6 Zinc Toxicity

Ott *et al*. (1966) conducted a study to evaluate the effects of high dietary Zn on performance by lambs. Dietary levels of supplemental Zn in this study were 0, 0.5, 1.0, 2.0, and 4.0 g/kg of diet. Results showed that as level of dietary Zn increased, feed consumption decreased, weight gain decreased, and feed efficiency (reported as feed: gain) increased.

Just as Zn deficiency can result in poor production by animals, excess dietary Zn also can be problematic for producers. For most species, overt toxicosis of Zn first appears when the levels of dietary Zn approach 1,000 mg/kg (NRC, 1980). Some of the problems observed with Zn toxicity include decreased weight

gains, anemia, and reduced bone ash, decreased tissue concentrations of Fe, Cu, and Mn, and diminished utilization of Ca and P (NRC, 1980).

In most studies in which dietary Zn concentrations were less than 600 mg/kg, no adverse physiological effects were observed (NRC, 1980).

Campbell and Mills (1979) showed that pregnant ewes fed 750 mg/kg zinc produced no viable lambs compared with lambs supplemented with 30 and 150 mg of Zn/kg. Other studies have shown that sheep given intraruminal doses of zinc sulfate displayed diarrhea, weight loss, and death (McDowell, 1992).

2.1.7 Bioavailability of zinc

Many studies have been conducted to evaluate the bioavailability of organic minerals. The term bioavailability refers to the proportion of an ingested nutrient that is absorbed and utilized (O'Dell, 1989).

Spears (1989) conducted two balance trials to evaluate the influence of Zn source on the bioavailability and metabolism of Zn. In the first experiment, lambs were fed a semi-purified diet deficient in Zn, and treatments consisted of Zn oxide and Zn methionine added to the basal diet to provide 15 mg of Zn/kg of diet. Results showed that lambs fed Zn methionine had a lower fecal excretion of Zn than lambs fed Zn oxide, but apparent Zn absorption did not differ between for the two Zn sources. In addition, urinary excretion of Zn tended to be less by lambs receiving Zn methionine, which resulted in a greater retention of Zn by lambs fed Zn methionine than by lambs fed Zn oxide. In the second experiment, lambs were fed an orchard grass hay-based diet containing 30 mg of Zn/kg and a supplement formulated to supply 20 of mg Zn/kg from either Zn oxide or Zn methionine. In this experiment, apparent absorption and retention of Zn did not differ between the

two sources. Urinary Zn excretion tended to be less by lambs receiving Zn methionine, but the difference was not significant. Results of these two experiments suggest that Zn methionine might be a more bioavailability source of Zn than Zn oxide.

2.1.8 Effect of Zinc on live weight gain

Pond (1983) reported that, feeding of 0.5% Ca and19 to 26 ppm Zn is adequate for normal weight gain in Columbia and Suffolk lambs.

Spears (1989) observed that daily gain and efficiency of feed use were significantly lower in lamb fed the control diet. Growth and performance of lamb fed ZnO was similar to lamb fed Zn-Methionine.

Feeding trials showed a significant 3.26% increase in daily gain and a 4.05% improvement in feed conversion when zinc methionine was added to the control diet (Spears and Kegley, 1991). Kegley and Spears, (1994) reported that steers fed a finishing diet supplemented 25 mg/kg Zn with Zn protein tended to gain faster and more efficiently than steers supplemented with ZnO or unsupplemented controls. Spears, (1989) supplementation of growing cattle with Zn methionine has positive effect on ADG and gain:feed relative to controls group or supplemented with inorganic Zn.

The Zn supplemented lambs gain weight significantly higher than control group. Subsequent weight gains by the lambs receiving 15 mg of zinc/kg of diet were below those of the lambs fed at the 2 higher zinc levels. Hatfield *et al*. (1993) reported no effect of zinc methionine added to a basal diet when lamb average daily gain was measured as compare to control group.

Puchala *et al*., (1999) reported that, supplementation of the diet with Zn-Met (1, 3 and 5 g/day of Zn-Met) increased ADG (65.5 versus 55.9 g/day for control) of goats as compare to control group. ADG for goats receiving ZnO was lower than for goats receiving a similar amount of Zn from Zn- Met but it is not significantly differ .

Malcolm-Callis *et al*. (2000) found that, no significant differences in body weight were observed among steers across all zinc treatments. A linear decrease in ADG was observed among steers receiving increasing zinc concentrations on day 28 to day56. Overall, no differences were noted among treatments for ADG.

Nunnery (2002) found that the heifers fed the control supplement had a greater body weight on day 35 than heifers in any of the other three Zn supplemental groups. This increased final body weight was reflected in a tendency for control heifers to have an increased ADG compared with the other three treatments for the 35 day receiving period. Average daily gain by heifers in all treatments did not differ from day 0 through 14 or from day 0 through 28. Average daily feed intake, ADG, and gain.:feed were not affected by dietary Zn treatment.

Garg *et al.* (2003) reported that, average daily gain of the lambs and feed conversion efficiency were also significantly higher in Zn-methionine group as compared to control and ZnSO4 groups, suggesting a positive role of organic zinc supplementation on the performance of lambs.

Wang *et al.* (2006) reported that, there was no significant difference in body weight gain and feed/gain between zinc supplemented group and control group.

Zali *et al.* (2008) reported that the ewe body weight gain and ADG at the end of the study and ewe body weight gain and ADG change from beginning to end of the study did not differ between ewes supplemented with sulfate zinc and control ewes.

2.1.9 Effect of Zinc on feed consumption

Wagner *et al*. (1999) reported that, there was not significant source of variation for dry matter intake between zinc supplemented group and control group. Treatment had no effect on feed to gain ($P > 0.94$) or gain to feed ($P > 0.73$) ratio.

Malcolm-Callis *et al*. (2000) observed that, there was a linear ($P < 0.10$) decrease in daily DM intake with increasing zinc concentration, suggesting that higher concentration of ZnSO4 may have a negative influence on palatability. Feed efficiency of steers for d 56 to 84 exhibited a linear ($P < 0.10$) increase and a positive quadratic ($P < 0.10$) response with supplemental zinc.

Nunnery (2002) observed during the first collection period, there were no differences in DMI or fecal DM excretion between treatments groups and control group in lambs; however, DM digestibility was lower for the control treatment than for the average of the other three treatments. Dry matter intake did not differ among treatments during the second collection period. Lambs supplemented with Zn sulfate had a greater fecal DM excretion than lambs in the Zn methionine and Zn propionate treatments during the second collection period, and DM digestibility was lower for the Zn sulfate-supplemented lambs than for the Zn methionine- and Zn propionate-supplemented Iambs. There were no differences among treatments

for the following measurements; N intake, fecal N (g/d), urinary N (g/d), urinary N (% of intake), N absorption (g/d), and N retained (% of absorbed).

Garg *et al*. (2003) reported that, intake of dry matter, organic matter, crude protein, digestible CP and total digestible nutrients and digestibility of DM, OM, CP, ether extract, neutral detergent fiber and hemi cellulose were significantly higher in Zn-methionine group as compared to control group lambs. However, digestibility of cellulose and acid detergent fiber was significantly higher in Zn-methionine group as compared to control group.

Aliarabi and Chhabra (2005) reported that the average intake concentrate mixture, maize hay, milk and skimmed milk on DM basis was similar in Zn supplemented groups and control group. Average TDN and CP intake of different treatment groups were also similar in all groups. The result indicated that zinc supplementation did not have any significant effect on intake of DM and other nutrients in cross bred calves.

2.1.10 Effect of Zinc on biochemical parameters

Pond (1983) reported that, feeding of 0.5% Ca and19 to 26 ppm Zn is adequate for normal plasma concentrations of total protein, albumin and alkaline phosphatase in Columbia and Suffolk lambs.

Malcolm-Callis *et al*.(2000) found that, serum cholesterol concentrations were not affected significantly by added zinc (0.26, 0.25, and 0.27 mg/ml) for 20, 100, and 200 mg of added zinc/kg, respectively. In addition, no differences ($P > 0.10$) were observed in fatty acids.

Ozcel *et al*. (2001) observed that supplementing the lamb diets with 30 ppm of zinc bacitracin , the total serum protein and albumin, level was not significantly effected to control group.

Spears *et al*. (2004) reported that, plasma alkaline phosphatase activity was not affected by dietary Zn concentration or source. Alkaline phosphatase activity, across sampling days, averaged 49.6, 57.5, 53.8, and 54.1 Units/l for steers fed the control, ZnSO4, Zn-methionine, and Zn-glycine treatments, respectively.

Wang *et al*. (2006) reported that, no significant difference was observed in biochemical values between the control, the low, and the medium-Zn supplemented groups. Plasma folate concentration and glucose were not influenced by dietary zinc level.

Zali *et al*. (2008) reported that the serum alkaline phosphatase activity was differ ($P<0.05$) between control and supplemented ewes and alkaline phosphatase activity tended to be greater in supplemented ewes than control group.

2.1.11 Effect of Zinc on mineral profile

Pond (1983) reported that, feeding of 0.5% Ca and19 to 26 ppm Zn is adequate for normal blood plasma concentrations of Ca, P, Zn in Columbia and Suffolk lambs.

Puchala *et al.* (1999) reported that supplementation of the diet with Zn-methionine increased plasma Zn concentration (0.92 versus 0.72 mg/l for control) and there were no differences in plasma Zn concentration between goats receiving the ZnO supplement and goats receiving a similar amount of Zn from Zn-methionine (0.87 versus 0.92 mg/l; $P > 0.56$).

Eryavuz *et al* (2002) reported that, the plasma Cu concentration in the defaunated+zinc supplemented group was significantly lower than other groups in goats. Plasma zinc level in the faunated + Zn and defaunated +Zn groups was higher than other groups. There were no significant differences in the plasma Mg, Ca, inorganic P and K levels among the groups.

Ozcel *et al.* (2001) observed that supplementing the Kıvırcık lamb diets with 30 ppm of zinc bacitracin raised the blood serum inorganic phosphorus (Pi) level and especially Cu level of liver and kidney tissues. Total serum Ca, Mg, Cu and Zn level was not affected by zinc bacitracin supplementation.

Garg *et al*. (2003) reported that, though the balance of calcium was adversely affected in both the Zn supplemented groups, but it was significantly higher in Zn-methionine group compared to ZnSO4 group. While apparent absorption and retention of nitrogen, phosphorus, copper, iron and manganese were similar among different groups, retention of Zn as well as its concentration in the serum was highest in Zn-methionine group, followed by ZnSO4 group and lowest in the control group, suggesting higher bioavailability of Zn from Zn-methionine as compared to ZnSO4.

Kumar *et al.* (2003) reported that significantly higher plasma zinc level in zinc supplemented kids. The Ca intake was similar in treatment group and control group but its balance and retention was maximum in treatment group probably due to its better utilization as a result of zinc supplementation.

Spears *et al*. (2004) reported that, plasma Zn concentrations were significantly higher for the Zn-glycine and Zn-methionine treatments compared to the control group.

Aliarabi and Chhabra (2005) reported that after supplementing zinc, significant difference was observed in plasma zinc concentration at 2nd fortnight among the treatments with a significantly lower value in control group. Zinc plasma level of calves increased sharply and this increase in zinc content continued till 4th fortnight of the experiment and after that it become almost constant. However, no significance difference was observed in treatment groups, while animals in chelated zinc supplementation, tended to have a higher value of plasma zinc. This shows the bioavailability of chelated zinc is higher than that of inorganic zinc.

Zali *et al* (2008) reported that the blood Zn concentration was higher in Zn supplemented than control ewes although this increase was not significant ($p>0.05$). Blood Cu, Fe, Na, K and Ca concentration did not differ ($p>0.05$) between ewes supplemented with sulfate zinc and control ewes.

2.1.12 Effect of zinc on hematological parameters

Onder and Kecec (1998) reported that, the erythrocyte count of the Zn group was significantly higher than those of the other groups at the 2nd month. The haemoglobin amounts in the Zn+Cu groups increased compared to those in the control group at the 2nd and 3rd months. In addition, at the 2nd month of the study, the mean corpuscular volume of the Zn group was lower than those in the other groups. But, there were no significant effects of Zn and/or Cu supplements on other investigated parameters.

Wang *et al* (2006) reported that, dietary treatment of zinc did not affect white blood cell count (WBC) , hemoglobin (Hb) , mean corpuscular volume(MCV) , mean corpuscular hemoglobin (MCH) , mean corpuscular

hemoglobin concentration (MCHC) , packed-cell volume (PCV) , or percentage neutrophils, lymphocytes, monocytes, and eosinophils.

3.1.13 Effect of zinc on rumen fermentation pattern

Eryavuz *et al.* (2002) found that, defaunation and adding zinc to the ration did not affect the pH level of the rumen content. NH^3-N levels of the rumen contents in supplemental zinc group were found to be significantly higher than control group.

Bateman *et al.* (2004) observed that, mean ruminal pH remained above 5.5 and was not affected by zinc supplementation. There were no time dependent changes in ruminal pH due to supplementation of zinc. Mean ruminal concentrations of $NH4^+$ were not affected by treatment. The postprandial pattern of ruminal NH^{4+} release or production was not affected by treatment. Average ruminal concentrations of NH^{4+} were above 10 mg/dl at all sampling times. Urea N in plasma averaged 12.96 mg/dl and was not affected by the addition of Zn or monensin to diets.

Spears *et al.* (2004) reported that, total VFA concentrations were significantly higher in steers fed the control and $ZnSO_4$ treatments than in those receiving Zn-Glycine or Zn-Methionine Steers supplemented with ZnMet had a significantly higher molar proportion of propionate and lower molar proportions of butyrate and valerate than controls.

2.2 Chromium

The fact that Cr is an essential mineral was first demonstrated by Schwarz and Mertz (1959) in rats. Trivalent Cr is an essential trace mineral that is involved in carbohydrate, lipid, and protein metabolism (Mertz *et al*., 1974). 1990s Cr was

studied intensively as an essential mineral in livestock animals (cattle, sheep, goat, horses, pigs and poultry). Schwarz and Mertz (1959) showed that chromium increased serum glucose and later research established chromium as a cofactor with insulin, necessary for normal glucose utilization and for animal growth (Rosebrough and Steele, 1981, Uusitupa *et al*., 1992). In rats and calves, chromium supplementation increased serum insulin, glucose, total protein and albumin (Holdsworth and Neville.1990, Chang and Mowat, (1992).

2.2.1 Sources of Dietary Chromium

Generally, forages and byproducts seem to contain more Cr than grains the basal Cr concentrations reported for experimental diets for ruminants (range: 0.3 to 1.6 ppm) is usually higher than the supplemental Cr added to these diets (0.25 to 0.5 ppm). There is little information on the biological activity/availability of Cr in feedstuffs for livestock. Generally, the Cr in most ruminant feedstuffs should be considered poorly available.

Table 1. The chromium content of feedstuffs is as given below:

S. No.	Feedstuff	Cr, ppm
1.	Dehydrated alfalfa	0.20
2.	Corn silage	2.03
3.	Ryegrass	0.44
4.	Barley	0.83
5.	Corn	0.91
6.	Wheat bran	0.63
7.	Meat meal	0.80
8.	Fish meal	0.63
9.	Soybean meal	0.15
10.	Brewers yeast	1.00
11.	Brewers grain	0.23

(Subiyatno, 1994)

2.2.2 Chemical properties of chromium

Elemental chromium is not naturally present in the earth crust and is biologically inert. Almost all naturally found Cr is trivalent while hexavalent Cr is mostly of industrial origin. Most Cr compounds are halides, oxides or sulphides.

2.2.2.1 Divalent chromium (Cr^{2+})

Divalent chromium is a strong reductant; the form is readily oxidised when in contact with air, producing $Cr3^{+}$. This explains why divalent Cr is not available in biological systems.

2.2.2.2 Hexavalent chromium(Cr^{6+})

Hexavalent chromium is the second most stable form and a strong oxidising agent, especially in acidic media. Hexavalent chromium is bound to oxygen as chromate (CrO^{42-}) or dichromate ($Cr2O^{72-}$) with a strong oxidative capacity. This form of Cr crosses biological membranes easily, reacting with protein components and nucleic acids inside the cell while being deoxygenated to Cr^{3+}. The reaction with genetic matter provides for the carcinogenic properties of Cr^{6+}.

2.2.2.3 Trivalent chromium (Cr^{3+})

Trivalent chromium is the most stable oxidation state in which Cr is found in living organisms. It does not have the capacity to cross cell membranes easily (Mertz, 1992) and has a low reactivity, which is the most significant biological feature distinguishing it from Cr^{6+}. Trivalent Cr forms a number of coordination complexes, hexadentate ligands being the basic form. Some forms of Cr^{3+} (e.g. Cr2O3) are, thanks to their low reactivity and absorption from the gastrointestinal system, used as markers in the study of digestion processes (Furnival *et al*., 1990 a,b).

Table 3.2: Ingredient composition of starter diet

Ingredients	Group					
	T_1	T_2	T_3	T_4	T_5	T_6
Maize	58.60	55.70	54.90	55.17	54.63	54.1
Soya DOC	35.74	35.85	35.57	35.71	35.57	35.44
DORB	0.12	1.48	2.51	2.14	2.37	3.48
Crude soy oil	1.00	-	-	-	-	-
Crude soy lecithin	-	3.00	-	-	-	-
Soy acid oil	-	-	2.50	-	-	-
L75 : A 25	-	-	-	3.00	-	-
L 50: A 50	-	-	-	-	3.00	-
L 25 : A 75	-	-	-	-	-	3.00
DCP	2.18	2.17	2.18	2.17	2.16	2.16
LSP	0.94	0.94	0.95	0.94	0.95	0.95
Methionine	0.34	0.35	0.35	0.35	0.35	0.35
Lysine	0.08	0.07	0.08	0.08	0.08	0.08
Salt	0.33	0.33	0.33	0.33	0.33	0.33
Sodium bicarbonate	0.11	0.11	0.11	0.11	0.11	0.11
Choline chloride	0.07	-	0.07	-	-	-
Premix	0.49	0.45	0.45	0.45	0.45	0.45
Total	100	100	100	100	100	100

L crude soy lecithin
A soy acid oil
Mineral premix per kg diet contained $CoCo_3$ 0.20 mg, ZnO112.0 mg, Fe $(So_4)_3$ 85 mg, MnSo4 105 mg, $CuSo_4$ 22.5, Sodium selenite 0.30 mg and potassium iodide 2.5 mg.
Vitamin premix per kg diet contained Vit A 15.60 MIU, Vit D_3 5.25 MIU, Vit B_1 3.60 mg, B_2 12.0 mg, B_6 8.0 mg, B_{12} 0.02 mg, Biotin 0.18 mg, Ca D pentothenate 15.0 mg, Vit E 120 mg, folic acid 5 mg, Vit K 4 mg and niacin 57.5 mg

Table 3.3: Ingredient composition (%) of grower diet

Ingredients	Group					
	T_1	T_2	T_3	T_4	T_5	T_6
Maize	61.44	58.96	59.21	59.49	58.59	58.43
Soy DOC	30.27	29.29	29.26	29.42	29.91	29.15
MBM	3.50	3.50	3.50	3.50	3.50	3.50
DORB	-	2.36	2.59	1.73	2.11	3.03
Crude soy oil	1.89	-	-	-	-	-
Crude soy lecithin	-	3.00	-	-	-	-
Soy acid oil	-	-	2.50	-	-	-
L75 : A 25	-	-	-	3.00	-	-
L 50: A 50	-	-	-	-	3.00	-
L 25 : A 75	-	-	-	-	-	3.00
DCP	1.35	1.34	1.34	1.35	1.33	1.34
LSP	0.43	0.44	0.44	0.44	0.44	0.44
Methionine	0.27	0.28	0.27	0.28	0.28	0.28
Lysine	0.05	0.04	0.07	0.04	0.06	0.04
Salt	0.31	0.31	0.31	0.31	0.31	0.31
Sodium bicarbonate	0.03	0.07	0.04	0.07	0.03	0.07
Choline chloride	0.04	-	0.05	0.04	-	-
Premix	0.41	0.41	0.41	0.41	0.41	0.41
Total	100	100	100	100	100	100

L crude soy lecithin
A soy acid oil
Mineral premix per kg diet contained $CoCo_3$ 0.20 mg, ZnO112.0 mg, Fe $(So_4)_3$ 85 mg, MnSo4 105 mg, $CuSo_4$ 22.5, Sodium selenite 0.30 mg and potassium iodide 2.5 mg.
Vitamin premix per kg diet contained Vit A 15.60 MIU, Vit D_3 5.25 MIU, Vit B_1 3.60 mg, B_2 12.0 mg, B_6 8.0 mg, B_{12} 0.02 mg, Biotin 0.18 mg, Ca D pentothenate 15.0 mg, Vit E 120 mg, folic acid 5 mg, Vit K 4 mg and niacin 57.5 mg

Table 3.4: Ingredient composition (%) of broiler finisher diet

Ingredients	Group					
	T_1	T_2	T_3	T_4	T_5	T_6
Maize	67.92	67.2	66.38	66.67	66.14	65.6
Soy DOC	22.63	21.91	21.60	21.77	21.63	21.5
MBM	5.00	5.00	5.00	5.00	5.00	5.00
DORB	0.30	0.54	2.15	1.24	1.90	2.57
Crude soy oil	2.20	-	-	-	-	-
Crude soy lecithin	-	3.00	-	-	-	-
Soy acid oil	-	-	2.50	-	-	-
L75 : A 25	-	-	-	3.00	-	-
L 50 : A 50	-	-	-	-	3.00	-
L 25 : A 75	-	-	-	-	-	3.00
DCP	1.05	1.08	1.05	1.06	1.05	1.05
LSP	0.21	0.21	0.22	0.22	0.22	0.22
Methionine	0.23	0.23	0.23	0.23	0.23	0.23
Lysine	0.11	0.13	0.13	0.13	0.13	0.13
Salt	0.26	0.26	0.26	0.26	0.26	0.26
Sodium bicarbonate	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.03	0.04	0.04	-	-	-
Premix	0.39	0.39	0.39	0.39	0.39	0.39
Total	100	100	100	100	100	100

L crude soy lecithin
A soy acid oil
Mineral premix per kg diet contained $CoCo_3$ 0.20 mg, ZnO112.0 mg, Fe $(So_4)_3$ 85 mg, MnSo4 105 mg, $CuSo_4$ 22.5, Sodium selenite 0.30 mg and potassium iodide 2.5 mg.
Vitamin premix per kg diet contained Vit A 15.60 MIU, Vit D_3 5.25 MIU, Vit B_1 3.60 mg, B_2 12.0 mg, B_6 8.0 mg, B_{12} 0.02 mg, Biotin 0.18 mg, Ca D pentothenate 15.0 mg, Vit E 120 mg, folic acid 5 mg, Vit K 4 mg and niacin 57.5 mg

2.2.3 Chromium Absorption

Chromium may be present in diets in the form of inorganic compounds or organic complexes. Elemental Cr is not absorbed and has no nutritional value (Ducros, 1992).

Cr is supplemented orally; most Cr^{6+} seems to be reduced to Cr^{3+} before reaching the site of absorption in the small intestine (Doisy *et al*., 1976). The main path for Cr^{3+} to get into the organism is through the digestive system. The most active absorption site in rats is the jejunum; absorption is less efficient in the ileum and the duodenum (Chen *et al*., 1973).

The mechanism of Cr intestinal absorption is not fully known yet. Some papers give evidence of passive diffusion (Stoecker, 1999). Absorption of inorganic Cr^{3+} is indirectly proportional to dietary content. The percentage of Cr absorbed from diet decreases until it reaches 40 μg/day after which the absorption stabilises at 0.5% (Anderson and Kozlowski, 1985; Bunker *et al*., 1984). Lyons (1994) claims that the bioavailability of inorganic Cr is < 3% while organic Cr is over ten times more available. The causes of the low bioavailability of inorganic Cr are numerous and they are likely to be in connection with the formation of non-soluble Cr oxides, Cr binding to natural chelate-forming compounds in fodders, interference with ion forms of other minerals (Zn, Fe, V) (Borel and Anderson, 1984). Suboptimal level of dietary niacin lowers the conversion of inorganic Cr to the bioactive Cr (Ranhotra and Gelroth, 1986).

Cr absorption from food is enhanced by the presence of amino acids, the ascorbic acid, high carbohydrate, oxalate and aspirin levels in the diet, while

phytates and antacids (natrium hydrogen carbonate, magnesium hydroxide) reduce Cr concentrations in blood and tissues (Hunt and Stoecker, 1996).

2.2.4 Excretion of Chromium

Absorbed Cr is excreted primarily in urine by glomerular filtration, or bound to a low-molecular organic transporter (Ducros, 1992). A small amount is nevertheless eliminated in hair, perspiration and bile. Urinary excretion of Cr also heavily depends on the form of Cr supplementation.

2.2.5 Chromium deficiency

Papers dealing with the experimental study of Cr deficiency are relatively scarce and most of the existing ones quote results of experiments on laboratory animals. Frank *et al*. (2000a, b) have studied experimentally induced Cr deficiency in goats. The population with a Cr deficiency showed higher weight gains (31.1 ± 11.7 vs. 20.0 ± 7.3 kg) for the period of monitoring (84 weeks) compared with the control group. The authors explain this unexpected effect by the possibility that Cr deficiency has impaired glucose tolerance and increased insulin release subsequently leading to hyperinsulinemia. Cr deficiency has also led to an increase in haematological parameters (haemoglobin, haematocrit, erythrocytes, leucocytes and mean erythrocyte volume); increased total protein concentrations and hyperinsulinemia were observed compared with the group of controls as well.

2.2.6 Chromium toxicity

Cr toxicity is associated mainly with hexavalent chromium, whole trivalent Cr is believed to be a highly safe mineral. Hexavalent Cr is more soluble than trivalent Cr and at least five times as toxic (Barceloux, 1999). The safety limit for Cr^{3+} is approximately 1:10 000. Cr^{3+} toxicity is in fact lower than the toxicity of all

other essential elements such as Cu, I, Zn, Mn and especially Se (Lindemann, 1996). The details of Cr^{6+} toxic activity are however not known. The main protection mechanism against Cr^{6}+ activity in the lungs and the stomach is the reduction of Cr^{6+} to Cr^{3+} by an NADPH-dependent mechanism involving ascorbate.

2.2.7 Effect of chromium on live weight gain

In cattle, a positive effect of Cr supplementation on weight gain has been recorded by Chang and Mowat (1992). Cr supplementation during periods of increased stress has a positive effect on weight gain.

Page *et al*. (1993) reported a linear reduction in daily feed intake and an increase in daily gain for pigs supplemented with 50, 100 and 200 parts per billion (ppb) chromium picolinate. . Other studies (Mooney and Cromwell, 1995, 1997) have confirmed the positive effect of supplementing 0.2 mg/kg of Cr on weight growth, but not on nutrient conversion.

Harris *et al*. (1995) reported no differences in daily gain, daily feed intake, gain: feed ratio and loin muscle area for pigs when supplemented with chromium picolinate. Supplemental chromium did not affect the average daily gain and dry matter intake of weaned growing rabbits observed by Shain *et al*. (2001).

Kitchalong *et al*. (1995) reported that the mean ADG (average 272 g/lamb) and DMI (average 1.92 kg lamb) were not affected (P >.l0) by Cr-Picolinate supplementation. There was no effect of Cr supplementation on, ADG in beef steers during the 21 to 23 day feeding period reported by Kegley *et al*. (2000).

Green (1997) observed that chromium had no significant effect on any growth, carcass, or muscle quality characteristics, although chromium-fed pigs

were slightly fatter. Barrows gained faster (P < .001) and consumed more feed (P< .001) than gilts, yielding heavier (P < .001) carcasses, and heavier (P < .05) wholesale cuts.

Uyanik (2001) reported that, chromium supplementation had no significant effect on live weight in sheep, but subcutaneous fat was reduced significantly in both chromium groups.

Pechova *et al.* (2002) demonstrated favorable effects of chromium supplementation on growth rate in bulls. The difference in weight gains between the experimental and the control bulls was highly significant in the first phase of the fattening period, but diminished to become no significant in the second phase.

Paul *et al*. ((2005) reported that the total body weight gain and live weight gain to DM intake ratio was higher (P < 0.001) in the Cr supplemented goats. However, similar performance of the 0.2 and 0.4 mg Cr supplemented bucks suggested that there was not much benefit of supplementing these animals with Cr above the level of 0.2 mg.

The initial body weight of the goats was similar between control groups and Cr supplemented groups. The goats gained body weight during the experiment but neither the ADG nor the final body weight was affected by dietary treatments observed by Haldar *et al.* (2007). Supplemental organic chromium can increase rate of gain anywhere from 0 to 30% depending upon level of stress and/or disease challenge.

2.2.8 Effect of Chromium on feed consumption

Moonsie - Shageer and Mowat (1993) did show improved ADG and DMI in calves supplemented with high-Cr yeast in a corn-silage diet. Whereas Boleman

et al. (1995) reported reduced ADG and DMI in pigs fed Cr tripicolinate from the growing to the finishing phase. There was no effect of Cr supplementation on, DMI, and gain:feed ratio in beef steers during the 21 to 23 day feeding period reported by Kegley *et al*.(2000).

Mean DMI were not affected by Cr-Picolinate supplementation. Supplemental Cr-Pic did not influence any measure of N balance during week 3 or 11. During week 3, the wether lambs had an average N intake of 28.3 g/d, with an average daily absorption of 18.5 g, resulting in 7.69 g of N retained per day. During wk 11, the wether lambs had an average N intake of 34 g/d, with 21.7 g of N absorbed daily, and an average of 7.67 g of N retained. This resulted in an average of 65.3 and 63.7% of the N intake being absorbed, and 27 and 22.6% of the N intake being retained during week 3 and 11, respectively. (Kitchalong *et al*.,1995).

Chromium with CP interaction was observed for ADG, DMI, and gain:feed ratio; Cr increased all three variables in lambs fed high protein and decreased all three variables in lambs fed low protein. (Gentry *et al*., 1999).

Paul *et al*. ((2005) reported that the Intake of DM, CP and NDF was higher in the Cr supplemented bucks compared to the control ones though the difference between the 0.2 and 0.4 mg Cr supplemented bucks was not significant ($p > 0.05$). The higher intake of Cr and other trace elements from the basal diet in the Cr supplemented bucks, it is due to the difference in DM intake among the treatment groups. DM intake in the Cr supplemented bucks was higher resulting more Cr intake in these animals compared to the control ones.

Paul *et al*. ((2005) found that the Intake of OM and ADF, on the other hand, was higher in the 0.4 mg Cr supplemented group only. The apparent total tract digestibility of DM, OM, CP and ADF increased and that of NDF showed an increasing trend ($P < 0.1$) when the diet was supplemented with Cr. However, the effect of increasing the level of supplemental Cr beyond 0.2 mg on the apparent digestibility of nutrients was not evidenced ($P > 0.05$) in this study.

2.2.9 Effect of Chromium on biochemical parameters

Supplemental Cr altered lipid metabolism. Reductions in circulating concentrations of cholesterol and (or) nonesterified fatty acids (NEFA) are frequently reported in ruminants (Bunting *et al*., 1994; De Pew *et al*., 1998; Kitchalong *et al*., 1995; Yang *et al*., 1996). It is likely that the more dramatic hypolipidemic effects of organic Cr may be attributable simply to increased glucose tolerance with corresponding reductions in lipolysis.

Kitchalong *et al*. (1995) reported that overall, mean total cholesterol (average 44.6 mg/dL) was not affected by Cr-Picolinate; however, during week 2, total cholesterol was 16.7% lower in the Cr-Picolinate supplemented lambs. Moreover, plasma cholesterol exhibited a quadratic effect with time. Plasma albumin and total protein concentrations (average 33.8 and 59 g/L, respectively) were not affected by treatment, but circulating albumin concentrations exhibited a quadratic response over week independent of dietary treatment (diet x week). Mean plasma glucose did not differ between the dietary treatments (average 3.68 mmol/l).

Hayirli *et al*. (2001) is demonstrated that Plasma glucose and serum glucagon concentrations and molar ratio of serum insulin to plasma glucose were similar across all levels of Cr-methionine supplementation.

Uyanik (2001) reported that, there was a slight decrease in glucose concentrations in the 200-ppb chromium group, although only the differences on day 55 were significant. Triglyceride levels in both chromium groups were lower than the control group with marked differences in the 400-ppb chromium group. HDL cholesterol levels increased in both treatment groups compare to control, although the differences in the 400-ppb chromium group on day 40 were significant. No significant differences were found in total and LDL cholesterol, total protein, albumin, ALT, AST, and GGT levels.

Pechova *et al*. (2002) demonstrate a significantly higher concentration of total blood plasma proteins found in chromium supplemented bulls. A significance between-group difference was also found in the concentration of total cholesterol, which was lower in the control than in the experimental group on day 136.

Paul *et al*. ((2005) found that the preprandial and postprandial plasma glucose on days 0 and 65 was in all the experimental groups. However, a day x dose interaction suggested that as supplementation progressed plasma glucose concentration got elevated especially in the bucks supplemented with 0.4 mg Cr. The elevated plasma glucose in the 0.4mg Cr supplemented bucks apparently contradicted the general hypothesis governing the mode of Cr action. Taking into account the effect of Cr on the potentiation of insulin action a lower plasma glucose concentration in this group was expected. However, in Cr supplemented ruminants, an enhanced gluconeogenesis from propionate was reported (Subiyatno

et al., 1996), which at the dose level of 0.4 mg, might have had overridden the effect of insulin on the cellular uptake of glucose and resulted an elevated plasma glucose concentration.

Paul *et al*. ((2005) found that the higher alkaline phosphatase activity in the chromium supplemented bucks because, Cr augmented new bone crystal formation by enhancing the uptake of circulatory Ca into the bone matrix. This was supported by the lower concentration of Ca in the serum of the Cr supplemented bucks (2.18 and 2.17 mmol/ L respectively in the 0.2 and 0.4 mg groups respectively) compared to the control group of bucks (2.34 mmol/L).

Haldar *et al.* (2007) found that serum cholesterol on day 120 was lower in the goats fed added dietary Cr from Cr chloride and Cr yeast relative to the control group of goats and the overall mean serum cholesterol concentration also tended to be lower in the Cr supplemented goats. Serum glucose in the control group of goats increased while that in both the Cr supplemented groups decreased. Dietary Cr supplementation improved post-prandial utilization of glucose and that of cholesterol with lower serum levels of these metabolites being detected in the Cr chloride and Cr yeast dietary groups than in the control group. Dietary treatment had no effect on the post-prandial changes in serum levels of triacylglycerol and total protein.

2.2.10 Effect of Chromium on hematological parameters

Gentry *et al*. (1999) reported that dietary treatment of chromium did not affect white blood cell count (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed-cell volume (PCV) or percentage neutrophils,

lymphocytes, monocytes and eosinophils. Lambs fed high protein diets had higher red blood cell count (RBC) when they were also fed supplemental Cr; however, lambs fed low protein diets had lower RBC when they were fed supplemental Cr. Platelets and heat-precipitable protein (HPP) were higher in lambs fed supplemental Cr than in lambs fed diets without Cr supplementation.

2.2.11 Effect of Chromium on mineral profile pattern

Uyanik (2001) reported that, Serum Cr concentrations slightly but not significantly increased in both chromium groups as compare to control group.

Paul *et al*. ((2005) found that the intake and apparent absorption of Cr in the bucks increased in almost a parallel fashion as the level of supplemental Cr increased in the treatment groups. The intake of Cu, Zn, Fe and Mn and the apparent absorption of Cu and Zn increased due to Cr supplementation though the increment in the dose level from 0.2 to 0.4 mg did not appreciably affect their apparent absorption. Apparent absorption of Mn was similar among the treatment groups. Neither the intake nor the apparent absorption of Fe in the bucks was affected by Cr supplementation.

Paul *et al*. ((2005) observed that the plasma Cr concentration increased in the Cr supplemented bucks. The difference between the 0.2 and 0.4 mg Cr supplemented animals was not conspicuous, however. Concentration of Cu, Zn and Mn in plasma was similar in all the treatment groups prior to the start of the experiment. Though Cr supplementation had no apparent effect on the plasma Cu concentration, levels of plasma Zn and Mn were elevated due to Cr supplementation. No definite effect of supplemental Cr feeding on plasma Fe

concentration could be ascertained though on day 65 it was found to be higher in the 0.4 mg Cr group of bucks.

Pechova *et al* (2002) demonstrate that the Cr supplementation had no significant effect on whole blood concentration of Cr and no marked fluctuations were observed during the observation period in Dairy Cows. No significant between-group differences indicative of effects of supplemental Cr on the metabolism of Ca, P and Mg in Dairy Cows. Certain differences between the experimental and the control groups were found also in the metabolism of minerals in control and Cr supplemental bulls. Compared with controls, the experimental group showed significantly lower concentrations of phosphorus. Interesting data resulted also from the monitoring of trace element concentrations. Compared with the experimental group, copper concentration in blood plasma of controls was significantly lower. The copper concentration decreased throughout the experimental period, the decrease was less marked in the experimental group. Interactions of copper with zinc, selenium, cadmium, mercury, lead, and iron were investigated, but little is known about relations between copper and chromium. Zinc concentration remained constant during the experimental period indicating the absence of effects of chromium supplementation. Supplemental chromium protects the stress-induced urinary losses of zinc, copper, iron and manganese in ruminants. Zinc, vanadium and iron can decrease inorganic chromium absorption in animals.

A decreased loss of some micro elements (Zn, Fe, Cu and Mn) during stress after Cr supplementation to mice has been reported by Schrauzer *et al*. (1986). Interaction between Cr and Cu was studied by Stahlhut *et al*. (2006), Cr supplementation had no effect on the liver or plasma Cu concentrations in cows,

although, supplemental Cr resulted in higher plasma Cu concentrations in calves on Day 279. The relation between Cr and Fe has been investigated most since both these minerals are transported as transferrin-bound. At low Fe saturation, Cr and Fe bind preferentially to different binding sites. When, however, the Fe concentration is higher, the two minerals compete for the same binding sites. (Sargeant *et al*., 1979).

2.2.12 Effect of chromium on rumen fermentation pattern

Besong *et al* (2001) reported that, the molar proportion of propionate decreased, whereas butyrate and isobutyrate increased linearly with increasing Cr content at 12 h of incubation. Molar proportion of valerate alone increased linearly with increasing Cr content at 24 h of incubation. A second in vitro study evaluated the supplementation of Cr at concentrations of 0, 0.8, 1.6, 3.2, 6.4, 12.8, or 25.6 mg/kg. Molar proportions of acetate, propionate, and isovalerate, and total VFA production responded quadratically with increasing Cr content at 24 h.

CHAPTER- III

MATERIALS AND METHODS

The present investigation was carried out in the Department of Animal Nutrition, College of Veterinary Science & A. H., Anjora, Durg. The experiment was designed to assess the storage quality of meat when supplemented with crude soy lecithin and soy acid oil alone or in combination in the diet of broiler chickens from 0-42 d. A brief description of the planning and execution of the experiments are presented hereunder along with the methodologies for various analytical techniques.

3.1. Procurement of feed sample

The required feed ingredient and supplements were procured from the Indian Broiler group feed plant, Indamara, Rajnandgaon. The feed ingredients and supplements included maize, deoiled soybean cakes, deoiled rice bran, maize gluten, meat and bone meal, dicalcium phosphate (DCP), limestone (LS), crude soy oil, soy acid oil (acidulated soybean soapstock), crude soy lecithin, laboratory reagent grade inorganic salts of trace minerals (copper sulphate pentahydrate, ferrous sulphate septahydrate, manganese sulphate, zinc oxide, cobalt carbonate, sodium selenite, potassium iodide) and premixes containing vitamins and feed additives.

3.2. Birds and housing

Day old Ven cobb 400 strain broiler chicks (n=180) from the same hatch were procured from M/s Indian Broiler Group, Baldeobagh, Rajnandgaon. Chicks

were wing banded, weighed and distributed randomly into 6 treatment groups of 30 chicks; each group had 3 replicates of 10 chicks in each. The chicks were reared under deep litter system. Throughout the experiment, all the test groups received similar management practices like vaccination, lighting and watering. The birds were provided with weighed quantity of experimental diet ad libitum.

The pre-starter (0-14 days) diets were formulated to contain 23% crude protein and 2900 kcal ME/kg feed, starter (14-28 days) diet with 21.5% CP and 3000 kcal ME/kg feed and finisher (28-42 days) diet contained 20% crude protein and 3100 kcal ME/kg feed. Level of inclusion in all the dietary treatments during starter, grower and finisher stage remain same.

Table 3.1. Experimental design

Particulars	Treatment					
	T_1	T_2	T_3	T_4	T_5	T_6
Total chicks	30	30	30	30	30	30
Replicate	3	3	3	3	3	3
Chicks per replicate	10	10	10	10	10	10
Soy oil	+	-	-	-	-	-
Crude soy lecithin	-	+	-	-	-	-
Soy acid oil	-	-	+	-	-	-
Crude soy lecithin + soy acid oil	-	-	-	75 : 25	50 : 50	25 :75

3.3. Experimental diet

Six treatment diets were formulated for each phase *i.e*. starter, grower and finisher, diet 1 as control (T_1) in which soy oil was used, diet 2 was formulated

using 3% crude soy lecithin (T_2), diet 3 contained 2.5% soy acid oil (T_3). In diet 4-6 (T_4-T_6) different combination of crude soy lecithin and soy acid oil (*i.e.* 75:25, 50:50 and 25:75, respectively) were used. The ingredient composition of different diet is presented in table 3.2-3.4.

3.4. Sample Collection and Preservation

At the end of 42 days 3 birds from each replicate (*i.e*. 9 birds /treatment) were selected randomly and starved for 12 h before sacrifice for collection of samples & then sacrifice as per standard procedure. Pectoralis major muscle *i.e*. breast muscle from the carcass were separated and packaged in low density polyethylene, each containing 200 g of meat sample and stored at -18°C in deep freezer for one month. One portion of meat was analyzed in fresh state on 0 day. The fresh & stored samples were analyzed for proximate composition, physico chemical changes, microbial quality and the lipid content.

3.5. Proximate analysis of meat

To assess the nutrient composition of meat, the moisture, protein, ether extract and ash contents of meat were determined by the methods of AOAC, 2000. The meat samples were analyzed for proximate principles on 0^{th} day (fresh) and 30^{th} day after storage in deep freezer at -18°C.

3.5.1. Dry matter (DM)

About 20g of meat sample was taken in a previously weighed thimble. It was dried at 100±5°C in hot air oven for over night. The remaining weight after loss of moisture gave rise to the dry matter.

3.5.2. Total Protein (TP)

Total Protein of fresh and stored meat samples were estimated by micro Kjeldahl method as per AOAC, 2000.

3.5.3. Ether extract (EE)

About 5g of dried powdered samples of meat were taken in a readymade thimble. The samples were extracted continuously for 60 minutes with petroleum ether (40°C) at 250°C in a modified Soxhlet extraction apparatus (Socs plus-Pelican India Ltd). The ether extract was calculated by difference in the weight of oil flask before and after extraction.

3.5.4. Total ash (TA)

About 5g of dried meat sample was taken in previously weighed silica crucible and charred on a low flame of the gas burner. Thereafter it was ignited in furnace at 600°C for 30 minutes. After cooling it in desiccators it was weighed. The ignited material left in the crucible was considered as total ash of meat.

3.6. Physico-chemical parameters

3.6.1. pH

pH of the finely minced meat sample was determined by the method of Gillespie (1960). The meat samples were analyzed for pH on 0^{th} day (fresh) and 30^{th} day after storage in deep freezer at -18°C. Measurement of pH using pH meter is accomplished by determining the potential developed by an electric cell. The cell consists of an electrode system immersed in a test solution. The electrode system is pH sensitive and develops an electric potential proportional to the pH of the solution in which it is immersed. The pH meter was standardized by adjusting the pH meter to the value of the buffer solution. Then the electrodes after proper

Table 4.1. Moisture (%) of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
	Moisture (%)		
T_1	$77.23^{abx} \pm 0.38$	$75.72^{by} \pm 0.39$	*
T_2	$77.33^{bx} \pm 0.69$	$75.62^{by} \pm 0.15$	*
T_3	$74.17^{a} \pm 0.69$	$74.06^{ab} \pm 0.39$	NS
T_4	$75.88^{ax} \pm 0.44$	$75.26^{by} \pm 0.39$	*
T_5	$76.1^{a} \pm 0.24$	$75.46^{b} \pm 0.94$	NS
T_6	$74.64^{a} \pm 1.00$	$73.91^{a} \pm 1.65$	NS
Sig	*	*	

T

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
Mean values with different superscript a, b within column are significantly different
Mean values with different superscript x, y within row are significantly different
NS – Non Significant, * Sig at 5% level,

Table 4.2: Total Protein (%) of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
T_1	$18.79^{x} \pm 0.68$	$17.74^{y} \pm 0.32$	**
T_2	$18.94^{x} \pm 0.14$	$17.78^{y} \pm 0.34$	**
T_3	$18.93^{x} \pm 0.49$	$18.60^{y} \pm 0.39$	**
T_4	$19.15^{x} \pm 0.20$	$18.49^{y} \pm 0.19$	**
T_5	$18.27^{x} \pm 0.22$	$17.78^{y} \pm 0.18$	**
T_6	$19.26^{x} \pm 0.28$	$17.99^{y} \pm 1.13$	**
Sig	NS	NS	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
Mean values with different superscript x, y within row are significantly different ($p<0.05$)
NS – Non Significant, ** Sig at 1% level

washing is immersed in the test solution, pH meter then indicate the pH value of the test solution directly.

3.6.1.1. Procedure

10 g of meat sample were homogenised with 50 ml of distilled water using mortar and pestle for 30-60 seconds. Then the slurry is taken into a beaker and the electrode is dipped into the slurry and the pH of the suspension was recorded using digital pH meter.

3.6.2. Extract Release Volume (ERV)

Extract Release Volume is the volume of extract released by a homogenate of meat when allowed to pass through the filter paper for a given period of time. It is inversely proportional to the extent of spoilage. Extract Release Volume is of value in determining spoilage of meat as well as in predicting shelf life of meat.

Extract Release Volume was determined as per the procedure outlined by Pearson, 1968. The meat samples was analyzed for Extract release volume on 0^{th} day (fresh) and 30^{th} day after storage in deep freezer at -18°C.

3.6.2.1. Chemicals required

a) 0.2M Potassium dihydrogen ortho phosphate

b) 0.2 M Sodium hydroxide

Table 4.3: Ether Extract (%) of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
T_1	$4.70^{ax} \pm 0.15$	$3.84^{y} \pm 0.18$	**
T_2	$4.68^{ax} \pm 0.08$	$3.98^{y} \pm 0.23$	**
T_3	$5.42^{cx} \pm 0.13$	$3.84^{y} \pm 0.15$	**
T_4	$4.86^{abx} \pm 0.13$	$4.16^{y} \pm 0.09$	**
T_5	$4.74^{ax} \pm 0.07$	$3.85^{y} \pm 0.14$	**
T_6	$5.16^{bcx} \pm 0.04$	$4.00^{y} \pm 0.27$	**
Sig	*	NS	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.

Mean values with different superscript a, b, c within column are significantly different ($p<0.05$)
Mean values with different superscript x, y within row are significantly different ($p<0.05$)
NS – Non Significant, * Sig at 5% level, ** Sig at 1% level

Table 4.4: Total Ash (%) of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
T_1	1.09 ± 0.07	1.04 ± 0.02	NS
T_2	1.20 ± 0.06	1.17 ± 0.01	NS
T_3	1.23 ± 0.05	1.21 ± 0.03	NS
T_4	1.10 ± 0.11	1.07 ± 0.13	NS
T_5	1.10 ± 0.06	1.09 ± 0.03	NS
T_6	1.14 ± 0.03	1.14 ± 0.01	NS
Sig	NS	NS	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.

NS – Non Significant

Fig 1: The effect of crude soy lecithin and soy acid oil alone or their different combination on the moisture content of chicken meat at different period

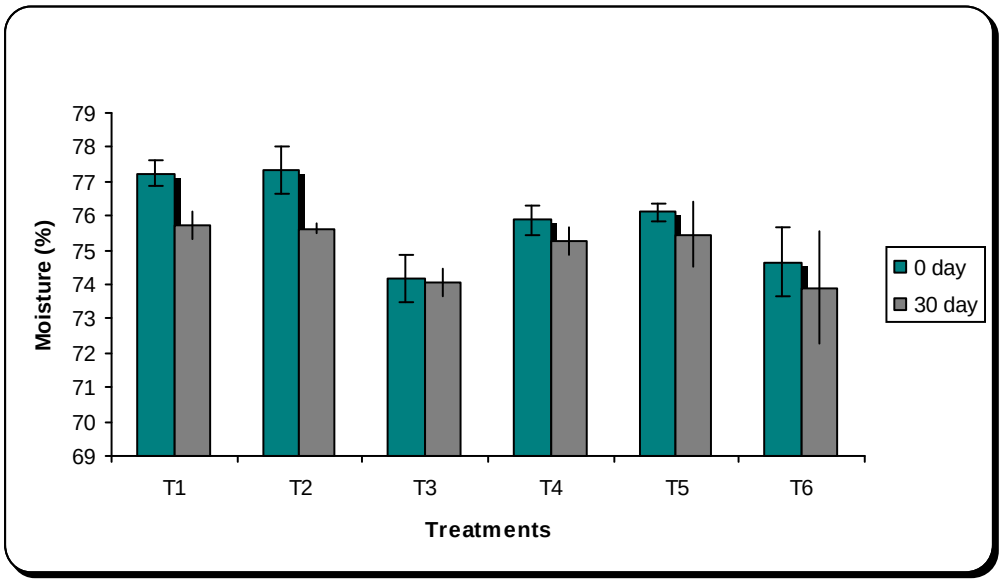
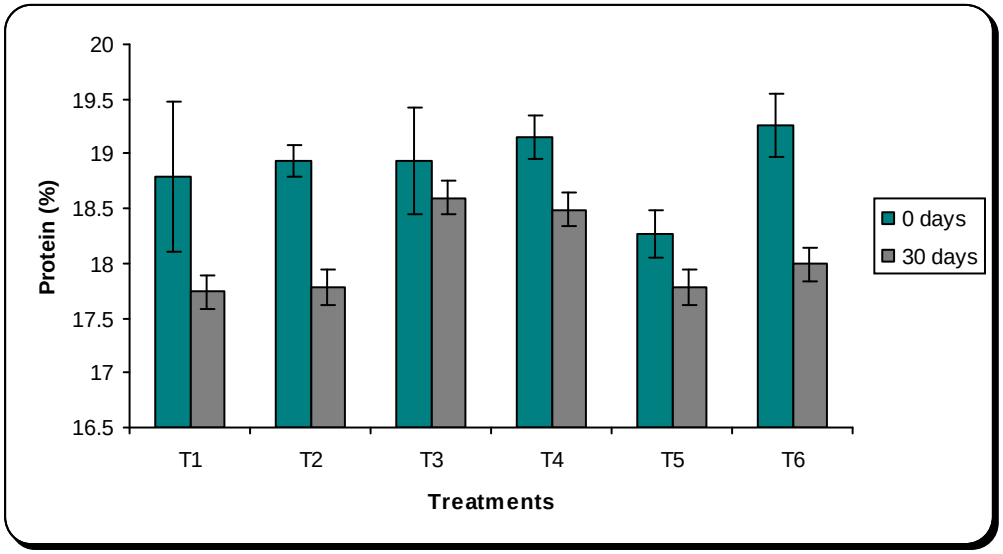


Fig 2: The effect of crude soy lecithin and soy acid oil alone or their different combination on the protein content of chicken meat at different period



3.6.2.2. Preparation of extraction reagent

An extraction reagent was prepared by mixing 50 ml of 0.2 M Potassium di hydrogen orthophosphate with 3.72ml of 0.2 M NaOH and then diluted with distilled water to 200 ml. The pH of the reagent was corrected to 5.8.

3.6.2.3. Procedure

1) Fifteen gram of meat sample was blended with 60 ml of extraction reagent for 2 min.
2) The blended contents are quantitatively transferred to a glass funnel provided with filter paper (Whatman No.1, 18.5cm diameter).
3) The filter paper is folded thrice so as to make 8 sectors and the filtrate was collected in a 100 ml measuring cylinder.
4) The volume of the filtrate (in ml) collected within first 15 min at a temperature of 20°C after pouring the homogenate into the funnel is reported as the extract release volume.

3.6.3. Water Holding Capacity (WHC)

Water-holding capacity of meat is described as the ability of the post-mortem muscle to retain water even though external pressures are applied to it.The method used for determining the WHC was a modification of the high speed centrifugation method (Harris and Shorthose, 1988). The meat samples were analyzed for WHC on 0^{th} day (fresh) and 30^{th} day.

3.6.3.1. Procedure

1. 2 g accurately weighed meat sample was centrifuged at 6000 rpm for 10 min using a centrifuge machine (REMI model).

2. No water added to the samples and, after centrifuging, the juice expressed is decanted off

3. The meat sample was then removed carefully from the tubes with forceps dried with tissue paper and then reweighed to determine liquid loss. The water content of the muscles when raw and after centrifugation was determined by oven drying (105°C for 24 h).

3.6.4. Thio Barbituric Acid (TBA) value

The TBA value is the measure of oxidative rancidity in food and is expressed as a value or number. Rancidity of fat in stored food is defined as the development of stale off flavour as a result of oxidation of unsaturated fatty acids. Thio barbituric acid value measures the carbonyl residues resulting from lipid peroxidation. The rate of this change depends on initial bacterial load, physical and bio chemical change, availability of oxygen, temperature of storage and muscle composition.

Thio barbituric acid number of meat samples were determined as per Strange *et al.,* (1977) with slight modification. The meat samples were analyzed for TBA number on 0^{th} day (fresh) and 30^{th} day after storage in deep freezer at -18°C.

Table 4.6: pH of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
T_1	$5.62^{c} \pm 0.05$	$5.59^{b} \pm 0.25$	NS
T_2	$5.55^{bc} \pm 0.02$	$5.34^{ab} \pm 0.15$	NS
T_3	$5.39^{abc} \pm 0.02$	$5.07^{a} \pm 0.14$	NS
T_4	$5.22^{a} \pm 0.11$	$4.99^{a} \pm 0.05$	NS
T_5	$5.26^{ab} \pm 0.17$	$5.05^{a} \pm 0.04$	NS
T_6	$5.23^{a} \pm 0.14$	$4.93^{a} \pm 0.04$	NS
Sig	*	*	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
Mean values with different superscript a, b, c within column are significantly different
NS – Non Significant, * Sig at 5% level,

Table 4.7: ERV of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
T_1	18.67 ± 2.33	17.67 ± 1.45	NS
T_2	18.67 ± 1.2	19.00 ± 1.15	NS
T_3	19.00 ± 1.15	17.67 ± 2.72	NS
T_4	20.00 ± 2.31	25.33 ± 5.61	NS
T_5	19.00 ± 2.31	17.00 ± 2.08	NS
T_6	25.67 ± 8.29	24.00 ± 6.08	NS
Sig	NS	NS	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
NS- Non Significant

Fig 3: The effect of crude soy lecithin and soy acid oil alone or their different combination on the ether extracts content of chicken meat at different period

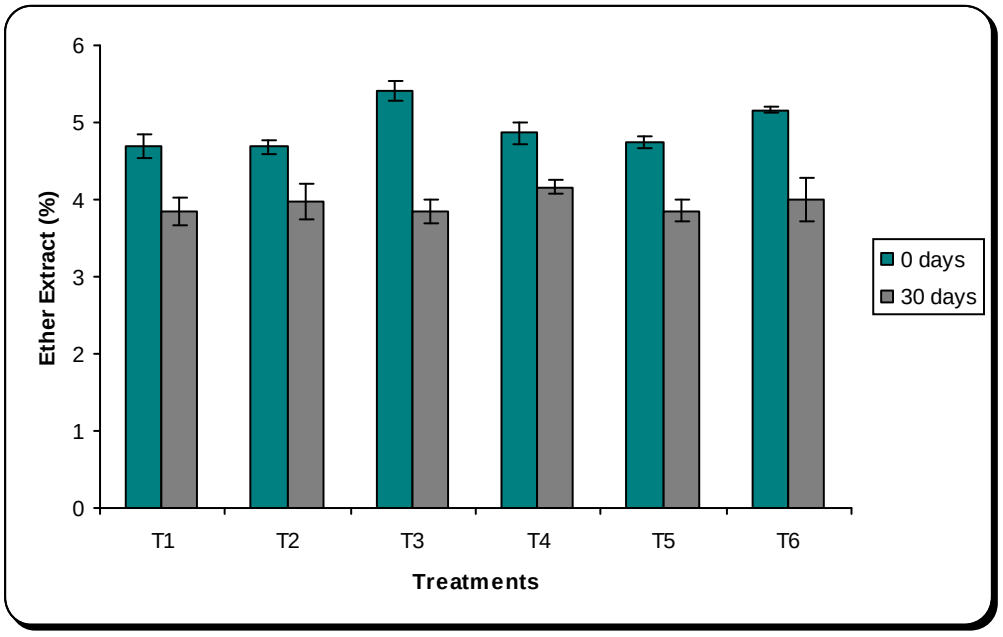
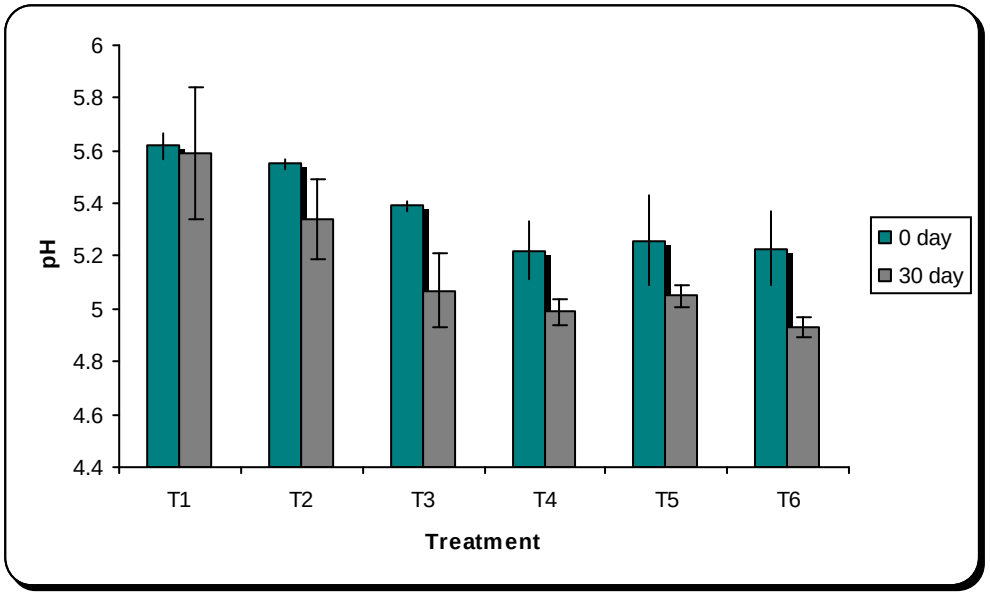


Fig 4: The effect of crude soy lecithin and soy acid oil alone or their different combination on the pH content of chicken meat at different period



3.6.4.1. Reagents Required

1. Thiobarbituric acid
2. Acetic acid
3. Trichloro acetic acid
4. Distilled water

3.6.4.2.Preparation of Thio barbituric acid reagent

TBA reagent was prepared by dissolving 0.2883 g of Thiobarbituric acid in sufficient quantity of 90% acetic acid by slightly warming the solution. The volume is then made up to 100 ml with 90 % acetic acid.

3.6.4.3.Preparation of Trichloro acetic acid extract

1. 20 g of meat was blended in 50 ml cold 20 % trichloro acetic acid for 2 minutes.
2. The blended contents was rinsed with 50 ml of distilled water, mixed together and filtered through Whatman filter paper no.1 and the volume of filtrate was collected in a 100 ml measuring cylinder.
3. The filtrate is termed as trichloro acetic acid extract and is used in the estimation of TBA number and tyrosine value.

3.6.4.4. Procedure

1. 5 ml of TCA extract is mixed with 5 ml of 0.01 M thiobarbituric acid
2. After mixing, the test tube is placed in boiling water bath (100°C) for 30 minutes
3. A blank constitutes 5 ml of 10 % trichloro acetic acid in another test tube and placed in boiling water bath along with the sample

4. After 30 minutes the test tubes were removed from the water bath and cooled in running water for about 10 minutes
5. The developed colour was measured as absorbance value at 532 nm and expressed as thiobarbituric acid number.

3.6.5. Tyrosine value

Tyrosine value can effectively monitor the meat quality to indicate proteolysis and to measure the amino acid tyrosine and tryptophan present in a non polar extract of meat. In the presence of tyrosine, folin ciocalteu phenol reagent produces blue colour, the intensity of which is a measure of protein cleavage.

The meat samples were analyzed for tyrosine value on 0^{th} day (fresh) and 30^{th} day after storage in deep freezer at -18°C as per procedure of Strange *et al.*, (1977) with slight modification.

3.6.5.1. Reagent Required

1. Folin ciocalteu phenol reagent
2. Tyrosine
3. Trichloro acetic acid
4. 0.5 N sodium hydroxide
5. Distilled water

3.6.5.2. Preparation of TCA Extract

Same as in estimation of TBA number

Table 4.8: WHC (%) of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
T_1	$76.24^{bcx} \pm 0.15$	$64.18^{y} \pm 0.98$	**
T_2	$72.21^{ax} \pm 0.19$	$66.27^{y} \pm 0.72$	**
T_3	$78.99^{c} \pm 0.34$	61.70 ± 7.31	**
T_4	$76.81^{bcx} \pm 0.36$	$63.29^{y} \pm 0.50$	**
T_5	$77.39^{cx} \pm 0.23$	$65.85^{y} \pm 1.33$	**
T_6	$73.46^{ab} \pm 2.53$	67.25 ± 1.97	**
Sig	*	NS	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.

Mean values with different superscript a, b, c within column are significantly different ($p<0.05$)

Mean values with different superscript x, y within row are significantly different ($p<0.05$)

NS – Non Significant, * Sig at 5% level, ** Sig at 1% level

Table 4.9: TBA no of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
T_1	$0.22^{abx} \pm 0.08$	$0.55^{aby} \pm 0.04$	*
T_2	$0.08^{ax} \pm 0.007$	$0.46^{ay} \pm 0.07$	*
T_3	$0.12^{ax} \pm 0.02$	$0.59^{aby} \pm 0.02$	**
T_4	$0.19^{abx} \pm 0.03$	$0.74^{by} \pm 0.02$	**
T_5	$0.19^{abx} \pm 0.05$	$0.69^{by} \pm 0.12$	*
T_6	$0.33^{bx} \pm 0.04$	$0.70^{by} \pm 0.02$	**
Sig	**	**	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.

Mean values with different superscript a, b within column are significantly different ($p<0.05$)

Mean values with different superscript x, y within row are significantly different ($p<0.05$)

* Sig at 5% level, ** Sig at 1% level

3.6.5.3. Procedure

1. 2.5 ml of TCA extract was diluted with equal amount of distilled water
2. 10 ml of 0.5N freshly prepared sodium hydroxide and 3 ml of diluted Folin and ciocalteu's reagent (1:2 with distilled water) was then added in extract
3. After mixing it was kept for 15 minutes at room temperature
4. The developed blue colour was measured as absorbance value at 660 nm in a spectrophotometer using a blank (5 ml of 5% TCA) for comparison
5. With reference to the standard graph, the tyrosine value was calculated and expressed as mg of tyrosine/ 100 gm of meat sample.

3.6.5.4. Preparation of standard graph for the estimation of Tyrosine value

1. 100 mg of pure tyrosine was dissolved in 500 ml 5% trichloroacetic acid in a volumetric flask
2. Following volume of tyrosine solution was then transferred to a series of 100 ml volumetric flask: 0, 1 ml, 3 ml, 5 ml, 7 ml, 9 ml, 10 ml, 12 ml, 15 ml and 20 ml
3. The volume of each volumetric flask was then made up to the mark with distilled water and mixed thoroughly
4. To 5 ml of each of the solution in a test tube 10 ml of 0.5 N sodium hydroxide and 3 ml of diluted Foline ciocalteu phenol reagent was added and well mixed then kept for 15 minutes at room temperature
5. The developed colour was measured as absorbance at 660 nm in spectrophotometer

6. The value of absorbance was recorded for various dilution of tyrosine and plotted on a graph sheet.

3.7. Microbial quality of fresh and stored meat

Microbial quality of meat samples were determined by Standard Plate Count Method as per the procedure outlined by David, 2009. The meat samples were analyzed for microbial quality on 0^{th} day (fresh) and 30^{th} day after storage in deep freezer at -18°C.

3.7.1. Preparation and dilution of samples

1. 1 g of ground meat sample was taken and macerated with sterile distilled water using mortar and pestle and 10% suspension of meat was prepared
2. Serial 10 fold dilutions was made by 1ml of the solution transferred from the 10^{-1} dilution to another test tube containing 9 ml of the diluent to obtain the 10^{-2} dilution from which 1ml was transferred to another test tube containing 9ml of the diluents to obtain the 10^{-3} dilution. The process was repeated to obtain 10^{-4}, 10^{-5} dilutions, so on and so forth.

3.7.2. Plating

1. 1ml aliquots of the diluted samples were placed using sterile pipettes in pre identified Petri dishes. This should be done immediately after the next higher dilution is made *i.e* once 1ml of the test sample was transferred to make the next higher dilution. Similarly plating is continued till the required dilution. For each dilution separate sterile pipette was used

Fig 5: The effect of crude soy lecithin and soy acid oil alone or their different combination on the Water Holding Capacity (WHC) of chicken meat at different period

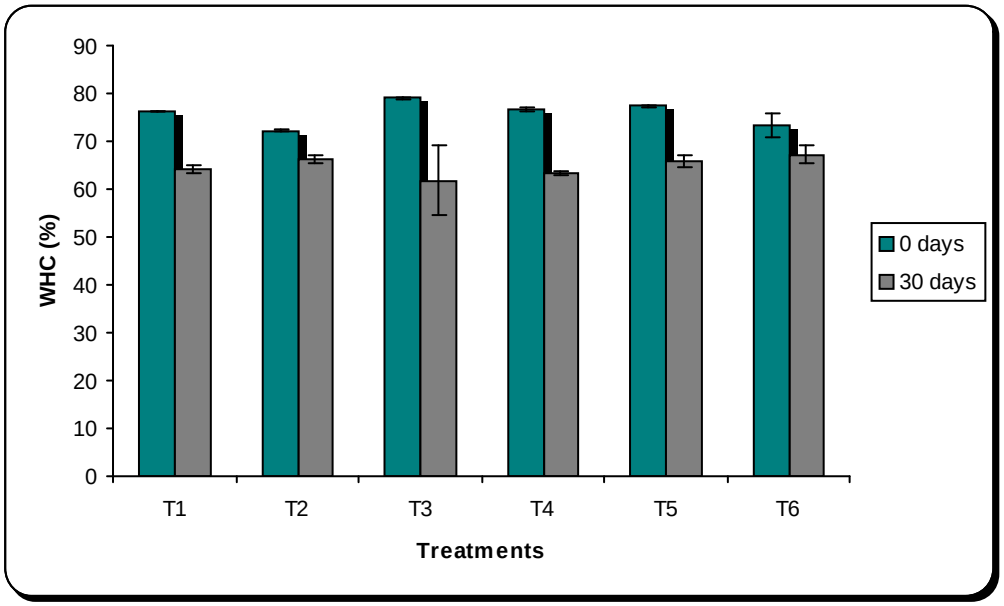
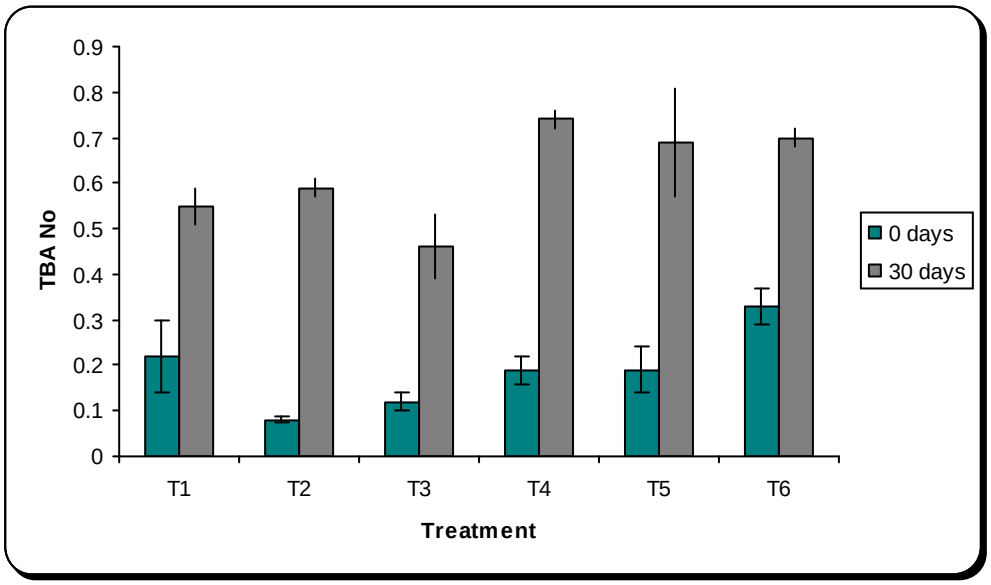


Fig 6: The effect of crude soy lecithin and soy acid oil alone or their different combination on the Thiobarbituric Acid (TBA) Number of chicken meat at different period



2. Lift the cover of the petri plate just high enough to insert the pipette while measuring diluted samples of meat suspension into petri plates
3. Hold the pipette at about 45˚ angle with the tip touching the inside bottom of the petri plate. Deposit the sample away from the centre of the plate to aid in mixing
4. Allow 2 to 4 seconds for the samples to drain from the 1ml graduation mark to the rest point in the tip of the pipette; then, holding the pipette in a vertical position, touch the tip once against a dry spot on the plate. Do not blowout. Replicate plates may be prepared for each dilution plated.
5. Prepare sterilized molten plate count agar media and hold it at 44-46˚C in a water bath until use. Remove the agar from the water bath; blot it dry with a clean towel to prevent water from contaminating the plates
6. Pour 12 to 15 ml of liquefied medium at 44˚C to 46˚C into each plate by lifting the cover of the petri plate just high enough to pour the medium. Avoid spilling the medium on the outside of the container or on the inside of the plate lid when pouring
7. As each plate is poured, thoroughly mix the medium with the test portions in the petri plate, taking care not to splash the mixture over the edge, by rotating the plate first in one direction and then in the opposite direction, by tilting and rotating the plate, or by using mechanical rotators

Table 4.10: Tyrosine value (mg %) of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
T_1	$4.6^{ax} \pm 1.06$	$5.49^{ay} \pm 2.17$	*
T_2	$6.03^{abx} \pm 0.44$	$9.02^{aby} \pm 1.94$	**
T_3	$4.84^{ax} \pm 0.96$	$7.23^{ay} \pm 0.67$	*
T_4	$8.68^{bcx} \pm 0.77$	$8.86^{aby} \pm 1.23$	**
T_5	$7.46^{abcx} \pm 0.639$	$16.20^{bcy} \pm 0.82$	**
T_6	$9.74^{cx} \pm 1.25$	$18.87^{by} \pm 5.15$	*
Sig	*	**	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.

Mean values with different superscript a, b within column are significantly different

* Sig at 5% level, ** Sig at 1% level

Table 4.12: Total Bacterial Count (log_{10} CFU/g) of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
	Total Bacterial Count (log_{10} CFU/g)		
T_1	$5.17^{ax} \pm 0.02$	$5.27^{ay} \pm 0.008$	**
T_2	$5.21^{bx} \pm 0.005$	$5.31^{by} \pm 0.01$	*
T_3	$5.25^{cx} \pm 0.02$	$5.38^{cdy} \pm 0.008$	**
T_4	$5.23^{bcx} \pm 0.01$	$5.41^{dy} \pm 0.03$	**
T_5	$5.19^{abx} \pm 0.01$	$5.37^{cy} \pm 0.008$	**
T_6	$5.26^{cx} \pm 0.02$	$5.44^{ey} \pm 0.005$	**
Sig	**	**	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.

Mean values with different superscript a, b, c, d, e within column are significantly different ($p<0.05$)

Mean values with different superscript x, y within row are significantly different ($p<0.05$)

* Sig at 5% level, ** Sig at 1% level

Table 4.5: Overall effect of crude soy lecithin, soy acid oil and their combination on proximate composition of chicken meat at different period

Particulars	Treatment Effect						Sig	Storage Effect		Sig	Interaction
	T1	T2	T3	T4	T5	T6		0 day	30 day		Treatment* Storage
Moisture (%)	76.48^{bc} ±0.41	76.92^{c} ±0.86	74.12^{abc} ±0.36	75.19^{ab} ±0.37	75.78^{abc} ±0.46	74.28^{a} ±1.2	*	75.89 ±0.37	74.92 ±0.35	**	*
Protein (%)	18.27 ±0.41	18.11± 0.41	18.77 ±0.29	18.82 ±0.19	18.03 ±0.17	18.63 ±0.59	NS	17.98 ±0.21	18.89 ±0.15	**	NS
Ether extract (%)	4.27^{a} ±0.22	4.33^{b} ±0.19	4.63^{ab} ±0.36	4.51^{a} ±0.17	4.29^{a} ±0.21	4.58^{b}± 0.28	**	4.93 ±0.72	3.95 ±1.65	NS	**
Total ash (%)	1.06^{a} ±0.03	1.19^{ab} ±0.03	1.22^{b} ±0.03	1.09^{ab} ±0.11	1.10^{ab} ±0.03	1.14^{ab} ±0.02	NS	1.14 ±0.03	1.13 ±0.02	NS	NS

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
Mean values with different superscript a, b, c within row are significantly different
NS – Non Significant, * Sig at 5% level, ** Sig at 1% level

Table 4.11: Overall effect of crude soy lecithin, soy acid oil and their combination on physico chemical characteristics of chicken meat at different period

Particulars	Treatment Effect						Sig	Storage Effect		Sig	Interaction
	T1	T2	T3	T4	T5	T6		0 day	30 day		Treatment* Storage
pH	5.6^{c} ±0.11	5.44^{bc} ±0.08	5.23^{ab} ±0.1	5.1^{a} ±0.07	5.16^{a} ±0.08	5.08^{a} ±0.09	**	5.38 ±0.05	5.16 ±0.07	*	NS
ERV (ml)	17.67 ±1.31	18.83 ±0.75	18.33 ±1.36	22.67 ±2.96	18 ±1.46	24.83 ±4.61	NS	20.17 ±1.44	19.94 ±1.53	NS	NS
WHC (%)	70.21 ±2.73	69.37 ±1.42	70.35 ±5.06	70.05 ±3.03	71.62 ±2.65	70.35 ±1.99	NS	69.24 ±0.66	70.34 ±1.19	**	NS
TBA no	0.38^{abc} ±0.08	0.29^{ab} ±0.11	0.35^{a} ±0.08	0.46^{cd} ±0.12	0.44^{bcd} ±0.13	0.52^{d} ±0.08	**	0.19 ±0.04	0.62 ±0.05	**	NS
Tyrosine value (mg %)	5.05^{a} ±1.1	7.53^{a} ±1.11	6.04^{a} ±0.75	8.77^{ab} ±3.25	11.83^{bc} ±6.69	18.87^{c} ±0.69	**	6.89 ±0.55	10.94 ±2.91	**	**

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
Mean values with different superscript a, b, c, d within row are significantly different
NS – Non Significant, * Sig at 5% level, ** Sig at 1% level

Table 4.13: **Overall effect of crude soy lecithin, soy acid oil and their combination on Total Bacterial Count of chicken meat at different period**

Particulars	Treatment Effect						Sig	Storage Effect		Sig	Interaction
	T1	T2	T3	T4	T5	T6		0 day	30 day		Treatment* Storage
Total Bacterial Count (log_{10} CFU/g)	5.22^{a} ±0.05	5.25^{ab} ±0.02	5.32^{ab} ±0.04	5.32^{b} ±0.05	5.28^{ab} ±0.04	5.35^{a} ±0.005	*	5.22 ±0.008	5.36 ±0.01	**	**

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
Mean values with different superscript a, b within row are significantly different ($p<0.05$)
* Sig at 5% level, ** Sig at 1% level

8. Allow agar to solidify (no longer than 10 min) on a level surface. Select the number of samples to be plated in anyone series so that not more than 20 min (preferably 10 min) elapse between diluting the first sample and pouring the last plate in the series.

3.7.3. Incubation

After solidification, invert the plates to prevent spreaders, and promptly place them in the incubator. Culture characteristics can be read after 18 to 24 hours of incubation at 35 to 37˚C.

3.7.4. Counting colonies

Count colonies with the aid of magnification under uniform and properly controlled artificial illumination, using a tally. Routinely use a colony counter equipped with guide plate ruled in square centimetres. Count all colonies on selected plates containing 25 to 250 colonies promptly after the incubation period.

3.7.5. Counting and Reporting

Colony counts may be computed by multiplying the total number of colonies (or the average number if replicate plates of the same dilution are used) per plate by the reciprocal of the dilution used. Counts are reported as Colony Forming Unit (CFU) per g of meat.

3.7.6. Calculation

CFU/g meat = CFU/plate x meat suspension factor (10 ml/g) x dilution factor x aliquot factor (1)

3.8. Determination of total lipid

The meat samples were analyzed for the total lipid content on 0^{th} day (fresh) and 30^{th} day after storage in deep freezer at -18°C.Total lipid content in breast muscles were determined as per the procedure of Bligh and Dyer (1959) with minor modification.

3.8.1. Purification of solvent

- Chloroform: Chloroform was distilled over anhydrous sodium sulphate discarding the first and last 10 per cent fractions.
- Methanol: Methanol was distilled same as chloroform

3.8.2. Extraction of lipid

1. For lipid extraction 10 g sample (minced meat) from breast was taken in a beaker.
2. 2 ml distilled water, 10 ml chloroform and 20 ml of methanol was added to the beaker and then homogenized for 2 minutes. Another 10 ml of distilled water was added and blended for 30 seconds and the homogenate was filtered first through Whatmann filter paper no.1
3. Another 10 ml of chloroform was poured into the beaker for washing the walls and dissolving the residual meat sample and finally the chloroform along with residual meat sample was filtered through Whatmann filter paper no.1 with slight suction.

Fig 7: The effect of crude soy lecithin and soy acid oil alone or their different combination on the Tyrosine Value of chicken meat at different period

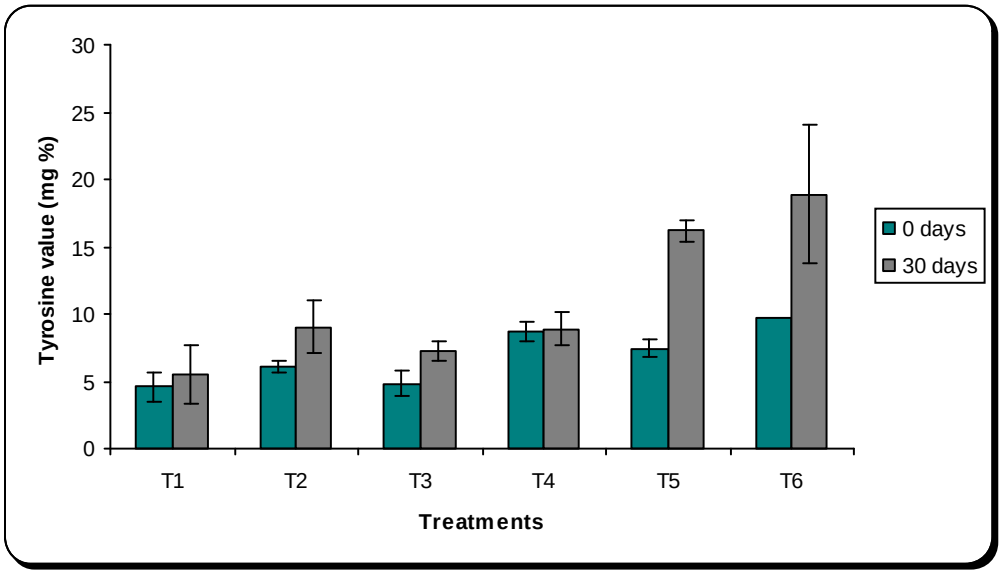
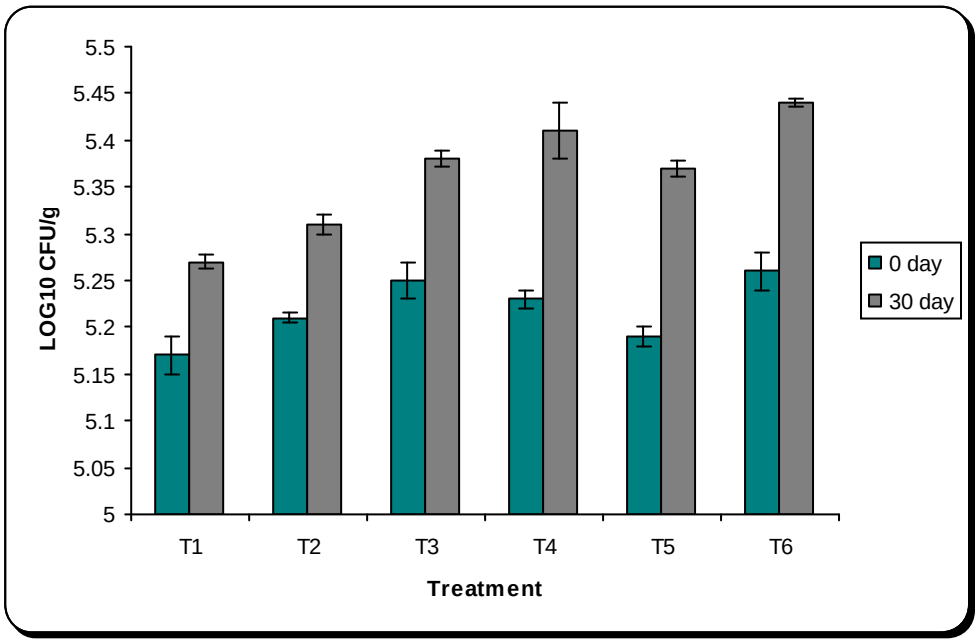


Fig 8: The effect of crude soy lecithin and soy acid oil alone or their different combination on the Total Bacterial Count of chicken meat at different period



4. The filtrate was transferred to a 50 ml measuring cylinder and after 10 minutes the volume of chloroform layer (bottom) was recorded and then it was transferred to a separating funnel and collected which contained the total lipid.

5. In a beaker 5 ml of the lipid extract was evaporated to dryness in an oven at 80^0C. Beaker is then cooled in a desiccators and the weight of the beaker with lipid was recorded.

6. The lipid content of the sample was calculated as below

$$\text{Total lipid (\%)} = \frac{\text{Wt. of lipid in chloroform layer X volume of chloroform extract}}{\text{Volume of aliquote taken X wt. of sample}} \times 100$$

3.9. Statistical analysis

For interpretations of results, the data were analyzed by ANOVA two way classification to test the significant difference between the treatment and within treatment group using the standard statistical formulae given by Snedecor and Cochran (1994) using SPSS package (SPSS ver. 10.0).

Table 4.14: Lipid (%) of the chicken meat when fed with crude soy lecithin and soy acid oil alone or in different combination at different period.

Treatments	Storage		Sig
	0 days	30 days	
	Lipid (%)		
T1	1.2^{abx}±0.01	0.67^{y}±0.01	**
T2	1.19^{abx} ±0.02	0.70^{y} ±0.06	**
T3	1.2^{bx}±0.02	0.56^{y} ±0.06	**
T4	1.18^{abx} ±0.01	0.73^{y} ±0.11	*
T5	1.15^{ax}±0.02	0.64^{y} ±0.05	**
T6	1.24^{bx}±0.02	0.64^{y} ±0.06	**
Sig	*	NS	

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
Mean values with different superscript a, b, c within column are significantly different
Mean values with different superscript x, y within row are significantly different
NS – Non Significant, * Sig at 5% level, ** Sig at 1% level

Table 4.15: **Overall effect of crude soy lecithin, soy acid oil and their combination on Total Lipid content of chicken meat at different period**

Particulars	Treatment Effect						Sig	Storage Effect		Sig	Interaction
	T1	T2	T3	T4	T5	T6		0 day	30 day		Treatment* Storage
Total Lipid (%)	0.94 ±0.39	0.94 ±0.16	0.89 ±0.15	0.96 ±0.11	0.89 ±0.11	0.94 ±0.14	NS	1.19 ±0.01	0.66 ±0.02	**	*

T_1- control diet, T_2 -CSL 3%, T_3 - SAO 2.5%, T_4 – CSL 75: 25 SAO, T_5- CSL 50:50 SAO and T_6 - CSL 25:75 SAO.
Mean values with different superscript a, b within row are significantly different ($p<0.05$)
NS-on Significant, * Sig at 5% level, ** Sig at 1% level

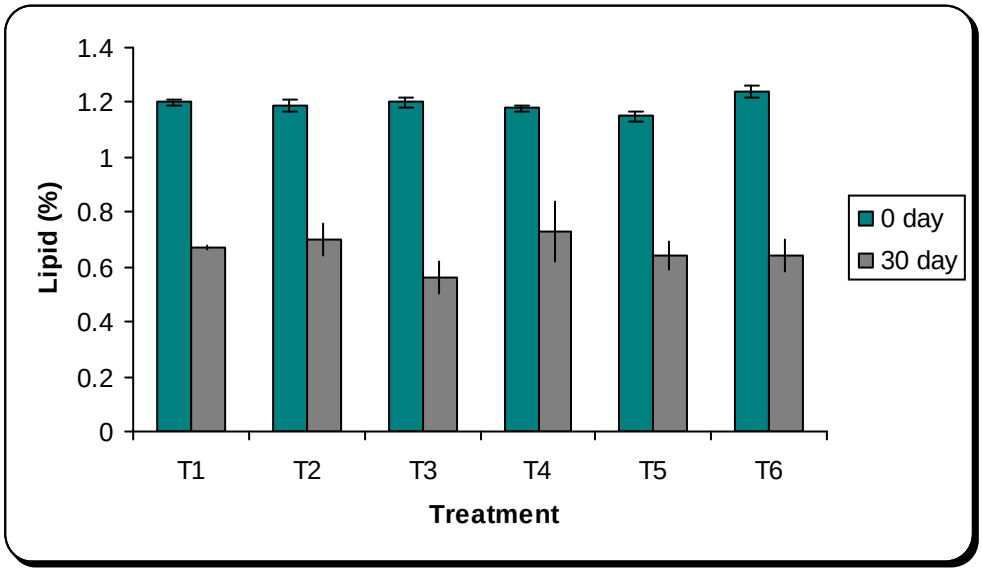
CHAPTER- IV

RESULTS AND DISCUSSION

4.1 Chemical composition of diet

The ingredient composition and chemical composition of the diet is presented in table 3 and 4 respectively. The diet consisted of chopped sola (*Aeschynomene indica*) grass hay as a source of roughage. Sola grass and concentrate mixture was given in the ratio of 60:40 (basal diet) a*d lib* to all kids. Concentrate mixture consisted of commonly available feed ingredients: crushed yellow maize 43%, soya deoiled cake 32%, deoiled rice bran 22%, mineral mixture 2% and salt 1%. In group I (control – basal diet) there was no supplementation of Cr and Zn, group II received the basal diet and 0.5ppm Cr picolinate + 20ppm Zn methionine, in group III basal diet was supplemented with 1 ppm Cr picolinate and 35 ppm Zn methionine where as in group IV basal diet was supplemented with 1.5 ppm Cr picolinate and 50 ppm Zn methionine. Total zinc received by group I, II, III and IV were 28.78, 48.78, 63.78 and 78.78 ppm respectively while total Cr received by the animals in the respective groups were 0.48, 0.98, 1.48 and 1.98 ppm respectively. Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), total ash (TA), nitrogen free extract (NFE), acid insoluble ash (AIA), total calcium (Ca) and phosphorus (P) contents of the diet were presented in table 4. The NDF, ADF, hemicellulose (HC), cellulose (C) and lignin of the diet were 44.60, 30.63, 13.97, 21.51 and 10.56% respectively (Table 4). The concentration of zinc (Zn), chromium (Cr), manganese (Mn), copper (Cu), iron (Fe), molybdenum (Mo), and cobalt (Co)

Fig 9: The effect of crude soy lecithin and soy acid oil alone or their different combination on the Total Lipid content of chicken meat at different period



have been given in table 4. DCP and TDN requirement of goat kid was 10% and 65%, respectively (Ranjhan, 1997). In present experiment diet contain almost similar percentage of DCP and TDN for feeding of kids.

4.2 Effect on feed intake, growth performance and feed conversion ratio

4.2.1 Dry matter intake (DMI)

The average DMI (g/day) by kids in various groups is presented in table 5 and depicted in figure 1. No significant difference was observed amongst groups. Although the average DMI during the first (0-15day) fortnight was recorded highest in group II (280 g/day) where there was dietary supplementation of 20 ppm Zn and 0.5 ppm Cr. However during 2^{nd} (15-30) and 5^{th} (60-75) fortnights it was highest (296g/day and 426g/day respectively) in group I where no supplements were there in the diet. During third (30-45 d) and sixth (76.90 d) fortnights highest DMI (318 g/day and 440 g/day respectively) was recorded in group III. While in group IV the maximum intake (368 g/day) was noted during fourth fortnight. This shows that supplementing Cr-picolinate and Zn-methionine together in the diet has no marked effect in DMI irrespective of the period of growth in kids. Wagner *et al.* (1999) and Aliarabi and Chhabra (2006) also reported the similar trend in DM intake in crossbred calves when diet was supplemented with Zn. However they did not studied the combined effect of Zn and Cr supplement in the diet. It indicated that Cr in no way has negative effect in over all DMI when supplemented along with Zn. However, Garg *et al.* (2003) reported that, DMI was significantly ($P>0.05$) higher in Zn-methionine group as compared to control group in lambs. Malcolm-Callis *et al.* (2000) observed

that, there was a linear ($P < 0.10$) decrease in daily DMI with increasing zinc concentrations, suggesting that higher concentration of $ZnSO_4$ may have a negative influence on palatability. Mean DMI were not affected by Cr-Picolinate supplementation in lambs (Kitchalong *et al*.1995). Moonsie - Shageer and Mowat (1993) did show improve DMI in calves supplemented with high-Cr yeast in a corn-silage diet. Whereas Boleman *et al.* (1995) reported reduced DMI in pigs fed Cr tripicolinate from the growing to the finishing phase. The DMI per 100 kg body weight varies from 4.33 to 4.82kg in group IV as against 4.94 to 5.20kg in control (Table 6). This shows that the animals in control group has to eat more to meet out their requirement and balance physiological norms normally. However the animals of mineral supplemented groups meet out their requirement in relatively less amount of feed due to its better utilization in these groups. Similar trend has been observed when DMI was calculated in terms of $kg^{0.75}$ (table 7). The current study suggests that the form and the combination of these two elements exerts the specific effect in altering the palatability of the diets which reflected the over all DMI. In no above reports the combined effect of Zn and Cr was studied which was undertaken in the current study.

4.2.2 Growth Performance

The growth performance of kids from the day of experiment upto 90 day at the interval of 15 day, due to combined dietary supplement of Zn methionine and Cr picolinate was recorded and presented in table 8. The change of body weights at every fortnights and the pattern of ADG in body weight during the entire period was depicted in figure 2 and 3 respectively. There was no significant difference amongst

groups upto 45 day of growth trial thereafter the change in the body weight was recorded till 90 days. At 60 days the highest weight (7.88 Kg) was recorded in group IV. It was significantly higher than control group (I) at 5% probability level. However no significant difference was observed either between groups I, II and III or groups II, III and IV. Similar trend was continued upto 75 day, the highest being in group IV (8.9 Kg) and lowest (7.75 Kg) in group I. It shows that combined supplementation of Zn and Cr increased the body weight of kids to the extent of 12.41 % in group IV as compared to group I (without supplement) at 60 day and 14.83 % at 75 day of growth. At 90 day group IV differed significantly at 5% probability ($P<0.05$) level with groups I, II and III however the later all the three groups did not differ significantly. The difference was worked out to 17.99% higher than that of control group (unsupplemented). The growth pattern of daily gain in body weight was different at different growth period. It has been given in table 9. The gain at the beginning of the trial in all groups was toward increasing order however the difference due to dietary treatments was non significant. This picture was noticed upto 45 days and thereafter a drastic increased in daily gain was noticed between 45-60 day and 60-75 days. It was 50 % and 44.1% increased in the respective periods in group IV as compared to control. No significant difference was observed amongst groups I, II and III and II, III and IV. The data suggest that a level of 50 ppm Zn with 1.5 ppm Cr (group IV) supplement in the diet could give better gain in kids. This indicates that Zn plus Cr supplement was almost depending on physiological stage of kid. During the peak growth period these elements increase the efficiency of nutrient utilization across the

gastro-intestinal tract, as the secretion of gastric enzymes during such period is in the state of optimum levels. The synergistic action of these elements helps each other in their absorption. Their optimum levels in body tissues exert a positive action on the growth by activating the enzyme system in the body responsible for the synthesis of anabolic hormones required for the synthesis of body protein and the over all development of skeletal tissues (Chester, 1992). Garg *et al.* (2003) also reported that, average daily gain of the lambs was significantly higher in Zn-methionine group as compared to control group. Puchala *et al.* (1999) reported an increase of 17 % in daily gain as compared to control when Zn-methionine was supplemented alone at the rate of 1, 3 and 5 g/day in goat. However Malcolm-Callis *et al.*(2000) and Zali *et al.*, (2008) found no significant differences in steers and ewes respectively when the diets were supplemented with zinc sulphate as compared to the control. In contrary, Paul *et al.* ((2005) and Pechova *et al.* (2002) reported that supplementation of Cr picolinate in the diet increase body weight gain in goat and bulls respectively. However no significant difference was recorded in sheep due to dietary supplement of Cr (Uyanik, 2001). In the current study supplementation of Zn and Cr showed an additive effect in the daily gain in body weight of kids when supplemented in combination in the diet. This may be due to the fact that Zn plays a significant role in digestion, glycolysis, synthesis of DNA and nucleic acid and protein metabolism (Underwood and Suttle, 2001). Similarly Cr helps in carbohydrate, protein and lipid metabolism (Mertz et al, 1974).

4.2.3 Feed Conversion Ratio (FCR)

The FCR at various physiological stages of growth due to dietary treatments of Zn and Cr in various combinations have been presented in table 10 and depicted in figure 4. Data suggest that there was no significant difference due to treatments from 0- 30 days which varied from 7.83 to 10.26. However it varied significantly at 5 % probability level amongst groups from 30 – 75 days. The maximum FCR from 30 – 45 day was recorded in group I and the minimum in group IV (10.07 *vs.* 6.72, respectively) which differed significantly. During this period no significant difference of FCR was noticed amongst groups I, II and III, and II, III and IV. Similar trends of results were observed from 45 – 60 days. During the period of 60–75 days supplementation of Zn and Cr exerted significantly higher difference either between groups I and II or I and IV. However no significant difference was observed between groups I and III, and amongst II, III and IV. At the last stage of growth period (75-90 day) the values did not differ significantly. This shows that as the FCR increases the rate of growth and the over all change in body weight of kids decrease and *vice-versa*. It indicates that narrow the ratio better the growth performance and wider the ratio poor the utilization of diets. The minimum FCR was obtained in group IV irrespective of the stage of growth indicating better utilization of diets due to a supplement of 50 ppm Zn + 1.5 ppm Cr. Garg *et al.* (2003) reported better feed conversion efficiency in Zn-methionine supplemented diets as compared to un supplemented diets. Spears and Kegley (1991) also found a significant (4.05%) improvement in feed conversion when zinc methionine was added to the control diet. In contrary, Wang *et al.* (2006) reported

no significant difference in feed: gain ratio between zinc supplemented group and control group. The FCR changed drastically due to supplementation of Cr in the diet and gave better growth performance in goats due to better utilization of nutrients (Paul *et al.*, 2005).The results were found highly significant ($P < 0.001$). However Kitchalong *et al.* (1995) and Uyanik (2001) reported no significant change in FCR in sheep due to supplementation of chromium in the diet. It is well evident that feed conversion ratio is a better indicators for the growth performance of kids. In current study the FCR was better in group IV. It may be inferred that for each kilogram of body weight gain 5.83 to 8.91 kilogram of diet is required at various stages of growth period due to dietary supplementation of Zn plus Cr. Overall ADG in body weight and FCR was significantly higher in group IV (54.62g/day; 6.88 respectively) than control group (table 11). However no significant difference was observed amongst mineral supplemented groups (II, III, IV).

4.3 Nutrient Utilization

The nutrient utilization of diets due to supplementation of Zn-methionine and Cr-picolinate in various groups were studied and presented in table 12. The nutritive value of diets was calculated with respect to digestible protein and digestible energy in kids. There was no significant difference observed amongst groups with respect to digestible protein (DCP) and digestible fat (DEE) due to supplementation of combined chelated Zn-methionine and Cr-picolinate in the diet while digestible energy in terms of DCF, DNFE and TDN differed significantly ($P<0.05$) amongst groups. These were highest in group IV and followed a linear trend of decreasing as the levels of Zn and

Cr reduced in the diet. There was no significant difference either between groups I and II or groups II and III. However a significant difference could be recorded between groups I and IV, I and III, and III and IV. This shows that as levels of chelated Zn +Cr in the diet increased the levels of available energy from the diets also increased. The value of TDN varied from 55.21 to 61.12 % from group I to group IV which is about 11% increase as compared to control where there was no supplementation. Garg *et al.* (2003) and Paul *et al.* ((2005) also reported the increase digestibility of various nutrients including TDN in lambs supplemented with Zn-methionine and Cr individually, respectively in the diet. However they did not studied their combined effect. From the current study it could be inferred that Zn and Cr together derived more energy from the carbohydrate fraction of the diet than other nutrients fraction for improving the available energy to the kids.

4.4 Balance Study

The change in nutrient balance pertaining to N, Ca and P due to the exogenous dietary supplementation of Zn and Cr in the chelated forms has been presented in table 13. It has been noted that all balances were positive in kids and no significant differences were noticed due to supplementation of these elements on N and Ca balance at different period of growth except on P balance which differs at 5% probability level ($P<0.05$). N balance varied from 53 to 58 %, Ca from 75 to 87 % and P from 23 to 46% % of N, Ca and P intake, respectively. The N excreted through faeces ranged from 27 to 31 % as against 12 to 15 % in urine which is under the normal physiological limits of small ruminants. The results were in accordance to

Nunnery (2002) who reported no significant difference in the control and the Zn supplemented groups in lamb. Similarly Kitchalong *et al.* (1995) reported an average of 65.3 and 63.7% of the N intake being absorbed and 27 and 22.6% of the N intake being retained during week 3 and 11, respectively due to supplementation of Cr-picolinate in the diet of lamb. No significant difference was observed in P balance amongst groups II, III and IV due to the dietary treatments however it differed significantly between Zn plus Cr supplemented (II,III and IV) and control group (I). It was higher where Zn plus Cr was supplemented at the rate of 20 ppm plus 0.5 ppm respectively. It may be indicated here that Zn and Cr help in the synthesis of body protein and nucleic acid which increased the utilization of P and also stored the energy in the form of high energy phosphate bond (phospho creatine) thereby increasing the P balance. The ratio of Ca and P in the complete mixed diets varied from 1: 1.48 to 1: 1.51 which reflected better utilization of these minerals in the biological system by favoring the absorption of each other. The percent balance of Ca and P in the body envisaged the maximum availability of these minerals to kids for their better growth. In all it can be indicated that combined supplement of Zn and Cr has no adverse effect on nutrient utilization of either Ca or P across the gastro-intestinal tract and the balances in all treatments were at par with the control.

4.5 Biochemical Study

4.5.1 Total Serum Protein

The average concentrations of total serum protein in kids supplemented with different levels of Zn methionine and Cr picolinate have been presented in table 14

and depicted in figure 5. There was no significant difference amongst groups due to dietary treatments from the day of start of experiment to 30 days; and 75 days to 90 days. However it differed significantly ($P<0.05$) from 45 to 60 days. The concentrations during these periods were significantly different between groups I and II, III and IV; and II and IV. These were higher in Zn and Cr supplemented groups than the control group. However no significant difference could be noticed between II and III; and III and IV. It increased from 3.15 mg/dl to 7.10 mg/dl due to the supplementation of elements. There was a linear decrease in the concentration of serum protein from 0 to 45 day and thereafter it follows an increasing trend continuously till 90 days irrespective of the dietary treatments. It indicates that Zn and Cr both influence at the cellular level for the synthesis of nucleic acids and protein synthesis (Underwood and Suttle, 2001). It was reflected by higher serum protein and lesser residual nitrogen, detoxified in the liver within the urea synthetic cycle. It was evident through the mechanism of chromium action for the improvement of amino acid entry into the muscular cells for the protein synthesis (Evans and Bowman 1992). Pechova *et al.* (2002) found significantly higher concentration of total plasma proteins in chromium supplemented bulls. However Kitchalong *et al.* (1995) and Ozcel *et al.* (2001) reported that total protein concentrations (59g/l) was not affected due to supplementation of Cr and Zn respectively in the diet of lamb. Plasma proteins, transferrin and albumin can increase inorganic chromium absorption in animals (Mowat, 1997).

4.5.2 Serum Albumin

Supplementation of Zn methionine and Cr picolinate in the diets result in significant ($P<0.05$) rise of serum albumin concentrations amongst groups in kids from 45 to 60 days however no significant difference was recorded during the rest of the periods upto 90 days. The results are presented in the table 15 and depicted in figure 6. At 45 day group I significantly differed from group IV and other groups did not differ significantly ($P>0.05$) from each other. However at 60 days Zn plus Cr supplementation result in a significant rise of serum albumin as compared to control. Amongst supplemented groups no change in albumin levels could be noticed due to the difference in the levels of supplementation. However the change in the serum albumin levels due to supplementation of these elements in the diet was 1 to 1.97 fold more as compared to the control. The period of growth also effect the concentration of serum albumin. It was toward decreasing trend from day 0 to 60 and thereafter no definite trend was observed. It was in accordance to the report of Moonsie-Shageer and Mowat (1993) who conducted study in beef supplemented with Cr. It might be due to fact that insulin being anabolic in nature help in more synthesis of amino acid in liver in the presence of Cr (Schroeder *et al.,* 1956). This could be reflected by increase in muscular tissue of kids during the period of growth and simultaneously increasing the levels of albumin in the serum. Neto *et al.* (2003) reported that Zn has direct effect on insulin synthesis and its secretion as it is a component of insulin molecule responsible for the synthesis of albumin.

4.5.3 Serum Globulin

The mean values of serum globulin in different groups have been presented in table 16 and depicted in figure7. It was observed that supplementation of Zn and Cr in the diet significantly increased the level of globulin as compared to control group. However, there was no significant change amongst groups II, III and IV. It has also been observed that as growth period of kids increased the level of globulin decreased however, it could again regained after 60^{th} day till 90^{th} day. The change of globulin level varied from 1 to 2.06 times more due to an additive effect of growth period and level of supplementation. This change was expected due to the improvement of immunological status of kid by dietary supplementation of Cr. It was evident through the *in vitro* measures of cellular and humoral immune function (Burton *et al.*, 1993; Moonsie-Shageer and Mowat, 1993) of kids. Chang and Mowat (1992) also reported an increase serum immunoglobulin- M and total immunoglobulins due to supplementation of high-Cr yeast in calves. De Pasquale-Jardies and Fraker (1979) suggested that Zn is required for normal growth of thymus so as to improve the immune system. Kyriaszakis *et al.* (1994) noticed that increase in globulin levels probably reflects an increased rate of immunoglobulin synthesis associated with the development of immunity.

4.5.4 Albumin Globulin Ratio

The data pertaining to albumin: globulin ratio has been presented in table 17 and depicted in figure 8. In spite of change in the values of albumin and globulin at different stage of growth the albumin: globulin ratio did not change much and

therefore no significant (P>0.05) results could be obtained in different groups due to supplementation of Zn and Cr. No definite trend was also recorded during the entire period of growth.

4.5.5 Blood Glucose

The change in blood glucose levels in kids due to dietary supplementation of organic Zn and Cr have been given in table 18, while the values of such change at different growth period was depicted in figure 9. No significant difference was observed amongst groups due to supplementation of organic minerals from day 1 to 30 and thereafter a significant change in the levels of glucose was measured. At day 45 the supplementation of Zn with Cr (50 ppm + 1.5 ppm, respectively) drastically reduced the level of glucose from 54.2 (I) to 45.05 mg/dl (IV). The levels were non significant amongst supplemented groups. Similarly there was also no significant change observed in groups I, II and III. The levels of blood glucose after 45 days followed almost similar trends. It was significantly higher (P<0.05) in the supplemented groups and lower in control group. Although the level of blood glucose in supplemented groups was lower than control it was within the limit of physiological norms (40-60 mg/dl). The low level may be due to relatively more synthesis of insulin in pancreas utilizing glucose for the production of energy inside the cells. This could be achieved due to dietary supplement of Zn and Cr which might have affected the synthesis of insulin. The higher utilization of glucose is reflected by the better growth performance of kids in these groups. The exact mechanism of action of Cr for the synthesis of insulin is not very clear however it enhances the binding of insulin to cell

membrane receptors and optimizes the insulin activity resulting better regulation of glucose uptake by cells. Thereby improved the control of blood glucose concentration and maximize the energetic potential (Anderson, 1997). Zn has also direct effect on insulin synthesis and its secretion as it is a component of insulin molecule. (Neto *et al.*, 2003). As a result more insulin is secreted which causes low blood glucose levels in Zn and Cr supplemented groups. The current findings could be confirmed with the reports of Haldar *et al.* (2007) and Uyanik (2001) who found low blood glucose levels in kids supplemented with dietary Cr. In contrary Paul *et al*. ((2005) found no significant change in glucose concentration of bucks in Cr supplemented groups.

4.5.6 Serum Alkaline Phosphatase

The levels of alkaline phosphatase in the serum of kids at different growth periods due to combined supplement of Zn methionine and Cr picolinate have been presented in table 19 and depicted in figure10. There was no significance difference amongst groups due to dietary supplement of these minerals in kids upto 30 days although the values were toward higher side in supplemented groups. After 30 days significant ($P<0.05$) change in alkaline phosphatase levels was recorded in group IV which was 38.18 units more than control. There was no significant difference amongst groups I, II and III. It indicates that the optimum level of 50 ppm Zn and 1.5 ppm Cr is required to influence the level of alkaline phosphatase in serum (135.98U/l). After 45 days no definite trend in alkaline phosphatase level could be recorded due to dietary supplements of minerals though the level was significantly higher in mineral supplemented groups. Within the same period of growth as the level of Zn and Cr in

the diet increased the level of alkaline phosphatase increased almost linearly. Earlier workers (Zali *et al*., 2008., Wan *et al*., 1993., Kraus *et al*., 1997) used blood alkaline phosphatase as an indicator of Zn status of animals. Paul *et al*. (2005) found in bucks higher activity of alkaline phosphatase in the Cr supplemented group than control and it was explained that Cr augmented new bone crystal formation by enhancing the uptake of circulatory Ca into the bone matrix. It was evident by lower concentration of Ca in serum of treated groups. Zn also interfere the absorption of Ca which lowers its level in blood. In fact the low level of blood Ca stimulate parathyroid hormone to maintain the normal blood Ca by activating osteoblast cells to release alkaline phosphatase for calcification of bone. This happens especially when the animal is in the active stage of growth. In this process a little amount of alkaline phosphatase gets released in blood and elevating its level (Tiwari *et al.,* 1994).

4.5.7 Serum Cholesterol

The concentration of total cholesterol in kids due to the supplementation of different level of Zn and Cr at various stages of growth periods (0-90 day) have been presented in table 20 and figure 11. There was significant difference ($P<0.05$) amongst groups due to mineral supplements in the level of cholesterol. The level of cholesterol decreased significantly ($P<0.05$) from the day 60 to 90. However, no difference in the level was observed during 0-45 days. Although the level of cholesterol in groups III and IV were almost similar but it was higher than group II, though not significant. Earlier workers (De Pew *et al*., 1998; Kitchalong *et al*., 1995; Yang *et al*., 1996; Bunting *et al*., 1994) reported the reduction in circulating concentrations of cholesterol

and/or non esterified fatty acids (NEFA) due to supplementation of Zn in ruminants. However in another study (Kitchalong *et al*. 1995) total cholesterol was not affected due to supplementation of Cr- picolinate. Similarly Malcolm-Callis *et al* (2000) found that supplementation of Zn at 20, 100 and 200 ppm in feed did not effect serum cholesterol concentration. In current study the decrease level of total cholesterol was more due to Cr and less due to Zn as Cr act through the linkage of chromodulin with the insulin receptor and increase the net synthesis of fat in adipose tissue thereby decreased its net release in serum (McNamara and Valdez, 2005).

4.5.8 Serum Triglyceride

The total serum triglyceride concentration in kids of various groups have been given in table 21 and depicted in figure 12. Similar to the level of total cholesterol total triglyceride also follow the decreasing trend in Zn and Cr supplemented groups as compare to control group (I). Its level was also affected from the day 60 onwards. During these periods its level drastically reduced almost to 43% in group III and IV and 31% in groups II. It clearly indicates that as the level of supplement increased the level of triglyceride decreased. Although there was no significant difference between the higher levels of supplements (group III and IV). The reduction in the triglyceride can be explained through the mechanism operating the reduction in the blood glucose level which lowers the activity of insulin and in turns releases the activity of capillary endothelial lipase causing break down of triglyceride and release of free fatty acids and glycerol (Murray *et al.*, 2003).

4.5.9 Serum HDL and LDL-Cholesterol

The level of HDL and LDL-cholesterol in serum was not affected due to the periods of growth; however it was significantly ($P<0.05$) influenced by supplementation of Zn and Cr in the diet (Table 22, 23 and figure 13, 14, respectively). Higher the level of mineral supplements lowers the level of HDL and LDL-cholesterol. There was no significant difference amongst the Zn and Cr supplemented groups. However it was significantly different ($P<0.05$) from the control group. The change in the levels was pronounced after day 60 of growth period. The reason of lowering their levels could be similar to that as of total cholesterol.

4.6 Mineral Profile

4.6.1 Serum Calcium

The mean serum calcium values at 90^{th} day of experiment for groups I, II, III and IV, were presented in table 24 and depicted in figure 15. The highest serum calcium was recorded in group I (9.41 mg/dl) whereas the lowest in group IV (6.46 mg/dl). There was significant difference ($P<0.05$) in Ca levels amongst groups. Though the value of group I was significantly ($P<0.05$) higher than groups II, III and IV but the mineral supplemented groups did not differ significantly with each other. It was supported by the findings of Paul *et al*. ((2005) who found lower level of serum Ca in Cr supplemented bucks due the fact that Cr augmented new bone crystal formation by enhancing the uptake of circulatory Ca into the bone matrix.

4.6.2 Serum Phosphorus

The mean serum phosphorus values at 90th day of experiment for groups I, II, III and IV were given in table 24 depicted and in figure 16. The highest serum phosphorus was recorded in group IV (3.11 mg/dl) whereas the lowest in group I (2.68 mg/dl).Though the results were non significant amongst groups. The level of phosphorus in the serum always go parallel to the calcium and these minerals maintain a normal physiological relationship with each other in the serum too.

4.6.3 Serum Zinc and Chromium

The level of serum Zn and Cr in kids due to exogenous supplementation of their organic chelets methionine and picolinate respectively were significantly ($P<0.05$) different amongst groups (table 25 and 26; figure 17 and 18, respectively). It has been observed that as the level of these minerals in the diet increased their levels in serum also increased. It may be indicated that serum Zn and Cr depends more due to associative effect of diet supplemented with these elements and less due to their interaction in the biological system. No definite trend was observed amongst groups at different periods of growth. The higher intake of Cr and Zn from the basal diet in the Cr and Zn supplemented bucks might be due to the difference in DM intake among the groups. DMI in the mineral supplemented bucks was higher resulting more Cr intake as compared to the control. It was reported (Anderson, 1988) that inorganic trivalent Cr is absorbed at very low levels (0.4 to 3 %) though in the current experiment the apparent absorption of these minerals was higher than these values. In spite of the higher apparent absorption of the minerals, the bucks supplemented with 50 ppm Zn

and1.5 ppm Cr failed to outperform those supplemented with 20 to35 ppm Zn and 0.5 ppm to 1 ppm Cr and probably a widened molar ratio between the glucagons and insulin might explain this phenomenon. The difference in the intake of Cu, Zn, Mn and Fe in the supplemented groups can be explained as a function of DMI that was augmented due to Cr supplementation. The intake of these trace elements in all the three experimental groups was within the normal range. The dietary levels of Zn and Cr revealed their levels accordingly in serum (*Paul et al* (2005). Puchala *et al.* (1999) reported that supplementation of diet with Zn-methionine increased plasma Zn concentration (0.92 versus 0.72 mg/l for control). Garg *et al*. (2003) also reported higher Zn concentration in the serum in Zn-methionine supplemented group. Supplemental chromium protects the stress-induced urinary losses of zinc, copper, iron and manganese (Mowat, 1997). Scott and Ziegler (1963) demonstrated in chicks that adding distillers dried soluble and liver extract to soybean protein based diets improved the availability of the dietary Zn, though the reason remained unknown. In current study dietary supplement of Zn methionine complex improve bioavailability of Zn in kids which was in accordance to the study of Nielsen et al., (1966), and Hortin *et al.*, (1991) in chicks. At the alkaline pH, found in the intestine it is likely that little free Zn cation exists in solution. One action of beneficial chelates is to form Zn complexes that are soluble within the small intestine permitting soluble Zn to reach the brush border membrane for absorption. The absorption of many essential metals is controlled by these mechanisms, such as Zn, whose absorption can vary from less than 10 to over 80% depending on the animal's status (Underwood and Suttle, 1999).

4.6.4 Serum Cobalt and Molybdenum

The levels of serum Co and Mo in kids at different growth periods amongst groups due to dietary treatment of Zn and Cr have been presented in table 27 and 28, and depicted in figure 19 and 20, respectively. No significant ($P>0.05$) difference was observed in their levels in serum either due to supplementation of Zn and Cr or due to period of growth. It indicates that Co and Mo have no active interaction either with Zn and Cr.

4.6.5 Serum Manganese

The level of Mn in the serum of kids due to dietary supplementation of Zn and Cr have been presented in table 29 and depicted in figure 21. No significant ($P>0.05$) difference was observed amongst groups upto day 30 of growth and thereafter a significant ($P<0.05$) difference was noticed upto 60 day and after that again non significant increase was observation amongst groups. Though there was no significant difference amongst groups II, III and IV due to mineral supplementation from 30 to 60 days, it differed significantly ($P<0.05$) with the control group. No definite trend of Mn level was observed during the entire growth period irrespective of the dietary treatments. The data suggest that Zn and Cr might have influenced the level of Mn in a positive direction and maintaining its level up than the control group.

4.6.6 Serum Iron

Dietary supplementation of Zn and Cr greatly influenced the level of Fe in the serum as presented in the table 30 and depicted in figure 22. From day 45 onward a pronounced decreased of Fe level amongst groups was observed. Though there was no

significant (P>0.05) difference amongst mineral supplemented groups it was significantly lower than the control group. It might be due to the fact that Zn and Cr contribute a synergistic action against Fe in the transport mechanism across the intestinal wall as the Cr competes with iron for the common binding sites on the brush border of the intestine. There are few documented reports on the effect of Cr supplementation on the metabolism of other mineral substances. The relation between Cr and Fe has been investigated most since both these minerals are transported as transferrin-bound. At low Fe saturation, Cr and Fe bind preferentially to different binding sites. When, however, the Fe concentration is higher, the two minerals compete for the same binding sites. This seems to be the reason why a lower Cr retention has been identified in patients suffering from hemochromatosis than in healthy subjects with a Fe deficiency (Sargeant *et al.*, 1979). Evidence that Cr may impair Fe metabolism has been published by Ani and Mostaghie (1992). Fe homeostasis alteration has been reported by other authors too, the most significant alteration being detected in association with Cr-picolinate supplementation (Lukaski *et al.*, 1996). Alteration of Fe metabolism in association with Cr supplementation has also been reported by Anderson *et al.* (1996), decreased tissue Fe concentrations was detected in response to Cr supplementation. Mineral metabolism in experimentally induced Cr deficiency, using goats, has been explored in detail by Frank *et al.* (2000a,b) on the basis of determining Ca, Co, Cr, Cu, Fe, Mn, Mo, P, and Zn concentrations in the blood plasma. The workers attribute the increased concentrations of these minerals to a decreased Cr concentration causing subsequent freeing of

binding sites on the transferrin, competed for by the individual minerals. A decreased loss of some microelements (Zn, Fe, Cu and Mn) during stress after Cr supplementation to mice has been reported by Schrauzer *et al.* (1986).

4.6.7 Serum Copper

As such there was no significant difference amongst groups due to the supply of Zn-methionine and Cr-picolinate in the diets of kids. Also there was no definite trend of concentration with the increase of growth period (0-90 days) in kids (table 31 and depicted in figure 23) in any groups including mineral supplemented groups. Interactions of copper with zinc and iron were investigated, but little is known about the relation between copper and chromium. Zinc concentration remained constant during the experimental period indicating the absence of effects of chromium supplementation (Pechova et al, 2002). Unfortunately a primary regulator of enterocyte metallothionein concentration is the Zn status of the animal. A diet high in Zn can induce high intestinal metallothionein concentrations which blocks Cu absorption leading to Cu deficiency. From a practical standpoint the research suggests diet zinc only interferes with copper absorption when dietary zinc is greater than 1000 ppm (Goff, 2000). In current study the levels of dietary Zn was far below this range. Interaction between Cr and Cu was studied by Stahlhut *et al.* (2006), Cr supplementation had no effect on the liver or plasma Cu concentrations in cows, although, supplemental Cr resulted in higher plasma Cu concentrations in calves on day 279. Similarly Pechova *et al.* (2002) have detected higher plasmatic Cu concentrations in response to Cr supplementation in fattening bulls which is different

as compared to the results of current study. The current study was however in accordance to Ozcel *et al.* (2001) and Paul *et al.* (2005) who observed that total serum Cu level was not affected by zinc bacitracin supplementation in bucks. Though Cr supplementation had no apparent effect on plasma Cu concentration.

4.7 Haematological Study

4.7.1 Total Erythrocyte Count (TEC)

The data pertaining to effect of Zn and Cr on TEC have been given in table 32 and depicted in figure 24). Significantly ($P<0.05$) higher number of RBC from 45^{th} day onwards were recorded and before that the difference amongst the group were non significant. After 45^{th} day though there was no significant difference amongst the supplemented groups, it was significantly ($P<0.05$) higher than control (I). At certain stages of growth, the low levels of Zn and Cr supplement (II) did not differ with control and followed an indefinite trend though higher levels of supplement (IV) did differ significantly ($P<0.05$) from the control. The current finding was in accordance to the report of Onder and Kecec (1998) and Schrauzer *et al*. (1986). The former reported higher level of TEC due to Zn supplementation and later higher due to Cr supplementation in the diet of beef and mice respectively. The reason may be attributed to the fact that these two minerals enhance the process of erythropoesis resulting into more formation of RBC and Hb. Although the exact mechanism of Cr is not very clear but it causes reduction in the loss of body Fe which is utilized for the synthesis of RBC (Schrauzer *et al.,* 1986).

4.7.2 Haemoglobin (Hb)

The level of Hb in blood was greatly influenced by supplementation of Zn and Cr in the diet. The effect was predominant and significantly higher ($P<0.05$) after 45 days of growth trials (table 33 and depicted in figure 25). Although there was no significant difference amongst Zn and Cr supplemented groups (II, III and IV) it was significantly higher ($P<0.05$) than the control group. There was almost 45% to 65% increased in the Hb concentration due to the supplementation of Zn and Cr in the basal diet. At some stage (75 day) the difference between control group with groups II and III were non significant. The reason for higher level of Hb in kids due to dietary supplementation of Zn and Cr remain the same as previously discussed under TEC.

4.7.3 Packed Cell Volume (PCV)

The PCV was significantly higher ($P<0.05$) in the Zn and Cr supplemented groups as compared to the control where no dietary supplementation of Zn and Cr was given (table 34 and depicted in figure 26). The levels of Zn and Cr did not effect much to the PCV of kids however, it was significantly ($P<0.05$) higher than un supplemented group (I). No definite trend of PCV was noticed from 0 to 90 days in any groups. Due to more RBC and high level of HB the PCV of kids increased accordingly, in the Zn and Cr supplemented groups.

4.7.4 Mean Corpuscular Volume (MCV), Mean Cell Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH)

The date pertaining to MCV, MCHC and MCH has been presented in table 35, 36, 37 and figure 27, 28, 29 respectively. There was no significant ($P>0.05$) difference

amongst groups due to supplementation of Zn and Cr in the diet for the above haematological attributes. Also there was no definite trend of these characteristics of RBC/Hb at any stage of growth periods. It shows that Zn and Cr supplementation affects the number of RBC and Hb concentration and not their other characteristics like MCH, MCV and MCHC.

4.8 Rumen fermentation pattern

4.8.1 Rumen Fermentation

The rumen fermentation characteristics especially pH, ammonia-N and microbial counts (bacterial and protozoal) did not differ significantly amongst groups due to supplementation of Zn and Cr in the diet (table 38). The pH of rumen liquor in different groups remains stationary indicating no change in the buffering capacity and the redox potential of the rumen. It is reflected by the microbial population in the rumen as there was no significant change in bacterial and protozoal population in the rumen due to supplementing Zn and Cr in the diet. The solubility of N-fraction in the rumen remains normal and no significant release of ammonia-N amongst groups in the rumen could be observed. However, the total-N concentration TCA perceptible-N and microbial protein differs significantly ($P<0.05$) amongst groups due to supplementation of these minerals in the diet. The total-N concentration was significantly ($P<0.05$) more in group III where Zn and Cr was supplemented at the rate of 35 and 1 ppm respectively. However there was no significant ($P>0.05$) difference amongst groups II, III and IV. The change toward higher values of total-N, TCA precipitated-N and microbial-N in mineral supplemented groups were recorded,

probably due to more synthesis of protein inside the microbial cells, as a result of more utilization of energy, where Zn and Cr play a significant role in the process. Hume *et al,* (1970) claimed that the increase in total-N concentration in rumen was due to associative effect of the diet in sheep; however it is not always true as there are many factors which influence the rumen environment and favors the condition for more synthesis of microbial protein. Out of these the available ammonia-N and keto acid is the important substrates favored the situation.

4.8.2 Differential protozoal count

Though the microbial protein yield increased due to mineral supplementation, the number of different protozoa in the rumen remains unchanged (table 39). The population of *Holotricha* sp, *Diplodenium* sp. and *Epididenium* sp. increased, however the population of *Entodenium* sp.and *Ophyroscolex* sp. decreased due to mineral supplementation but change was not significant (P>0.05).

4.9 Economics

The economics of raising kids with different types diets supplemented with Zn-methionine and Cr-picolinate is given in table 40 and depicted in figure 30. In spite of higher expenditure incurred in the mineral supplemented groups than control and the income received in such groups were also significantly higher. It has been reflected by higher net profit. The maximum net profit was obtained in group IV where Zn and Cr were supplemented 50 ppm and 1.5 ppm, respectively in the diet of kids. The maximum net profit obtained in this group may be due to efficient conversion of feed

nutrient into body nutrient. It indicated that, body retained more balance of P required for utilization of other nutrient through the energy metabolism.

CHAPTER–V

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK

SUMMARY

Goat farming is one of the most important subsidiary occupations of majority of farmers in Chhattisgarh state. The livelihood of a large section of people including tribal in the state is subsistence by the goat farming. Besides, the agro-climatic condition of the state and the ample availability of green vegetation in the forest areas favor goat raising. Looking to the need of the people, the current investigation was carried out in this species of animal.

Due to heavy rainfall in state there is leaching of most of the essential minerals from the soil resulting in deficiency of these minerals in the vegetation which affect the livestock productivity. Out of the minerals deficient in the feeding material of goat, Zn and Cr being the growth promoters, lack predominantly. In the present investigation the experiment was carried out to determine the optimum level of inclusion of these two minerals in combination in the diet of goat and to study their effect on growth performance, nutrient utilization, blood biochemical characteristics, change in the hematological profile, rumen fermentation characteristics and mineral interactions in serum of goat.

Twenty four kids (3-5 months; av. body wt. 5.1 kg) of either sex were randomly allotted on body weight basis to four dietary groups (I, II, III and IV) following completely randomized design (CRD). Group I (control) was fed *ad libitum* basal complete feed diet constituted sola (*Aeschynomene indica*) grass hay 60 parts and

concentrate mixture 40 parts. Group II, III and IV were fed as in control along with a combined supplement of Zn-methionine and Cr-picolinate at the rate of 20 ppm and 0.5 ppm; 35 ppm and 1 ppm; and 50ppm and 1.5 ppm respectively. Basal complete feed contain 11.07% DCP and 54.21% TDN. A growth trial of 90 days was conducted. In mid of experiment (45-50 days) a metabolism trial of five days was conducted to study the nutrient utilization and balance of nutrients (N, Ca, P) in kids. Daily record of DMI and body weight of kid was recorded at fortnight interval from 0-90 days to calculate the growth performance and FCR in kids.

Blood samples were collected from kids at 0, 15, 30, 45, 60, 75 and 90 day for determination of blood biochemical constituents including essential mineral elements and haematological profile. Strained rumen liquor was collected only once at day 45 to study the important rumen fermentation characteristics.

No significant difference was obtained in DMI of kids amongst groups due to dietary supplementation of minerals. Similarly no significant change in body weight gain and FCR was recorded amongst groups from day one to day 30 of the experiment. Thereafter a significant ($P<0.05$) change was noticed between mineral supplemented and control group. The gain in body weight was recorded higher (55 g/day) in group IV than control which is almost 42% higher. Though there was no significant difference in growth rate amongst mineral supplemented groups it was higher in group IV. Comparatively narrower FCR was recorded in mineral supplemented groups than control indicating best in group IV. Supplementation of Zn and Cr improved the nutrient utilization of diets as reflected by 11.29% DCP and 61.12% TDN in group IV which is better amongst groups. The digestibility coefficient of nutrient did not differ significantly

except IV and I. The growth performance, FCR and the value of DCP and TDN in different groups determine the optimum level of inclusion of Zn and Cr in the diet. It was found optimum in group IV (50 ppm and 1.5 ppm).

Supplementation of Zn-methionine and Cr-picolinate in the diet results in significant increase of alkaline phosphatase, total protein, albumin and globulin levels in serum of kids. However blood glucose and total cholesterol, triglyceride, HDL and LDL cholesterol in serum were significantly reduced due to mineral supplementation. The above changes in blood and serum profile were more marked after a growth period of 30 days however no consistent results were obtained before 30 days. The level of blood glucose and total serum cholesterol falls by 10.18 and 26 units respectively due to supplementation of minerals in group IV from control. In contrary, alkaline phosphatase and total serum protein increased by 59 and 3.2 units respectively in group IV from control. The fraction of serum cholesterol (HDL and LDL) and total protein (albumin and globulin) were also affected accordingly. However no significant change was observed in albumin: globulin ratio amongst group.

The supplementation of Zn-methionine and Cr-picolinate also increased significantly ($P<0.05$) the number of RBC, Hb concentration and values of PCV in kids of group IV as compared to control group (I). However no significant change was observed in respect to MCH, MCV and MCHC amongst groups.

There was no change in $_{P}H$, ammonia-N concentration and microbial (bacteria and protozoa) population due to supplementation of Zn and Cr in the diet. However there was a drastic increase in total-N (6.95mg/dl), TCA precipitated-N (2.62mg/dl) and microbial protein (5.71%) concentrations in the rumen liquor of goat. Also there was no

significant change in the various sp. of protozoa due to mineral supplementation in different groups.

The levels of of Fe in the serum were drastically reduced due to effect of Cr present in the supplement. Where as dietary supplementation has no significant effect on Co, Mo and Cu levels in serum. The serum levels of Zn, Cr and Mn were significantly higher in mineral supplemented groups than control however they did not differ significantly among themselves. In all the increase or decrease level of minerals in serum was noticed after 30 day of growth trial and continued till 60 day or in even in some case up to 90 days.

CONCLUSION

In the current study, the effect of dietary supplementation of Zn-methionine and Cr-picolinate in the basal complete feed, fed to kids, was studied and the following conclusions were drawn:

(I) Mineral supplementation results in increased gain in body weight and nutrient utilization (DCP and TDN) and better feed conversion ratio in kids over control diet.

(II) Supplementation of minerals also increased the utilization of glucose and cholesterol for energy production and for production of free fatty acids in the blood circulation, respectively.

(III) The level of total serum protein and alkaline phosphatase were significantly increased in mineral supplemented groups indicating more uptake of Ca for development of skeleton and synthesis of body tissue protein, respectively.

(IV) Supplementation drastically decreased the level of Fe in serum than control indicating it better utilization for synthesis of Hb and production of more number of RBC in kids without affecting the normal serum Fe concentration.

(V) Dietary supplement of Zn methionine and Cr picolinate also elevate their levels in serum which is considered as the better index of health status of animals.

(VI) Dietary supplementation of Zn methionine and Cr picolinate to the level of 50 ppm and 1.5 ppm respectively, improved better growth of kids. The net profit/kg live weight due to mineral supplementation was Rs. 5.53 as against Rs. 3.03 in unsupplemented group (control).

SUGGESTIONS FOR FUTURE RESEARCH WORK

1. There is need to explore the possibility of extending this research under the field conditions where the feed resources as well as the availability of essential minerals are limited.
2. The similar experiment should also be designed in the pregnant ewe to study the effect of Zn methionine and Cr picolinate, both being growth promoter as well as enhancer of immunity.
3. There is need to establish an interactive relationship between these minerals with the enzyme profile and hormone levels at various physiological stages of goat and to explore the possibility of work at cellular level.

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THESIS ABSTRACT

Title of the Thesis:

Quality characteristics of fresh and stored meat as influenced by supplementation of soy acid oil and crude soy lecithin alone or in combination in vencobb broiler diet

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The study was conducted to find out the effect of soy acid oil and crude soy lecithin alone or in combination on the proximate composition, physico chemical characteristics, microbial quality and the total lipid content of ven cobb broiler meat on fresh state and after storage for 30 days at -18˚C. Day old broiler chicks (n=180) were divided into 6 groups of 30 chicks in each. Each group had 3 replicates of 10 chicks in each. Diet for prestarter phase contained 23% CP and 2950 kcal ME, starter 21.5% CP and 3025 kcal ME and finisher 20% CP and 3100 kcal ME/kg feed. The diets were formulated using maize, soy DOC, maize gluten, meat and bone meal and de-oiled rice bran along with premixes of vitamins, minerals and feed additives. Six diets were formulated, T_1 as control with soy oil, T_2 3% crude soy lecithin (CSL), T_3 contained 2.5% soy acid oil (SAO), T_4-T_6 contained CSL and SAO in the ratios of 75:25, 50:50 and 25:75 respectively.

The birds were fed for 42 days. At the end 9 birds were randomly selected from each treatment, starved for 12 hrs and sacrificed under standard procedure. The breast muscle from the carcass were separated and packaged in low density

polyethylene, each containing 200 g of meat sample and stored at -18°C in deep freezer for one month. One portion of meat was analyzed at fresh state (0 d).

The moisture content in fresh meat was significantly higher in group fed crude soy lecithin as compared to other treatment groups. There was significant ($P<0.05$) reduction in the moisture level in one month stored meat samples as compared to fresh meat. The protein content of fresh as well as stored meat does not vary significantly amongst the groups. Storage of meat significantly reduced the protein content in all the groups irrespective of treatment. The ether extract content of fresh meat was significantly higher in group fed soy acid oil. There was significant ($P<0.01$) difference in the ether extract content of fresh and one month stored meat irrespective of treatment. Addition of soy acid oil and crude soy lecithin alone or in different combination did not influenced the ash content of fresh as well as one month stored meat sample.

The pH value of fresh meat samples in control group was significantly ($P<0.05$) higher as compared to groups fed CSL and SAO in different ratios (T_4, T_5 and T_6). Addition of SAO and CSL alone or in combination does not significantly influence the ERV values amongst various treatment groups at fresh as well as after one month storage period. The water holding capacity of fresh meat was significantly ($P<0.05$) lower in group fed soy acid oil alone (T_3) or with crude soy lecithin in 75:25 ratios (T_6) as compared to other groups. Storage of meat results into significant ($P<0.01$) reduction in the WHC of meat in all the groups irrespective of treatment. The TBA value of fresh meat was significantly ($P<0.05$) lower in group fed diet containing crude soy lecithin (T_2) as compared to T_6 (SAO 75:25 CSL). Storage of

meat leads to significant increase in the malonaldehyde concentration (TBA value) in meat in all the groups, however difference was less in group fed CSL. There was significant increase in the tyrosine value of stored meat as compared to fresh meat in all the groups irrespective of treatment. The total bacterial count (TBC) in fresh as well as stored meat was significantly ($P<0.01$) lower in control group as compared to all treatment groups except T_5.There was significant ($P<0.01$) increase in the total bacterial count of stored meat as compared to fresh meat in all the groups irrespective of treatment. Addition of soy acid oil increases the total lipid content in fresh meat. Storage of meat result into significant reduction in the lipid content in all the groups irrespective of treatment. Based on the above findings it can be concluded that SAO and CSL alone or in different combination does not influence the proximate composition *viz.* moisture, total protein, ether extract and total ash and physico-chemical properties *viz.* pH and ERV of meat in fresh as well as after storage period of 30 days. Soy acid oil alone or in higher proportion along with crude soy lecithin decreases the water holding capacity of fresh meat. Storage of meat result into decrease in total protein, ether extract, total ash, pH and water holding capacity and increased tyrosine value, TBA value and total bacterial count irrespective of treatment.

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