REVIEW OF LITERATURE

The literature reviewed during this study focused on the utilization of iron (Fe) and citric acid (CA) as supplements that influenced the performance of broilers. Previous work reviewed is presented below under the following headings:

- Iron as a supplement in broiler diets.
- Citric acid as a supplement in broiler diets

2.1 Iron as a supplement in broiler diets

McNaughton and Day (1978) determined the interrelationship between Fe and Cu requirements of 1 to 21 day old broiler chicks. The heaviest 21 day old chicks were found by feeding 80 ppm dietary Fe. A 10:1 dietary Fe to Cu ratio with an Fe requirement of 80 ppm appeared to maximize hemoglobin levels, whereas 5:1 ratio Fe to Cu maximized 21 day old chick weights. Iron requirements for hematological and growth responses are essentially critical.

Phual and Hutagalung (1980) assessed the effects of supplemental levels of zinc in combination with iron. Inclusion of increasing amounts of zinc in combination with iron or copper had inconsistent effects on fat and protein content of the carcass. Raising the zinc content of the diet resulted in reducing copper retention in the liver, regardless of its combination with iron and copper. On performance, incorporation of graded levels of zinc and iron in diet did not significantly affect daily gain, feed intake or feed conversion ratio; nor was there significant zinc x iron interaction for these criteria. For body composition, increased dietary intake of iron significantly reduced the carcass protein content in a linear trend.

Southern and Baker (1982) investigated the effects of duodenal coccidiosis infection on iron status of chicks fed iron levels ranging from deficient to excess. Excess supplementation of iron (500 – 1500 mg/kg) did not affect performance of uninfected chicks, but 1500 mg/kg iron exacerbated the coccidiosis-induced growth depression. Dietary iron increased intestinal iron content, but the increase was partially ameliorated.
by the coccidial infection. Performance and hematological parameters were maximized by approximately 0.9 mg iron per day in both control and infected chicks.

Fajmonova et al., (2004) studied the effect of age upon iron retention. The dependence of Fe content in weight gains on age was highly significant. The growth rate of total amount of Fe in the body was by 6% lower than that of live weight of chickens.

Bao et al. (2006) conducted an experiment to study the effect of three levels of Fe (20, 40 80 mg/kg), among other minerals on the broiler performance and tissue trace mineral contents. The diets organically supplemented with 40 mg/kg Fe achieved a superior feed conversion and lower mineral excretion than the inorganic control. The other trace minerals had similar effects at specific levels. So organically complexed Cu, Fe, Mn and Zn appeared to meet broiler requirements at lower levels than inorganic supplements and did not compromise broiler growth.

Fairchild et al. (2006) conducted trials to evaluate the effects of Fe concentration on broiler performance. No differences were noted in body weight, feed consumption, water consumption, mortality, or manure-soluble P in any of the trials. None of the birds in the treatments exhibited any signs of flushing or any other intestinal problems. These results indicate that Fe concentrations in water do not affect performance or soluble P in broilers. Broiler performance was not affected by elevated SO₄ levels or reduced water pH.

Oguz et al. (2006) determined the effects of iron sulphate supplementation of diet containing 15% cottonseed meal on the live weight gain, feed consumption, feed efficiency and some haematological parameters of broilers. At the end of the 4 weeks experiment, mean live body weights in Fe group was significantly greater than that of the control group. The values of feed conversion ratio of control and Fe groups were 2.72 and 2.40, respectively. Although heamatocrit and heamoglobin levels were not significant, mean corpuscular heamoglobin concentration were significantly different. Consequently, iron sulphate supplementation has a significant effect on live body weights for diet containing cottonseed meal.
Boa et al. (2007) included diets with supplemental trace minerals from organic and inorganic sources based on a trace mineral deficient control diet to examine the possible responses of broiler chickens to organic mineral supplements. The results showed that supplementation with 4mg of Cu and 40mg each of Fe, Mn and Zn from organic sources may be sufficient for normal broiler growth of chicks up to 29 days old. It was possible to use these lower levels of organic trace minerals in broiler diets to avoid high levels of trace mineral excretion.

Seo et al. (2008) compared the effects of duration and level of iron-methionine (Fe-Met) chelate supplementation on the iron, copper and zinc content of broiler meat. Production performance was not significantly affected by treatments. It was concluded that iron content of broiler meat can be effectively enriched by supplementation of 200 ppm of Fe as Fe-Met for 5 weeks.

Seo et al. (2008) compared the effects of supplementary iron source and levels on the iron content of broiler meat. There were no significant differences among treatments in parameters related to production performance. Liver contained approximately 10 times more iron than the leg muscle which contained approximately 3 times more iron than either breast muscle or wing muscle. Significant differences in iron content in the broiler meat were observed. It was concluded that iron-methionine (Fe-Met) chelate is more efficient than iron sulfate and 200 ppm iron supplementation as Fe-Met is recommended for maximum iron enrichment in broiler meat.

Svetlana et al. (2008) investigated the influence of organic and inorganic Fe supplementation on immune response and quantity of iron in organs of broiler chickens. Addition of organic iron supplements resulted in increased erythrocyte count, hemoglobin concentration and hematocrite value. Different iron forms did not change the concentration of non-heme iron in the liver.

Petrovic et al. (2010) studied the effects of dietary supplementation of different doses and forms of Cu, Zn, Fe, Mn and Se on the growth performance and concentration of
these elements in the breast and thigh muscle of broiler chickens. The diet supplemented with the restricted doses of trace elements in proteinated forms (50% Cu, 20% Fe, Zn, Mn and a regular level of Se) had the same effect on the indices of growth performance, such as, body weight, total feed intake, feed conversion ratio, carcass yield and abdominal fat of chickens, as well as on the concentration of these elements in the various body parts as did the diets with the recommended doses of minerals in the form of inorganic salts.

**Ramadan et al. (2010)** studied the effect of iron, copper and zinc supplementation on egg iron concentration and performance. Iron supplementation either at the level of 100 or 200 mg/kg improved feed conversion. The best value of feed conversion was detected for those fed 100 mg Fe/kg and Zn supplementation. Iron addition with Cu or/and Zn improved the economical efficiency as compared to the control.

**Yang et al. (2011)** evaluated the effects of additional supplementation of the diet with Cu, Fe, Zn and Mn on the performance, meat quality, and immune responses of broiler chickens fed on corn-soya based diet. The addition of these elements to the diets did not improve the growth performance of broilers, but it could influence the general meat quality by affecting the lightness and yellowness values, as well as the water-holding capacity of certain carcass parts.

### 2.2 Citric acid as a supplement in broiler diets

**Zyla et al. (2000)** determined the cumulative effects of citric acid among enzymes and other minerals on feed intake, body weight gain, feed conversion, intestinal viscosity and toe ash of broilers fed on wheat-based diets. The supplementation of the diet having 0.17% available P with the enzymes and citric acid, significantly improved body weight gain, feed intake, feed conversion and intestinal viscosity above the diet with 0.41% available P.

**Boling-Frankenback et al. (2001)** evaluated the effects of citric acid on Ca and P utilization for chicks fed on corn-soybean diet. The results indicated that citric acid did
not significantly affect the Ca requirement. However, citric acid at 4 and 6% produced the largest responses in growth and tibia ash. This study indicated that citric acid increased P utilization and reduced available phosphorus requirement by approximately 0.10% of the diet.

Snow et al. (2004) evaluated the effects of all combinations of citric acid and some enzymes on phytate P used as assessed by growth performance and tibia ash of chicks fed on P deficient corn-soya based diet. It was found that the levels of citric acid (3 and 4%) and 1α-(OH)D₃ were probably too high to critically allow potential additive or synergistic effects to be evaluated as was evident by the marked tibia ash and weight gain responses. However, with lower 1α-(OH) D₃, the levels of citric acid were obtained enough to critically evaluate synergistic effects. Between the citric acid and 1α-(OH)D₃ there were improvements in tibia ash, which suggested the synergism between them.

Sukria and Liebert (2004) investigated the effect of microbial phytase in combination with citric acid in the presence of different native phytase activity. The results showed feeding corn-soya diet with citric acid improved feed intake and growth, protein deposition and P deposition significantly.

Afsharmanesh and Pourreza (2005) determined the effects of citric acid, among other additives, on broiler performance and nutrient digestibility in wheat-based diet. The data showed that the additives improved feed conversion ratio and body weight in both (low and adequate P) diets. As such, citric acid, among the others, can result in a considerable reduction in the amount of excreted phosphorus and nitrogen into the environment.

Ataputtu and Nelligaswatta (2005) studied the effects of two levels of citric acid (1 and 2%) on the performance and the utilization of phosphorous and crude protein in broiler chickens fed on rice by-product based diets. Growth performance and feed conversion ration were not significantly affected by the inclusion of citric acid. Though not significant, 2% citric acid increased the feed intake and thus resulted in poor feed conversion ratio. Toe ash percentage was significantly increased by 2% citric acid.
Atapattu and Nelligaswatta (2005) studied the effects of two levels of citric acid (1 and 2%) on the performance and the utilization of phosphorous and crude protein in broiler chickens fed on rice by-produce based diets. Growth performance and feed conversion ratio were not significantly affected by the inclusion of citric acid. Though not significant, 2% citric acid increased the feed intake and thus resulted in poor feed conversion ratio, as well as, improved mineral retention.

Rafacz-Livingston et al. (2005) studied if citric acid is also effective on phytate P utilization in broiler chicks. They found that feed intake was not depressed by citric acid and in some cases feed intake was improved. They realized that citric acid improved P utilization in chicks as observed by increased tibia ash responses.

Rahmani and Speer (2005) conducted studies to compare the effects of using citric acid and natural additives on broiler performance and its relation with gut circumstances. The studies showed that micro-flora content of the intestine was dynamically changed by adding citric acid and decreased the pH of the intestine significantly, which affected broiler performance positively by acting on microbial population of the digestive system.

Rafacz-Livingston et al. (2005) conducted experiments to determine if citric acid is effective on improving phytate P utilization in commercial broiler chicks. They found that chick weight gain and tibia ash were significantly increased. The results indicated that citric acid markedly improved phytate P utilization in commercial broiler chicks.

Martinex-Amezcua et al. (2006) evaluated the effectiveness of phytase and citric acid for releasing P that is not available in distillers dried grains with soluble (DDGS). They concluded that broiler diet supplemented with phytase and citric acid increased tibia ash and it was estimated that phytase and citric acid could release from 0.04 to 0.07% P from DDGS. In terms of bioavailability, P in DDGS was increased from 62 to 72%. These results indicated that phytase and citric acid increased the bioavailability of P in DDGS.
**Moghadam et al. (2006)** investigated the effects of different levels of dietary citric acid (1.5 and 3%) on calcium and phosphorus requirements in broiler chicks and on their performance. The results showed that the effects on body weight, feed consumption, calcium and phosphorus concentrations in bone ash and plasma were significant but they had no significant effects on bone ash percentage, feed conversion efficiency and feed ratio. It suggested that citric acid plays a role in calcium and phosphorus utilization by poultry and may increase the absorption of these minerals.

**Abdel-Fattah et al. (2008)** assessed the effect of using citric acid, among other organic acids, on thyroid activity, some serum constituents, organs morphology and performance of broiler chicks. The results indicated that there were clearly improvements in performance index, economical efficiency and relative economic efficiency in acidifier groups compared with control ones. Birds fed diets containing citric acid at 1.5 and 3% levels had the best values of either economic or relative economic efficiency compared with other sources and levels of organic acids.

**Biggs and Parsons (2008)** evaluated the effects of citric, gluconic, fumaric and malic acids on growth and nutrient digestibility for growth performance in chicks. The results indicated that these organic acids did not have any consistent effects on growth performance and the other measured parameters.

**Islam et al. (2008)** investigated the effects of feeding citric acid, acetic acid and their combination to broiler chicks on their performance and to determine the economic competence of using these acids in broiler ration. The performance showed significant increase in body weight gain when compared with the control during 0 – 5 week of age on 0.5% citric acid, as well as, feed consumption increased at 2nd and 3rd weeks of age. It was concluded that use of 0.5% citric acid in the diet of broilers may have better performance in respect of live weight gain and feed conversion.
**Liem et al. (2008)** determined the effect of various organic acids, including citric acid, on phytate P utilization. They found that the addition of citric acid significantly increased the retention of P and phytate P. In addition, citric acid improved phytate P utilization.

**Ao et al. (2009)** studied the effects of simultaneous application of citric acid and α-galactosidase on nutrient digestion and growth performance of broiler chicks fed on corn-soya based diets. It was found that body weight gain and feed intake were significantly increased during the overall 21 day period and were significantly decreased by citric acid supplementation during 1 to 14 and 1 to 21 day periods. In the first 2 weeks, there was a significant enzyme and citric acid interaction on weight gain and gain to feed ratio. Significant main effects of citric acid and enzyme supplementation but not energy level were observed. Citric acid significantly decreased the pH of the crop content. The crop contents from chicks fed citric acid also contained more reducing sugars than did the crop contents from chicks fed no citric acid.

**Chowdhury et al. (2009)** determined the effect on supplementation of citric acid (0.5%) on the growth, feed efficiency, carcass yield, tibia ash and immune status of broilers. Body weight gained was significantly higher than the control, 1318g and 1094g, respectively. Total feed intake and feed conversion efficiency were higher with citric acid fed chicks when compared with the control. This supplementation significantly increased tibia ash and reduced pH, as well as improved the chicks’ immune status. It was concluded that citric acid at 0.5% had positive effects on growth, feed intake, feed efficiency, carcass yield, bone ash and immune status of broilers. Also, that citric acid is a suitable alternative to replace antibiotic growth promoters.

**Nourmohammadi et al. (2010)** investigated the effects of adding citric acid (3 and 6%) and microbial phytase (500 and 1000 IU/kg) supplementation on growth performance on broiler chickens fed on corn-soya base diets. Citric acid at 6% decreased feed intake and body weight gain but improved feed conversion ratio. They concluded that depression of performance was differently affected by citric acid levels.
Tollba (2010) determined the effect of citric acid to reduce or control the prevalence of pathogenic bacteria and parasite in the intestine of broiler chicks reared under heat stress and normal conditions. His results showed that citric acid had statistical effects regarding the decrease in the counts of pathogenic intestinal bacteria and parasite under normal temperature or high temperature as compared to the control. In addition, there was significant improvement in body weight gain, mortality rate, feed consumption, feed conversion, and carcass characteristics of the broilers.

Wayengo et al. (2010) determined the effect of supplementing a corn-soyabean based diet with phytase alone or in combination with citric acid on growth performance, nutrient utilization and bone mineralization. Results showed that the addition of citric acid to a phytase supplemented diet further increased P digestibility. While phytase improved the kcal/kg from 2959 to 3068, there was a tendency to increase further with the addition of citric acid to 3150.

Abas et al. (2011) investigated the effects of the addition of citric acid, zeolite, or both to broiler diets with microbial phytase containing low and adequate levels of P on performance, among other parameters. It was determined that the difference in growth performance was dependent on the level of P and the addition of citric acid and zeolite mixture to the diet. Citric acid alone did not have an effect on feed consumption or body weight.

Ali et al. (2011) examined the ability of citric acid (0.2%) to increase nitrogen retention and utilization of the low protein low energy (LPLE) diet. At the end of the grower period, the addition of citric acid improved weight gain and feed conversion by 9.89 and 11.01%, compared with LPLE. In general, it seemed that addition of citric acid to LPLE diet recorded the better feed conversion ration.

Arjona-Roman et al. (2011) investigated the effect of citric acid (12.5, 25 and 50g/kg) on certain thermal and pasting properties of poultry ration. The results showed that acidity level affected the energy requirements for swelling and gelatinization processes.
Enthalpy, heat capacity, and initial and maximal gelatinization temperature decreased as citric acid concentration increased in the ration. Moreover, the degree and rate of conversion for starch gelatinization was faster as the citric acid concentration increased and also, significantly affected the temperature at initial viscosity increase. They concluded that citric acid can be used to improve feed efficiency, poultry health, as well as, to promote growth performance in young broiler chickens.

Deepa et al. (2011) studied the effect of phytase and citric acid on the broiler growth performance, phosphorus, calcium and nitrogen retention. They found that the weight gain of the chicks was significantly higher for the chicks that received phytase plus citric acid supplementation. Feed intake and feed conversion ratio were significantly higher for those that received both phytase and citric acid. These supplements individually and in combination significantly improved phosphorus, calcium and nitrogen retention.

Esmaeilipour et al. (2011) studied the effects of xylanase and citric acid (20 and 40 g/kg) on the performance, nutrient retention, among other parameters on broiler chicks fed on low P wheat based diet. No interaction effect was observed between xylanase and citric acid in any measured response. The inclusion of 40 g/kg of citric acid decreased body weight gained and feed intake by 8.6 and 12.5%, respectively. The study showed that citric acid increased P and decreased pH of crop contents. It was concluded that adding 20 g/kg of citric acid, especially in the starter period, and 200 mg/kg of xylanase to low P wheat-based diets can be helpful.

Ghazalah et al. (2011) conducted experiments to study the effects of different levels of citric acid (1, 2 and 3%). They found that citric acid significantly increased body weight gained and the European Production Efficiency Index as compared with the control. Feed conversion ratio was also significantly improved with citric acid supplementation. They concluded that 2% citric acid could be used safely to improve performance and health of broiler chicks.
Nezhad et al. (2011) studied the combined effects of citric acid (2.5 and 5%) and microbial phytase on digestibility of Ca, P and mineralization parameters of tibia bone in broiler chickens. The results showed that interaction effects of citric acid and phytase on tibia Ca content with low available P diets was significant. Adding citric acid to P deficient diet increased tibia P content when compared to the control, as well as, significantly increased the digestibility of P.

Nourmohammadi et al. (2011) studied the effect of microbial phytase supplementation and citric acid on thyroid activity, relative weight of lymphoid organs and pH values of segments of the gastro-intestinal tract in broiler chickens fed on corn-soybean based diets. The addition of citric acid caused significant decrease in pH values in the gastro-intestinal track segments studied and caused significant increase thyroid activity, as well as, relative weight of lymphoid organs. It revealed that broiler chicks fed on acidifiers diets had better immune response resistance that lead to immunological advances. Also, that the decreased pH in the gastro-intestinal tract by citric acid caused a beneficial effect in the inhibition of intestinal bacteria competition.

Nourmohammadi et al. (2011) studied the effect of microbial phytase supplementation and citric acid on thyroid activity, relative weight of lymphoid organs and pH values with the gastro-intestinal tract in broilers. They found that addition of citric acid (3 and 6%) caused significant decrease in pH values and caused significant increase on thyroid activity.

Nourmohammadi et al. (2011) determined the effect of microbial phytase supplementation and citric acid (3 and 6%) in broiler chicks fed on corn-soya base diets on enzyme activity, among other parameters. The results indicated that addition of citric acid to diets caused significant decrease in alkaline to the enzyme activities, as well as, cholesterol and iron concentrations. They concluded that the enzyme with citric acid could modify some serum enzyme activities and increase the availability and use of minerals for growth and performance improvement.
Ebrahimnezhad et al. (2012) evaluated the combined effects of citric acid and microbial phytase on the serum concentration and digestibility of some minerals in broiler chicks. It was found that the interaction effect on concentration of copper, zinc and manganese in serum of broilers fed with low available phosphorus diets was significant. Adding 2.5% citric acid to low available phosphorus diets increased digestibility of zinc in comparison to diets without citric acid or with 5% citric acid in broiler. Adding citric acid into low available phosphorus diets increased manganese digestibility on corn-soya based diets.

Kopecky et al. (2012) evaluated the effect of citric acid (0.25%) in the drinking water on broiler performance. The results showed not significant effects on body weight. Supplementation of citric acid caused decrease in total feed consumption. However, there was positive effect on total mortality of the chicks.

Nourmohammadi et al. (2012) studied two main factors of citric acid (3 and 6%) and microbial phytase (500 and 1000 IU/kg) for their possible interaction on body weight, average daily gain, average daily feed intake and feed conversion ration. The results indicated that diets containing 3% citric acid caused significant increase in P content in tibia ash, ileal digestibility of crude protein, apparent metabolizable energy total phosphorus and body weight. Addition of 6% citric acid, when compared with diets of 0 and 3%, significantly decreased body weight, average daily gain, average daily feed intake, Ca content in tibia ash, ileal digestibility of crude protein, apparent metabolizable energy and total phosphorus.

Saki et al. (2012) compared the influence of antibiotic, citric acid and herbal additives on broiler chickens. Their results showed that citric acid as an additive contributed to significantly higher feed intake as compared with the control during the starter (0 to 21 days) period. Significantly increased body weight gain was obtained by supplementation of each of the additives during the grower period. In addition, better feed conversion ratio was observed for all treatments compared to the control in the starter period. Overall, supplementation of citric acid, among the other additives, improved performance and morphological indices of broiler chickens.
In conclusion of this review, it can be stated that supplementation of iron and citric acid to the diets of broiler chicks may or may not influence their performance. It is important to ascertain the amounts to be added under different growing conditions and base diet nutrition, as well as their effects in combination with other minerals or elements. It is established that their interaction with other minerals or elements can be antagonistic or synergistic. As such, this experiment was timely conducted to evaluate the performance of broilers while using iron and citric acid as diet supplements.