EFFECT OF DIFFERENT DIETARY AMINO ACIDS AND ZINC AMINO ACID CHELATES ON THE PERFORMANCE, GUT MORPHOLOGY, IMMUNE RESPONSE AND MEAT QUALITY OF BROILER CHICKS

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ABSTRACT

The impact of different dietary amino acids and chelated zinc amino acid on the performance, gut morphology, immune response and meat quality of broiler chicks was studied in five experiments. Two experiments were conducted to evaluate the effect of supplemental effects of Arg, Gln and Trp on growth performance, gut morphology, immune response and meat quality of broiler chicks. In Exp 1, birds were allotted to 4 dietary treatments (6 replicates per treatment and 6 birds per cage), C (control); Arg (a diet with 0.5% L-Arg); Gln (a diet with 0.5% L-Gln); and Trp (a diet with 0.5% L-Trp). In Exp 2, 312 1-d-old broiler chicks (Ross×Ross 308) were weighed and randomly assigned to similar treatments as of Exp 1. In a 42 d experiment, chicks had an ad libitum access to basal starter (d 0 to 14), grower (d 15-28) and finisher diet (d 29 to 42) based on corn and soybean meal. Body weight and feed intake were measured at the end of each phase. On d 11, 2 birds from each cage were sacrificed to measure gut morphology and circulating level of serum immunoglobulins (IgG and IgA). In both experiments, 2 birds from each pen were sacrificed at 6 weeks of age to measure carcass trait and meat quality. In Exp 1, results showed that dietary Arg, Gln and Trp supplementation significantly increased ($P < 0.05$) the BW gain of broilers during 0 to 42 d. Feed intake and FCR were also favorably influenced by the supplementation of AA in the diet ($P < 0.05$). Warm and chilled carcass weight was significantly increased by the AA supplementation compared to those of control group. Consequently, weights of breast and thigh meat in Arg, Gln and Trp groups were higher ($P < 0.05$) than those of C group. Breast and thigh meat in C group had lower pH, a* value and had higher L* and b* value than those of AA

supplementation group. No significant difference was observed among the AA treatments. Morphometric analysis showed increased ($P < 0.05$) villus height and villus to crypt ratio and decreased crypt depth in chickens treated with AA groups. Improved gut morphology and growth performance was very well supported by the Exp 2. Significant increased weight gain, feed intake and FCR in all phases were also observed among the same groups, suggesting beneficial effects of AA supplementation into the diet. Dietary supplementation with 0.5% Arg, Trp or Gln improved meat quality of broiler chicks. In addition, supplementation of AA into the basal diets did not influence serum IgA and IgG content. As a result, dietary supplementation of Arg, Trp or Gln at 0.5% would enhance growth performance, gut morphology, meat and carcass quality of broiler chicks.

In the chelated zinc amino acid feeding experiment, three trials were conducted consecutively to evaluate bioavailability of Zn from various Zn amino acid chelates and their tissue accretion until 3 weeks of age. Each experiment used 447 broiler chicks and fed experimental diets for 21 d. In Exp 1, dietary treatments were NC, negative control diet without Zn supplement; PC, positive control diet with 40 mg Zn/kg from Zn-sulfate; Zn-gly, NC diet + 40 mg Zn/kg from Zn glycine; Zn-met-gly, NC diet + 40 mg Zn/kg from Zn methionyl-glycine. In Exp 2, dietary treatments were CON, a basal diet with 40 mg Zn/kg from Zn-nitrate; Zn-gly1, a basal diet with 40 mg Zn/kg from Zn glycine; Zn-gly2, a basal diet with 40 mg Zn/kg from Zn glycinyl-glycine; and Zn-arg, a basal diet with 40 mg Zn/kg from Zn arginine. In Exp 3, dietary treatments were ZS, a basal diet with 60 mg Zn/kg from Zn-sulfate; ZA1, a basal diet with 60 mg Zn/kg from Zn-sulfate and Zn-methionyl-glycine at 2:1 ratio; ZA2, a basal diet with 60 mg Zn/kg from Zn-sulfate and Zn-methionyl-glycine at 1:2 ratio; and ZAA, a basal diet with 60 mg Zn/kg

from Zn-methionyl-glycine. Growth performance was not affected by dietary Zn sources in all experiments. In Exp 1, birds fed diets with inorganic Zn or Zn-AA had higher ($P < 0.05$) Zn content in whole body than those fed a diet without Zn supplementation on d 5, 7, 14 and 21. Zn-met-gly group had a higher ($P < 0.05$) Zn bioavailability than PC group during d 7-14. From d 0 to 14, Zn bioavailability of chicks fed Zn-met-gly diet was higher ($P < 0.05$) than that of chicks fed PC diet. During the entire 21-d, Zn bioavailability of chicks fed Zn-AA diets was higher than that of chicks fed PC diet ($P < 0.05$). In Exp 2, whole body Zn contents were higher ($P < 0.05$) in birds fed Zn-gly2 diet than in those fed CON or Zn-gly1 diets at d 1. At d 7, Zn contents of the birds fed Zn-gly1 diet were increased ($P < 0.05$) compared to the birds fed CON diet. At the final day of experiment, birds fed Zn-Arg diet had higher ($P < 0.05$) Zn content in whole body than those fed other diets. In Exp 3, birds fed ZS or ZAA diets ingested more Zn ($P < 0.05$) than those fed ZA1 or ZA2 diets from d 0 to 7. Also, birds fed ZS diet ingested more Zn ($P < 0.05$) than those fed ZA1 or ZA2 diets for d 0-14. Zn retention ratio for d 0 to 1 was higher ($P<0.05$) in chicks fed ZA1 diet than in those fed ZAA diet. Also, chicks fed ZA2 diet had the highest ($P < 0.05$) Zn retention ratio for d 0 to 3 compared to those fed ZAA or ZS diets. The ZAA had a greater ($P < 0.05$) Zn bioavailability than ZS and ZA1 during d 7-14. Also the ZAA had a greater ($P < 0.05$) Zn bioavailability than other treatment groups from d 0 to 14. In conclusion, this study shows that Zn from Zn AA chelates is more bioavailable than Zn from inorganic source by broiler chickens during the starter period (d 0 to 14) when Zn was supplemented at 40 mg/kg to a basal diet containing 28 mg endogenous Zn/kg.

The results of the present study indicate that dietary supplementation of L-Arg, L-Trp and L-Gln at 0.5% would enhance growth performance, gut morphology, meat and carcass quality of broiler chicks. On the other hand, supplementation of Zn from Zn amino acid chelates would reduce dietary Zn supplementation levels to meet the Zn requirement especially for young broiler chicks.

(Key words: Amino Acid, Tryptophan, Arginine, Glutamine, Chelated Zinc, Performance, Meat Quality, Intestinal Morphology and Immune Response, Broiler Chicks)

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LIST OF ABBREVIATIONS

a*	Redness
AA	Amino acid
ADFI	Average daily feed intake
ADG	Average daily gain
Arg	L-arginine
b*	Yellowness
BW	Body weight
CON	Control
D	Day
DM	Dry matter
Exp	Experiment
FCR	Feed conversion ratio
G	Gram
GIT	Gastrointestinal tract
Gln	L-glutamine
IgA	Immunoglobulin A
IgG	Immunoglobulin G
Kg	Kilogram
L*	Lightness
ME	Metabolizable energy
mg	Milligram

mL	Milliliter
µm	Micrometer
NC	Negative control
NRC	National research council
Trp	L-tryptophan
Wk	Week
Zn	Zinc
Zn-AA	Zinc-amino acid
Zn-gly	Zinc-glycine
Zn-met-gly	Zinc-methionyl-glycine
ZS	Zinc sulphate

CHAPTER Ⅰ

LITERATURE REVIEW

Introduction

In recent years, the growth performance of modern commercial broiler strains has been continually improved with the advancement of genetic selection (Leeson 2005; Havenstein et al., 1994 and 2003) and such improvement is likely to affect the maintenance and growth needs of the birds for amino acids and trace minerals. During the past decade, it is reported that breeding companies has increased 0.55 kg/year body weight within 6 weeks (Fancher, 2006). Therefore, amino acid and trace mineral requirements of broilers suggested by the NRC (1994) might not be optimal to support the maximal growth potential of high yielding strains. Researchers have reported that feeding high-level amino acid diets throughout the life of the broiler optimizes breast meat yield and body weight, whereas decreasing the amino acid level causes reduced body weight, breast meat yield, feed conversion efficiency, and meat yields (Kidd et al., 2004; Corzo et al., 2005b; Bartov and Plavnik, 1998; Dozier et al., 2008). Additionally, excessive dietary amino acids have been shown to decrease feed consumption, which directly influences the ingested amino acids of the broiler (Skomial et al., 2002). On the other hand, considering the high cost of feed, it is important to optimize nutrition to maximize growth and meat yield of the broiler chicks (Ensminger et al., 2004). Therefore, adequate amino acid level is important for a successful feeding program. In avians, many of the classic neurotransmitters, including amino acids, have shown to affect food intake when injected directly into the central nervous system (Denbow, 1985 and 1999).

Recently, research findings indicate that a specific amino acid requirement may also need to consider some other special functions of amino acids. In particular, Gln, Trp and Arg are among the important 'bioactive amino acids', and participate in many important and diverse biochemical reactions associated with the normal physiology of the organism.

L-tryptophan (Trp) is an essential amino acid and plays a rate limiting role in protein synthesis. Trp is also a precursor of serotonin and melatonin (Voet and Voet, 1995; Emadi et al., 2010), which control of circadian rhythms and is associated with blood pressure, body temperature, feed intake, growth and repair of tissues (Henry and Seve, 1993; Corzo et al., 2005b). It has also been involved with niacin biosynthesis in chicken (Corzo et al., 2005). NRC (1994) pointed out the requirement of Trp for 1-3 week broiler chickens has been reduced from 0.23 to 0.2%. A recent report mentioned that dietary inoculation of Trp in different growth stage (starter 0.20%, grower 0.15 % and finisher 0.13%) significantly increased weight gain, feed intake, blood albumin, total protein, glucose, urea and uric acid and decreased FCR, aspartate amino-transferase, lactic dehydrogenase, triglycerides and cholesterol (Emadi et al., 2010).

L-glutamine (Gln) is a free, neutral, non-essential amino acid. Gln may be a vehicle for nitrogen exchange between tissues, and may play an essential role in several important metabolic pathways (Marliss et al., 1971; Smith, 1990). Gln is recognized as a crucial energy substrate in rapidly dividing cells, and may act on the humoral immune response, that is, in certain sites of mucosa membranes such as the respiratory and GIT's, with increase in the number of lymph nodes in mammals (Newsholme, 2001). The potential use of Gln in broiler diets has been discussed, and many benefits have been noted in different studies (Murakami et al., 2007; Yi et al., 2005; Bartell and Batal, 2007;

Sakamoto et al., 2006). According to Wu (2009), Tapiero et al. (2002) and Newsholm et al. (2003a and 2003b), Gln is related to the development of GIT of broiler chickens. Glutamate was previously demonstrated to be an endogenous agent involved in the neural control of food intake and body weight in mammals (Zeni et al., 2000; Bisaga et al., 2008). Yi et al. (2005) observed the importance of Gln supplementation (1%) in the diet of broilers up to 28 days of age, which presented better performance (weight gain, feed efficiency, and viability) than the non-supplemented birds. However, these results are not in agreement with the study of Maiorka et al., (2000), who did not find any effect of Gln on the performance of broilers supplemented at the same levels and during the same period.

L-arginine (Arg), a dibasic amino acid, (Boorman & Lewis, 1971) is essential to birds. It is also essential for optimal growth and nitrogen balance in growing animals (Borman et al., 1946; Milner et al., 1974). Most mature mammals can synthesize Arg to meet their requirements. Due to the lack of some enzymes in the urea cycle, broiler chickens are unable to biosynthesize Arg from ornithine; thus, this amino acid must be supplemented in diets (Wu et al., 1995) to meet their needs for protein synthesis and other functions (Tamir and Ratner, 1963). It is one of the metabolically versatile amino acids, giving rise to nitric oxide (NO) which is recognized as one of feeding-regulatory factors in the brain of mammals (Morris, 2004). It is also important for a series of biological and physiological functions including protein biosynthesis, nitrogen transport and excretion, production of polyamides and NO, stimulation of several endocrine glands (Efron and Barbul, 1998 and 2000) and immune regulatory action (Tayade et al., 2006a and 2006b; Lee et al., 2002). Based on the above observations, the objective of the

present research was, in the first instance, to investigate impact of two essential (Trp and Arg), and one non-essential (Gln) amino acids on growth performance, gut morphology, immune response and meat quality of broiler chicks and, secondly, to examine zinc bioavailability of various zinc amino acid chelates in young broiler chickens by the several experiments.

Dietary protein and amino acids in broiler chicks

Proteins have been described as complex organic compounds of high molecular weight composed of 22 different amino acids and derivatives that were linked by peptide bonds to form a primary chain structure. It was realized that chickens require the essential amino acids plus some other amount of non-essential amino acids to synthesize protein at acceptable rates. Therefore, it was clear that chickens require not only the essential amino acids but also some other amino acids, which have been referred to as the "non-essential" amino acids. Clearly, some quantity of these non-essential amino acids is seriously considered for maximal growth. The sum of the essential and nonessential amino acids may also be referred to as the CP requirement. NRC (1994) sets the CP requirement for 0-3 wk-old broilers at 23%. It was recognized that the amino acid requirements of the birds are proportional to the CP content of the diet (NRC, 1994 and Almquist, 1947). Because protein synthesis at the point of mRNA translation has been shown to require all 22 amino acids, these must all be considered physiologically essential to the animals (Bedford and Summers, 1985; NRC, 1994). Poultry have been shown to be able to synthesize only 12 among the 22 amino acids required for protein synthesis (D'Mello, 1994). Amino acids that cannot be synthesized by an animal have been termed "indispensable" or "essential" amino acids, while those that an animal can synthesize

have been termed “dispensable” or “non-essential” amino acids (D’Mello, 1994; Leeson and Summers, 2001). Therefore, these essential amino acids must be met entirely by dietary means, while the dietary content of non-essential amino acids need not accurately reflect the exact requirement.

However, the distinction between essential amino acids and non-essential amino acids may, in some instances, be dependent upon the concentration of other amino acids in the diets as well as the variable responses used to determine the degree of adequacy. This has led to a third classification of amino acids termed “conditionally indispensable” or “conditionally essential” amino acids which under specific conditions such as rapid early growth, the rate of amino acid synthesis may not be able to meet the requirements of the broiler. Therefore, by definition, this must lead to the conclusion that under specific conditions at least part of the requirement for these amino acids must be met by dietary sources (Leeson and Summers, 2001). In certain cases young and fast growing poultry may not be able to synthesize sufficient quantities of these amino acids to achieve maximal BW gain (Leeson and Summers, 2001). While individual non-essential amino acids may be removed from a diet without deleterious effects on performance, there has been data to suggest that poultry have a requirement for non-essential amino acids proportional to the dietary essential amino acid concentration. In support of this, Bedford and Summers (1985) showed a significant quadratic effect of the ratio of essential amino acids to non-essential amino acids on BW gain of broiler chickens grown to 21 d of age with the optimum ratio of essential:non-essential amino acids determined to be 55:45. An important aspect of this study was that the same ratio of essential:non-essential amino acid treatments were repeated across three different levels of dietary CP ranging from

14.0 to 22.0%, while in each instance, the essential amino acid requirement was calculated as a fixed percentage of the dietary CP level. Based on the levels of CP employed, the degree of adequacy of treatments in meeting the calculated requirement ranged from 79% to 147% as the ratio of essential amino acids to non-essential amino acids was varied from 35:65 to 65:35, respectively.

Functional amino acids

Nutritional studies have shown that dietary supplementation with several AAs (e.g., arginine, glutamine, glutamate, leucine, and proline) modulate gene expression and enhance growth of the small intestine and skeletal muscle (Geng et al., 2011; Jobgen et al., 2009; Wang et al., 2008; Wu et al., 2011a and 2011b; Yao et al., 2008; Yin et al., 2010). Wu (2010) proposed the new concept of functional AA, which is defined as those AAs that participate in and regulate key metabolic pathways to improve health, survival, growth, development and reproduction of the organisms. Metabolic pathways include: (1) intracellular protein turnover (synthesis and degradation) and associated events (Bertrand et al., 2013; Kong et al., 2012; Wauson et al., 2013; Xi et al., 2011 and 2012; Yao et al., 2012), (2) AA synthesis and catabolism (Brosnan and Brosnan, 2012; Lei et al., 2012a and 2012b), (3) generation of small peptides, nitrogenous metabolites, and sulfur-containing substances, e.g., H_2S (Mimoun et al., 2012), (4) urea cycle and uric acid synthesis (Wu, 2013), (5) lipid and glucose metabolism (Dai et al., 2013; Go et al., 2012; Satterfield et al., 2013), (6) one-carbon unit metabolism (Wang et al., 2012), and (7) cellular redox signaling (Hou et al., 2012a). Functional AA can be nutritionally ‘‘essential’’, ‘‘nonessential’’, or conditionally essential AA. It is noteworthy that the concept of functional AA takes into consideration, the animal’s metabolic needs for

dietary AA beyond serving as the building blocks for proteins, large peptides, and small peptides.

The ideal amino acid concept in broiler chicks

The amino acid requirements of broilers, expressed either as a percentage of the diet or as a minimum daily requirement, can be expected to vary considerably as a result of differences in physiological, dietary, environmental, and genetic factors, thereby making an accurate estimate of broiler nutritional requirements for all amino acids under all combinations of conditions and genotypes almost impossible to determine (D'Mello, 1994; Mack et al., 1999; Baker et al., 2002). However, it has been generally accepted that the dietary requirement for each of the essential amino acids can be expressed as a fixed proportion of the requirement for the other essential amino acids (Schutte and de Jong, 1998). Therefore, in an effort to simplify the determination of the amino acid requirements, research efforts in poultry have been directed at determining the requirements of a single reference amino acid under a range of different conditions with the assumption that the dietary requirement of all other amino acids can be expected to change in a fixed proportion to a reference amino acid. Therefore, by definition, the "ideal ratio" of amino acids refers to the blend of essential amino acids in a diet known to satisfy, but not exceed the amino acid requirements for protein accretion and maintenance of an animal, with no deficiencies or excesses (Emmert and Baker, 1997). The advantage of applying such an ideal ratio of amino acids in meeting the amino acid requirements of poultry was that once this ideal ratio had been established for a certain age or period. Researches determining the amino acid requirements under a variety of different conditions need only focus on a single reference amino acid, with the requirement for the

remaining amino acids being calculated relative to the reference amino acid. The important assumption of such an approach was that the requirement for the reference amino acid, as determined using the respective test diet, would not change once an ideal amino acid profile was applied in practical feed formulation. From the outset, most of the ideal amino acid ratios in poultry were specified on a digestible amino acid basis, since differences in amino acid digestibility between ingredients could lead to changes in the ratios of absorbed amino acids relative to the ratio of total amino acids in the diet (Emmert and Baker, 1997). However, in contrast, the NRC (1994) listed the amino acid requirements for broilers in terms of total amino acids without providing digestible ideal amino acid ratios separately.

Amino acid imbalance on broiler performance

Unbalanced dietary protein was defined by Harper (1970) as protein with a single amino acid deficiency, while a protein imbalance refers to the adverse effect of an amino acid deficiency that was the direct result of supplementation of amino acids other than the first limiting amino acid. In accordance with the definition of an amino acid imbalance, supplementation of excess dietary threonine to a diet marginal in tryptophan was shown to cause a severe depression in growth that was alleviated by the supplementation of Trp in a dose dependent manner to the degree of threonine excess (D'Mello and Lewis, 1970). The authors further demonstrated a linear relationship between the Trp requirement of chicks and threonine concentrations over a range from 0.8 to 2.3% dietary threonine. Sugahara and Kubo (1992) showed that a single amino acid deficiency resulting a reduced feed intake and lower BW gain. The effects of an amino acid deficiency on BW gain, however, were not attributable only to feed intake but were also due to an amino

acid imbalance independent of feed intake. Nonetheless, a reduction in feed intake has been one of the primary mechanisms whereby an amino acid imbalance or absence of one essential amino acid was shown to negatively affect performance (Harper et al., 1970). Burnham et al. (1992) attributed the decreased feed intake to a combination of a reduced growth rate that resulted from a severe amino acid deficiency as well as possible imbalances between the limiting amino acid and ME concentrations. However, under conditions of marginal amino acid deficiencies and unbalanced protein, researchers have shown that birds over-consumed feed in an attempt to satisfy their essential amino acid requirements (Lipstein et al., 1975; Smith and Austic, 1978; Summers et al., 1992). In particular, imbalance of Trp decreased protein synthesis, niacin biosynthesis (Corzo et al., 2005) and serotonin and melatonin secretion (Voet and Voet, 1995; Emadi et al., 2010), and imbalance of circadian rhythms is associated with blood pressure, body temperature, FI, growth, and repair of tissues (Henry and Seve, 1993; Corzo et al., 2005). Due to the lack of some enzymes in the urea cycle, broiler chickens are unable to biosynthesize arginine from ornithine; thus, this amino acid must be supplied in diets (Wu et al., 1995) to meet their needs for protein synthesis and other functions (Tamir and Ratner, 1963).

Responses of balanced AA on broiler chicks

Several studies in broilers have shown protein and amino acid accretion to increase linearly with either dietary CP or amino acid intake up to the point of maximal protein retention and BW gain (Velu et al., 1971; Chung and Baker, 1992; Baker et al. 1996; Edwards et al. 1997; Edwards et al., 1999). More recently substantial improvements in BW, FCR and breast meat yield of Ross 308 broilers fed high CP diets formulated to contain an ideal balance of all amino acids were also shown by Kidd et al.

(2005). A study showed differential responses between male and female broilers that demonstrated optimum BW and FCR by a feeding of high CP diets to 33 and 35 d for male and female broilers, respectively. Feed intake is one of the important factors which influence broiler growth performance and carcass characteristics. The original ideal protein hypothesis is based on the balance between amino acids and their final products. However, today's research findings indicate that the specific amino acid requirement may also need to consider some other special functions of amino acids. Two attractive examples for livestock and poultry production are: first, whether some amino acids control feed intake; and second, whether amino acid balance of feed depends on the specific requirements of antibodies. Trp is the precursor of serotonin (Voet and Voet, 1995), which plays an important role in feed intake (Henry and Seve, 1993). NRC pointed out the requirement of Trp for 1-3 week broiler chickens has been reduced from 0.23 to 0.2%.

AA and gut morphology of broiler chicks

The avian intestinal tract is a multilayered tube containing a serosal layer, a longitudinal muscular layer, a circular muscle layer, a submucosal layer, and a mucosal layer (Turk, 1982). Absorption takes place primarily through the mucosa of the small intestine. Most of the digestion of the bird's intestinal tract occurs in the lumen of the intestine under the influence of the digestive enzymes secreted by the pancreas and intestinal wall and the bile secreted by the liver. Digestion of sugars and peptides, however, takes place within the brush board by the enterocytes, facilitated by membrane-bound enzymes (Turk, 1982). The interior surface of the intestine is folded into many structures called villi (Romanoff, 1960), which greatly increases its absorptive surface

area. Between the villi are the crypts of Lieberkuhn, in which crypt cells proliferate and then migrate up to the tip of villus (Turk, 1982). These cells have a life cycle of 48 to 96 hours under normal conditions (Imondi and Bird, 1966; Cook and Bird, 1973; Fernando and McCraw, 1973; Turk, 1982; Moran, 1982). As the crypt cells move up to the tip of villus, they differentiate into principal (absorptive) or goblet (secretory) cells. The absorptive epithelial cells are most abundant along the length of the villi, and goblet cells are intermittently dispersed.

After hatching, the birds start to have exogenous diets. This change necessitates an adaptation period for the GIT of the birds, because the GIT undergoes a posthatch maturation process that can significantly affect performance, mainly in the first 2 wk posthatch, which represents approximately 30% of the useful life of the bird. The GIT of the birds possesses the functions of feed content storage, secretion, digestion, and absorption of nutrients. The small intestine (duodenum, jejunum and ileum) has a primordial function in the processes of digestion and nutrient absorption. The structure and the function of the intestinal mucosa, which has the highest turnover rate of all the tissues of the body, depend on the balance among proliferation, cell migration, and apoptosis. Some nutrients are essential for intestinal homeostasis. According to Ruemmele et al. (1999), the lack of Gln and polyamines inhibits proliferation, migration, and apoptosis. In addition, Gln is an essential substrate in the construction of the passive barrier of mucin to bacteria because it is necessary for the synthesis of nitrogen bases and amino sugars of the extracellular matrix, N-acetylglucosamine and N-acetylgalactosamine, and for the glycosylation of mucins (Reeds and Burrin, 2001). Therefore, Coates et al., (1954), Izat et al., (1989) and Yi et al., (2001) indicated that

increased villi height has been proposed to increase performance by improving nutrient absorption. It also stimulates gut mucosal proliferation in rats (Inoue et al., 1993).

Tryptophan (Trp)

Trp metabolism

Trp (Fig. 1.1) is an essential amino acid in poultry and is required for a wide variety of metabolic activity.

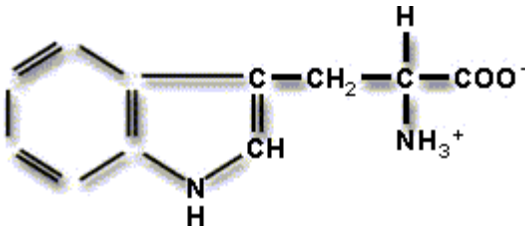

Figure 1.1 Structure of tryptophan

Trp is involved in many pathways that yield various end products. A small proportion of Trp serves as the precursor for the neurotransmitter and vasoconstrictor, serotonin. Serotonin is responsible for smooth muscle contraction as well as affecting various other physiological functions. Production of serotonin occurs in the brain stem (1-2% of the total body serotonin) and in serotonergic nerves, enterochromaffinic cells, thrombocytes and mast cells. It is also widely distributed in the hypothalamus (Guyton and Hall, 1996). Serotonin is produced by the hydroxylation of Trp (by tryptophan hydroxylase) in position 5 of the indol-ring, resulting in the formation of 5-hydroxytryptophan, which is then converted by decarboxylation to 5- hydroxytryptamine (or serotonin). Subsequent metabolism of serotonin results in the formation of melatonin, a neurohormone in the pineal body. Quantitatively, the most important pathway for Trp metabolism, after protein synthesis, is the kynurenine pathway (via tryptophan 2,3-dioxygenase), which is responsible for over 90% of Trp catabolism. Metabolites from this

pathway include: nicotinic acid ribonucleotide (where ≈60 mg of Trp are equivalent to 1 mg of nicotinic acid), picolinic acid, tryptamine, anthranilic acid, kynurenic acid, and acetyl-CoA. Trp originating from food proteins and from endogenous sources is degraded to indole, skatol, indolacetate, indolpyruvate, and indican when subjected to microbial degradation in the large bowel. Lastly, serotonin can be metabolized to 5-hydroxyindole acetic acid by the enzyme monoamine oxidase, which is excreted in the urine. Normally however, this pathway excretes only about 1% of the ingested Trp (Heine et al., 1995; Sainio et al., 1996).

Role of Trp on broiler chicken

Trp is an essential amino acid in poultry and is required for a wide variety of metabolic activities. Because its concentration in organisms is among the lowest of all amino acids, it can easily play a rate-limiting role in protein synthesis. Apart from being a structural component of all proteins it is a precursor for synthesis of two hormones, serotonin and melatonin. These hormones generally act in the classic check and balance mode with serotonin predominating during periods of activity (usually daylight) and melatonin predominating during periods of rest (usually nighttime). In chickens, the adaptive control of circadian rhythums by serotonin and melatonin is well known particularly that related to rhythms associated with blood pressure, body temperature, feed intake, growth and repair of tissues. In addition to all aforementioned, Trp has also been linked with niacin biosynthesis in chickens (Corzo et al., 2005a). More recently it was demonstrated that Trp can affect lipid levels in chickens (Akiba et al., 1988; Rogers and Pesti, 1990b). The Trp requirement to minimize lipid levels is almost double that to maximize body growth of broilers (Rogers and Pesti, 1990). Optimal dietary Trp for male

chicken has been reported at a wide range from 1.4 g kg^{-1} (Shan et al., 2003), 1.6 g kg^{-1} (Rosa et al., 2001), 1.9 g kg^{-1} (Steinhart and Kirchgessner, 1984) to 2.2 g kg^{-1} (Han et al., 1991). The quantification of Trp may allow for a reduction in dietary protein and thus, nitrogen excretion. Rogers and Pesti (1990b) conducted three experiments to determine how dietary protein and Trp influence the lipid metabolism of growing broiler chicks. Gain was maximized when the dietary levels of Trp were 0.83±0.03, 0.77± 0.04, 0.77 ±0.05, and 0.78±0.05% of the protein for 16, 20, 24, and 28% dietary protein, respectively. Therefore, requirement of the chicks for Trp was estimated to be 0.80±0.01% of the dietary protein for the growing chick. NRC (1994) pointed out the requirement of Trp for 1-3 week broiler chickens has been reduced from 0.23 to 0.2%. Recently, it is mentioned that dietary inoculation of Trp in different growth stage (starter 0.20%, grower 0.15 % and finisher 0.13%) significantly increased weight gain, feed intake, blood albumin, total protein, glucose, urea and uric acid and decreased FCR, aspartate amino-transferase, lactic dehydrogenase, triglycerides and cholesterol (Emadi et al., 2010). West et al. (1952) and Childs et al. (1952) found the Trp requirement for birds to be 0.19% of the diet. Fischer et al. (1955) showed that birds need diets with only 0.15% of Trp. Klain et al. (1960) and Hewitt and Lewis (1972) estimated that the ideal level is 0.17% of the diet. Woodham and Deans (1975) found that the requirement of Trp is around 0.14% of the diet. Freeman (1979) determined that requirements for males and females, from 0 to 7 d of age, are 0.24 to 0.22% of the diet. Smith and Waldroup (1988) estimated that the requirement of Trp for male broilers, from 1 to 20 d of age, is not greater than 0.16%. The level of Trp recommended for broiler chickens by the NRC (1994), from 0 to 3 wk of age, is 0.20% of the diet.

Arginine (Arg)

Arg (Fig. 1.2), a dibasic amino acid, (Boorman & Lewis, 1971) is essential for birds. It is also essential for optimal growth and nitrogen balance in growing animals (Borman et al., 1946; Milner et al., 1974).

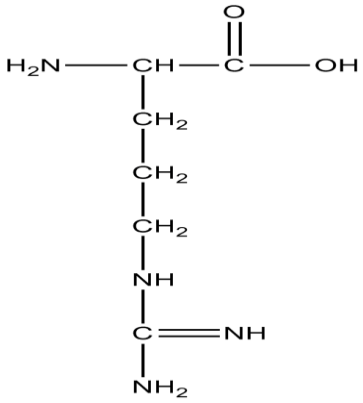

Figure 1.2 Structure of arginine

Most mature mammals can synthesize Arg to meet their requirements. Due to the lack of some enzymes in the urea cycle, broiler chickens are unable to biosynthesize Arg from ornithine; thus, this amino acid must be supplemented in diets (Wu et al., 1995) to meet their needs for protein synthesis and other functions (Tamir and Ratner, 1963). Indeed, Arg is a precursor of proline, which plays a key role in collagen synthesis (Barbul et al., 1990). Arg supplementation enables the synthesis of polyamines, which are implicated in mucosal repair (Moinard et al. 2005). A large number of evidence from various animal studies indicates that adequate provision of Arg is required for lymphocyte development and that dietary Arg supplementation enhances immune function in various models of immunological challenges (Field et al., 2000; Calder and Yaqoob, 2004). Inadequate intake of dietary Arg reduces the immune response in growing chickens (Konashi et al. 2000).

Arg Synthesis

Gln, glutamate and proline serve as major precursors for intestinal Arg and citrulline synthesis (Wu, 1998). They are also extensively catabolized by the small intestine (Wu, 1998), which releases appreciable amounts of citrulline (Wu et al., 1994). There is no significant uptake of arterial glutamate or proline by the small intestine (Wu et al., 1994). Thus, dietary Gln, glutamate, and proline must be the major substrates for intestinal citrulline synthesis (Murphy et al., 1996; Wu, 1998). Indeed, relatively high activities of proline oxidase, the enzyme responsible for converting proline to pyrroline-5-carboxylate, have been found in porcine enterocytes (Wu, 1997). Furthermore, intestinal activity of this enzyme is greater than in other organs such as the liver and kidneys (Wu et al., 1997; Samuels et al., 1985). Citrulline is an effective precursor of Arg (Wu and Morris, 1998). A high rate of Arg synthesis, which occurs via the hepatic urea cycle, is possible with the continuous supply of substrates such as ornithine. However, net synthesis of Arg in the liver is absent due to the high levels of arginase, the enzyme that degrades Arg (Wu and Morris, 1998). In adults, endogenous arginine synthesis occurs in the small intestine and kidneys (Morris, 1992; Rabier, 1995; Reyes et al. 1994; Wu and Morris, 1998) through the intestinal-renal axis (Morris, 2002). Namely, citrulline is released into the circulation from the small intestine and is extracted by kidneys for Arg production (Dhanakoti et al., 1990). Around 60% of net Arg synthesis occurs via this metabolic reaction. Furthermore, whole body protein turnover contributes to the majority of the de novo Arg synthesis accounting for 5 to 15% of endogenous Arg flux (Wu and Morris, 1998).

Arg Catabolism

Two pathways for Arg catabolism are initiated by nitric oxide (NO) synthase (which produces NO and citrulline) and arginase. Three NO synthase isoforms have evolved in animals, including neuronal and endothelial NO synthase, which are both constitutively expressed, and the inducible NO synthase (Knowles and Moncada, 1994; Morris and Billiar, 1994; Wu and Meininger, 2002). NO synthesis also requires cofactors such as heme, tetrahydrobiopterin (BH_4), flavin mononucleotide, flavin adenine dinucleotide, NADPH and calmodulin (MacMicking et al., 1997). On the other hand, there are two distinct arginase isoenzymes that are encoded by different genes. The type I arginase is a cytosolic enzyme and the type II is a mitochondrial enzyme. The type I arginase is highly expressed in the liver (Wu and Morris, 1998). In enterocytes and other mammalian cells, type II arginase is located in the mitochondria.

Role of Arg on performance of broiler chicks

Due to the lack of a complete urea cycle, broiler chicks are unable to synthesize Arg de novo (Cohen and Hayano, 1946; Tamir and Ratner, 1963). Therefore, it is important to supply adequate amount of dietary Arg for normal body function. Several studies have been able to recognize the importance of Arg on growth (Allen and Baker, 1972; Burton and Waldroup, 1979; Cuca and Jensen, 1990; Kidd et al., 2001; Corzo et al., 2003) and immunity (Kidd et al., 2001). Kidd et al. (2001) conducted three experiments to evaluate growth and immunity of broiler fed different level of Arg and mentioned that increasing dietary Arg from 100 to 120% of the NRC recommendation would increase body weight gain. Cuca and Jensen (1990) conducted experiments in broiler chicks evaluating Arg needs at varying dietary CP levels. Although an Arg requirement of 1.28%

or less was estimated for BW gain and feed conversion, feed conversion was not affected by Arg addition to the Arg deficient test diets in some experiments (Cuca and Jensen, 1990). In another experiment, Corzo et al. (2003) mentioned that 0.98 % dietary Arg optimized BW gain and FCR of broiler chicks, whereas weight of the chilled carcass was optimized at 1.00%. Although meat quality in terms of blood contamination was unaltered, but lightness (L*) and yellowness (b*) values were increased with Arg content in diet.

Glutamine (Gln)

Gln is the most abundant free alpha-AA in the body and turnovers rapidly in plasma (Van Acker et al., 1998; Watford, 1999), which reflects a crucial role of this AA in whole-body nutrient metabolism and health (Wu, 2009). Studies over the last three decades indicated that Gln is a major fuel for the small-intestinal mucosal cells and is crucial for maintaining the integrity and function of the small intestine (Burrin and Davis, 2004; Wang et al., 2008; Wu et al., 1996a). It is an important constituent of proteins and is a precursor for the synthesis of AAs, nucleotides, nucleic acids, amino sugars, and several other biologically important molecules (Souba, 1993; Roth, 2008). Furthermore, Gln is the main energy substrate for rapidly proliferating cells such as enterocytes and activated lymphocytes in the intestinal epithelium (Calder and Yaqoob, 1999). As an immunonutrient, Gln is important for promoting the integrity and maturation of intestinal microflora associated with the immune system, for enhancing mucin synthesis to maintain intestinal mucosa structure, and for reinforcing the epithelial barrier against bacterial attacks (Khan et al., 1999; Yi et al., 2005).

Gln metabolism

Gln is a non-essential amino acid which is quantitatively the most abundant free amino acid in blood plasma compared to other free amino acids (Tapiero et al., 2002; Newsholme et al., 2003a; Murakami et al., 2007; Bartell and Batal, 2007). Gln is important for different physiological functions and maintenance of cell functions (Fig. 1.3) (Newsholme et al., 2003a; Tapiero et al., 2002). It acts as the substrate for several aminotransferases involved in the syntheses of purines, glucosamine, pyrimidines and asparagine (Watford, 2008; Li et al., 2007). Gln is also involved in protein, peptide, and nucleic acid syntheses. It is available as a source of oxidative energy and in the biosyntheses of glucose, amino sugars, and glutathione (Tapiero et al., 2002; Newsholme et al., 2003a). The end products of Gln catabolism could be either carbon dioxide or glucose (Watford, 2008; Herbert et al., 1975). However, in most cells, Gln metabolism results in the production of L-glutamate and ammonia by the action of glutaminase (Newsholme et al., 2003a; Tapiero et al., 2002; Watford, 2008). On the other hand, Gln can be produced by the combination of an amino group and glutamate (Glu) by the action of Gln synthetase. Gln is involved in ammonia exchange among numerous tissues, and Gln acts as a precursor for ammoniagenesis in the gut and kidneys (Tapiero et al., 2002). Gln thus plays a role in the regulation of ammonia level in the body. In birds, ammonia is excreted in feces in the form of uric acid, and Gln is involved in uric acid synthesis (Soltan, 2009; McDonald et al., 2002). The major site of dietary Gln absorption is small intestine (Souba, 1993; Tapiero et al., 2002). According to Souba et al. (1990), however, the absorption of Gln may increase by up to 10 folds in sepsis (potential severe

inflammatory condition or blood poisoning). Skeletal muscles and lungs could be the major sites for Gln excretion (Tapiero et al., 2002).

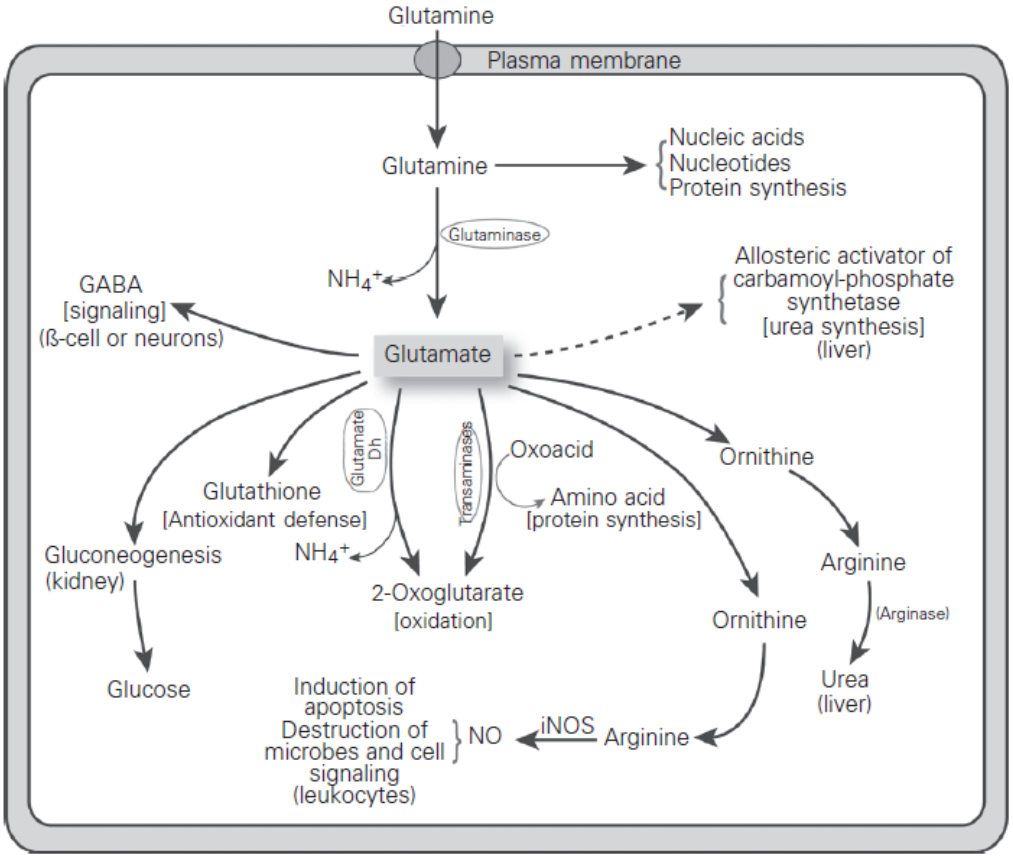

Figure 1.3 A summary of glutamine and glutamate metabolism in mammalian cells (Newsholm et al., 2003b).

Gln is known as non-essential amino acid but it also seems that they might be considered as conditionally essential amino acid. Bartell and Batal (2007) observed that weight gain in 21 day old broiler chickens fed a diet supplemented with 1% Gln was significantly higher (11%) in comparison with chickens fed a control diet. Positive effects of Gln on broiler performance have been also reported in other studies. For instance, Dai et al. (2009) found that broilers fed diets containing 0.5 and 1.0% Gln had better performance during heat stress than birds fed diets without Gln supplementation.

Effects of Gln on the small intestine

The small intestine is known as the principal digestion and absorption site of dietary nutrients such as proteins and amino acids (Wu, 1998). In adult rats, the small intestine was able to extract 25-30% of arterial Gln in a single pass. Wu et al. (1996a) observed that by addition of 1% Gln in the basal diet, the concentration of Gln in digesta fluid was raised up to 8 folds. It has been found that dietary Gln may be the most important fuel for small intestinal mucosa (Wu, 1998) connected with the formation of glutamate (Newholms et al., 2003a; Reeds et al., 2000). Different studies have shown that addition of Gln to the diet of broilers could increase the relative weights of duodenum and jejunum (Bartell and Batal, 2007; Soltan, 2009). Newsholm et al. (2003a) and Li et al. (2007) pointed out that dietary Gln is known as an important constituent for maintenance of the gut integrity. It is believed that longer villi in the small intestine could increase the food utilization efficiency in early stages of chicken life and result in better performances of broilers. Several studies have shown that Gln supplementation to the diet could increase the villi length in different segments of the small intestine. Soltan (2009) observed that broilers fed a diet containing Gln had significantly longer villi in duodenum and jejunum compared to the control group.

Gln on immune response of broiler chicks

Gln is an essential substrate for development of lymphocytes and macrophages. Moreover, activity of immune cells (e.g. T-cell proliferation, cytokine production, B-lymphocyte differentiation, antigen presentation and macrophage phagocytes) was increased in the presence of Gln. In vivo studies have shown that supplementation of Gln improves the function of the immune system. Soltan (2009) demonstrated that broilers

fed a diet containing 1% Gln had significantly higher counts of red blood cells, white blood cells and percentage of hemoglobin in comparison with broilers fed a diet without Gln. Calder and Yaqoob, (1999) reported that glutaminase (enzyme involved in glutamine deamination) activity was increased in all lymphoid organs (e.g. spleen, thymus and lymph nodes) in response to pathogens. Bartell and Batal (2007) observed an increase in the relative weights of thymus and spleen of broilers fed a diet containing 1% Gln in comparison with the group fed a control diet. Similarly, Sakamoto et al. (2006) showed that in 7 days old broilers fed a diet containing 1% Gln, only the relative weight of the spleen was increased but that other lymphoid organs had the similar weights among the treatments. Further, Bartell and Batal (2007) observed that broilers fed Gln had a higher IgA concentration level in serum, bile and intestine. The IgA acts as a barrier against bacterial bonding to mucosal cells. Therefore, inclusion of Gln in the diet could induce the IgA production, increasing the immunity against bacteria and parasitic antigens.

Zinc (Zn) in Poultry Nutrition

Zn is a trace element that is necessary for normal growth and maintenance and is included for bone development, feathering, enzyme component and function, and appetite regulation for all avian species (Batal et al., 2001). Zn at 0.012-0.018% on a total-weight basis is commonly added as a supplement to all formulated poultry diets (Batal et al., 2001; Leeson and Summers, 1997). Currently, there are two inorganic feed-grade Zn sources commercially available and commonly used by the poultry feed industry (Batal et al., 2001; Wedekind and Baker, 1990): zinc oxide (ZnO: 72% Zn) and zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$: 36% Zn). Of the supplemental Zn feeds, 80-90% is ZnO,

which is less bioavailable for poultry than reagent-grade or feed-grade Zn sulfate (Sandoval et al., 1997; Edwards and Baker, 2000; Fosmire, 1990). However, the sulfate (acid salt) is highly water soluble, allowing reactive metal ions to promote free-radical formation, which can facilitate reactions that lead to the breakdown of vitamins and ultimately to the degradation of fats and oils, decreasing the nutrient value of the diet (Batal et al., 2001). Oxide is not only less reactive, but also less bioavailable (Batal et al., 2001). Dietary Zn is relatively nontoxic to animals and humans; both exhibit considerable tolerance to high intakes of Zn (Fosmire, 1990). However, high levels of zinc in the diets can result in a reduced growth rate (Dewar et al., 1983), an increased mortality (Blalock and Hill, 1988), and a reduced FI (Hermayer et al., 1977) in chicken. Zn toxicity is responsive to supplemental copper, and both iron and zinc interfere with copper and iron metabolism (Cox and Harris, 1960). Metallothionein is a nonspecific metalbuffering ligand to sequester or displace Zn from normal sites (Richards and Cousins, 1975). Dietary Zn-Met supplementation (80 mg/kg for old broilers and 40 mg/kg for young broilers) in the broiler diet improves immunity in the progeny of old and young broiler breeders. In poultry, Zn deficiency causes reduction in weight gain, skeletal malformations, poor bone mineralization, and immunological dysfunctions (Kidd et al., 1996, Blamberg et al., 1960). Therefore, Zn is often supplemented in practical poultry diets to elicit a positive response in broiler chickens, particularly during early age. Tissue uptake of Zn in chicks is linearly related to Zn levels in the diet (Sandoval et al., 1997 and Bartlett et al., 2003). The Zn was found to be essential for normal function of the immune system by increasing the counts of thymocytes and peripheral T cells, an activity of natural killer cells (Dardenne and Bach, 1993). It also enhances the production of

neutrophils and antibodies, in addition to improving the functions of macrophages (Kidd et al., 1996). Because Zn supplementation was found to be essential, the NRC (1994) recommended 40 ppm for broiler chickens, which appeared to be based on the results that considered growth performance as the only criterion (Stahl et al., 1986 and Burrell et al., 2004). However, higher Zn levels (60 to 180 ppm) produced a better immune status in broiler chickens (Bartlett et al., 2003; Bertuzzi et al., 1998; Kidd et al., 2000) but clear information on Zn requirements for growth, mineral bioavailability, and immune response is scanty. In recent research, it was shown that the levels of phytate and calcium in corn-soybean meal diets can increase the requirement of dietary Zn up to levels that are twice the current NRC recommendation of 40 mg of Zn/kg of diet (Suttle, 2010; Huang et al., 2007; Linares et al., 2007).

Chelated Zn and broiler performance

"Chelate" comes from a Greek word meaning "claw". Chelate can refer to a variety of chemical compounds that are bound to Zn and it can increase the absorption of Zn. Chelates has centered on the theory that they are more bioavailable or similar to forms naturally occurring in the body than inorganic source (Spears et al., 1991). If the chelate is stable in the digestive tract, then it would be protected from forming complexes with other dietary components that inhibit absorption, and would thus allow a greater absorption. Some studies reported that chelated form of Zn is more bioavailable than its inorganic form (Spears, 1996). It has also been reported that chelated forms of minerals have higher absorbability than traditional forms, which leads to better absorbance through AA transport system (Mazzoni et al., 2010). More recently, interest has been directed

from inorganic trace mineral sources to minerals that are bound, often in a chelated structure, to an organic ligand, resulting in trace minerals with a greater bioavailability.

Richards et al. (2010) showed that the bioavailability of chelated Zn is between 160 and 250% that of inorganic Zn as sulfate. The increased bioavailability of chelated trace minerals is likely due to reduced antagonistic reactions with other dietary constituents in the GIT. Feeding organic or chelated trace minerals, either with partial replacement of inorganic trace mineral or as top-dressed supplementation, compared with industry levels of inorganic trace mineral (sulfates) resulted in an improvement in good-quality paws in broilers reared up to 49 to 54 d of age (Saenmahayak et al., 2010; Zhao et al., 2010). The use of more bioavailable trace minerals in the form of chelates allows nutritionists to reduce the trace mineral content of the feed while still meeting bird requirements. Zn plays an integral part in the synthesis of two important functional proteins, collagen and keratin (Underwood and Suttle, 2001). Collagen is the major structural protein of internal tissues, including cartilage and bone, whereas keratin is the structural protein of the feathers, skin, beak and claws. Research has documented that mineral bioavailability varies considerably between sources (oxide vs. sulfates). Wedekind and Baker (1990) showed that the bioavailability of Zn from ZnO (zinc oxide) was only 0.44 that from the sulfates, based on research conducted in chicks.

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CHAPTER II

EFFECT OF TRYPTOPHAN, ARGININE, AND GLUTAMINE ON GROWTH PERFORMANCE, GUT MORPHOLOGY, IMMUNE RESPONSE AND MEAT QUALITY OF BROILER CHICKS

Abstract

Two experiments were conducted to evaluate the effect of supplemental effects of Arg, Gln and Trp on growth performance, gut morphology, immune response and meat quality of broiler chicks. In Exp 1, birds were allotted to 4 dietary treatments (6 replicates per treatment and 6 birds per cage), C (control); Arg (a diet with 0.5% L-Arg); Gln (a diet with 0.5% L-Gln); and Trp (a diet with 0.5% L-Trp). In Exp 2, 312, 1-d-old broiler chicks (Ross×Ross 308) were weighted and randomly assigned to similar treatments as of Exp 1. In a 42 d experiment, chicks had an ad libitum access to basal starter (d 0 to 14), grower (d 15 to 28) and finisher diets (d 29 to 42) based on corn and soybean meal. BW and FI were measured at the end of each phase. On d 11, 2 birds from each cage were sacrificed to measure gut morphology and circulating level of serum immunoglobulins (IgG and IgA). In both experiments, 2 birds from each pen were sacrificed at 6 weeks of age and to measure carcass traits and meat quality. In Exp 1, results showed that dietary Arg, Gln and Trp supplementation significantly increased ($P < 0.05$) the BW gain of broilers during 0 to 42 d. Feed intake and FCR were also favorably influenced by the supplementation of AA in the diet ($P<0.05$). Warm and chilled carcass weights were also significantly higher in the AA supplemented groups than that in control group. Consequently, weight of breast and thigh meat in Arg, Gln and Trp groups were higher

(P<0.05) than C group. Breast and thigh meat in C group had lower pH, a* value and had higher L* and b* values. No significant difference was observed among the AA treatments. Morphometric analysis showed increased (P<0.05) villus height and villus to crypt ratio and decreased crypt depth in chickens treated with AA groups. Improved gut morphology and growth performance was very well supported by the Exp 2. Significant increased weight gain, feed intake and FCR in all phases were also observed among the same groups, suggesting beneficial effects of AA inoculation into the diet. Supplementation with Arg, Trp or Gln at 0.5% improved meat quality of broiler chicks. In addition, supplementation of AA into the basal diets did not influence serum IgA and IgG contents. As a result, dietary supplementation of Arg, Trp or Gln at 0.5% would enhance growth performance, gut morphology, meat and carcass quality of broiler chicks.

(Key words: Amino Acids, Performance, Gut Morphology, Meat Quality and Broiler Chicks)

Introduction

To attain optimal animal performance and quality meat, it is important to formulate diets to meet AA requirements. Therefore, an optimum AA concentration in broiler chicks is a strategy to maximize the performance and mitigate diet cost. Amino acids are known as organic compounds comprising both an amino and an acid group (Wu, 2009). For a long period of time it has been cost benefit to supplement broiler diets with pure (synthetic) amino acids (Dozier et al., 2008). Feeding low protein diets results in impaired performances of broilers (Berres et al., 2010). But, when low protein diets are supplemented with certain amino acids, broiler performance might be recovered (Berres et al., 2010; Corzo et al., 2005). Besides the role of amino acids as proteins and peptides

constituents, some amino acids (e.g. Gln, Arg and Trp) are also involved in regulation of metabolic pathways, thereby affecting growth GIT morphology and immunity (Wu, 2009). The European Union has banned the use of antibiotics as a growth promotor in animal diets due to the post-negative effects on consumer health (Mateos et al., 2002). Hence, amino acids have become an alternative to antibiotics, to improve structure of GIT (Mateos et al., 2002).

Gln, Trp and Arg are among the important 'bioactive amino acids', and participate in many important and diverse biochemical reactions associated with the normal physiology of the organism. Due to the lack of some enzymes in the urea cycle, broiler chickens are unable to biosynthesize Arg from ornithine; thus, this amino acid must be supplemented in diets (Wu et al., 1995). Therefore, birds are dependent on dietary Arg to meet their needs for protein synthesis and other functions (Tamir and Ratner, 1963). Different studies have been able to recognize the importance of Arg (Kidd et al., 2001, Corzo et al., 2003). On the other hand, Trp is an essential amino acid for protein synthesis of chickens and significantly elevated BW gain and FI of broiler (Bungo et al., 2008). It also affects lipid levels in chickens (Akiba et al., 1998; Rogers and Pesti, 1990). The Trp requirement to minimize lipid levels is almost double that to maximize body growth of broilers (Rogers and Pesti, 1990). Gln is a free, neutral, non-essential amino acid and it contains two nitrogen groups that can be mobilized. Gln may be a vehicle for tissue nitrogen exchange, and may play an essential role in several important metabolic pathways (Marliss et al., 1971; Smith, 1990). Yi et al. (2005) mentioned that Gln supplementation in the diet enhanced weight gain, feed efficiency, and viability of broiler chicks. A few investigations have been made on the effects of

several essential amino acids on the performance, gut morphology, meat quality and immune responses of broiler chicks. Therefore, two experiments were conducted to clarify those amino acids have the most potential to modulate the performance, GIT morphology, metabolic response and meat quality as well as immunity in broiler chickens.

Materials and methods

Birds and management

All experimental procedures using animals were approved by the Institutional Animal Use and Care Committee at Texas Tech University. For each experiment, 312, 1-day old Ross×Ross 308 broiler chicks were purchased from a local hatchery and weighed and placed onto 48 cages (4 dietary treatments × 12 replications) and each cage contained 6 birds. The chicks were housed in separate cages and a ventilation fan in the central arena that circulated fresh air inside the cages. The room temperature was maintained at 33℃ for the first week, and then reduced by 2-3℃ per week until it reached 22℃, which was maintained until the end of the experiment. Mean relative humidity was maintained at 60–65% throughout the experiment. The broilers had free access to feed and water at all the time. Each cage was cleaned daily to prevent any familiar odors interfering with the treatments. BW, feed consumption, and FCR were measured weekly throughout the trial. Birds were individually weighed weekly to calculate the mean of population weight. All management of chicks and experimental procedures were conducted in accordance with the commercial breeding company, Aviagen, AL, USA.

Experiment 1:

Upon arrival, 312, 1-d-old chicks were weighed (44.18 ± 0.31 g) and divided equally into four dietary treatments with twelve replications. All birds were randomly

assigned to four different treatments with twelve replications in each (6 × 12 × 4 = birds per cage × replications × treatments) and fed a diet containing a corn-soybean based meal. All basal diet ingredients were mixed based on the recommendation of NRC and then divided into four different treatments as follows: 1) negative control (corn-soybean meal based diet); 2) a diet including 0.5% Trp supplement from L-Trp; 3) a diet including 0.5% Arg supplement from L-Arg 4) a diet including 0.5% Gln supplement from L-Gln as Table 2.1 and Table 2.2.

Carcass traits and meat quality of broiler chicks

At the end of the experiment after a 12 h of withdrawal of feed and water, 1 bird per cage (12 birds/treatment) was randomly selected, weighed, and sacrificed by cervical dislocation, and then scalded, feathers mechanically plucked in a rotary drum picker and eviscerated to determine carcass yields and dressing percentage. Breast and thigh muscles were removed from the carcass and placed into individually labeled Ziploc bags (Ziploc freezer bags, S. C. Johnson & Son Inc., Racine, WI) and stored at 2℃ overnight. The pH values of both breast and thigh muscle samples were determined using a digital pH meter (Seven Easy pH, Mettler-Toledo AG, Schwerzenbach, Switzerland). Five grams of the breast and thigh muscle were thoroughly ground with 45 mL of double distilled water for 30 seconds at 13,500 rpm, the pH was measured in triplicate, and the mean values are calculated.

The complete International Commission on Illumination (CIE) system color profile of lightness (L*), redness (a*) and yellowness (b*) was measured for breast and thigh muscle samples collected at 6 weeks of experiment using a Minolta colorimeter (Minolta Chroma Meter CR-300; Ramsey, NJ, USA). The color values were determined after calibration using a white tile expressed as y = 92.8, x = 0.3134, and y = 0.3193, and

duplicate readings per sample. Each treatment reading was averaged and expressed as the CIE L*, a* and b* color space value, respectively.

Intestinal morphological analysis

At d 11, 1 bird from each cage was sacrificed under deep Nembutal anesthesia (50 μg/g of BW, intraperitoneally; n = 72). Their jejunum (Meckel's diverticulum), and ileum (0.5 cm from the ileocecal orifice) segments (1 cm long) were taken and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4, 4℃) for 48 h, and paraffin sections (5 μm thick) were made. For histological studies, sections were stained with hematoxylin and eosin. Eight cross-sections for each intestinal segment were prepared for each bird. Villus height (from the tip of villus to the junction of villous and crypt) and crypt depth (from the base of crypt to the level of crypt opening) were measured from 5 vertically oriented crypts and villi structures of each section by using image analysis software (Scion Image, Version Beta 4.0.2; Scion Co., Frederick, MD, USA).

Experiment 2:

In experiment 1, we demonstrated that the amino acid treatments did enhance broiler chick performance and meat quality. Therefore, further follow up experiment was designed with similar treatments, including a control diet (C); diet including 0.5% Trp; diet including 0.5% Arg and diet including 0.5% Gln. A total of 312, 1-d-old broiler chicks (Ross×Ross 308) were weighed (41.54±0.25 g) and randomly assigned to similar cages in Exp 1 (4 dietary treatments, 12 replicate cages/treatment; 6 chicks/cage). All other procedures were as described in Exp 1. Meat samples were collected and analyzed using the procedures as described in Exp 1.

Serum IgA and IgG

Two blood samples per cage were obtained at d 11 by puncturing the wing vein. The blood was allowed to clot for 2 h at room temperature and was centrifuged at 400 × g for 8 min at 4℃. Sera were collected and stored at 4℃ for 4 h and then stored at -70℃ until IgG and IgA analyses were conducted using chicken IgG and IgA ELISA quantitation kits (Bethyl Laboratories, Inc. Montgomery, TX, USA) as described by Perez-Carbajal et al. (2010).

Statistical analysis

All data were analyzed by one-way analysis of variance using the GLM procedure in SAS (9.1., Cary, NC, USA, 2002). Duncan's new multiple-range test was performed to identify differences (Steel and Torrie, 1980). The P-values of less than 0.05 were considered significant.

Results

Growth performance

The performance of the broilers in experiment 1 was influenced by the amino acid treatments (Table 2.3). During the first two weeks (0–14 days), the 0.5% Arg treated group showed increased body weight compared to that in control group. A significant ($P < 0.05$) increase in weight gain was found in the Arg and Gln treatments compared to that of control treatment during the starter period (0-2 weeks). However, no significant difference was observed in weight gain among the 0.5% Arg, Gln and Trp treatments. Consequently, weight gain was significantly higher ($P < 0.05$) in the treatment groups (Arg, Gln and Trp) during both 3-4 weeks and 5-6 weeks period than that in the control group. Weight gain was also different by amino acid treatments in a follow-up experiment conducted during the 0–14 d period (Table 2.3). Extending the grow-out

period from d 15 to 28 resulted in the increased weight gain in chicks in the Gln treatment. However, no significant difference was observed among the Arg, Trp and Gln treatments. When the growth period was extended further (d 29-42), weight gain was increased when chicks were exposed to a diet including Trp. The Trp treatment had a similar effect to that of Arg and Gln but was higher than that of the control treatment.

In Exp 1, amino acid treatment affected FI during 0-2 wks and 5-6 wks of age, whereas it remained unaffected during the 3-4 weeks of the experimental period (Table 2.3). During week 0-2, amino acid treated birds consumed more feed, whereas control birds had the lowest FI in all period. During the overall experimental period (1–6 wk), significantly higher FI was recorded under Arg, Trp and Gln treatments, respectively. Consequently in Exp 2, dietary Gln, Trp and Arg supplementations significantly increased ($P < 0.05$) FI of broilers during 0-14 d, 15-28 d and 29-42 d of age (Table 2.7). Overall, birds fed diets supplemented with 0.5% AA (Gln, Trp and Arg) had significantly increased FI compared with the birds fed the control diet.

With the growth and development of broilers, feed consumption was increased gradually. In the early stage (d 0-14), the FCR was not influenced by the dietary treatments in Exp 1 (Table 2.3). In the middle stage (d 15-28), feed conversion ratios were increased in the control group and were the lowest in Arg group. No significant difference was observed in feed conversion ratios among treatments during the later stage (d 29-42) ($P < 0.05$). And, no significant difference was observed during the d 0-42 period. In the follow up Exp 2, the FCR was not altered by the dietary treatments during d 0-14, d 29-42 (Table 2.7). During d 15-28 of age, the lowest FCR values were noted

under control group. Therefore, no difference ($P > 0.05$) in FCR was observed during the overall experimental period from d 0-42 of age.

Gastrointestinal tract morphology

The intestinal villus had a considerable integrity in various dietary groups (Table 2.4), and its height was increased gradually during the entire experiment of broilers. Though there was no significant ($P < 0.05$) difference was observed in GIT weight and percentage with their body weight, but numerically increased in AA treatments. Chicks fed corn-soybean meal based diets supplemented with 0.5% Arg, Trp and Gln preparation had the highest ($P < 0.05$) villus height and villus height:crypt depth ratio and consequently the low crypt depth in comparison to the control group. However, there was no difference among the Arg, Trp and Gln groups at d 11 in the jejunum and ileum respectively. In contrast, the crypt depth of the Arg and Trp groups in the jejunum, and ileum was lower than that of the control group at d 11 of age. There was no significant difference in the crypt depth among the AA treatment groups (Table 2.4).

Carcass traits and meat quality of broiler chicks

In Exp 1, there were significant effects of different amino acid on liveweight, carcass weight, dressing percentage, breast and thigh muscle weights and breast and thigh muscle percentage among broiler treatments (Table 2.5). Warm and chilled carcass weights were significantly increased by the AA supplemented groups than that of control group. Dressing percentage of broilers fed 0.5% Arg and Trp diets were higher ($P < 0.05$) than that of broilers fed control diet. Similarly, breast muscle weight and percentage were significantly increased by the amino acid treatment groups. On the other hand, thigh muscle percentage was increased by the control diet. In the second follow-up studies, liveweight and carcass weight were also increased by the AA supplemented groups ($P <$

0.05) and which is similar with the Exp 1. But both in breast and thigh muscle weights and percentage were not influenced by the AA treatments. On the other hand, dressing percentage was not changed by the AA supplemented diet as showd Table 2.9 in Exp 2.

The meat quality properties of 42-day old chicks exposed to the different dietary amino treatments were analyzed in Exp 1. Supplementation of AA had no significant effect on DM and moisture contents of breast and thigh muscles. The results showed that breast muscle pH and CIE (L*, a* and b*) were affected by the treatments (Table 2.6). However, no significant differences were observed among the Arg, Trp and Gln groups. The pH values of breast meat were increased in chicks exposed to the Trp treatments and decreased in those exposed to the Arg, Gln and control treatments. These differences were not observed in thigh muscles. On the other hand, CIE (a*) value was not influenced by the Trp treatment. The control group had a higher CIE (L* and b*) than those in the AA treatments ($P < 0.05$). Only control group had a decreased a* value of breast muscle, but no difference the b* value of thigh muscle. Apart from these changes, AA treatment did change the color properties of muscle samples. The L* values were higher in thigh muscle when compared to those in breast muscle. In the follow up studies, breast meat CIE (L* and b*) color was also significantly increased by the control group (Table 2.10), but this difference were not visible in thigh muscle. On the other hand, pH in thigh meats was increased by the Arg feeding group and no difference in breast meat.

Serum IgA and IgG

We measured total antibody levels (IgG and IgA) in serum at 11 days of age (Table 2.8). Chickens (11-days of age) reared under the Arg treatment had numerically higher levels of IgG than those of birds reared under the control treatment, whereas birds reared under the Gln and Trp treatments had intermediate levels of IgG. Serum IgA levels

in 11-day old birds under the different amino acid treatments did not show a different distribution pattern, but birds reared under control group had lower IgA levels. However, serum IgA levels were not different among birds reared under Arg, Trp and Gln treatments.

Discussion

In both experiments, significant improvements in weight gain, FI and feed:gain ratio were observed when 0.5% Gln, Trp and Arg was supplemented in the feed for 42 d as compared with the birds fed the corn-soybean meal based control diet. Our results also shown that broiler chicks supplemented with 0.5% Trp significantly increased growth performance and FI during the overall experimental period. It might be due to the function of Trp as a precursor of the neurotransmitter serotonin and this serotonin formed in the brain and influences FI. Thus the present results correspond with the findings of Tackman et al., (1990) and Denbow et al., (1993). In a previous study, Harms and Russell (2000) reported that dietary Trp increased BW, weight gain and FI of broiler chicks. Also, Peganova and Eder (2003) observed that feed consumption was increased 6% in broiler chickens which received a higher concentration of dietary Trp in the diet. In the present results, Arg also influences growth performance by stimulating the release of pituitary and pancreatic hormones (glucagon, insulin, and growth hormone) and thus enhances protein synthesis and feed consumption (Floyd et al., 1966; Rocha et al., 1972; Franchimont and Burger, 1975; Palmeret al., 1975; Davila et al., 1987) of broiler chicks. In a recent work, Yi et al. (2005) evaluating the influence of Gln and reported better feed efficiency, weight gain, and livability of broiler chicks. But, Murakami et al. (2007) and Maiorka et al. (2000) observed no effect of supplementing Gln on the FI, BW gain, or

FCR in any of the development phases in broiler chickens. Therefore, dietary inoculation of Trp, Arg and Gln is a potential way to improve growth performance of broiler chicks.

Chickens reared under the Arg treatment had numerically higher levels of IgG, IgA than those of birds reared under the control treatment. This may indicate that the birds fed diets supplemented with 0.5% Gln, Trp or Arg had a better gut barrier function because the birds had higher IgA concentrations in the small intestines and thus may be more resistant to infection. In previous, Mathers and Cuff (2004) mentioned that IgG levels did increase in birds fed diets supplemented with Gln and which corresponds with the present findings. In another experiment, Bartell and Batal (2007) reported that the birds fed diets supplemented with 1% Gln for 7 days or more had significantly higher IgA concentrations in the serum and bile than the control, which indicated better health and resistant to infection.

If the intestinal villi height can be increased early in chick's life, then chicks may be able to utilize nutrients more efficiently earlier in their life and thus have improved growth performance. Diets supplemented with 0.5% Arg, Trp and Gln preparation had the highest ($P<0.05$) villus height and villus height:crypt depth ratio and consequently the lowest crypt depth in comparison to the control group. The present results indicated that birds fed diets supplemented with Arg had a longer villi height than the control diet. The longer villi height should increase surface area and consequently greater nutrient absorption by the Arg treatment and thus improved weight gain of broiler chicks. In previous experiment, Kelly et al. (1995) observed that villus height is positively correlated with BW gain and FI and which corresponds with the present findings. In consequent, Bartell and Batal (2007) mentioned that broiler fed diets supplemented with

Gln had significantly longer intestinal villi than the control corn-soymeal diet. Coates et al. (1954) and Izat et al. (1989) indicated that increased villi height has been proposed to increase performance by improving nutrient absorption. Therefore, chicks might be able to utilize nutrients more efficiently in the early stage under the AA treatments and thus improved growth performance (Lilja, 1983; Nitsam et al., 1991).

Generally, pH value is a direct reflection of muscle acid content, and affects on color in meat and these color is a major criterion to judge meat quality, storage characteristics and shelf life of meat (Jakobsen and Bertelsen, 2000). Several authors also mentioned that CIE (L*, a* and b*) values are important in poultry muscles and correlate with pH, water holding capacity and chemical composition (Allen et al., 1998; Qiao et al., 2002). In the present two consecutive experiments, pH values of breast meat increased in chicks exposed to the Trp treatments. It might be due to supplementation of dietary Trp may play a regulatory role of serotonin and thus improve meat quality. More clearly, serotonin reduced the release of cathecholamines (cortisol, epinephrine, etc.) which might be reduced metabolic acidosis and thus increased pH and CIE (a*) value. But in another experiment, Dai et al. (2009) mentioned that addition of Gln at the levels of 0.5% and 1.0% to the broiler diets also has beneficial effects on breast muscle pH and color values. Therefore, 0.5 % dietary inoculation of Trp would be effective for enhancing meat quality of broiler chicks.

Conclusions

Based on two experiments, dietary supplementation of Arg, Trp or Gln at 0.5% improves growth, gut morphology and carcass quality of broiler chicks. Circulating levels of IgG and IgA in chicks increased under the Arg treatments at the early growth stage.

The results of this study indicate that diets supplemented with 0.5% Arg, Gln or Trp provided a better development of the intestinal mucosa in broiler chickens. Therefore, these results suggested that dietary supplementation of Arg, Trp or Gln at 0.5% may be a potential feed additive used to enhance performance and improve meat quality of broiler chicks.

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APPENDIX Ⅰ

TABLE 2.1 Ingredients and composition of the broiler diets

Ingredients	Starter (0-2 wks)	Grower (3-4 wks)	Finisher (5-6 wks)
Corn	55.90	59.25	61.00
Soybean Meal	35.40	31.20	29.40
Poultry Fat	1.91	3.42	4.00
Limestone	1.32	1.07	1.03
Dicalcium Phosphate	2.00	1.78	1.63
DL-Methionine	0.37	0.30	0.20
L-Lysine	0.32	0.18	0.03
Threonine	0.10	0.07	0.00
Salt	0.33	0.38	0.36
Mineral Premix[1]	0.10	0.10	0.10
Choline Chloride	0.10	0.10	0.10
Vitamin Premix[2]	0.05	0.05	0.05
Selenium Premix	0.10	0.10	0.10
Amino Acids Supplement	2.00	2.00	2.00
Total	----------100----------		
Calculated value			
ME (kcal/kg)	3000	3125	3167
CP (%)	24.0	20.7	20.3

TABLE 2.1 Continued

Lysine (%)	1.30	1.23	1.20
Methionine (%)	0.60	0.57	0.60
Ca (%)	0.90	0.85	0.85
Available phosphate (%)	0.50	0.45	0.40

[1]Contains per kg: vit. A, 16,000,000 IU; vit D_3, 4,000,000 IU; vit E, 44,000 mg; vit K_3, 4,000 mg; vit B_1, 4,000 mg; vit B_2, 8,000 mg; vit B_6, 9,000 mg; vit B_{12}, 20 mg; biotin, 240 mg; pantothenic acid, 24,000 mg; folic acid, 1,200 mg; nicotinic acid, 7,000mg; niacin, 70,000mg.

[2]Contains per kg: Fe, 72,727 mg; Cu, 21,818 mg; Mn, 90,909 mg; Zn, 54,545 mg; I, 909 mg; Se, 182 mg.

TABLE 2.2 Composition of amino acids in broiler diets

Treatment	Gln	Arg	Trp	Ala	Carrier	Total
Control	0.000	0.000	0.000	1.023	0.977	2.000
G[1]	0.500	0.000	0.000	0.413	1.087	2.000
A[1]	0.000	0.500	0.000	0.000	1.500	2.000
T[1]	0.000	0.000	0.500	0.150	1.350	2.000

[1]G(L-Gln), A(L-Arg), and T(L-Trp) are 98.5% pure. It should be L-Arg but not L-Arg HCl. NC means Negative Control.

TABLE 2.3 Effect of different amino acids on the performance of broiler chicks (Exp 1)

Treatment	C	A	G	T	SEM[1]	P value
Body weight (g)						
At 14 d	350.97±15.99[c]	422.86±12.92[a]	417.25±10.27[ab]	384.11±8.40[bc]	8.28	0.002
At 28 d	1046.65±23.07[b]	1216.95±33.93[a]	1166.92±24.01[a]	1164.01±28.25[a]	24.83	0.013
At 42 d	2231.63±89.94[b]	2547.45±41.85[a]	2400.24±58.85[a]	2530.72±30.63[a]	44.07	0.003
Weight gain (g)						
0-14 d	306.87±15.99[c]	379.16±12.86[a]	372.79±10.23[ab]	339.66±8.46[bc]	8.29	0.001
15-28 d	695.68±13.12[b]	794.09±23.74[a]	749.67±16.98[a]	779.88±22.57[a]	13.56	0.001
29-42 d	1184.99±70.52[c]	1330.50±31.19[ab]	1233.32±36.02[bc]	1366.72±10.89[a]	25.12	0.025
0-42 d	2187.53±89.99[b]	2503.75±41.78[a]	2355.77±58.76[a]	2486.28±30.43[a]	44.07	0.0003
Feed intake (g)						
0-14 d	440.89±13.83[b]	526.67±13.21[a]	513.63±13.96[a]	504.59±10.62[a]	9.17	0.0007
15-28 d	1189.36±22.67	1243.60±26.57	1270.05±16.92	1232.54±14.98	10.45	0.383
29-42 d	2128.39±58.19[b]	2381.28±62.09[a]	2239.52±71.76[a]	2399.33±75.19[a]	39.81	0.004

TABLE 2.3 Continued

0-42 d	3688.62±95.43[b]	4151.54±77.09[a]	4023.20±84.67[a]	4136.46±78.85[a]	55.29	0.003
Feed conversion ratio						
0-14 d	1.443±0.03	1.393±0.03	1.378±0.01	1.488±0.03	0.02	0.088
15-28 d	1.71±0.03[a]	1.568±0.04[b]	1.698±0.04[ab]	1.587±0.04[b]	0.02	0.001
29-42 d	1.795±0.06	1.796±0.07	1.815±0.03	1.755±0.04	0.03	0.683
0-42 d	1.653±0.04	1.661±0.05	1.710±0.03	1.663±0.02	0.02	0.132

[1]Pooled standard error means.

[a,b,c] Values in a row with no common superscripts differ significantly ($P < 0.05$); C, control; A, Arginine; G, glutamine; T, tryptophan; d, days.

TABLE 2.4 Effect of different amino acid on gut morphology of broiler chicks (Exp 1)

Treatment	C	A	G	T	P value
Gastrointestinal tract weight (g)	35.13±8.33	53.37±1.82	46.57±3.74	50.10±1.03	0.104
GIT weight (% live weight)	5.32±0.77	6.42±0.39	6.15±0.43	6.78±0.82	0.463
Jejunum					
Villus height (µm)	59.25±9.02^{b}	69.79±15.62^{a}	64.39±11.25^{ab}	65.92±16.54^{a}	0.027
Crypt depth (µm)	18.26±3.58	16.90±4.76	17.33±3.57	17.43±3.46	0.432
Villus height-to-crypt depth ratio	3.51±0.77^{b}	3.83±0.59^{a}	3.78±0.64^{ab}	3.99±0.76^{a}	0.041
Ileum					
Villus height (µm)	44.38±7.10^{c}	52.28±11.47^{a}	49.53±10.95^{ab}	45.63±10.31^{bc}	0.018
Crypt depth (µm)	15.01±2.02^{a}	13.45±4.28^{bc}	14.69±3.68^{ab}	12.23±2.32^{c}	0.034
Villus height-to-crypt depth ratio	3.09±0.43^{b}	3.49±0.67^{ab}	3.85±0.81^{a}	3.75±0.58^{ab}	0.329

^{a,b,c} Values in a row with no common superscripts differ significantly ($P < 0.05$); C, control; A, Arginine; G, glutamine; T, tryptophan.

TABLE 2.5 Effect of different amino acid on carcass characteristics of broiler chicks (Exp 1)

Parameter	N	A	G	T
Liveweight (g)	2123±414[b]	2547±279[a]	2397±364[a]	2530±303[a]
Dressing percentage, %	76.05±2.42[b]	77.34±1.30[a]	76.72±1.42[ab]	77.17±1.83[a]
Warm carcass weight (g)	1559±303[b]	1888±235[a]	1748±285[a]	1877±230[a]
Chilled carcass weight (g)	1642±318[c]	1967±229a	1821±296[ab]	1983±241a
Breast muscle weight (g)	361±86[b]	451±56[a]	427±84[a]	451±78[a]
Breast muscle (%)	21.84±1.76[b]	23.15±1.70[a]	23.62±1.85[a]	23.24±1.96[a]
Thigh muscle weight (g)	263±67	296±47	267±43	277±34
Thigh muscle (%)	15.97±1.88[a]	15.18±1.51[ab]	14.93±1.45[b]	14.41±1.15[b]

[a,b,c] Values in a row with no common superscripts differ significantly ($P < 0.05$); C, control; A, Arginine; G, glutamine; T, tryptophan.

TABLE 2.6 Effect of different amino acid on meat quality of broiler chicks (Exp 1)

Meat quality properties	Treatment							
	Breast muscle				Thigh muscle			
	C	A	G	T	C	A	G	T
pH	5.94 ±0.2[b]	6.02±0.2[b]	5.97 ±0.32[b]	6.43 ±0.4[a]	6.35±0.1	6.32±0.3	6.26±0.4	6.38±0.2
DM (%)	32.56±1.2	32.70±0.5	32.45±1.21	32.46±2.2	30.39±1.2	28.86±0.9	31.13±1.5	30.79±1.9
Moisture (%)	67.43±1.2	67.29±0.5	67.54±1.21	67.53±2.2	69.60±1.2	70.13±0.9	68.86±1.5	69.20±1.9
L* values	53.6 ±3.1[a]	54.00±2.9[a]	53.06±4.4[a]	51.67±2.1[b]	58.38±8.6[a]	57.02±4.6[ab]	53.62±3.8[c]	55.03±4.1[bc]
a* values	2.18 ±0.9[b]	3.23±1.8[ab]	3.52±2.0[ab]	4.27±1.6[a]	8.43±2.5	8.84±3.4	9.49±3.2	8.64±3.5
b* values	6.54 ±1.6[a]	6.38±2.8[ab]	5.70 ±2.0[b]	4.31 ±1.3[c]	7.95 ±3.4[a]	7.49±2.3[ab]	7.25±2.1[ab]	6.56±1.9[b]

[a,b,c] Values in a row with no common superscripts differ significantly ($P < 0.05$); C, control; A, Arginine; G, glutamine; T, tryptophan.

TABLE 2.7 Effect of different amino acids on the performance of broiler chicks (Exp 2)

Treatment	C	A	G	T	SEM[1]	P value
Body weight (g)						
At 14 d	300.49±14.79[b]	368.95±7.47[a]	362.50±11.31[a]	386.40±9.24[a]	8.51	0.0001
At 28 d	965.72±34.87[b]	1106.35±27.80[ab]	1140.13±28.28[a]	1117.06±70.69[ab]	24.26	0.047
At 42 d	1949.14±58.73[b]	2135.56±40.23[a]	2199.66±52.25[a]	2225.22±93.91[a]	36.87	0.023
Weight gain (g)						
0-14 d	268.72±15.38[b]	328.32±7.53[a]	321.95±11.38[a]	343.16±8.83[a]	8.50	0.0002
15-28 d	665.24±21.73[b]	737.41±22.33[a]	777.63±18.61[a]	730.66±62.73[a]	17.62	0.034
29-42 d	975.42±36.27[b]	1029.21±35.59[ab]	1059.53±23.50[ab]	1108.16±20.64[a]	17.16	0.035
0-42 d	1909.37±58.47[b]	2094.93±40.27[a]	2159.11±52.33[a]	2181.97±92.71[a]	37.78	0.022
Feed intake (g)						
0-14 d	410.05±15.57[b]	522.77±10.29[a]	511.23±14.98[a]	549.25±20.23[a]	11.46	0.027
15-28 d	1050.65±39.01[b]	1249.13±48.97[a]	1293.27±44.14[a]	1255.90±123.71[a]	43.90	0.014
29-42 d	1701.47±29.32[b]	1863.98±58.73[ab]	1958.26±51.47[a]	2003.21±99.22[a]	38.65	0.018

TABLE 2.7 Continued

0-42 d	3162.16±71.61[b]	3635.88±78.23[a]	3762.76±93.54[a]	3808.36±229.35[a]	91.06	0.002
Feed conversion ratio						
0-14 d	1.526±0.08	1.593±0.04	1.586±0.11	1.601±0.07	0.04	0.298
15-28 d	1.579±0.03[b]	1.694±0.06[a]	1.663±0.05[a]	1.718±0.09[a]	0.03	0.019
29-42 d	1.753±0.06	1.813±0.04	1.848±0.02	1.806±0.08	0.02	0.671
0-42 d	1.619±0.03	1.703±0.03	1.741±0.04	1.745±0.05	0.02	0.137

[1]Pooled standard error means.

[a,b] Values in a row with no common superscripts differ significantly ($P < 0.05$); C, control; A, Arginine; G, glutamine; T, tryptophan.

TABLE 2.8 Effect of different amino acid on serum immunoglobulins G and A concentrations of broiler chicks (Exp 2)

Treatment	N	A	G	T	SEM[1]	P value
IgG (mg/ml)						
2nd week	196.16	213.79	192.23	158.96	14.27	0.635
IgA (µg/ml)						
2nd week	367.06	366.903	297.709	316.96	15.10	0.248

[1]Pooled standard error means.

TABLE 2.9 Effect of different amino acid on carcass characteristics of broiler chicks (Exp 2)

Parameter	N	A	G	T
Liveweight (g)	1950±321[b]	2160±193[a]	2206±273[a]	2217±347[a]
Dressing yield, %	71.94±1.79	72.27±3.0	71.76±2.26	71.10±2.84
Warm carcass weight (g)	1352±229[b]	1492±138[a]	1521±203[a]	1517±270[a]
Chilled carcass weight (g)	1405±243[b]	1552±149[a]	1585±211[a]	1581±279[a]
Breast muscle weight (g)	356±83	394±8	401±72	409±89
Breast muscle (%)	25.03±2.51	25.31±2.00	25.22±1.88	25.73±1.91
Thigh muscle weight (g)	214±38	231±28	231±34	234±43
Thigh muscle (%)	15.28±1.33	14.88±1.05	14.58±0.84	14.82±1.04

[a,b] Values in a row with no common superscripts differ significantly ($P < 0.05$); C, control; A, Arginine; G, glutamine; T, tryptophan.

TABLE 2.10 Effect of different amino acid on meat quality of broiler chicks (Exp 2)

Meat quality properties	Treatment							
	Breast muscle				Thigh muscle			
	C	A	G	T	C	A	G	T
pH	5.71±0.16	5.72±0.18	5.68±0.15	5.78±0.15	5.90±1.66^{ab}	6.07±0.22^{a}	5.81±0.24^{b}	5.69±0.43^{b}
DM	33.64±0.92	33.24±1.10	33.22±1.02	33.13±0.62	33.28±0.82	33.35±1.10	32.72±1.27	33.31±1.42
Moisture	66.35±0.92	66.75±1.10	66.77±1.02	66.86±0.62	66.71±0.82	66.62±1.10	67.27±1.27	66.68±1.42
L* values	52.80±3.19^{a}	51.62±2.34^{b}	52.57±2.49^{a}	51.04±2.66^{b}	57.68±5.62	58.38±5.00	59.73±5.26	59.05±4.70
a* values	3.78±1.93^{ab}	4.51±2.34^{a}	4.36±2.03^{a}	3.05±2.08^{b}	10.88±4.22	10.34±3.67	10.60±3.37	10.52±3.53
b* values	9.34±1.69^{a}	8.77±1.10^{b}	8.99±1.76^{ab}	6.99±1.61^{b}	11.18±3.59^{ab}	10.85±2.70^{ab}	11.70±2.69^{a}	10.21±2.46^{b}

^{a,b} Values in a row with no common superscripts differ significantly ($P < 0.05$); C, control; A, Arginine; G, glutamine; T, tryptophan.

CHAPTER III

ZINC BIOAVAILABILITY OF VARIOUS ZINC AMINO ACID CHELATES IN YOUNG BROILER CHICKENS

Abstract

Three experiments were conducted to evaluate bioavailability of Zn from various Zn amino acid chelates and their tissue accretion until 3 weeks of age. Each experiment used 447, 1-d-old broiler chicks and fed experimental diets for 21 d. In Exp 1, dietary treatments were NC, negative control diet without Zn supplement; PC, positive control diet with 40 mg Zn/kg from Zn-sulfate; Zn-gly, NC diet + 40 mg Zn/kg from Zn glycine; Zn-met-gly, NC diet + 40 mg Zn/kg from Zn methionyl-glycine. In Exp 2, dietary treatments were CON, a basal diet with 40 mg Zn/kg from Zn-nitrate; Zn-gly1, a basal diet with 40 mg Zn/kg from Zn glycine; Zn-gly2, a basal diet with 40 mg Zn/kg from Zn glycinyl-glycine; and Zn-arg, a basal diet with 40 mg Zn/kg from Zn arginine. In Exp 3, dietary treatments were ZS, a basal diet with 60 mg Zn/kg from Zn-sulfate; ZA1, a basal diet with 60 mg Zn/kg from Zn-sulfate and Zn-methionyl-glycine at 2:1 ratio; ZA2, a basal diet with 60 mg Zn/kg from Zn-sulfate and Zn-methionyl-glycine at 1:2 ratio; and ZAA, a basal diet with 60 mg Zn/kg from Zn-methionyl-glycine. Growth performance was not affected by dietary Zn sources in all experiments. In Exp 1, birds fed a diet with inorganic Zn or Zn-AA had a higher ($P < 0.03$) Zn content of whole body than in fed without Zn supplementation at d 5, 7, 14 and 21. Zn-met-gly group had a higher ($P=0.048$) Zn bioavailability than PC group during the d 7-14. During the d 0 to 14, Zn bioavailability of chicks fed Zn-met-gly diet was higher ($P < 0.05$) than that of chicks fed

PC diet. During the entire 21-d, Zn bioavailability of chicks fed Zn-AA diets was higher than that of chicks fed PC diet ($P < 0.05$). In Exp 2, whole body Zn contents were higher ($P < 0.05$) in birds fed Zn-gly2 diet than in those fed CON or Zn-gly1 diets at d 1. At d 7, Zn contents of the birds fed Zn-gly1 diet were increased ($P < 0.05$) compared to the birds fed CON diet. At the final day of experiment, birds fed Zn-arg diet had higher ($P < 0.05$) Zn content in whole body than those fed other diets. In Exp 3, birds fed ZS or ZAA diets ingested more Zn ($P < 0.05$) than those fed ZA1 or ZA2 diets for d 0 to 7. Also, birds fed ZS diet ingested more Zn ($P < 0.05$) than those fed ZA1 or ZA2 diets for d 0-14. Zn retention ratio for d 0 to 1 was higher ($P < 0.05$) in chicks fed ZA1 diet than in those fed ZAA diet. Also, chicks fed ZA2 diet had the highest ($P < 0.05$) Zn retention ratio for d 0 to 3 compared to those fed ZAA or ZS diets. The ZAA had a greater ($P < 0.05$) Zn bioavailability than ZS and ZA1 during d 7 to 14. The ZAA had a greater ($P < 0.05$) Zn bioavailability than other treatment groups during the d 0 to 14. In conclusion, this study shows that Zn from Zn AA chelates is more bioavailable than Zn from inorganic source by broiler chickens during the starter period (d 0 to 14) when Zn was supplemented at 40 mg/kg to a basal diet containing 28 mg endogenous Zn/kg.

Key words: Bioavailability, Broiler, Growth, Zn Amino Acid Chelates

Introduction

Inorganic form of trace minerals have traditionally been used in poultry diets. Among these trace minerals, Zn and Cu have been used at sub-therapeutic levels for their beneficial effects on growth (Jenkins et al., 1970; Pimentel et al., 1991a; Edwards and baker, 2000). Typical commercial broiler diets include 100 to 120 mg/kg of Zn (Dozier et

al., 2003) which is much higher than Zn requirement (40 mg/kg, NRC, 1994). However, it has been shown that more than 90% of inorganic form of dietary Zn is not bioavailable but excreted in a commercial production condition (Mohanna and Nys, 1997 and 1999). Thus, sub-therapeutic level supplementation of inorganic Zn in poultry diets may cause soil phytotoxicity.

Use of organic form of minerals has been introduced for their possible benefits on high bioavailability. The American Association of Feed Control Officials (AAFCO, 1997) defines organic trace minerals as organically-bound mineral compounds. A metal amino acid (AA) complex results from complexing a soluble metal salt with an AA in a ratio of 1 mol of metal to 1 mol of AA whereas a metal AA chelate is a product resulting from the reaction of soluble metal salt with AA in the ratio of 1 mol of metal to 1 to 3 mol of AA to form covalent bonds with molecular weight less than 800. Zinc from AA chelates has been reported to be more bioavailable than Zn from inorganic sources (Wedekind et al., 1992; Hudson et al., 2005) whereas some others did not show any benefits (Pimentel et al., 1991b; Kidd et al., 1993). Wedekind et al. (1992) determined on the basis of tibia Zn content that Zn in Zn methionine had a considerably higher bioavailability than Zn sulfate fed corn-soybean meal diets for chicks. Hudson et al. (2005) also reported broilers fed diets with Zn AA complex had a higher body weight than broilers provided diets with Zn sulfate at d 17. However, there is a limited information showing bioavailability of various Zn AA chelates. The purpose of this study was to evaluate the efficacy of Zn from four types of Zn AA chelates and combinational use with inorganic Zn for their bioavailabilities and effects on growth of broiler chickens.

Materials and methods

Zn sources

Inorganic Zn sources were Zn sulfate (35.7 % Zn, Terramicro Nutrients, Henan, China) for Exp 1 and 3, and Zn nitrate (24.3 % Zn, Sigma, St. Louis, MO 63178, USA) for Exp 2. Zinc amino acid chelates (Albion Advanced Nutrition, Clearfield, UT, US) used in this study were Zn glycine (10.9% and 26.2% Zn for Exp 1 and 2, respectively), Zn glycinyl glycine (22.3 % Zn for Exp 2), Zn methionyl glycine (20.4 % Zn for Exp 1 and 3), and Zn arginine (12.8 % Zn for Exp 2).

Birds and dietary treatments

Three experiments were conducted in this study. Each experiment used 447, 1-d-old, Fast Cornish male broiler (Privett Hatchery, Portales, NM, US) for 21-d feeding period. Fifteen birds were sacrificed at d 0 and ground for carcass sampling. Remaining 432 birds were allotted to 4 dietary treatments. There were 6 replicates per treatment and 18 birds per cage at the beginning. In Exp 1, dietary treatments were consisted of NC, a basal diet without Zn supplementation; PC, a basal diet with 40 mg Zn/kg from Zn sulfate; Zn-gly, a basal diet with 40 mg Zn/kg from Zn glycine; and Zn-met-gly, a basal diet with 40 mg Zn/kg from Zn methionyl glycine. In Exp 2, dietary treatments were consisted of CON, a basal diet with 40 mg Zn/kg from Zn-nitrate; Zn-gly1, a basal diet with 40 mg Zn/kg from Zn glycine; Zn-gly2, a basal diet with 40 mg Zn/kg from Zn glycinyl glycine; and Zn-arg, a basal diet with 40 mg Zn/kg from Zn arginine. In Exp 3, dietary treatments were consisted of ZS, a basal diet with 60 mg Zn/kg from Zn sulfate; ZA1, a basal diet with 60 mg Zn/kg from Zn sulfate and Zn methionyl glycine at 2:1 ratio; ZA2, a basal diet with 60 mg Zn/kg from Zn sulfate and Zn methionyl glycine at 1:2 ratio; and ZAA, a basal diet with 60 mg Zn/kg from Zn methionyl glycine.

The basal diets contained 3.2 Mcal ME/kg, 23.1% CP, 1.30% lysine, and 0.50% methionine (Table 3.1). Birds had an access to feed and water ad libitum during 21 d feeding period. Birds were housed in a stainless steel brooder cage battery with a heater (Petersime Incubator Co., Gettysburg, OH, US) for each cage. The temperature of each cage was maintained at 32 ± 1^{o}C during the first 7 days and decreased by 2^{o}C weekly. Illumination was maintained for 24 hours for the first 4 days and 12 hours thereafter. All experimental procedures using animals were approved by the Institutional Animal Use and Care Committee at Texas Tech University.

Sample collections and chemical analysis

Broiler chickens and feeders were weighed on d 1, 3, 5, 7, 14, and 21 to calculate average daily gain, average daily FI, and gain:feed ratio. Total excreta were collected on d 1, 3, 5, 7, 14, and 21, dried under 60^{o}C in a drying oven, and ground to determine Zn content. Groups of 3 birds were randomly selected and euthanized at d 1, 3, 5, 7, 14, and 21. Whole body from each group (3 birds) were ground together using a waring blender, sampled, freeze dried, and ground to determine Zn content in whole body. Experimental diets were also sampled to determine Zn content. The Zn content in feed was determined by collecting a 2-g sample from the basal diet and finely ground to digest at 120℃ using 5 mL of concentrated HNO_3 for 1 h using the Tecator digestion system-2000 (Digestion system, Tecator 2000, Perstorp Analytical Tecator, Hoganas, Sweden). Content of Zn was determined by ICP (ICP, Perkin Elmer, Shelton, CT, US). Bioavailability of Zn (%) was calculated as [(Zn intake – Zn excretion) / Zn intake] × 100.

Statistical analysis

The data from all experiments were subjected to ANOVA as a completely randomized design using the general linear model (GLM) procedure of SAS (1996) with

a cage as the experimental unit. Main effect was the treatment. Tests for least significant differences were applied with a level of statistical significance of 5%. Orthogonal contrasts were also used to compare 1) NC vs. PC, 2) NC vs. Zn-AA, 3) PC vs. Zn-gly, 4) PC vs. Zn-AA, 5) PC vs. Zn-gly, and 6) PC vs. Zn-met-gly in Exp 1.

Results

Experiment 1

The initial BW of birds was not different among treatments indicating that the allotment was done correctly (Table 3.2). The ADG of birds during 21-d was 29.0 ± 1.0 g/d, indicating that birds grew normally during the 21-d period (NRC, 1994). The ADG of birds did not differ among treatments during wk 1, 2, 3, and the entire 3 wk period. The ADFI of birds did not differ among treatments during wk 1, 2, 3, and the entire 3 wk period. Gain:feed ratio of birds did not differ among treatments during the 21-d feeding period.

Whole body Zn contents did not differ among treatments until d 3 (Table 3.3). However, birds fed diet with Zn sulfate, Zn glycine, and Zn methionyl glycine had a higher ($P < 0.03$) whole body Zn content than those fed without Zn supplementation on d 5, 7, 14 and 21. There was no difference on whole body Zn content between birds in PC and Zn-gly.

Bioavailability of Zn did not differ among treatments during wk 1 (Table 3.4). However, Zn bioavailability of Zn-met-gly was greater ($P < 0.05$) than that of PC and Zn bioavailability of Zn-gly and Zn-met-gly together tended to be greater ($P < 0.10$) than that of PC during wk 2. There was no difference in Zn bioavailability among treatments during wk 3. During wk 1 to 2, Zn bioavailability of Zn-met-gly was greater ($P < 0.05$)

than that of PC and Zn bioavailability of Zn-gly and Zn-met-gly together tended to be greater ($P < 0.10$) than that of PC. During the entire 21-d, Zn bioavailability of Zn-met-gly and Zn-gly together was greater ($P < 0.05$) than that of PC.

Experiment 2

The initial BW of birds was not different among treatments indicating that the allotment was done correctly (Table 3.5). The ADG of birds during 21-d was 34.8±2.4 g/d, indicating that birds grew normally during the 21-d period (NRC, 1994). The ADG of birds were not different among treatments during wk 1, 2, 3, and the entire 3 wk period. The ADFI of birds did not differ among treatments during wk 1, 2, 3, and the entire 3 wk period. Gain:feed ratio of birds did not differ among treatments during the 21-d feeding period.

Zinc contents were greater ($P < 0.05$) in birds of Zn-gly2 than those of CON and Zn-gly1 on d 1 (Table 3.6). However, there were no differences in whole body Zn content among treatments on d 3 and 5. On d 7, Zn content in birds of Zn-gly1 was greater ($P < 0.05$) than those of CON. Whole body Zn content did not differ among Zn-gly1, Zn-gly2, and Zn-arg on d 7. Birds in Zn-arg had greater ($P < 0.05$) Zn content than those in other treatments on d 21. Bioavailability of Zn did not differ among treatments during wk 1, 2, 3, and the entire 3 wk period (Table 3.7).

Experiment 3

The initial BW of birds was not different among treatments, indicating that the allotment was done correctly (Table 3.8). The ADG of birds during 21-d period was 38.1±1.7 g/d, indicating that birds grew normally during the 21-d period (NRC, 1994). The ADG of birds were not different among treatments during wk 1, 2, 3, and the entire 3

wk period. The ADFI of birds did not differ among treatments during wk 1, 2, 3, and the entire 3 wk period. Gain:feed ratio of birds did not differ among treatment during the 21-d feeding period, except for the d 1.

Whole body Zn contents were greater ($P < 0.05$) in birds of ZS than those of ZAA on d 1 (Table 3.9). Whole body Zn content did not differ among treatments on d 3, 5, 7, 14, and 21. Bioavailability of Zn in ZAA was greater ($P < 0.05$) than ZS and ZA1 during wk 2 (Table 3.10). Bioavailability of Zn in ZAA was greater ($P < 0.05$) than other treatment groups during wk 1 to 2. However, when considering the entire period, Zn bioavailability did not differ among treatment.

Discussion

Zinc bioavailability of Zn-met-gly was consistently higher than Zn from other sources during d 0 to 14 of age. This result is similar to Lee et al. (2001) who reported that feces collected from birds fed diets with either metal-AA chelates or complexes contained lower Zn than those fed diets with Zn-sulfate when 80 mg/kg Zn were supplemented to the diets. However, Dozier et al. (2003) reported birds provided diets supplemented with Zn-sulfate had a lower amount of Zn output than that of birds given feed containing Zn-AA chelates when 40, 80 and 120 mg/kg Zn were supplemented to the diets. Results from Dozier et al. (2003) are similar to those found in Exp 2 where Zn bioavailability did not differ between Zn-glycine chelates and inorganic Zn. This inconsistency among studies may be due to different sources of Zn amino acid chelates used and different levels of Zn supplemented to diets. It is believed that endogenous Zn in typical feedstuffs are phytic acid bound (Oberleas et al., 1966). Bioavailability of Zn in phytic acid is shown to be poor (Oberleas et al., 1966) and thus not much of endogenous

Zn would be bioavailable depending on the composition of basal diets. Thus, types of basal diets would also affect bioavailability of Zn supplemented to basal diets.

Optimal requirement of Zn for broiler chickens is 40mg/kg (0 to 6 wk of age; NRC, 1994). On the other hand, INRA (1989) recommends dietary supplementations of 40 and 20 mg Zn/kg to basal diets containing 30 to 40 mg Zn/kg in growing and finishing diets, respectively. Zinc requirement for young broiler chickens is higher than mature birds due to growth performance, normal bone, skin and feather development (Liao et al., 2013). In this study, endogenous Zn in the basal diets was 28 mg/kg which provides 140% and 210% of Zn requirements for starter and finisher, respectively. Supplementation of 40 (Exp 1 and 2) or 60 (Exp 3) mg/kg additional Zn to the basal diets may partly explain why benefits of Zn AA chelates in improving Zn bioavailability were obtained only in starter period. In this study, it was hypothesized that 40 or 60 mg/kg Zn from various Zn sources would have beneficial effects on performance and which correspond with NRC (1994) requirements. The current NRC Zn requirement for chicks (40 mg of Zn/kg from 0 to 3 wk) was based on the level of Zn needed to maximize growth. Recently Huang et al. (2007) examined the effects of various dietary Zn levels and mentioned that the requirement for early chick growth was satisfied when chicks were fed diets containing 40 mg of Zn/kg.

Of the supplemental zinc feed, ZnO is less bioavailable for poultry than feed-grade Zn sulfate (Sandoval et al., 1997; Edwards and Baker, 2000; Fosmire, 1990; Wedekind and Baker, 1990). Therefore, in the present study, Zn sulfate and Zn nitrate were used as inorganic sources of Zn. Supplementation of various dietary Zn sources used in this study did not affect growth performance of broiler chickens during 21 d

experimental period. Amounts of Zn in the diets used in this study were 170 to 220 % higher than Zn requirements for broiler chickens (NRC, 1994) partly explain why Zn supplementation did not affect the growth performance of broiler chickens in this study. Growth is a measure of utilization but is generally considered as a fairly unresponsive criterion for many mineral elements (Ammerman, 1995). Several studies have been conducted to determine the supplemental effect of various Zn sources on growth performance in broilers, but the results were inconsistent. Pimentel et al. (1991a) reported dietary Zn methionine complex supplementation at 8 to 88 mg/kg did not affect growth performance of broilers compared to dietary Zn oxide supplementation at 8 to 88 mg/kg which provides similar results from Mohanna and Nys (1999) where they reported supplementation of Zn methionine complex at 30 to 60 mg/kg had no effect on growth performance compared to Zn sulfate supplementation at xx mg/kg for broiler chickens when those feed were fed from 5 to 21 d of age. Dozier et al. (2003) also found no differences of weight gain in broilers fed the diets with Zn sulfate, Zn AA chelates, or the mixtures of both Zn sulfate and Zn AA chelates at 40 to 120 mg Zn/kg. However, Hudson et al. (2005) reported broilers fed diets with mixture of Zn sulfate and Zn AA complex had a higher body weight than broilers provided diets with Zn sulfate when Zn supplementation were 140 mg/kg. These inconsistencies may be due to the difference in Zn content in diets. When a typical broiler diet is based on corn and soybean meal, xxx, and xxx, Zn contents often meet the requirements for broiler chickens (40 mg/kg; NRC, 1994), and additional Zn to these diets with adequate amount of Zn may not improve weight gain of broiler chickens (Wedekind et al., 1992). Growth response to Zn was observed when total dietary Zn contents were increased from 10 to 30 mg/kg (Roberson

and Shaible, 1958; Wedekind et al., 1992), and 13 to 21 mg/kg (Aoyagi and Baker, 1993). Other researchers also confirmed the positive effect of Zn on growth performance when total dietary Zn contents were 35 mg/kg (Watkins and Southern, 1993) and 40 mg/kg (Yi et al., 1996).

In this study, dietary supplementation of 40 or 60 mg Zn/kg from Zn AA chelates did not increase whole body Zn content compared to Zn supplementation from inorganic Zn sources. These results are similar to those from Mohanna and Nys (1999) who reported dietary Zn-methionine complex supplementation at 10 to 40 mg/kg to the diets with 20 mg/kg endogenous Zn did not affect to whole body Zn content compared to Zn sulfate. However, there is a limited research conducted to estimate whole body Zn content when chicks were fed different Zn sources. Pimentel et al. (1991b) found no differences of tibia Zn concentrations between birds fed diets with Zn oxide or Zn methionine, whereas pancreatic Zn concentrations were greater in chicks fed Zn methionine than chicks fed Zn oxide. Lee et al. (2001) observed a higher Zn concentration in serum of birds fed diets with 40 mg/kg Zn from metal AA chelates compared to those fed diets with inorganic Zn sources. This study did not evaluate Zn contents in various tissues affected by dietary supplementation of various Zn sources. It warrants further studies to see if supplementation of Zn AA chelates would improve Zn retention to specific tissues such as bone or muscle.

Conclusions

Based on three experiment, this study shows that Zn from Zn methionyl glycine was more bioavailable to broiler chickens than Zn from Zn sulfate especially during starter period (d 0 to 14) when Zn supplementation was 40 mg/kg to a basal diet with 28

mg/kg of endogenous Zn mainly from corn and soybean meal. However, this benefit disappeared when broiler chickens are older than 14 d of age. There was no improvement in Zn bioavailability when two Zn sources (i.e., Zn AA chelates and Zn sulfate) were supplemented together. Supplementation of Zn from Zn AA chelates may allow the nutritionists to reduce dietary Zn supplementation levels to meet the Zn requirement especially for young chickens.

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APPENDIX II

TABLE 3.1 Composition of the basal diet used in Exp 1, 2, and 3

Ingredients	%
Corn, yellow	55.64
Soybean meal, dehulled	31.00
Spray dried animal plasma	4.65
Soybean oil	4.95
Dicalcium phosphate	1.50
Limestone	1.50
Salt	0.18
DL-Met	0.18
Trace mineral mix[1]	0.17
Vitamin mix[2]	0.12
Choline chloride	0.01
Zn supplement and cornstarch[3]	0.10
Calculated composition	
ME, Mcal/kg	3.17

TABLE 3.1 Continued

CP, %	23.11
Lysine, %	1.30
Methionine, %	0.50
Zinc, mg/kg	28.0

[1]The trace mineral mix provided the following per kilogram of complete diet: 1.93 mg manganese as manganese sulfate, 2.76 mg iron as iron sulfate, 0.39 mg copper as copper sulfate, 11.2 mg magnesium as magnesium oxide, 0.005 mg EDDI and 0.75 mg selenium as sodium selenite.

[2]At 0.12% of diet, the vitamin premix provided 4,582 IU vitamin A as vitamin A acetate, 529 IU vitamin D_3, 40 IU vitamin E, 1.8 mg vitamin K as menadione sodium bisulfate, 34 g vitamin B12, 8.8 mg riboflavin, 28 mg α-pantothenic acid as calcium pantothenate, 35 mg niacin, 0.2 mg biotin, 1.1 mg folic acid, 3.5 mg pyridoxine and 3.5 mg thiamine.

[3]Zn supplements were added replacing equivalent amount of cornstarch. In Exp 1, 0 ppm Zn was supplemented to NC diet; 40 ppm Zn from Zn-sulfate was supplemented to PC diet; 40 ppm Zn from Zn-glycine was supplemented to Zn-gly diet; and 40 ppm Zn from Zn-methionyl-glycine was supplemented to Zn-met-gly diet. In Exp 2, 40 ppm of Zn from Zn-nitrate was supplemented to CON diet; 40 ppm of Zn from Zn-glycine was supplemented to Zn-gly1 diet; 40 ppm of Zn from Zn-glycinyl-glycine was supplemented to Zn-gly2 diet; and 40 ppm of Zn from Zn-arginine was supplemented to Zn-arg diet. In Exp 3, 60 ppm Zn from Zn-sulfate was supplemented to ZS diet; 60 ppm Zn from Zn-sulfate and Zn-methionyl-glycine were supplemented at 2:1 ratio to ZA1 diet; 60 ppm Zn from Zn-sulfate and Zn-methionyl-glycine were supplemented at 1:2 ratio to ZA2 diet; 60 ppm Zn from Zn-methionyl-glycine was supplemented to ZAA diet.

TABLE 3.2 Growth performance of chicks fed the diets with Zn sulfate or Zn amino acid chelates (Zn-AA) for 21-d (Exp 1)

Item	NC[1]	PC[1]	Zn-AA		SEM[2]	Probabilities		
			Zn-gly[1]	Zn-met-gly[1]		NC vs. PC	NC vs. Zn-AA	PC vs. Zn-gly
Initial BW, g/chick	32.3	32.3	32.2	32.3	0.1			
Average daily gain, g/d								
0 to 1 d	9.3	10.4	9.2	8.1	0.7	0.583	0.712	0.562
1 to 3 d	10.0	9.8	10.1	11.0	0.2	0.694	0.324	0.613
3 to 5 d	15.3	15.5	14.4	14.7	0.3	0.753	0.343	0.184
5 to 7 d	22.0	21.8	22.0	21.3	0.3	0.812	0.602	0.851
Wk 1	14.8	15.0	14.6	14.6	0.2	0.856	0.613	0.523
Wk 2	38.2	37.7	38.9	36.4	0.7	0.803	0.761	0.574

TABLE 3.2 Continued

Wk 3	27.1	35.6	36.2	38.4	2.7	0.324	0.184	0.943
1 to 2 Wk	26.5	26.3	26.7	25.5	0.4	0.831	0.593	0.991
1 to 3 Wk	26.7	29.4	29.9	29.8	1.0	0.392	0.251	0.882
Average daily feed intake, g/d								
0 to 1 d	5.2	5.6	5.2	4.9	0.2	0.372	0.693	0.421
1 to 3 d	9.9	10.0	9.9	9.4	0.2	0.834	0.492	0.863
3 to 5 d	19.3	18.2	17.7	17.7	0.3	0.313	0.091	0.594
5 to 7 d	24.4	25.1	24.5	23.8	0.3	0.321	0.794	0.442
Wk 1	16.0	16.0	15.6	15.2	0.2	0.993	0.202	0.464
Wk 2	44.3	45.9	46.4	44.5	0.6	0.424	0.504	0.781

TABLE 3.2 Continued

Wk 3	67.4	82.4	80.8	76.7	3.0	0.112	0.161	0.864
1 to 2 Wk	30.2	31.0	31.0	29.9	0.3	0.401	0.743	0.932
1 to 3 Wk	42.6	48.1	47.6	45.5	1.1	0.114	0.181	0.883
Gain:feed ratio								
D 0 to 1	1.78	1.86	1.76	1.67	0.12	0.713	0.774	0.711
D 1 to 3	1.01	0.98	1.02	1.18	0.04	0.701	0.223	0.662
D 3 to 5	0.79	0.85	0.81	0.83	0.01	0.172	0.512	0.281
D 5 to 7	0.90	0.87	0.90	0.89	0.01	0.104	0.551	0.223
Wk 1	0.92	0.93	0.93	0.96	0.01	0.732	0.193	0.981
Wk 2	0.86	0.82	0.84	0.82	0.01	0.181	0.202	0.104

TABLE 3.2 Continued

Wk 3	0.40	0.43	0.45	0.50	0.03	0.333	0.104	0.822
1 to 2 Wk	0.87	0.85	0.86	0.85	0.05	0.792	0.953	0.761
1 to 3 Wk	0.62	0.61	0.63	0.66	0.01	0.921	0.392	0.634

[1]NC = a basal diet without Zn supplementation; PC = a basal diet with 40 ppm Zn from Zn-sulfate; Zn-gly = a basal diet with 40 ppm Zn from Zn-glycine; and Zn-met-gly = a basal diet with 40 ppm Zn from Zn-methionyl-glycine.

[2]Pooled standard error means.

TABLE 3.3 Whole body Zn content of chicks fed the diets with Zn sulfate or Zn amino acid chelates (Zn-AA) for 21-d (Exp 1)

Item	NC[1]	PC[1]	Zn-AA		SEM[2]	Probabilities		
			Zn-gly[1]	Zn-met-gly[1]		NC vs. PC	NC vs. Zn-AA	PC vs. Zn-gly
Zn, mg/chick								
1 d	0.67	0.74	0.87	0.64	0.01	0.978	0.937	0.876
3 d	0.87	1.08	0.87	0.64	0.04	0.644	0.488	0.323
5 d	1.28[a]	1.59[b]	1.58[b]	1.56[b]	0.04	0.016	< 0.001	0.418
7 d	1.99[a]	2.59[b]	2.86[b]	2.80[b]	0.04	0.003	0.002	0.851
14 d	5.40[a]	7.63[b]	7.95[b]	7.56[b]	0.10	0.024	0.001	0.281
21 d	10.78[a]	16.27[b]	15.85[b]	15.61[b]	0.24	< 0.001	< 0.001	0.391

[1]NC = a basal diet without Zn supplementation; PC = a basal diet with 40 ppm Zn from Zn-sulfate; Zn-gly = a basal diet with 40 ppm Zn from Zn-glycine; and Zn-met-gly = a basal diet with 40 ppm Zn from Zn-methionyl-glycine.

TABLE 3.3 Continued

[2]Pooled standard error means.

[ab]Means within a row lacking a common superscript differ ($P < 0.05$).

TABLE 3.4 Bioavailability of Zn by chicks fed the diets with Zn sulfate or Zn amino acid chelates (Zn-AA) for 21-d (Exp 1)

Item, %		Zn-AA		SEM[2]	Probabilities		
	PC[1]	Zn-gly[1]	Zn-met-gly[1]		PC vs. Zn-AA	PC vs. Zn-gly	PC vs. Zn-met-gly
Wk 1	62.4	66.4	71.3	3.01	0.261	0.534	0.186
Wk 2	48.3[a]	60.1[ab]	70.1[b]	5.13	0.070	0.237	0.048
Wk 3	47.2	52.2	44.4	3.24	0.906	0.745	0.599
Wk 1 to 2	51.4[a]	61.3[ab]	70.2[b]	4.21	0.057	0.222	0.036
Wk 1 to 3	49.3[a]	56.2[ab]	55.1[b]	2.34	0.041	0.072	0.068

[1]NC = a basal diet without Zn supplementation; PC = a basal diet with 40 ppm Zn from Zn-sulfate; Zn-gly = a basal diet with 40 ppm Zn from Zn-glycine; and Zn-met-gly = a basal diet with 40 ppm Zn from Zn-methionyl-glycine.

[2]Pooled standard error means.

[ab]Means within a row lacking a common superscript differ ($P < 0.05$).

TABLE 3.5 Growth performance of chicks fed different Zn amino acid chelates for 21-d (Exp 2)

Item	CON1	Zn-gly1[1]	Zn-gly2[1]	Zn-arg[1]	SEM[2]
Initial BW, g/chick	32.4	32.4	32.4	32.4	0.1
Average daily gain, g/d					
D 0 to 1	12.5	12.6	12.5	12.7	0.5
D 1 to 3	10.2	10.5	9.7	10.4	0.4
D 3 to 5	17.4	17.0	16.9	16.9	0.6
D 5 to 7	21.5	22.5	22.3	22.8	0.4
Wk 1	15.8	16.1	15.8	16.1	0.4
Wk 2	34.1	36.7	35.7	35.5	1.8
Wk 3	46.2	54.1	55.2	56.2	5.9
Wk 1 to 2	25.8	27.3	26.6	26.7	1.0
Wk 1 to 3	32.0	35.6	35.6	35.9	2.4
Average daily feed intake, g/d					
0 to 1 d	7.1	6.7	6.8	6.6	0.3
1 to 3 d	10.7	11.4	10.5	10.8	0.3
3 to 5 d	20.7	20.3	20.0	19.9	0.6

TABLE 3.5. Continued

5 to 7 d	23.8	24.0	24.4	25.2	0.5
Wk 1	16.8	16.9	16.6	16.9	0.3
Wk 2	42.7	44.8	43.1	43.5	1.1
Wk 3	68.4	77.0	77.3	78.0	5.3
1 to 2 Wk	30.2	31.3	30.4	30.7	0.6
1 to 3 Wk	42.6	46.2	45.7	46.1	2.0
Gain:feed					
0 to 1 d	1.77	1.90	1.84	1.95	0.06
1 to 3 d	0.95	0.92	0.93	0.97	0.03
3 to 5 d	0.84	0.83	0.85	0.85	0.02
5 to 7 d	0.90	0.94	0.92	0.90	0.01
Wk 1	0.94	0.95	0.95	0.95	0.01
Wk 2	0.79	0.82	0.83	0.81	0.03
Wk 3	0.65	0.69	0.71	0.71	0.04
1 to 2 Wk	0.85	0.87	0.88	0.87	0.02
1 to 3 Wk	0.74	0.77	0.78	0.78	0.02

TABLE 3.5 Continued

[1]CON = a basal diet with 40 ppm of Zn from Zn-nitrate; Zn-gly1 = a basal diet with 40 ppm of Zn from Zn-glycine; Zn-gly2 = a basal diet with 40 ppm of Zn from Zn-glycinyl-glycine; and Zn-arg = a basal diet with 40 ppm of Zn from Zn-arginine.

[2]Pooled standard error means.

TABLE 3.6 Whole body Zn contents of chicks fed different Zn amino acid chelates for 21-d (Exp 2)

Item	CON[1]	Zn-gly1[1]	Zn-gly2[1]	Zn-arg[1]	SEM[2]
Zn, mg/chick					
1 d	0.40[b]	0.46[b]	0.75[a]	0.60[ab]	0.10
3 d	0.50	0.66	0.59	0.49	0.07
5 d	1.34	1.33	1.24	1.26	0.11
7 d	1.89[b]	3.08[a]	2.30[ab]	2.84[ab]	0.25
14 d	8.71	8.01	8.66	6.19	1.04
21 d	13.24[b]	13.42[b]	14.28[b]	19.12[a]	1.57

[1]CON = a basal diet with 40 ppm of Zn from Zn-nitrate; Zn-gly1 = a basal diet with 40 ppm of Zn from Zn-glycine; Zn-gly2 = a basal diet with 40 ppm of Zn from Zn-glycinyl-glycine; and Zn-arg = a basal diet with 40 ppm of Zn from Zn-arginine.

[2]Pooled standard error means.

[ab]Means within a row lacking a common superscript differ ($P < 0.05$).

TABLE 3.7 Bioavailability of Zn by chicks fed different Zn amino acid chelates for 21-d (Exp 2)

Item, %	CON[1]	Zn-gly1[1]	Zn-gly2[1]	Zn-arg[1]	SEM[2]
0 to 1 d	60.98	57.69	57.72	56.80	3.74
1 to 3 d	59.33	53.05	54.76	53.82	3.62
3 to 5 d	54.85	53.88	58.80	61.76	3.26
5 to 7 d	51.12	48.02	50.33	53.48	3.47
Wk 1	58.96	53.98	55.07	56.73	3.42
Wk 2	53.24	51.20	50.17	53.83	2.36
Wk 3	44.89	45.49	45.30	42.95	3.98
1 to 2 Wk	53.72	50.94	48.09	52.52	2.54
1 to 3 Wk	43.10	42.91	43.29	46.63	2.61

[1]CON = a basal diet with 40 ppm of Zn from Zn-nitrate; Zn-gly1 = a basal diet with 40 ppm of Zn from Zn-glycine; Zn-gly2 = a basal diet with 40 ppm of Zn from Zn-glycinyl-glycine; and Zn-arg = a basal diet with 40 ppm of Zn from Zn-arginine.

[2]Pooled standard error means.

TABLE 3.8 Growth performance of chicks fed the diets with Zn from different ratios between Zn sulfate and Zn amino acid chelates for 21-d (Exp 3)

Item	ZS[1]	ZA1[1]	ZA2[1]	ZAA[1]	SEM[2]
Initial BW, g/chick	31.7	31.7	31.8	31.6	0.1
Average daily gain, g/d					
0 to 1 d	8.0	7.4	7.7	6.7	0.6
1 to 3 d	11.0	10.6	10.0	10.7	0.7
3 to 5 d	19.3	18.8	19.4	20.1	0.5
5 to 7 d	19.4	20.5	21.5	20.8	1.1
Wk 1	16.5	16.4	16.8	16.7	0.4
Wk 2	40.2	38.1	39.3	37.6	1.1
Wk 3	49.2	51.4	50.4	44.7	2.5
1 to 2 Wk	28.3	27.3	28.0	27.1	0.6
1 to 3 Wk	38.3	39.7	39.2	35.3	1.7
Average daily feed intake, g/d					
0 to 1 d	4.2	4.0	4.2	4.6	0.3
1 to 3 d	11.8	10.8	11.0	11.7	0.6
3 to 5 d	14.3	14.3	14.6	14.4	0.5

TABLE 3.8. Continued

5 to 7 d	24.5	23.4	24.0	23.7	0.7
Wk 1	15.7	15.0	15.4	15.5	0.4
Wk 2	46.5	45.3	45.5	45.6	0.9
Wk 3	57.9	59.0	58.3	53.8	1.8
1 to 2 Wk	31.1	30.2	30.5	30.5	0.5
1 to 3 Wk	43.8	44.3	44.0	41.0	1.3
Gain:feed					
0 to 1 d	1.94[a]	1.85[ab]	1.86[ab]	1.49[b]	0.10
1 to 3 d	0.93	0.98	0.91	0.92	0.04
3 to 5 d	1.36	1.32	1.33	1.42	0.06
5 to 7 d	0.80	0.88	0.89	0.88	0.05
Wk 1	1.05	1.09	1.09	1.08	0.03
Wk 2	0.86	0.84	0.86	0.83	0.02
Wk 3	0.85	0.87	0.87	0.81	0.03
1 to 2 Wk	0.91	0.90	0.92	0.88	0.01
1 to 3 Wk	0.87	0.90	0.89	0.85	0.02

TABLE 3.8 Continued

[1]ZS = a basal diet with 60 ppm Zn from Zn-sulfate; ZA1 = a basal diet with 60 ppm Zn from Zn-sulfate and Zn-methionyl-glycine at 2:1 ratio; ZA2 = a basal diet with 60 ppm Zn from Zn-sulfate and Zn-methionyl-glycine at 1:2 ratio; and ZAA = a basal diet with 60 ppm Zn from Zn-methionyl-glycine.

[2]Pooled standard error means.

[ab]Means within a row lacking a common superscript differ ($P < 0.05$).

TABLE 3.9 Zn content, Zn intake and Zn retention in whole body of chicks fed the diets with Zn from different ratios between Zn sulfate and Zn amino acid chelates for 21-d (Exp 3)

Item	ZS[1]	ZA1[1]	ZA2[1]	ZAA[1]	SEM[2]
Zn content, mg/chick					
1 d	0.97[a]	0.87[ab]	0.82[ab]	0.65[c]	0.05
3 d	1.14	1.11	1.25	1.19	0.06
5 d	1.35	1.32	1.25	1.25	0.12
7 d	2.42	2.26	2.59	2.75	0.15
14 d	7.69	7.33	7.04	7.32	0.32
21 d	14.70	14.48	14.10	14.27	1.04

[1]ZS = a basal diet with 60 ppm Zn from Zn-sulfate; ZA1 = a basal diet with 60 ppm Zn from Zn-sulfate and Zn-methionyl-glycine at 2:1 ratio; ZA2 = a basal diet with 60 ppm Zn from Zn-sulfate and Zn-methionyl-glycine at 1:2 ratio; and ZAA = a basal diet with 60 ppm Zn from Zn-methionyl-glycine.

[2]Pooled standard error means.

[abc]Means within a row lacking a common superscript differ ($P < 0.05$).

TABLE 3.10 Bioavailability of Zn by chicks fed the diets with Zn from different ratios between Zn sulfate and Zn amino acid chelates for 21-d (Exp 3)

Item, %	ZS[1]	ZA1[1]	ZA2[1]	ZAA[1]	SEM[2]
0 to 1 d	62.0	61.5	55.5	58.5	3.6
1 to 3 d	53.8	54.8	52.0	56.5	2.0
3 to 5 d	53.5	55.5	51.4	55.5	2.0
5 to 7 d	36.9	41.8	41.2	44.6	2.0
Wk 1	47.4	47.4	45.1	47.9	2.3
Wk 2	33.1[b]	33.9[b]	35.1[ab]	39.2[a]	1.4
Wk 3	32.3	30.2	29.7	30.2	2.0
1 to 2 Wk	34.5[b]	36.0[b]	35.8[b]	40.2[a]	1.2
1 to 3 Wk	35.2	36.2	31.4	33.6	2.0

[1]ZS = a basal diet with 60 ppm Zn from Zn-sulfate; ZA1 = a basal diet with 60 ppm Zn from Zn-sulfate and Zn-methionyl-glycine at 2:1 ratio; ZA2 = a basal diet with 60 ppm Zn from Zn-sulfate and Zn-methionyl-glycine at 1:2 ratio; and ZAA = a basal diet with 60 ppm Zn from Zn-methionyl-glycine.

[2]Pooled standard error means.

[ab]Means within a row lacking a common superscript differ ($P < 0.05$).