PHYSIOLOGICAL AND NUTRITIONAL FACTORS AFFECTING PROTEIN DIGESTION IN BROILER CHICKENS

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ABSTRACT

A series of experiments were conducted to examine protein digestion in the young bird and the physiological changes that affect protein digestion as the bird matures. Trial one determined the effect of age on pH of the gastrointestinal tract. The results showed that the pH of the proventriculus and gizzard decreases with age suggesting that gastric acid secretion increases proportionally with age and this may impact protein digestion. Experiment two examined the effect of age on ileal amino acid (AA) digestibility of feed ingredients. AA digestibility increased from 5 to 21 d and the degree of improvement differed among ingredients and specific AA. This finding coupled with the low AA content of some ingredients demonstrates the importance of using appropriate nutrient digestibility values for young birds when formulating pre-starter and starter diets. Experiment three determined the acid binding capacity (ABC) of feed ingredients and the effect of formulating diets based on ABC on diet ABC, gastrointestinal pH and performance. The research confirmed dramatic differences in ABC among ingredients and that diets could be formulated on the basis of ingredient ABC. However, the range in diet ABC was less than predicted suggesting interactive effects among ingredients. Intestinal pH was reduced however broiler performance was not improved when diets low in ABC were fed. Experiment four examined the effect of diet acidification with HCl on diet ABC, gastrointestinal pH, ileal amino acid digestibility and broiler performance. Improvements in performance and reductions in mortality were observed

when broilers were fed acidified diets. Adding acid to diets did not improve AA digestibility and therefore was not the reason for improved performance indicating an alternative mechanism of action. It is concluded that acid production by the proventriculus of young birds is low and increases with age however this does not impact protein digestion. Methods of improving performance of broiler chickens may include diet acidification however this is not the effect of remedying the low acid production by young birds. Therefore, alternative mechanisms are positively affecting broiler performance.

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LIST OF ABBREVIATIONS

AA	Amino acid
ABC	Acid binding capacity
AME	Apparent metabolizable energy
BW	Body weight
BWG	Body weight gain
CaCit	Calcium citrate
CDG	Dried corn distiller grains with solubles
CM	Canola meal
CYS	Cysteine
D	Dark
d	Day
Dical	Dicalcium phosphate
DW	Durum wheat
ECP	Embryonic chicken pepsinogen
FEM	Feather meal
FM	Fishmeal
GRP	Gastrin releasing peptide
HCl	Hydrochloric acid
ICPC	Insoluble canola protein concentrate
L	Light
LYS	Lysine
mEq	Milliequivelents
MET	Methionine
MM	Meat meal
NaCl	Sodium chloride/salt
PEA	Pea
PPC	Pea protein concentrate
SEM	Standard error of the mean
SBM	Soybean meal
SCPC	Soluble canola protein concentrate
THR	Threonine

1 INTRODUCTION

Broiler chickens have been extensively and successfully selected for ever more rapid growth rate. Because of the increases in growth rate, age at marketing has decreased in a proportional fashion. A consequence of broilers being marketed earlier is that the initial portion of their lives has become a larger proportion of the broiler life cycle. This is important because the digestive system of the young broiler chicken is characterized by limited nutrient digestibility. Subsequent growth and productivity depends upon the chick receiving adequate nutrients during the early period post-hatch. The unique nature of digestion by the young bird and the importance of the bird receiving adequate nutrients during the initial post-hatch period, make understanding the digestive system of the young bird and how it relates to digestion critically important for broiler production.

Immaturity of the digestive tract of young broilers results in limited nutrient digestion. Enzymes, transporters and other digestive secretions of the pancreas and small intestine increase with age. This suggests that they are limiting to digestion at a young age. Of the nutrients, protein is digested the poorest. Since protein is critically important for muscle development, improvements in protein digestion by the young bird may lead to improved broiler performance. Results have shown that some ingredients are digested better then others, which suggests that improvements can be made in the formulation of diets for young broilers.

Missing in the understanding of protein digestion is the initial steps that occur in the upper digestive tract. Gastric acid secretion occurs in the proventriculus and is responsible for protein denaturation and the activation of pepsinogen to pepsin, the first protease enzyme. Since acid secretion is reduced in young pigs and other digestive secretions are reduced in young broilers, acid secretion may be limited in the young bird as well. Inadequate acid secretion by the proventriculus of young birds could hinder protein digestion. Determining if acid secretions are limited in the young bird and developing methods to remedy this problem could be effective in improving broiler performance.

The objectives of this research were to determine if acid secretion is reduced in the young bird; determine if amino acid digestion is reduced in young birds and if the degree of reduction is similar for each ingredient tested; to examine methods of remedying the effects of reduced acid secretion by the young bird including formulating diets to reduce acid binding capacity and diet acidification; and to determine the effect of diet acidification when broilers are exposed to stress such as the initiation of a lighting program.

2 LITERATURE REVIEW

2.1 Gastrointestinal Physiology and Digestive Microorganisms

In most young animals the period immediately after birth is characterized by limited digestive ability due to digestive tract immaturity. Immediately post hatch, precocial birds must be prepared to use exogenous nutrients that are primarily carbohydrate based compared to the lipid based yolk that is the primary source of nutrients in the egg just prior to hatch (Parsons, 2004). To prepare for this change in nutrient sources, the gastrointestinal tract begins to change prior to hatch. Regardless of the changes pre-hatch, rapid changes in gastrointestinal morphology, function and microflora with age have been observed post-hatch suggesting that although broilers are considered precocial, they are not digestively mature at hatch (Moran, 1985; Uni et al., 1995a; Noy and Sklan, 1996a; Forder et al., 2007).

Morphological and functional changes in the crop of the broiler have not been studied extensively but changes in the microflora of the crop with age have been reported. These changes that occur as the broiler gets older indicate that the hatchling must develop the mature intestinal microflora post-hatch. As the bird matures, the crop microflora becomes predominantly acidogenic with lactobacilli being the most common bacterial species. With the increase in lactobacilli comes a decrease in the pH of the digesta in the crop.

As with the crop, examination of the morphology of the gastric stomach, including the proventriculus and gizzard, of the young bird is limited. Research conducted in piglets has indicated that the pH of the upper gastrointestinal tract decreases with age, which suggests that gastric acid secretion increases proportionately as the animal matures (Barrow et al., 1977). Research in older chickens has suggested a decrease in upper digestive tract pH may occur with age, however this has not been examined in very young chicks.

Immediately after hatching there is rapid morphological and functional development in the small intestine of a young bird. The morphological changes occur by maturation and differentiation of enterocytes, which cause villi growth and increased crypt depth (Uni et al., 1995b). This, in turn, results in an increase in surface area of the gastrointestinal tract that, along with an increase in gastrointestinal mucosal nutrient transporters, facilitates increased nutrient uptake by the growing bird (Moran, 1985). Functionally, a decrease in the digesta passage rate and a reduction in the presence of hydrophobic yolk in the gastrointestinal tract along with an increase in the secretion of bile acids and pancreatic and brush border enzymes allows for improved digestion of exogenous nutrients (Uni et al., 1995a; Noy and Sklan, 1995). These changes in the intestine have been found to plateau at 6-10 days of age (Noy and Sklan, 1996a; Parsons, 2004), which suggests that prior to a week of age, the chick is digestively unique and therefore to maximize the genetic potential of the broiler chicken, special attention must be paid to the nature of the feed during this time.

In the caeca, changes in the microflora occur as birds age and are exposed to more environmental bacteria. Like the crop, research has shown that as the animal gets older

the caeca are populated primarily by acid-producing bacteria therefore the pH of the caeca decreases with age (Cuche and Malbert, 1998).

The changes that occur in the gastrointestinal tract of the young bird as it matures allow the bird to attain full digestive capacity and recent research has found that there is an increase in the digestibility of nutrients by broilers with age (Sulistiyanto et al., 1999; Batal and Parsons, 2003). The delay in maturity after hatch, however, means that the young bird is digestively unique and special attention should be paid to nutrition during this time to ensure that the full genetic potential of the broiler is reached. When examining research that has been conducted on digestion of nutrients in young birds, it appears the digestion of proteins and absorption of amino acids are the most limiting (Noy and Sklan, 1995; 2001; Sulistiyanto et al., 1999); therefore, focusing on ways to improve protein digestion by the young bird may be the most dramatic means of improving performance. The exact reason for the lower ability to digest protein remains obscure. Protein digestion involves a number of sequential steps, which eventually result in amino acids being absorbed into the body by the small intestine.

2.2 Physiology of the Chicken Gastrointestinal Tract in Relation to Protein Digestion

2.2.1 Protein Digestion in the Crop

Little protein digestion occurs in the crop however the presence of acidogenic bacteria in the crop decreases the pH of the digesta which aids in the pH drop that must occur in the proventriculus for the initial steps of protein digestion (Hinton et al, 1990). As mentioned earlier, the crop microbiota is not fully developed in very young birds

therefore the reduction in digesta pH is not as notable in young birds as it is in older birds, which may affect protein digestion if upper digestive tract pH is critical (Bowen and Waldroup, 1969; Hinton et al., 2000).

2.2.2 Protein Digestion in the Proventriculus and Gizzard

The first step of protein digestion occurs in the proventriculus by exposing ingested proteins to hydrochloric acid, which denatures the protein and exposes peptide bonds for enzyme hydrolysis. Once feed is eaten, distention of the proventriculus occurs stimulating the release of acetylcholine. In turn, acetylcholine binds to G cells in the proventriculus, which stimulates the release of gastrin. Other stimulants of gastrin release are hypercalcemia and the presence of amino acids and gastrin releasing peptide (GRP), which is a neurocrine agent (Hersey and Sachs, 1995). The presence of gastrin promotes the release of histamine from enterchromaffin-like cells in the proventriculus. These stimulants, acetylcholine, gastrin and histamine, bind to parietal cells. Once bound, hydrochloric acid is secreted from these cells and acts to reduce the pH of both the proventriculus and gizzard environment to a range of 2 to 4 depending upon the amount and buffering capacity of feed present (Bohak, 1973; Khan and James, 1998). Gastrin also stimulates the release of pepsinogen, which at a pH of 2 and potentially a bit higher in birds, is converted to pepsin; therefore, adequate acid secretion is required for this conversion (Auer and Glick, 1984). Pepsin is the first enzyme responsible for protein digestion in the digestive tract. Specifically, pepsin preferentially cleaves the N-terminal of aromatic amino acids such as phenylalanine, tryptophan and tyrosine. The result of the acid denaturation and pepsin hydrolysis are smaller molecular weight peptides, which enter the small intestine.

Interestingly, it has been found that birds, like mammals, have a different pepsinogen as very young animals when compared to adult pepsinogen. In mammals this enzyme precursor, called chymosin, once converted to its enzyme, is responsible for clotting milk. The clotting slows the digesta passage rate and increases the time spent digesting the milk. In birds, the zymogen is called embryonic chicken pepsinogen (ECP) and the production and secretion begins soon after the initiation of incubation, but the use of this zymogen or its resulting enzyme is unknown (Sakamoto et al., 2000). Research has not established exactly when the secretion of ECP ceases, but it is just prior to or soon after hatch. Embryonic chicken pepsinogen may strictly be for the solidification or digestion of albumen or yolk proteins and may therefore be of little use once the yolk is no longer present and the bird is fed only solid exogenous feeds.

The gizzard facilitates the initial action of the secreted HCl and pepsin from the proventriculus. The passage time of digesta through the proventriculus is very short and therefore little time is afforded for contact of the ingested feed with the proventricular secretions. The gizzard acts only as a mechanical organ; therefore no digestive aids are secreted and absorption of nutrients does not occur. However, it is important for mixing ingested feed with water, saliva, hydrochloric acid and pepsin. Time spent in the gizzard by the digesta allows for increased contact between the feed and gastric acid and pepsin therefore facilitating the further denaturation and digestion of proteins prior to release into the small intestine.

2.2.3 Protein Digestion in the Small Intestine

In the small intestine, peptides from the proventriculus and gizzard are further degraded to amino acids by enzymes derived from the pancreas and the small intestine.

As with pepsin, each enzyme involved is capable of only hydrolyzing specific peptide bonds. The enzymes from the pancreas responsible for protein digestion are called proteinases. This class of enzymes includes trypsin, chymotrypsin, elastase, carboxypeptidase A and carboxypeptidase B (Lipscomb, 1970). Each of these enzymes is stored as zymogens in the pancreas (Puigserver and Desnuell, 1975). Once secreted into the intestine, enteropeptidase activates trypsinogen into trypsin (Light, and Fonseca, 1984.) The presence of trypsin causes the activation of chymotrypsinogen to chymotrypsin (Desnuelle, 1960), proelastase to elastase (Brown, and Wold, 1973), procarboxypeptidase to carboxypeptidase (Puigserver and Desnuell, 1975) as well as further conversion of typsinogen to trypsin (Huber and Bode, 1978). Each of these enzymes is specific in the reaction that it catalyzes. Trypsin binds only to the positively charged side chains of lysine and arginine residues. Once bound, trypsin facilitates the hydrolysis of those peptide bonds. Chymotrypsin is less specific than trypsin but still selective in the reactions that it catalyzes. Chymotrypsin binds to the side chains of aromatic or large hydrophobic amino acid residues. This includes the aromatic amino acids tyrosine, phenylalanine and tryptophan. It also catalyzes leucyl, methionyl, asparaginyl, and glutamyl residues (Desnuelle, 1960). Elastase is unique in that it binds small side chain amino acids such as glycine and alanine, which means that it is able to catalyze the degredation of elastin (Brown, and Wold, 1973). Carboxypeptidase A and B are different in that carboxypeptidase A hydrolyzes the peptide bond adjacent to the C-terminal end of a polypeptide chain, while carboxypeptidase B catalyzes the hydrolysis of basic amino acids including lysine, arginine and ornithine from the C-terminal end of polypeptide chains. Both carboxypeptidase A and B catalyzed reactions result in the

release of free amino acids from oligopeptides. The final step of protein digestion is membrane hydrolysis, which is accomplished by the previously mentioned pancreatic exopeptidases, carboxypeptidase A and B as well as two types of brush border enzymes called aminopeptidases and dipeptidases (Desnuelle, 1979.). These enzymes work to break the small oligopeptides and dipeptides resulting from the previous enzyme reactions into amino acids and peptides. The amino acids are then absorbed by several specific transport mechanisms through the small intestine wall.

2.3 Implications of Reduced Acid Secretion by the Proventriculus on Protein Digestion

Of the steps of protein digestion, the terminal aspects of the process and the changes that occur as the bird matures are relatively well understood, however little research has been done looking at the critical first step, which is acid production in the proventriculus. Since protein digestion is a sequential process, failure of the initial digestive step in the proventriculus and gizzard may affect the digestive process in the small intestine and reduce protein digestibility. In turn, the buffering capacity of feed ingredients may influence protein digestion in young birds with limited acid production capacity. As mentioned previously, acid secretion in young pigs increases with age and therefore the amount of acid secreted may be limiting at a young age (Barrow et al., 1977; Xu and Cranwell, 1990). If this is also the case for young birds, this first step may be limiting protein digestion within the immediate time post-hatch.

2.4 Methods of Improving Protein Digestion by Young Animals

2.4.1 Nutrient Digestibility

Not all protein sources are poorly digested indicating that careful selection of feed ingredients could improve broiler performance (Sulistiyanto et al., 1999; Batal and Parsons, 2002; Parsons, 2004). Protein sources that result in better performance may have protein or amino acids in a form that is more digestible. This difference in digestibility is significant as formulating feed on the basis of digestible amino acid content is fundamentally important in delivering the appropriate nutrient content to broiler chickens. Typically, amino acid digestibility values for young birds are derived from true amino acid digestibilities or ileal amino acid digestibilities, both of which are most commonly performed using older birds (Ravindran et al., 1999). The differences in the utilization of nutrients by young birds because of an immature digestive system strongly suggest that these amino acid values do not accurately predict amino acid digestibility for very young broilers and some research has found this to be the case (Sklan and Noy, 2004; Huang et al., 2005). Since digestible amino acid levels during this time have been shown to be of importance in growth rate and breast meat yield later in life (Mozdziak et al., 2002), it is important to evaluate the digestibility of amino acids in very young chicks.

2.4.2 Diet Acid Binding Capacity

Acid binding capacity (ABC) is the ability of a compound or solution to react with a strong acid. Diet ABC is determined as the amount of acid required to lower the pH of a solution to a desired pH. A higher ABC indicates an increased reactivity with

acid. It has been suggested that in young animals that have reduced secretions of gastric acid, lowering the ABC of the diet may improve performance. Ingredients have been found to have differing ABC with mineral ingredients being the highest followed by protein ingredients and then energy sources (Lawlor et al., 2005). More important is that differences within these groups of ingredients have been found, which suggests that diets can be formulated to affect acid binding capacity. Most research conducted has been in piglets with varied results (Evans and Ali, 1967; Blank et al., 1999; Mroz et al., 2000; Pickard et al., 2001). Research has examined the use of different calcium sources and levels of calcium (Mroz et al., 2000; Pickard et al., 2001) or adding a buffer to the diet (Blank et al., 1999) to modify the diet ABC. Little research has examined a combination of ingredients of lower ABC to create diets of low ABC.

2.4.3 Diet Acidification

Interest in acidification of pig and poultry diets has increased with concern over the use of antibiotic growth promotants in animal feed. It has been suggested that adding acid to the diet or water may beneficially influence the microbial populations inhabiting the gastrointestinal system. Little research has examined the effect of diet acidification on intestinal pH and nutrient digestibility. Most research has used a single or a combination of organic acids, bactericides or supplements and little research has used hydrochloric acid or other mineral acids (Patten and Waldroup, 1988; Krause et al., 1994; Biggs and Parsons, 2008). Results have varied with some organic acids resulting in better performance while others yielded no improvement or even reduced performance. A specific explanation of the reason for the variation of effects by organic acids has been poorly examined. It has been suggested that the varying characteristics of organic acids

may influence the acid's ability to pass through the bacterial wall (Dibner and Buttin, 2002). Additionally it has been suggested that each organic acid may be specific to the bacteria it affects. However, an examination of which acids work best to control specific bacteria has not been performed. It has also been suggested that acids lower the pH of the digesta therefore making the gastrointestinal environment less favourable to some bacterial populations. However, most organic acids that are used as feed additives have a pKa from 3 to 5, which is above the value that would be required to dramatically reduce the pH of the gut environment. Mineral acids such as hydrochloric acid have a very low pKa (-8), which would allow for increased reductions in digesta pH.

2.5 Conclusions

Regardless of the increased interest in the nutrition of the young bird, this area of study is young and some critical pieces of information are missing. Research has suggested that protein digestion is the most limiting nutrient digestibility. Some research examining the change in amino acid digestibility from the hatchling to the mature broiler of commonly used feed ingredients has been performed, however the research is limited. This research has suggested that although the digestibility of all ingredients increases with age, some ingredients are highly digestible by the hatchling and only improve modestly with age. Knowing which ingredients or the characteristic of ingredients that are highly digestible for young birds would improve the accuracy of diet formulation for birds during the early post-hatch period.

When examining the development of the digestive tract, research has focused on the small intestine. This misses the first step of protein digestion, acid secretion, which may be important to protein digestion if it is low in young birds. Research in piglets has

shown that gastric acid secretions are low in the young animal and steps to mitigate this problem are being researched. If this is also the case in young birds then similar steps such as diet acidification and changing diet ABC may improve performance and allow the broiler to reach full genetic potential. The objective of this study was to study changes in the pH of the digestive tract with age in young birds to determine if proventricular acid secretion is limited. Additional objectives were to develop methods to alter the pH of the young bird and to examine differences in the amino acid digestibility in young (5 d) and older (21 d) broilers.

3 THE EFFECT OF AGE ON INTESTINAL PH OF THE BROILER CHICKEN

3.1 Abstract

When considering protein digestion by young birds it is of interest to identify limiting factors. The first steps in digestion occur in the proventriculus and gizzard by means of hydrochloric acid and involve protein denaturation and activation of the protease, pepsin. If the production of hydrochloric acid is limited in young broilers, protein digestion may be hampered. Regression analysis was used to study the effect of age on the pH of intestinal contents of broiler chickens fed a common broiler starter diet. Ross x Ross 308 male broilers (200) were randomly assigned to 10 battery cages. Using one bird per cage per sample age, the pH of the crop, proventriculus, gizzard, duodenum, jejunum and ileum were measured from 2 to 10 and at 15 d of age. Crop pH increased linearly with age while the proventricular and gizzard pH decreased linearly. The relationships between age and intestinal pH for the duodenum, jejunum and ileum were quadratic with each section beginning high then dropping to a low at 6-8 d of age followed by a subsequent increase. In conclusion, during the first 15 d of age the pH of the gastrointestinal tract changes significantly and these changes may impact protein digestion and other digestive traits in broilers during the first week post-hatch.

3.2 Introduction

Previous research has suggested that the newly hatched chick has limited digestion and absorption of nutrients. This is mainly due to the immaturity of the intestinal tract and associated organs such as the pancreas. The young bird, although precocial and therefore fully independent at hatch, has low digestive secretions, such as bile and enzymes (Traber et al., 1991; Farraris et al., 1992; Noy and Sklan, 1995; Uni et al., 1999; Sklan and Noy, 2000). Beyond this, the chick has an immature intestinal mucosa, which also could limit both the digestion and absorption of nutrients (Uni et al., 1995b; Geyra et al., 2001; Parsons, 2004; Sklan 2001). Research examining the digestion of nutrient by young broilers has found that the digestion of proteins and absorption of amino acids is the most limiting of the nutrients (Noy and Sklan, 1995; 2001).

In swine, gastric secretion of HCl is reduced in piglets and this reduction has been suggested to negatively affect protein digestion (Barrow et al., 1977). Therefore, it can be hypothesized that the production and secretion of gastric acid is limited in the broiler hatchling as well. If this is the case, then this may affect digestion in the chick by reducing protein denaturation and limiting conversion of pepsinogen to pepsin, the initial protease enzyme; the latter conversion requires a low pH of 2-4 in the gastric lumen (Bohak, 1973; Khan and James, 1998). If protein digestion is limited in the young bird then early growth could be hindered, with potential negative consequences for the remainder of the broiler's life cycle. Therefore, it is hypothesized that gastric acid secretion by the proventriculus is low in the young broiler, and that the pH of the proventriculus and gizzard will decrease with age. The objective of this experiment was to determine whether age has an effect on the pH of the gastrointestinal tract of broilers.

3.3 Materials and Methods

3.3.1 Birds and Housing

Two hundred Ross x Ross 308 broilers were randomly housed on the day of hatch into 10 battery cages (46.25 cm long x 82.5 cm wide x 23.75 cm high). Birds were fed a commercial starter diet ad libitum for the entire experiment (Table 3.1). A standard room temperature curve starting at 35°C at d 0 and decreasing 0.5°C every day was used. To minimize development of circadian rhythms, birds were exposed to continuous light and bird care occurred at randomly selected times throughout the trial. Development of circadian rhythms in feed intake may influence acid production since acid secretion occurs in response to proventricular distention (Hersey and Sachs, 1995).

3.3.2 Chemical Analysis

The pH of the feed was determined by suspending 0.5 grams of diet in 50 mL double distilled water by continuous stirring using a stir plate. The pH of the solution was recorded once the pH stabilized for 3 minutes (stable to a pH of ± 0.001).

3.3.3 Intestinal pH Examination

In a preliminary experiment, a micro-pH meter was used in hopes of measuring the pH in the intestinal section rather than removing the contents and diluting with water, but it was found that the moisture content of some intestinal sections was not sufficient for accurate pH measures. Additionally, when beginning this experiment it was the objective to gather data from d 0 through d 10 and d 15. When taking samples on d 0 and

1 it was found that the contents in the gastrointestinal tract were not adequate to obtain accurate pH readings and therefore pH readings were initiated on d 2.

Daily from d 2 to 10 and on d 15, 1 bird from each of the 10 pens was randomly selected for pH sampling. Birds were killed by cervical dislocation, and then the crop, proventriculus, gizzard, duodenum, jejunum and ileum were removed and separated. Contents from the entire crop, proventriculus and gizzard and the middle two-thirds of the duodenum, jejunum and ileum were extracted with 0.9 mL of double distilled water. Once the contents were extracted, the contents were weighed taking into account the water added for rinsing. The contents were then diluted by 9 times with double distilled water minus the 0.9 mL water used to rinse the organs. Although water has a minor effect on the pH of a solution, care was taken to have all contents diluted equally to ensure that the effect of dilution was similar for all samples. Some organs, in particular the proventriculus, were found to have very little content and therefore a dilution of 9 times was chosen to ensure that all samples would have a minimum of 1 mL of solution. This minimum was required to allow the pH meter to make an accurate reading. After being weighed, diluted, and stirred for 1 minute using a stir plate, a pH probe[1] was inserted into the solution and a reading was recorded once the pH was stable to a pH of ± 0.001 for 1 minute.

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[1] Futura™ Refillable Combination Electrode Epoxy, Calomel, 7 x 245mL. Distributed by Beckman (part #511084)

Table 3.1. Ingredient composition (%) and formulated nutrient profile of broiler starter diet.

Ingredient	%
Wheat	45.57
Soybean meal	18.05
Corn	12.50
Meat and bone meal	7.50
Canola meal	7.00
Whole wheat	5.00
Animal/vegetable oil	2.37
Limestone	0.771
Salt	0.313
Lysine	0.289
Methionine (MHA-FA)	0.250
Dicalcium phosphate	0.202
Choline chloride	0.085
Vitamin mineral premix[1]	0.200
Threonine	0.054
Salinomycin sodium[2]	0.050
NSP enzyme[3]	0.034
Virginiamycin[4]	0.025
Calculated analysis	
pH	6.05
Calculated ABC[5]	883
ME, kcal/kg	2,985
Crude protein, %	22.37
Lysine, %	1.37
Methionine + Cysteine, %	1.04
Calcium, %	0.94
Available phosphorus, %	0.435

[1]The vitamin-mineral premix supplied per kilogram of feed: 10,000 IU vitamin A; 2,621 IU vitamin D3; 1377.5 IU Hy-D Premix 62.5; 40 IU vitamin E; 1.6 mg menadione; 2 mg thiamine; 5.72 mg riboflavin; 60 mg niacin; 3.2 mg pyridoxine; 0.0144 mg vitamin B_{12}; 12.32 mg D-pantothenic acid; 0.8 mg folic acid; 0.2 mg biotin; 2.5 mg ethoxyquin; 64 mg iron; 96 mg zinc; 96 mg manganese.
[2]Coccistac (Phibro Animal Health, Ridgefield Park, NJ).
[3]Avizyme 1302 (Danisco A/S, Copenhagen, DK distributed by PMT Inc., Regina, Canada).
[4]Stafac-44 (Philbro Animal Health).
[5] ABC = volume of HCl (mEq) per kg of feed sample to lower the solution to pH 3

3.3.4 Design and Statistical Analysis

This experiment was designed to examine the relationship between gastrointestinal pH and age for each gastrointestinal section. The independent variable for this experiment was day of age while the dependent variable was gastrointestinal section pH. Data were subjected to ANOVA and regression analysis to determine the relationship between age and intestinal content pH using the Proc GLM, Reg and RSReg procedure of SAS 9.1 (2002). Duncan's Multiple Range Test was used to separate means when the ANOVA was significant. Differences were considered significant when *P-value* ≤ 0.05.

3.4 Results

Age significantly affected the pH of the gastrointestinal tract but the effect differed between sections. As broilers got older, the pH of the crop linearly increases from 5.01 to 6.02 (Figure 3.1) while the pH of the proventriculus (Figure 3.2) and gizzard (Figure 3.3) decreased linearly from 5.20 to 3.37 and 3.49 to 3.27, respectively. Quadratic relationships between age and pH were found for the three sections of the small intestine. Day 2 pH was 6.57 in the duodenum (Figure 3.4), 6.82 in the jejunum (Figure 3.5) and 7.74 in the ileum (Figure 3.6) and then pH dropped to the lowest point of 6.07 on d 7 in the duodenum, 6.26 on d 8 in the jejunum and 6.74 on d 6 in the ileum. This low between 6 to 8 d of age in the small intestine was followed by a subsequent increase in pH to 6.40 in the duodenum, 6.50 in the jejunum and 8.15 in the ileum on the final day of measurement.

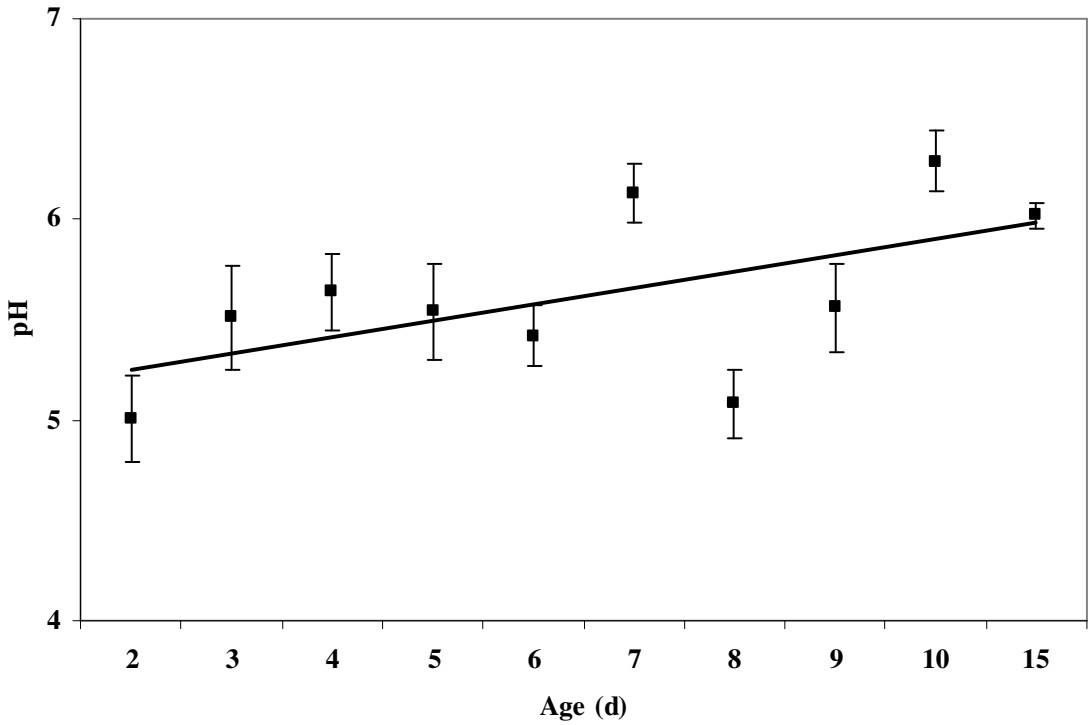

Figure 3.1. The effect of broiler age on crop content pH. Mean of 10 replicates. Vertical bars represent the SEM (d 2 = 0.2163; d 3 = 0.2587; d 4 = 0.1911; d 5 = 0.2416; d 6 = 0.1528; d 7 = 0.1503; d 8 = 0.1751; d 9 = 0.2185; d 10 = 0.1526; d 15 = 0.0654). The line represents the regression analysis. $P_{linear} = 0.0016$, $R^2 = 0.0973$, $y = 0.06390x + 5.18245$.

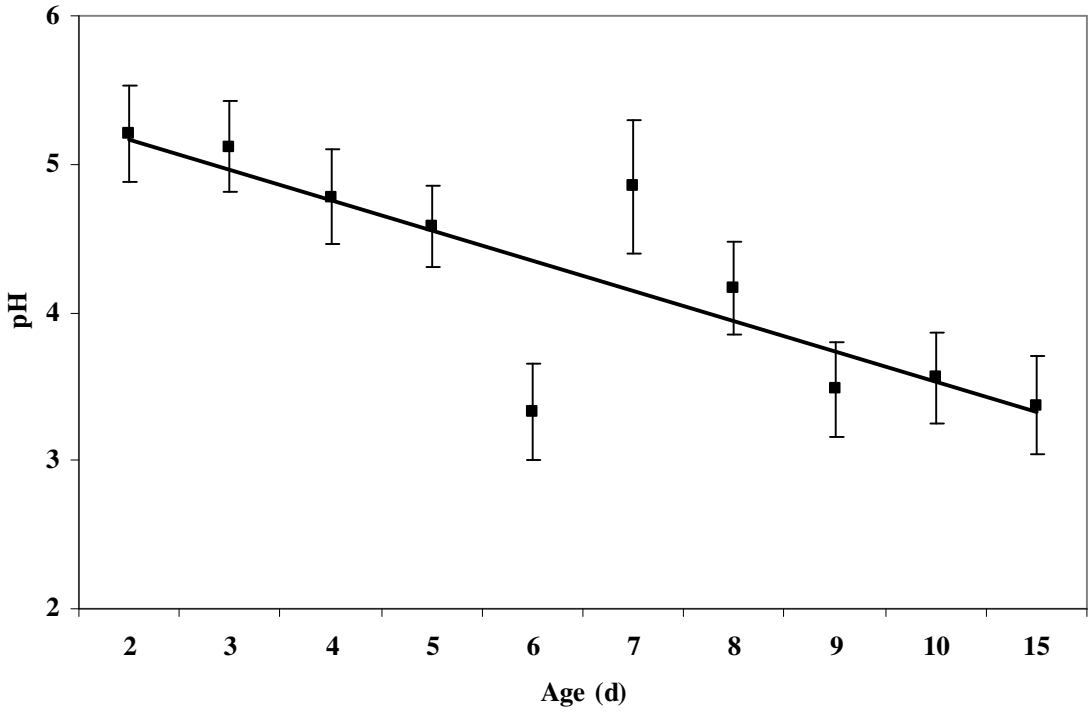

Figure 3.2. The effect of broiler age on proventricular content pH. Mean of 10 replicates. Vertical bars represent the SEM (d 2 = 0.3246; d 3 = 0.3026; d 4 = 0.3229; d 5 = 0.2708; d 6 = 0.3269; d 7 = 0.4526; d 8 = 0.3095; d 9 = 0.3151; d 10 = 0.3054; d 15 = 0.3339). The line represents the regression analysis. $P_{linear} < 0.0001$, $R^2 = 0.2045$, $y = -0.15253x + 5.29479$.

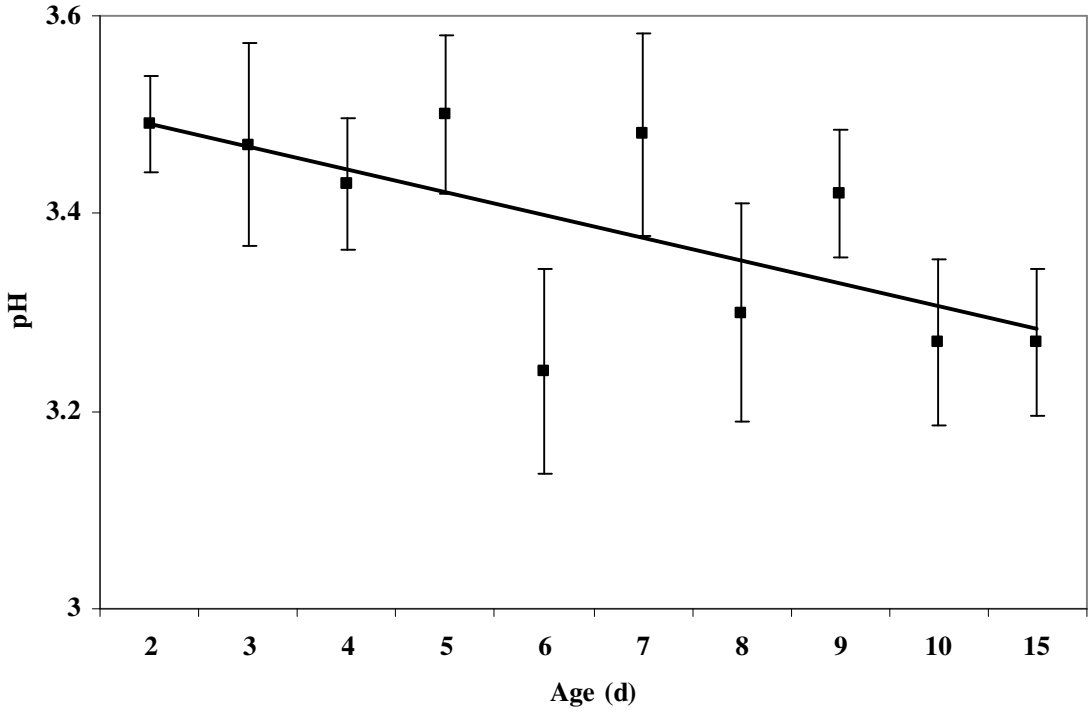

Figure 3.3. The effect of broiler age on gizzard content pH. Mean of 10 replicates. Vertical bars represent the SEM (d 2 = 0.0489; d 3 = 0.1018; d 4 = 0.0672; d 5 = 0.0801; d 6 = 0.1032; d 7 = 0.1025; d 8 = 0.1103; d 9 = 0.0639; d 10 = 0.0839; d 15 = 0.0745). The line represents the regression analysis. $P_{linear} = 0.0185$, $R^2 = 0.0542$, $y = -0.01795x + 3.51120$.

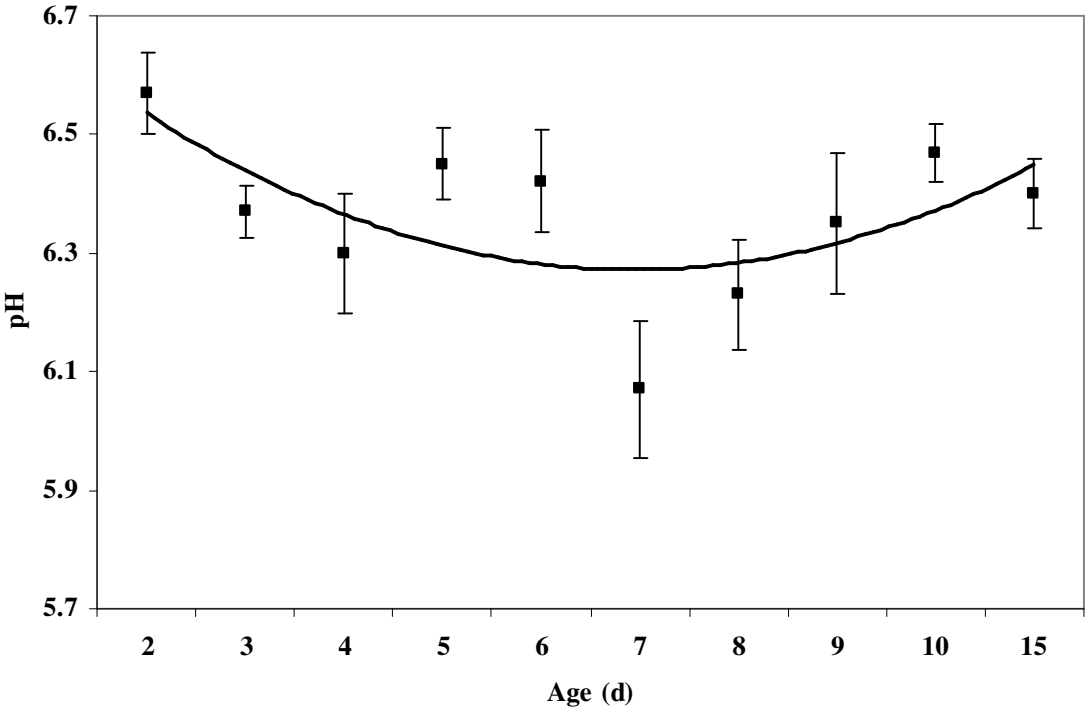

Figure 3.4. The effect of broiler age on duodenal content pH. Mean of 10 replicates. Vertical bars represent the SEM (d 2 = 0.0692; d 3 = 0.0435; d 4 = 0.1006; d 5 = 0.0600; d 6 = 0.0864; d 7 = 0.1147; d 8 = 0.0922; d 9 = 0.1203; d 10 = 0.0490; d 15 = 0.0591). The line represents the regression analysis. $P_{quadratic} = 0.0308$, $R^2 = 0.0509$, $y = 0.003840x^2 - 0.069017x + 6.607189$.

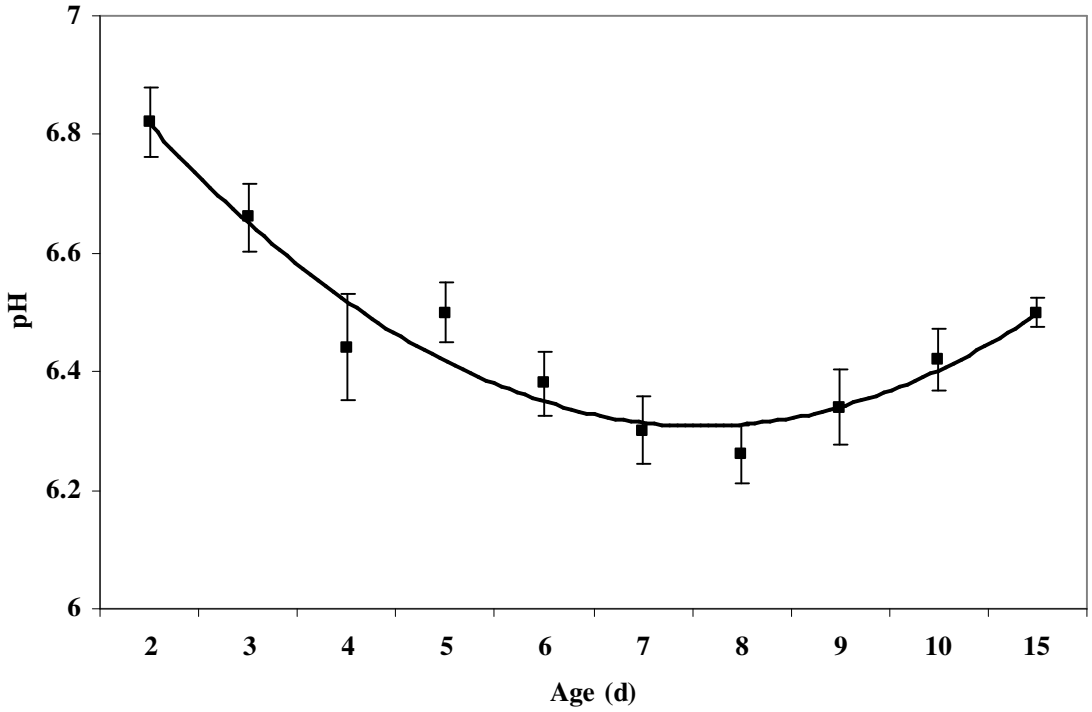

Figure 3.5. The effect of broiler age on jejunal content pH. Mean of 10 replicates. Vertical bars represent the SEM (d 2 = 0.0588; d 3 = 0.0562; d 4 = 0.0896; d 5 = 0.0508; d 6 = 0.0547; d 7 = 0.0569; d 8 = 0.0493; d 9 = 0.0624; d 10 = 0.0531; d 15 = 0.0245). The line represents the regression analysis. $P_{quadratic} < 0.0001$, $R^2 = 0.3858$, $y = 0.007929x^2 - 0.152239x + 7.028885$.

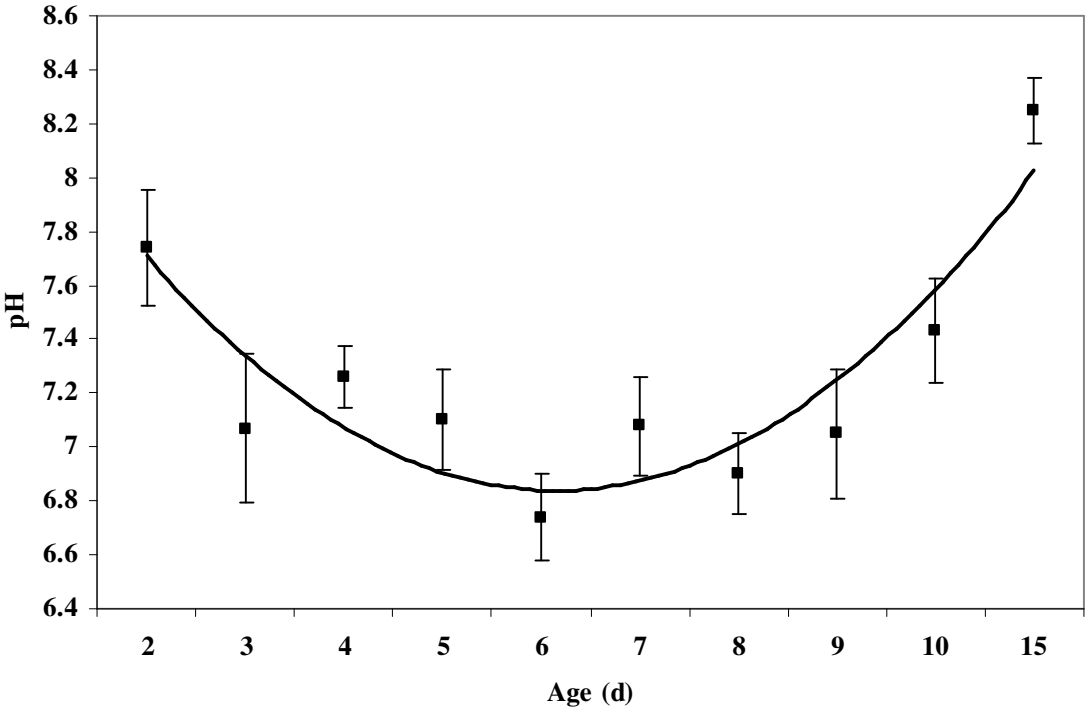

Figure 3.6. The effect of broiler age on ileal content pH. Mean of 10 replicates. Vertical bars represent the SEM (d 2 = 0.2118; d 3 = 0.2778; d 4 = 0.1127; d 5 = 0.1870; d 6 = 0.1614; d 7 = 0.1829; d 8 = 0.1503; d 9 = 0.2406; d 10 = 0.1926; d 15 = 0.1207). The line represents the regression analysis. $P_{quadratic} < 0.0001$, $R^2 = 0.1235$, $y = 0.036478x^2 - 0.472555x + 8.433560$.

3.5 Discussion

Throughout the digestive tract there are many factors affecting the pH of the digesta such as feed pH and acid binding capacity (amount of acid required to lower the pH of a solution), the duration between feeding and pH measurement, the type of microbial populations present, digestive secretions and mechanical actions of the digestive tract. Feed pH and acid binding capacity can vary depending upon feed ingredients and would therefore influence the effectiveness of the acidifiers and buffers produced by the gastrointestinal tract. Bacterial populations found in the crop are generally composed of acid-producing bacteria that reduce the pH of the crop contents (Hinton et al, 1990). In the proventriculus, HCl is produced which reduces the pH of the digesta. Mixing in the gizzard of ingested feed with gastric acid further reduces the pH of the digestive slurry. Once in the duodenum, pancreatic secretions of bicarbonate work to increase the pH of the digesta. Additionally, research suggests that bicarbonate mucus is also secreted from the intestinal mucosa and this secretion increases as the feed progresses down the small intestine to the jejunum and ileum (Flemström et al, 1982). Like the crop, microbial populations of the caeca are usually made up of acid producing bacteria, which can influence pH. Reverse peristalsis of digesta in the caeca to the jejunum can occur which would reduce the pH of the lower small intestine. With these pH influences in mind, the current research found that during the initial period post-hatch the pH of the gastrointestinal tract of broilers changes. Seeing a change in intestinal pH with age suggests that changes are occurring in the gastrointestinal tract as the bird matures, which could indicate that the digestive process is different immediately post-hatch compared to older birds.

Bowen and Waldroup (1969) found no change in crop pH from 19 to 28 d of age, with the pH at 19 d being 5.10, while research by Hinton et al. (2000) and Paul et al. (2007) found the crop pH at 42 d to be 5.5 and 5.0, respectively, compared to the current research at 15 d, which had a pH in the crop of 6.02. The difference in pH between the research in older birds compared to the current research suggests that the acid producing microbial population of the crop is still developing beyond 15 d of age but reaches maturity around 19 d of age. In the current study, the pH of the feed was initially higher than the pH of the crop, which suggests that the acid-producing bacterial populations were able to reduce the pH of the crop contents with the minimal feed intake. As the bird aged, the pH of the crop increased which may be the result of increased crop fill. In contrast, other research demonstrates that the crop microflora of older birds are able to reduce the pH of the crop contents regardless of the increased crop fill (Bowen and Waldroup 1969; Hinton et al., 2000).

Bowen and Waldroup (1969) also found no change in pH between 19 and 28 d of age in the proventriculus and gizzard. Paul et al. (2007) indicated that the pH in the proventriculus is lower at 42 d of age but gizzard pH is increased in comparison to the results of Bowen and Waldroup (1969) and the current study. Bowen and Waldroup (1969) found the proventricular pH at 19 d to be 3.75 and gizzard pH to be 2.47 while at 42 d of age Paul et al. (2007) found the pH of the proventriculus to be 2.7 and the gizzard pH to be 3.2 compared to the current study, which found proventricular pH to be 3.37 and gizzard pH to be 3.27 at 15 d of age. The change in the relationship between proventricular pH and gizzard pH in these experiments suggests that the time of pH measurement in relationship to feeding can have an effect on the pH and the relationship

between proventriculus and gizzard pH. Results of the current study show a decrease in pH from 2 d of age to 15 d in the proventriculus and gizzard from 5.20 to 3.37 and 3.49 to 3.27, respectively. The percentage daily decrease from 2 to 10 d (proventriculus 4%; gizzard 0.8%) is more dramatic than from 10 to 15 d (proventriculus 1%; gizzard 0%), which suggests a relative increase in acid production from the proventriculus but that intestinal maturity is being approached at 15 d of age. Studies in piglets (Cranwell, 1985) and humans (Vanzant et al., 1932) have also suggested an increase in gastric acid production with age. The low initial pH in the gizzard shows that although the pH of the proventriculus was high during the first few days post-hatch, hydrochloric acid was still being produced by the proventriculus to reduce the pH of the digesta in the gizzard. Regardless that the proventriculus is producing acid at an early age, the relatively smaller reduction in pH in the gizzard may still be of importance in protein denaturation and activation of pepsinogen to pepsin (Auer and Glick, 1984).

Paul et al. (2007) found intestinal pH to be 5.8 in the duodenum, 6.6 in the jejunum and 7.5 in the ileum at 42 d of age compared to the current study, which found intestinal pH to be 6.4 in the duodenum, 6.5 in the jejunum and 8.15 in the ileum at 15 d of age. Many factors could influence the pH of the digestive tract such as method of pH measurement, diet, housing, age and digestive tract secretions and microflora, therefore the differences between the results by Paul et al. (2007) and the current results are difficult to define.

The quadratic relationship between pH and age found in all the small intestinal sections from 2 to 15 d of age in the current study suggests that as the digestive system matures, the relationship between gastric acid production and pancreatic bicarbonate

secretions changes. Since the pH at 2 d of age in the intestine is higher than that found in the gizzard this suggests that there is some bicarbonate being produced by the pancreas, liver and intestines at this young age. The drop in pH of the intestine to approximately one week of age suggests that there is a delay between the increase in acid secretion in the proventriculus and the increase in pancreatic bicarbonate production. Possibly the upper digestive system is maturing quicker than the lower digestive system to approximately one week of age. The subsequent increase in the pH of the small intestine suggests a maturation of the digestive tract with pancreatic bicarbonate being secreted at an appropriate level to compensate for the low pH of the intestinal contents entering the duodenum.

Data from this research indicate that the production and secretion of gastric acid by the proventriculus of the broiler chicken is limited at a very young age and that it increases with age. This research also demonstrates significant changes in pH of other sections of the gastrointestinal tract. These changes in pH have potential to affect digestion and other characteristics of the gut such as its microbiota.

4 ILEAL AMINO ACID DIGESTIBILITY OF PROTEIN FEED INGREDIENTS AT 5 AND 21 DAYS OF AGE BY BROILER CHICKENS.

4.1 Abstract

The amino acid (AA) digestibility of feed ingredients by broiler chickens has most often been determined using older birds. However, these values are unlikely to predict AA digestibility in chicks during the initial period post-hatch because of their immature digestive tracts and lower nutrient utilization. Therefore, the objective of this research was to compare the ileal AA digestibility of selected protein sources using 5 and 21 d old broilers. Twenty-two (5 d sampling) or six (21 d sampling) Ross x Ross 308 broilers were randomly assigned to six battery cages per treatment. A 2 x 6 factorial arrangement was used to examine the effect of age (5 and 21 d) on the ileal AA digestibility of six protein feed ingredients (canola meal (CM), insoluble canola protein concentrate (ICPC), fishmeal (FM), meat meal (MM), pea (PEA) and soybean meal (SBM)). Diets were formulated to derive crude protein (approximately 18%) and AAs solely from the test ingredient. AA digestibility was higher for 21 d old broilers than their 5 d old counterparts for all AAs and significant differences were also observed among ingredients for all AAs. Interactions were observed for phenylalanine, proline (*P-value* $\leq$ 0.05) and interactions neared significance for isoleucine, lysine, methionine, valine and asparagine (*P-value* $\leq$ 0.10) between age and ingredient which suggests that the age-

associated improvement in digestibility was variable and dependent on feed ingredient and AA. In conclusion, ileal AA digestibility generally increases with age between 5 and 21 d of age but the response to age differs among ingredients and AAs. Therefore, accurate formulation of starter diets requires the use of AA digestibility values obtained in age-appropriate birds to ensure that diets meet AA requirements of broiler chicks for growth and other performance criteria.

4.2 Introduction

Broiler growth rate continues to increase as a result of genetic selection and as a consequence the first week can now represent over 20% of the bird's life and therefore is an increasingly important proportion of the total broiler life cycle. During this first week, chicks have been found to have an immature digestive system with reduced digestive ability with digestion of proteins and absorption of AAs being the most limiting (Noy and Sklan, 1995; 2001). Using digestibility values obtained in older birds when formulating pre-starter and starter diets assumes that the digestibility of the ingredients is at par with older birds. Mozdziak et al. (2002) suggested that hatchlings that did not have adequate nutrition early post-hatch, had reduced myofiber nuclei which leads to irreversible reductions in the size of the muscle cell and therefore breast meat yield. Additionally, Sklan and Noy (2004) illustrated the importance of proper AA balance by showing that if AAs are lacking or imbalanced, broiler carcass accretion is reduced. Therefore, if AA digestibility values are lower or different for young compared to older birds then pre-starter and starter diets may not be providing adequate digestible AAs to meet the requirements of the young bird so that they can reach their genetic potential for growth and production.

Additionally, during the first week digestive system growth is much faster than total body growth and therefore a higher proportion of nutrients consumed during this time are used directly for digestive development (Moran, 1985; Noy and Sklan, 1996a; Uni et al., 1995a). This early digestive development is important during subsequent growth and therefore providing nutrients with low bioavailability during this time may hamper intestinal development and therefore production.

There has been extensive research on the digestibility of AAs in feed ingredients for broiler chickens, however most research has used older birds such as mature cockerels or broilers older than 21 d of age (Ravindran et al., 1999). Very little research has examined ileal AA digestibility in young birds. Huang et al. (2005) examined ileal AA digestibilities for 14, 28 and 42 d old broilers and found that AA digestibility increased as the bird aged, but as previously mentioned, during the first week post-hatch digestive system growth is happening very quickly and therefore the period up to one week post-hatch the bird is digestively quite different than even a bird at 14 d of age. Therefore it is hypothesized that since the very young bird is digestively very different from older birds, values that are accurate for older birds may not be appropriate for diets formulated for the younger broiler.

Research in AA digestibility in young birds has determined ingredient digestibility is variable (Batal and Parsons, 2002). Additionally, the degree of improvement with age for ingredients is quite different. While chicks do not digest some protein sources very well, other protein sources are highly digestible and do not increase with age (Batal and Parsons, 2002; Parson, 2004). Sulistiyanto et al. (1999) found similar results using different feed ingredients fed to birds of one, three and ten d of age. These

studies reiterate the earlier suggestion that digestibility coefficients of feed ingredients for young birds are important to consider when formulating broiler pre-starter and starter diets. Therefore the objective of this experiment was to compare the ileal AA digestibility of protein feed ingredients at 5 and 21 d of age.

4.3 Materials and Methods

4.3.1 Animals and Housing

On day of hatch, Ross x Ross 308 male broilers were randomly placed in battery cages. Twenty-two (5 d sampling) or seven (21 d sampling) birds were randomly assigned to six battery cages per treatment. A standard room temperature curve (decreasing 0.5°C every day) starting at 35°C at d 0 was used. Birds were exposed to 24 h of light for the duration of the experiment.

4.3.2 Dietary Treatments

For the duration of the experiment birds were fed one of 6 dietary treatments, which included CM, ICPC, FM, MM, PEA or SBM. Diets were formulated to supply all crude protein (approximately 18%) and AAs from the test ingredient (Tables 4.1 and 4.2). Celite was used as the indigestible marker. The calcium to phosphorous ratio was maintained at 2:1. Water and diets were available ad libitum throughout the experiment. With the exception of AAs, diets were formulated to meet or exceed nutrient requirements specified by the National Research Council (1994).

4.3.3 Data Collection

Body weight gain and feed intake data were collected on the birds on day 0, 5 and 21. From this data, the feed conversion ratio (feed:gain), corrected for mortality, was determined for each period. Mortality and culls were collected and weighed daily. Ileal samples were collected from the middle two-thirds of the ileum from 20 birds at 5 d of age and 6 birds at 21 d of age per replicate. The ileum was defined as the section of intestine from the Meckel's diverticulum to the ileocecal junction. Birds were killed by cervical dislocation.

4.3.4 Chemical Analysis

Ileal samples from each replicate were pooled. Diets and ileal digesta were analyzed for crude protein, AAs and acid insoluble ash. Crude protein and AAs were analyzed by Evonik-Degussa Corporation (Hanau, Germany) using methods that conform to the Association of Official Analytical Chemists Official Method 994.12 (Llames and Fontaine, 1994). Crude protein was determined using a FP-2000 Nitrogen Analyzer (LECO Corp, St. Joseph, MI 49085-2396, USA) while AAs were determined with high performance liquid chromatography using a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd, Cambridge, UK). Dry matter was determined using a Foss NIRS 5000/6500 Feed and Forage Analyzer (FOSS Analytical A/S, DK 3400 Hillerod, Denmark).

Acid insoluble ash was analyzed using a modification of the method of Vogtmann et al. (1975). First, 1-2 g of sample were weighed into125 mm disposable borosilicate tubes which were then placed into an ashing oven at 500°C for 24 h or until contents were reduced to white ash. Following ashing, 5 mL of 4N HCl was slowly added to the

Table 4.1. Ingredient composition and formulated nutrient profile of test diets.

Item	CM	ICPC	FM	Pea	MM	SBM
Ingredients: %						
Dextrose	42.2	66.3	66.4	15.2	60.5	53.5
Canola meal	51	-	-	-	-	-
Insoluble canola protein concentrate	-	26.5	-	-	-	-
Fishmeal	-	-	30	-	-	-
Pea	-	-	-	76.6	-	-
Meat meal	-	-	-	-	36	-
Soybean meal	-	-	-	-	-	39.1
Corn oil	2.18	2	1.6	2.8	1.5	2
Dicalcium phosphate [1]	1.12	1.5	-	1.5	-	1.5
Limestone	1.09	1.5	-	1.5	-	1.5
Sodium chloride	0.4	0.22	-	0.4	-	0.4
Vitamin/mineral premix [2]	0.5	0.5	0.5	0.5	0.5	0.5
Celite	1.5	1.5	1.5	1.5	1.5	1.5
Formulated nutrient profile						
AME (kcal/kg)	2800	3930	3429	2800	3285	3178
Crude protein	18.25	18.03	18.00	18.00	18.00	18.00
Calcium	1.00	1.21	1.95	1.02	2.88	1.01
Non-phytate phosphorus	0.45	0.625	1.05	0.47	1.44	0.47

[1] Supplied per kilogram of diet: 11,000 IU of vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU of vitamin D3 (cholecalciferol), 30 IU of vitamin E (DL-α-tocopheryl acetate), 2.0 mg of vitamin K3 (menadione), 1.5 mg of thiamine, 6.0 mg of riboflavin, 60 mg of niacin, 4 mg of pyridoxine, 0.02 mg of vitamin B12, 10.0 mg of pantothenic acid, 6.0 mg of folic acid, 0.15 mg of biotin, 0.625 mg of ethoxyquin, 500 mg of CaCO3, 80 mg of Fe, 80 mg of Zn, 80 mg of Mn, 10 mg of Cu, 0.8 mg of I, and 0.3 mg of Se.

Table 4.2. Analyzed crude protein and AA composition of experimental diets (% as is basis).

	CM	ICPC	FM	PEA	MM	SBM
Crude Protein	21.25	17.61	22.08	16.07	21.82	19.30
Alanine	0.89	0.76	1.27	0.66	1.47	0.81
Arginine	1.14	1.05	1.39	1.23	1.36	1.32
Aspartate	1.47	1.33	1.89	1.75	1.61	2.18
Cysteine	0.46	0.35	0.18	0.23	0.23	0.28
Glutamate	3.64	3.07	2.63	2.52	2.57	3.40
Glycine	1.00	0.86	1.25	0.66	2.47	0.78
Histidine	0.51	0.44	0.42	0.36	0.40	0.48
Isoleucine	0.65	0.69	0.82	0.61	0.63	0.81
Leucine	1.36	1.30	1.52	1.09	1.35	1.42
Lysine	1.01	0.81	1.46	1.06	1.12	1.13
Met + Cys	0.84	0.68	0.76	0.37	0.55	0.53
Methionine	0.38	0.34	0.58	0.14	0.32	0.25
Phenylalanine	0.80	0.77	0.81	0.74	0.77	0.97
Proline	1.13	0.95	0.78	0.58	1.50	0.86
Serine	0.90	0.75	0.84	0.74	0.89	0.98
Threonine	0.86	0.71	0.88	0.58	0.73	0.75
Valine	0.84	0.85	1.00	0.70	0.89	0.85

CM = Canola meal; ICPC = Insoluble canola protein concentrate; FM = Fish meal; PEA = Pea; MM = Meatmeal; SBM = Soybean meal

ash and vortexed. After vortexing, tubes were covered with glass marbles and placed in an oven at 120°C for one hour. Finally samples were centrifuged at 2500 × g for 10 minutes. The supernatant was then removed and samples were washed repeatedly with 5 ml water (using the vortex/centrifugation method as described above). Samples were then dried at 80ºC overnight, followed by ashing at 500°C overnight. The percent acid insoluble ash was calculated as (total ashed wt - tube wt) / (original - tube wt).

4.3.5 Calculations

The digestibility of the tested AAs were calculated using the formula:

$$\frac{(\text{AA} \div \text{acid insoluble ash})^{\text{diet}} - (\text{AA} \div \text{acid insoluble ash})^{\text{digesta}}}{(\text{AA} \div \text{acid insoluble ash})^{\text{diet}}}$$

4.3.6 Design and Statistical Analysis

Data for ileal AA digestibilities were analyzed as a 2 (ages) x 6 (feed ingredients) factorial arrangement using the PROC GLM procedures of SAS (SAS Institute, 2002). The ANOVA assessed both main effects as well as interactions between age and test ingredient. Duncan's Multiple Range Test was used to separate means when the ANOVA was significant. Differences were considered significant when $P\text{-}value \leq 0.05$ and all differences were noted when $P\text{-}value \leq 0.10$.

4.4 Results

Diets were formulated to have approximately 18% crude protein however actual crude protein in the diet often did not match what was predicted. The diet that included

ICPC was close to 18% crude protein (17.61%) while PEA had less crude protein than expected and CM, FM, MM and SBM had more crude protein than expected (Table 4.2).

Increases in ileal AA digestibility from 5 to 21 d of age were observed for all AAs (Table 4.3). In addition, significant differences were found among test ingredients for all AAs.

Significant interactions between age and dietary protein source were found for phenylalanine and proline (*P-value* ≤ 0.05) while interactions approached significance (*P-value* ≤ 0.1) for isoleucine, lysine, methionine, valine and asparagine. All interactions are shown in Table 4.4. The average percentage improvement for ingredients shows that CM improves the most (average improvement 18.0%) while SBM improves the least (average improvement 6.4%) with age (Table 4.5).

4.5 Discussion

In the hatchling there are numerous factors that could reduce protein digestion and absorption. These factors include reduced proventricular acid secretions (Chapter 3), reduced pancreatic and intestinal mucosa secretions such as bile and proteolytic enzymes (Noy and Sklan, 1995) and juvenile intestinal AA transporters (Noy and Sklan, 2001).

Results for the current study for the 21-d AA digestibility are comparable to results found by Ravindran et al. (1999). Variations can be explained by the natural variability of these ingredients due to ingredient quality and processing that can affect digestibility (Adedokun et al., 2007).

Huang et al. (2005) examined the apparent ileal AA digestibility at 14, 28 and 42 d of age and found that, in general, digestibility increased with age however the increase

Table 4.3. The effect of age and protein source on broiler ileal AA digestibility coefficient[1].

	Age			Protein Source							SEM	Interaction
	5d	21d	*P-value*	CM	ICPC	FM	MM	PEA	SBM	*P-value*		
Alanine	0.74	0.85	<0.0001	0.78^{a}	0.73^{b}	0.82^{a}	0.80^{a}	0.83^{a}	0.81^{a}	<0.0001	0.0094	NS
Arginine	0.81	0.90	<0.0001	0.84^{cd}	0.83^{cd}	0.86^{bc}	0.81^{d}	0.92^{a}	0.88^{b}	<0.0001	0.0076	NS
Aspartate	0.68	0.79	<0.0001	0.74^{c}	0.66^{d}	0.76^{c}	0.59^{e}	0.86^{a}	0.81^{b}	<0.0001	0.0139	0.0552
Cysteine	0.56	0.69	<0.0001	0.75^{a}	0.68^{b}	0.60^{c}	0.34^{d}	0.71^{ab}	0.68^{b}	<0.0001	0.0197	NS
Glutamate	0.79	0.88	<0.0001	0.85^{b}	0.82^{c}	0.82^{c}	0.76^{d}	0.90^{a}	0.86^{b}	<0.0001	0.0085	NS
Glycine	0.72	0.83	<0.0001	0.75^{bc}	0.73^{c}	0.78^{b}	0.79^{ab}	0.82^{a}	0.78^{ab}	<0.0001	0.0089	NS
Histidine	0.77	0.87	<0.0001	0.82^{bc}	0.81^{c}	0.81^{c}	0.75^{d}	0.87^{a}	0.86^{ab}	<0.0001	0.0090	NS
Isoleucine	0.72	0.84	<0.0001	0.73^{b}	0.75^{b}	0.80^{a}	0.74^{b}	0.84^{a}	0.83^{a}	<0.0001	0.0108	0.0583
Leucine	0.75	0.86	<0.0001	0.80^{bc}	0.78^{c}	0.82^{ab}	0.77^{c}	0.85^{a}	0.84^{a}	<0.0001	0.0090	NS
Lysine	0.75	0.87	<0.0001	0.75^{c}	0.77^{c}	0.82^{b}	0.78^{c}	0.89^{a}	0.85^{ab}	<0.0001	0.0108	0.0985
Met + Cys	0.67	0.79	<0.0001	0.79^{a}	0.74^{ab}	0.77^{ab}	0.59^{c}	0.73^{b}	0.76^{ab}	<0.0001	0.0126	NS
Methionine	0.74	0.88	<0.0001	0.84^{a}	0.82^{ab}	0.83^{a}	0.77^{bc}	0.76^{c}	0.84^{a}	0.0031	0.0112	0.0763
Phenylalanine	0.58	0.75	<0.0001	0.66^{bc}	0.61^{c}	0.66^{bc}	0.65^{bc}	0.68^{b}	0.74^{a}	0.0005	0.0136	0.0134
Proline	0.71	0.83	<0.0001	0.74^{b}	0.76^{b}	0.75^{b}	0.75^{b}	0.80^{a}	0.81^{a}	0.0005	0.0095	0.0331
Serine	0.69	0.81	<0.0001	0.74^{bc}	0.70^{c}	0.76^{b}	0.66^{d}	0.81^{a}	0.81^{a}	<0.0001	0.0109	NS
Threonine	0.65	0.79	<0.0001	0.70^{b}	0.68^{b}	0.76^{a}	0.67^{b}	0.76^{a}	0.76^{a}	<0.0001	0.0112	NS
Valine	0.71	0.84	<0.0001	0.72^{b}	0.75^{b}	0.80^{a}	0.74^{b}	0.82^{a}	0.82^{a}	<0.0001	0.0105	0.0935
Total	0.73	0.84	<0.0001	0.78^{cd}	0.75^{d}	0.79^{bc}	0.75^{d}	0.85^{a}	0.82^{ab}	<0.0001	0.0096	NS

[1]Means of 6 replicates with 22 pooled ileal samples (5 d) or 6 pooled ileal samples (21 d) per replicate.

[a, b, c, d] Means within a common row and main effect with different superscripts differ significantly (*P-value* ≤ 0.05).

CM = Canola meal; ICPC = Insoluble canola protein concentrate; FM = Fish meal; PEA = Pea; MM = Meatmeal; SBM = Soybean meal.

Table 4.4. Effect of protein source on broiler ileal AA digestibility coefficients[1] at 5 and 21 d of age.

	5 d								21 d							
	CM	ICPC	FM	MM	PEA	SBM	SEM	*P-value*	CM	ICPC	FM	MM	PEA	SBM	SEM	*P-value*
Alanine	0.71^{ab}	0.66^{b}	0.77^{a}	0.76^{a}	0.76^{a}	0.78^{a}	0.012	0.0166	0.86^{b}	0.80^{c}	0.86^{b}	0.85^{b}	0.90^{a}	0.85^{b}	0.006	<0.0001
Arginine	0.78^{c}	0.78^{c}	0.82^{bc}	0.77^{c}	0.88^{a}	0.86^{ab}	0.010	0.0009	0.89^{b}	0.87^{bc}	0.90^{b}	0.86^{c}	0.96^{a}	0.90^{b}	0.006	<0.0001
Aspartate	0.66^{b}	0.58^{c}	0.71^{b}	0.53^{b}	0.81^{a}	0.79^{a}	0.020	<0.0001	0.82^{b}	0.73^{c}	0.82^{b}	0.66^{d}	0.81^{a}	0.83^{b}	0.014	<0.0001
Cysteine	0.69^{a}	0.62^{a}	0.51^{b}	0.28^{c}	0.61^{a}	0.66^{a}	0.026	<0.0001	0.80^{a}	0.74^{ab}	0.69^{b}	0.39^{c}	0.80^{a}	0.70^{b}	0.026	<0.0001
Glutamate	0.80^{ab}	0.78^{b}	0.77^{b}	0.70^{c}	0.86^{a}	0.84^{a}	0.011	<0.0001	0.90^{b}	0.87^{c}	0.87^{c}	0.81^{d}	0.94^{ab}	0.88^{bc}	0.008	<0.0001
Glycine	0.67^{bc}	0.67^{c}	0.73^{abc}	0.74^{ab}	0.76^{a}	0.76^{a}	0.011	0.0329	0.82^{b}	0.79^{c}	0.82^{b}	0.83^{b}	0.88^{a}	0.81^{bc}	0.007	0.0001
Histidine	0.76^{bc}	0.75^{b}	0.76^{bc}	0.69^{d}	0.82^{ab}	0.83^{a}	0.011	0.0004	0.89^{b}	0.86^{b}	0.86^{b}	0.81^{c}	0.93^{a}	0.88^{b}	0.007	<0.0001
Isoleucine	0.63^{c}	0.68^{bc}	0.74^{ab}	0.67^{bc}	0.77^{a}	0.80^{a}	0.014	0.0006	0.83^{bc}	0.81^{c}	0.85^{b}	0.80^{c}	0.90^{a}	0.86^{ab}	0.007	<0.0001
Leucine	0.72^{bc}	0.72^{c}	0.77^{abc}	0.71^{c}	0.79^{ab}	0.81^{a}	0.011	0.0165	0.87^{b}	0.83^{c}	0.87^{b}	0.82^{c}	0.91^{b}	0.86^{b}	0.006	<0.0001
Lysine	0.67^{c}	0.68^{c}	0.78^{ab}	0.72^{bc}	0.84^{a}	0.82^{a}	0.015	0.0001	0.84^{c}	0.83^{c}	0.87^{b}	0.84^{c}	0.94^{a}	0.88^{b}	0.001	0.0034
Met + Cys	0.73^{a}	0.68^{ab}	0.72^{a}	0.53^{c}	0.63^{b}	0.72^{a}	0.016	0.0001	0.85^{a}	0.80^{ab}	0.83^{ab}	0.65^{c}	0.83^{ab}	0.79^{b}	0.007	<0.0001
Methionine	0.77^{a}	0.76^{a}	0.78^{a}	0.71^{ab}	0.65^{b}	0.78^{a}	0.014	0.0292	0.91^{a}	0.88^{ab}	0.87^{b}	0.84^{c}	0.88^{ab}	0.89^{ab}	0.013	<0.0001
Phenylalanine	0.56^{b}	0.50^{b}	0.57^{b}	0.58^{b}	0.56^{b}	0.71^{a}	0.017	0.0052	0.76^{abc}	0.72^{c}	0.76^{abc}	0.72^{bc}	0.80^{a}	0.76^{ab}	0.007	0.0067
Proline	0.66^{b}	0.70^{b}	0.68^{b}	0.71^{b}	0.72^{b}	0.84^{a}	0.012	0.0142	0.81^{bc}	0.81^{bc}	0.82^{bc}	0.79^{c}	0.88^{a}	0.84^{b}	0.006	0.0007
Serine	0.66^{bc}	0.64^{bc}	0.71^{ab}	0.61^{c}	0.74^{a}	0.78^{a}	0.014	0.0002	0.81^{b}	0.77^{c}	0.82^{b}	0.72^{d}	0.88^{a}	0.83^{b}	0.010	<0.0001
Threonine	0.61^{bc}	0.61^{bc}	0.70^{ab}	0.60^{c}	0.67^{abc}	0.73^{a}	0.013	0.0120	0.78^{bc}	0.76^{cd}	0.82^{ab}	0.73^{d}	0.85^{a}	0.80^{bc}	0.008	<0.0001
Valine	0.63^{b}	0.68^{bc}	0.75^{ab}	0.68^{bc}	0.75^{ab}	0.79^{a}	0.014	0.0017	0.82^{bc}	0.81^{c}	0.85^{ab}	0.80^{c}	0.89^{a}	0.85^{ab}	0.007	0.0001
Total	0.70^{b}	0.69^{b}	0.74^{ab}	0.68^{b}	0.78^{a}	0.80^{a}	0.012	0.0029	0.85^{b}	0.81^{b}	0.85^{b}	0.82^{b}	0.91^{a}	0.84^{b}	0.008	0.0022

[1]Means of 6 replicates with 22 pooled ileal samples (5 d) or 6 pooled ileal samples (21 d) per replicate.

[a, b, c] Means within a common row and main effect with different superscripts differ significantly (*P-value* ≤ 0.05).

CM = Canola meal; ICPC = Insoluble canola protein concentrate; FM = Fish meal; PEA = Pea; MM = Meatmeal; SBM = Soybean meal

Table 4.5. Percentage increase of broiler ileal AA digestibility coefficient[1] of protein ingredients from 5 to 21 d.

	Protein Source					
	CM	ICPC	FM	MM	PEA	SBM
Alanine	17.4	17.5	10.5	10.6	15.6	8.2
Arginine	12.4	10.3	8.9	10.5	8.3	4.4
Aspartate	19.5	20.5	13.4	19.7	0.0	4.8
Cysteine	13.8	16.2	26.1	28.2	23.8	5.7
Glutamate	11.1	10.3	11.5	13.6	8.5	4.5
Glycine	18.3	15.2	11.0	10.8	13.6	6.2
Histidine	14.6	12.8	11.6	14.8	11.8	5.7
Isoleucine	24.1	16.0	12.9	16.3	14.4	7.0
Leucine	17.2	13.3	11.5	13.4	13.2	5.8
Lysine	20.2	18.1	10.3	14.3	10.6	6.8
Met + Cys	14.1	15.0	13.3	18.5	24.1	8.9
Methionine	15.4	13.6	10.3	15.5	26.1	12.4
Phenylalanine	26.3	30.6	25.0	19.4	30.0	6.6
Proline	18.5	13.6	17.1	10.1	8.5	0.0
Serine	18.5	16.9	13.4	15.3	15.9	6.0
Threonine	21.8	19.7	14.6	17.8	21.2	8.8
Valine	23.2	16.0	11.8	15.0	15.7	7.1
Average	18.0	16.2	13.7	15.5	15.9	6.4

[1]Means of 6 replicates with 22 pooled ileal samples (5 d) or 6 pooled ileal samples (21 d) per replicate.

CM = Canola meal; ICPC = Insoluble canola protein concentrate; FM = Fish meal; PEA = Pea; MM = Meatmeal; SBM = Soybean meal.

was not as dramatic as the current research found from 5 to 21 d. This suggests that the major changes in digestibility occur before 14 d of age therefore using birds that are 14 d or older is not appropriate when trying to determine the AA digestibility coefficients for young birds.

Batal and Parsons (2002) found that diets formulated using purified protein ingredients such as crystalline AA or dextrose casein were highly digestible at 3-4 d of age (lysine digestibility 93 and 97% respectively) with little increase to 21 d of age (lysine digestibility 98 and 98% respectively). Diets formulated with corn-SBM or corn-CM had lower digestiblities at 3-4 d of age (lysine digestibility 74 and 75% respectively) followed by larger increases to 21 d of age (lysine digestibility 88 and 80% respectively). The current study found significant interactions between age and ingredient for the AAs phenylalanine (*P-value* = 0.0134) and proline (*P-value* = 0.0331) while interactions for methionine, lysine, isoleucine, valine and asparagine neared significance (*P-value* $\leq$ 0.1). These interactions suggest that increases in digestibility with age occur regardless of ingredient however some ingredients are utilized more effectively by young birds than others and therefore result in smaller increases in digestibility with age. As an example, SBM is actually highly digestible at 5 d of age (lysine digestibility 82%) increasing only 6.8% by 21 d of age (lysine digestibility 88%) while CM has lower digestibility at 5 d of age (lysine digestibility 67%) increasing by 20.2% at 21 d of age (lysine digestibility 84%). Like the research by Batal and Parsons (2002), these interactions show that ingredients can be selected for use in the diets of young birds that are highly digestible while other ingredients should be used with caution.

The results of Batal and Parsons (2003) are comparable to the current study for SBM, which found the lysine digestibility of SBM to be 79% at 3-4 d of age and 84% at 21 d of age. The high digestibility of SBM at young ages suggests that SBM and purified ingredients such as casein and crystalline AAs are good protein sources for pre-starter and starter diets. On the other hand, PEA has very low methionine content (14% as is basis) coupled with low methionine digestibility at 5 d of age (65%), which suggests that PEA is not a good protein source for young broilers however the dramatic improvement in methionine digestibility of PEA with age (26.1%) to 88% at 21 d of age implies that PEA is a high quality protein source in older birds. Low digestibility of PEA at 5 d of age coupled with low methionine content exemplifies the necessity to use age-appropriate values for formulating diets for young broilers. Using ingredients such as PEA without considering the digestibility by young broilers would have a high potential to negatively affect early broiler growth and therefore subsequent growth and production.

The dramatic variation in increase of AA digestibility with age coupled with differences in AA content of feed ingredients demonstrates the importance of using accurate AA coefficients for young birds when formulating pre-starter and starter diets. To improve the formulation of diets for young birds more age specific digestibility information should be gathered. With this, the examination of the characteristics of protein and how it relates to protein digestibility capacity would provide information on determining ingredients that are suitable to pre-starter and starter broiler diets.

5 THE EFFECT OF FORMULATING DIETS BASED ON ACID BINDING CAPACITY ON DIET ACID BINDING CAPACITY AND INTESTINAL PH OF BROILER CHICKENS

5.1 Abstract

Research has suggested that gastric acid secretions may be limited in young birds. Reducing the amount of hydrochloric acid (HCl) required by chicks to lower the digestive environment to an optimal pH by providing diets formulated with reduced ABC may improve performance. Therefore, the objectives of this research were to determine the ABC of commonly used feed ingredients in broiler diets and to determine if diets could be formulated with different ABC. The acid binding capacity was determined for 24 feed ingredients. ABC was found to be greatest for the calcium sources followed by those containing higher protein. The differences found among ingredients suggested that diets could be formulated with reduced ABC. Using this information, two experiments were conducted. In the first experiment, diets were formulated with a combination of high or low ABC ingredients to determine the effect on diet ABC and pH of the broiler gastrointestinal tract. Results showed diets could be formulated with decreasing ABC and this linearly decreased crop and gizzard pH. In the second experiment the effect of calcium type (limestone or calcium citrate) and level of calcium (0.5, 0.75 and 1.00%) on diet ABC, broiler gastrointestinal pH, bone ash and performance was examined. These

modified diets were fed to 5 d of age followed by a diet with 1.00% limestone to 21 d of age. Results of the second experiment showed that calcium source had no effect on diet ABC, broiler gastrointestinal pH or performance. Reducing the level of dietary calcium reduced diet ABC, had no effect on gastrointestinal pH, broiler body weight and gain and increased overall feed intake and feed:gain. Bone ash was reduced at 5 d but no effect was observed at 21 d. In conclusion, feed ingredients have different ABC and diets can be formulated to have reduced ABC, however the ABC of ingredients are not additive when combined in a diet. Additionally, diets of low ABC do not necessarily improve performance.

5.2 Introduction

Young birds have reduced ability to digest nutrients and protein may be the most limiting (Chapter 4, Noy and Sklan, 1999; Sklan and Noy, 2000). Reduced protein digestion may be the result of reduced gastric acid secretions (Chapter 3). Gastric acid is responsible for the initial steps of protein digestion; protein denaturation and the activation of the first proteolytic enzyme, pepsin. The ABC of feed is the amount of acid required to reduce the feed to a chosen pH. Therefore if feed can be formulated to have reduced ABC, then the limitations of digestion due to reduced gastric acid secretion may be mitigated. With an increased interest in the early period of life in production animals, research has shown an interest in the ABC of feed ingredients and the subsequent formulation of swine and poultry diets based on ABC with the anticipation of improved performance.

Research has shown a large variation in ABC among feed ingredients. Commonly, results showed that mineral ingredients containing calcium had the highest

ABC followed by protein ingredients, while cereal grains and other energy sources had the lowest ABC (Lawlor et al., 2005). Interestingly, within these ingredient types were differences that suggest that balanced diets could be formulated to have reduced ABC.

Research in piglets examining the effect of dietary ABC on development and performance has had varied results. Most research has influenced the ABC of the diet by using different sources or levels of calcium (Mroz et al, 2000; Pickard et al., 2001) or by adding a buffer to the diet (Blank et al., 1999), while little research has tried to influence the ABC by using a combination of commonly used ingredients of different ABC. Lawlor et al. (2005) did find that diets could be manipulated using a combination of low ABC ingredients to result in a diet that is nutritionally adequate and of low ABC however the effect of these diets on animal performance was not examined. Some research has shown reductions in intestinal pH and improvements in intestinal development, (Pickard et al. 2001), ileal amino acid digestibility, calcium retention and performance with reduced dietary ABC (Blank et al., 1999; Evans and Ali, 1967) while other research has found no effect (Mroz et al., 2000).

The variability of results may be the effect of differing experimental methods used and suggests that the influence of diet ABC on animal performance is not fully understood. Additionally little research has examined the effect of dietary ABC on broiler performance and therefore it was hypothesized that dietary ingredients can influence the effect of reduced gastric acid secretion by young birds. The objective of this study was to determine the ABC of commonly used ingredients in broiler feed followed by an examination of the effect of reducing dietary ABC by using a

combination of low ABC ingredients and by reducing the level of calcium early in life on broiler intestinal pH and performance.

5.3 Materials and Methods

5.3.1 Experiment 1. Acid Binding Capacity of Feed Ingredients

5.3.1.1 Ingredient Samples

The ingredients tested included protein ingredients (canola meal (CM), corn gluten meal (CGM), feather meal (FEM), fishmeal (FM), insoluble canola protein concentrate[2] (ICPC), meat meal (MM), pea protein concentrate[3] (PPC), soybean meal (SBM), and soluble canola protein concentrate[4] (SCPC)), crystalline amino acids (lysine (LYS), methionine (MET) and threonine (THR)), cereal grains (barley, corn, dried corn distiller grains with solubles (CDG), durum wheat (DW), oats, pea, and wheat), minerals (calcium citrate (CaCit), dicalcium phosphate (dical), limestone and salt (NaCl)), and dextrose. Samples from 6 commercial feed mills were used for CM while samples from 5 mills were used for MM, FM, SBM and corn and samples from 4 mills were used for CGM and wheat. CM, corn, CDG, CGM, feather meal, FM, MM, SBM, and wheat were ground using a Wiley mill with 1 mm and 0.5 mm screens while DW, oats and barley were ground using only a 1 mm screen. All remaining samples were not ground because of their already fine nature.

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[2] CanPro IP (MCN Bioproducts, Saskatoon, SK, Canada).

[3] Prestige Protein (Parrheim Foods, Saskatoon, SK, Canada).

[4] CanPro SP (MCN Bioproducts, Saskatoon, SK, Canada).

5.3.1.2 Chemical Analysis

The pH of the ingredient was determined by suspending 0.5 grams of ground ingredient in 50 mL double distilled water by continuous stirring using a stir plate. The pH of the solution was recorded once the pH stabilized for 3 minutes (stable to $\pm$ 0.001). ABC was determined using the methods of Jasaitis et al. (1987) as modified by Lawlor et al. (2005). Tests for pH and ABC were performed twice for each sample. ABC is expressed as the amount of hydrochloric acid in milliequivalents (mEq) required to lower the pH of 1 kilogram of sample to pH 3.0.

5.3.1.3 Design and Statistical Analysis

This experiment was designed to determine the ABC of feed ingredients available for use in broiler feed and therefore sample means were not compared statistically. Using the PROC GLM procedures of SAS (SAS Institute, 2002), data for grind size was analyzed as a 2 (grind size) x 9 (ingredients) factorial arrangement for the ingredients that were ground using two screen sizes. The ANOVA analyzed both the individual effects as well as interactions that may have occurred between these factors. Duncan's Multiple Range Test was used to separate means when the ANOVA was significant. Differences were considered significant when *P-value* $\leq$ 0.05.

5.3.2 Experiment 2. Effect of Formulating Broiler Diets Based on Ingredient ABC on Diet pH and ABC and Broiler Intestinal pH

5.3.2.1 Animals and Housing

In this experiment, 400 day of hatch Ross x Ross 308 male broilers were fed 1 of 5 diets. Birds were housed in battery cages for the duration of the experiment. Treatments were replicated four times with 20 birds in each of 20 battery cages. A

standard temperature curve (decreasing 0.5°C every day) starting at 35°C at day 0 was used and birds were exposed to 24 hours of light per d.

5.3.2.2 Dietary Treatments

Five diets were created to have increasing ABC (Table 5.1). These diets were created from two extreme diets, one using a combination of ingredients with low ABC and the other with ingredients with high ABC. The three intermediate diets were created by blending the two extreme diets at 75% Low ABC:25% High ABC, 50% Low ABC:50% High ABC or 25% Low ABC:75% High ABC. Diets were pelleted using a cold pelleter that does not add water or steam during pelleting, therefore water was added manually prior to pelleting. Since water was added to the diets, the pellets had to be dried in an oven at 55°C. Diets were formulated to meet or exceed NRC (1994) nutrient requirements formulated on an ideal protein ratio of methionine to lysine (≥0.45 to 1.00) (Table 5.1). Calculated diet ABC was the summation of the ABC of ingredients multiplied by their percentage in the diet. Feed and water were provided ad libitum throughout the experiment.

5.3.2.3 Data Collection

Intestinal pH for the crop, proventriculus and gizzard was collected from three birds per replicate every other day from 2 to 12 d of age. Birds were killed by cervical dislocation and then the contents from the entire crop, proventriculus and gizzard were extracted using 0.9 mL of double distilled water to rinse the gastrointestinal section. Once the contents were extracted, the contents were weighed, taking into account the water added for rinsing. The contents were then diluted by 9 times with double distilled water minus the 0.9 mL water used to rinse the organs. After being weighed, diluted, and stirred for

Table 5.1. Ingredient composition (%) and formulated nutrient profile of diets with increasing ABC (Experiment 2).

	Diet (Calculated ABC (mEq/kg)[6])				
	556	637	718	799	880
Ingredients: %					
Wheat	55.52	41.64	27.76	13.88	0.00
Corn	14.03	27.26	40.50	53.73	66.96
Soybean meal	0.00	5.23	10.45	15.68	20.90
Fish meal	0.00	2.15	4.30	6.44	8.59
Feather meal	8.01	6.01	4.01	2.00	0.00
Canola meal	1.00	0.75	0.50	0.25	0.00
Corn gluten meal	14.95	11.21	7.48	3.74	0.00
Canola oil	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.27	0.95	0.64	0.32	0.00
Limestone	0.00	0.27	0.53	0.80	1.06
Calcium citrate	2.75	2.06	1.38	0.69	0.00
Sodium chloride	0.22	0.24	0.26	0.28	0.30
Vitamin/mineral premix [1]	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.00	0.00	0.00	0.00	0.00
DL-methionine	0.03	0.10	0.17	0.23	0.30
L-threonine	0.00	0.06	0.12	0.17	0.23
L-lysine HCL	0.64	0.49	0.35	0.20	0.05
Enzyme[2]	0.05	0.04	0.03	0.01	0.00
Pellet binder[3]	0.50	0.50	0.50	0.50	0.50
Salinomycin sodium[4]	0.01	0.01	0.01	0.01	0.01
Virginiamycin[5]	0.02	0.02	0.02	0.02	0.02
Formulated nutrient profile					
AME (kcal/kg)	3,100	3,100	3,100	3,100	3,100
Crude protein (%)	25.00	24.00	23.00	22.00	21.00
Lysine (%)	1.10	1.21	1.32	1.43	1.54
Methionine (%)	0.50	0.55	0.60	0.65	0.70
Calcium (%)	1.00	1.00	1.00	1.00	1.00
Non-phytate P (%)	0.45	0.45	0.45	0.45	0.45

[1] Supplied per kilogram of diet: 11,000 IU of vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU of vitamin D3 (cholecalciferol), 30 IU of vitamin E (DL-α-tocopheryl acetate), 2.0 mg of vitamin K3 (menadione), 1.5 mg of thiamine, 6.0 mg of riboflavin, 60 mg of niacin, 4 mg of pyridoxine, 0.02 mg of vitamin B12, 10.0 mg of pantothenic acid, 6.0 mg of folic acid, 0.15 mg of biotin, 0.625 mg of ethoxyquin, 500 mg of CaCO3, 80 mg of Fe, 80 mg of Zn, 80 mg of Mn, 10 mg of Cu, 0.8 mg of I, and 0.3 mg of Se.

[2] Avizyme 1302 (Danisco A/S, Copenhagen, DK distributed by PMT Inc., Regina, Canada).

[3] Pro-bond (pea starch) pellet binder (Parrheim Foods, Portage la Prairie, Manitoba, Canada).

[4] Coccistac (Phibro Animal Health, Ridgefield Park, NJ).

[5] Stafac-44 (Phibro Animal Health).

[6] ABC = volume of HCl (mEq) per kg of feed sample to lower the solution to pH 3.

one minute using a stir plate, a pH probe[5] was inserted into the solution and a reading was recorded once the pH was stable to $\pm$ 0.001 for 1 minute.

5.3.2.4 Chemical Analysis

The pH and ABC of the diets were determined as explained in experiment 1.

5.3.2.5 Design and Statistical Analysis

This experiment was designed to examine the effect of increasing calculated dietary ABC on actual diet ABC and broiler gastrointestinal pH. Using the Proc GLM, Reg and RSReg procedures of SAS (SAS Institute, 2002), data were subjected to ANOVA and regression analysis to determine the relationship between increasing calculated ABC and actual diet ABC and broiler gastrointestinal pH. Duncan's Multiple Range Test was used to separate means when the ANOVA was significant. Differences were considered significant when *P-value* $\leq$ 0.05.

5.3.3 Experiment 3. Effect of Reducing Dietary Calcium in Broiler Diets on Diet pH and ABC and Broiler Intestinal pH, Performance and Bone Ash

5.3.3.1 Animals and Housing

In this experiment, 240 Cobb 500 male broilers were housed in battery cages from 0-21 d of age. Treatments included one of 6 diets fed from 0-5 d of age. Treatments were replicated five times with 8 birds in each of 30 battery cages. A standard temperature curve (decreasing 0.5°C every day) starting at 35°C at d 0 was used and

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[5] Futura™ Refillable Combination Electrode Epoxy, Calomel, 7 x 245mL. Distributed by Beckman (part #511084)

birds were exposed to 24 hours of light per d. Feed and water was provided ad libitum throughout the experiment.

5.3.3.2 Dietary Treatments

Six pre-starter diets, fed from 0-5 d of age, were formulated to have either 0.50, 0.75 or 1.00% calcium using either limestone or calcium citrate as the calcium source (Table 5.2). After d 5, birds were all fed the diet with 1.00% limestone for the remainder of the experiment. With the exception of a calcium deficiency in the diets with 0.50 and 0.75% calcium, diets were formulated to meet or exceed NRC (1994) nutrient requirements. Diets were formulated using an ideal protein ratio of 0.43 (methionine to lysine) with lysine making up 1.25% of the diet.

5.3.3.3 Data Collection

To determine the effect of the type and level of calcium on broiler production, the parameters measured during this experiment included mortality, body weight and feed intake. Feed intake and bird body weight data were collected on 0, 7, 14 and 21 d of age. From this data, the feed conversion ratio (feed:gain), corrected for mortality, was determined for each period. Mortality and culls were collected, weighed and necropsied. Any abnormalities found during necropsy were recorded as they were identified. On d 5, intestinal pH for the crop, proventriculus and gizzard was collected from two birds per replicate by the methods explained in experiment 2. Left tibial bones were collected from 2 birds per replicate at 5 d of age and all remaining birds in a replicate at 21 d of age. Tibial bone ash was determined using methods that conform to the Association of Official Analytical Chemists Official Methods (1990).

Table 5.2. Ingredient composition (%) and formulated nutrient profile of diets different calcium level and type (Experiment 3).

	Diet (Calcium Source and % Calcium)					
	Limestone			Calcium Citrate		
	1.00%	0.75%	0.50%	1.00%	0.75%	0.50%
Ingredients: %						
Wheat	67.26	69.00	70.75	65.09	67.23	69.37
Soybean meal	15.72	15.45	15.18	16.06	15.73	15.39
Canola meal	10.00	10.00	10.00	10.00	10.00	10.00
Canola oil	2.88	2.36	1.84	3.52	2.88	2.25
Dicalcium phosphate[1]	1.40	0.71	0.03	1.41	0.73	0.04
Limestone	1.45	1.18	0.92	0.00	0.00	0.00
Calcium citrate	0.00	0.00	0.00	2.60	2.13	1.65
Sodium chloride	0.31	0.31	0.31	0.32	0.31	0.31
Vitamin/mineral premix [2]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.22	0.21	0.21	0.22	0.22	0.21
L-threonine	0.10	0.09	0.09	0.10	0.10	0.09
L-lysine HCL	0.40	0.40	0.40	0.40	0.40	0.40
Pellet binder[3]	0.50	0.50	0.50	0.50	0.50	0.50
Enzyme	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin sodium[4]	0.001	0.001	0.001	0.001	0.001	0.001
Virginiamycin[5]	0.025	0.025	0.025	0.025	0.025	0.025
Formulated nutrient profile						
AME (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000
Crude protein (%)	22.32	22.47	22.62	22.14	22.32	22.51
Lysine (%)	1.25	1.25	1.25	1.25	1.25	1.25
Methionine (%)	0.54	0.54	0.54	0.54	0.54	0.54
Calcium (%)	1.00	0.75	0.50	1.00	0.75	0.50
Chloride (%)	0.25	0.25	0.25	0.26	0.26	0.25
Non-phytate phosphorus (%)	0.50	0.38	0.25	0.50	0.38	0.25

[1] Supplied per kilogram of diet: 11,000 IU of vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU of vitamin D3 (cholecalciferol), 30 IU of vitamin E (DL-α-tocopheryl acetate), 2.0 mg of vitamin K3 (menadione), 1.5 mg of thiamine, 6.0 mg of riboflavin, 60 mg of niacin, 4 mg of pyridoxine, 0.02 mg of vitamin B12, 10.0 mg of pantothenic acid, 6.0 mg of folic acid, 0.15 mg of biotin, 0.625 mg of ethoxyquin, 500 mg of CaCO3, 80 mg of Fe, 80 mg of Zn, 80 mg of Mn, 10 mg of Cu, 0.8 mg of I, and 0.3 mg of Se.

[2] Avizyme 1302 (Danisco A/S, Copenhagen, DK distributed by PMT Inc., Regina, Canada).

[3] Pro-bond (pea starch) pellet binder (Parrheim Foods, Portage la Prairie, Manitoba, Canada).

[4] Coccistac (Phibro Animal Health, Ridgefield Park, NJ).

[5] Stafac-44 (Phibro Animal Health).

5.3.3.4 Design and Statistical Analysis

This experiment was set up as a 2 (type of calcium) x 3 (level of calcium) factorial arrangement in a completely randomized design. Statistical analysis was conducted as a factorial (interaction between type and level of calcium) using the Proc GLM, Reg and RSReg procedures of SAS. Duncan's Multiple Range Test was used to separate means when the ANOVA is significant. Differences will be considered significant when *P-value* ≤ 0.05.

5.4 Results

5.4.1 Experiment 1

Differences in ABC (mEq of HCl/kg of sample) were found with calcium products having the highest ABC followed by protein ingredients (Table 5.3). Differences between ingredient types were found with limestone having the highest ABC for calcium products at 20,169 mEq/kg and calcium citrate being lower at 8,319 mEq/kg. For protein ingredients, MM had the highest value at 2,873 mEq/kg and CGM had the lowest ABC at 283 mEq/kg. DW had the highest ABC for energy ingredients at 333 mEq/kg while dextrose was the lowest ABC at 138 mEq/kg. No differences were found for ABC as a result of grind size (Table 5.4).

5.4.2 Experiment 2

Diets were formulated on the basis of ingredient ABC and analyzed diet ABC followed a similar trend to the calculated ABC. However, the analyzed ABCs did not achieve the same range as the calculated ABCs (Table 5.5). Calculated ABCs of the diets ranged from 880 to 556 whereas the analyzed ABC of the diets ranged from 786 to 592.

Table 5.3. The pH and ABC[1] of feed ingredients available for use in broiler diets and ground in a Wiley mill using a 1mm screen (Experiment 1).

	pH	ABC[2]
Protein ingredients		
Meat meal	7.03 ± 0.06	2873 ± 202
Fishmeal	6.72 ± 0.03	2767 ± 224
Insoluble canola protein concentrate[3]	7.63 ± 0.03	1810 ± 10
Threonine[3]	5.68 ± 0.00	1392 ± 3
Canola meal	6.39 ± 0.03	1318 ± 48
Soybean meal	7.13 ± 0.01	1282 ± 37
Methionine[3]	5.75 ± 0.01	1215 ± 7
Pea protein concentrate[3]	6.60 ± 0.03	1035 ± 28
Soluble canola protein concentrate[3]	5.07 ± 0.01	924 ± 33
Lysine[3]	5.44 ± 0.13	767 ± 13
Feather meal	6.55 ± 0.03	602 ± 8
Pea	6.60 ± 0.03	554 ± 2
Corn distillers grain with solubles	4.30 ±0.01	554 ± 1
Corn gluten meal	4.21 ± 0.02	283 ± 29
Cereal grains and energy sources		
Durum wheat	6.91 ± 0.05	334 ± 29
Wheat	6.65 ± 0.03	295 ± 7
Oats	6.49 ± 0.01	269 ± 5
Corn	6.39 ± 0.10	256 ± 11
Barley	6.08 ± 0.07	245 ± 12
Dextrose[3]	6.80 ± 0.01	138 ± 7
Minerals		
Limestone[3]	9.26 ± 0.07	20170 ± 224
Calcium Citrate[3]	6.34 ± 0.04	8320 ± 24
Dicalcium Phosphate	6.01 ± 0.03	2335 ± 7
Sodium chloride[2]	6.00 ± 0.10	183 ± 66

[1]Means of 2 replicates having 6 (canola meal), 5 (meat meal, fishmeal, soybean meal and corn), 4 (corn gluten meal, wheat) or 1 sample(s) (all others) per ingredient.

[2] ABC = volume of HCl (mEq) per kg of feed sample to lower the solution to pH 3.

[3] Ingredients tested without grinding.

Table 5.4. ABC of feed ingredients ground in a Wiley mill using 0.5 and 1.0 mm screen sizes (Experiment 1).

	ABC[2]
Ingredient	
Canola meal	1317[c]
Corn	260[d]
Corn distillers grains	554[d]
Corn gluten meal	284[d]
Feather meal	623[d]
Fishmeal	2763[b]
Meat meal	3217[a]
Soybean meal	1264[c]
Wheat	306[d]
P-value	<0.0001
Grind Size	
0.5	1407
1	1313
P-value	NS
SEM	98.1
Interaction (ingredient x grind size)	NS

[1]Means of 2 replicates having 6 (canola meal), 5 (meat meal, fishmeal, soybean meal and corn), 4 (corn gluten meal, wheat) or 1 sample(s) (all others) per ingredient.

[2] ABC = volume of HCl (mEq) per kg of feed sample to lower the solution to pH 3.

[a,b,c,d] Means within a common column and main effect with different subscripts differ significantly (*P-value* ≤ 0.05).

Table 5.5. Analyzed ABC[1] of diets with increasing calculated ABC (Experiment 2).

Calculated Diet ABC[1]	Analyzed Diet ABC[2*]
556	592
637	672
718	677
799	687
880	786
P-value	NS
SEM	79.8

[1]Means of 2 replicates.

[2] ABC = volume of HCl (mEq) per kg of feed sample to lower the solution to pH 3.

[*] Significant linear regression (*P-value* ≤ 0.05) for increasing dietary ABC.

Increasing ABC increased crop and gizzard pH while proventricular pH was unaffected. A quadratic relationship was found between crop and proventriculus pH however the trend for all sections was a decreasing digesta pH with age (Table 5.6). Interactions were found between diet and age in the crop and therefore the effect of increasing dietary pH on the pH of all sections is shown in Table 5.7. For the diets of low ABC (556, 637 and 718), crop pH decreased to 6 d followed by an increase. The crop pH of birds fed the diets of higher ABC (779 and 880) decreased linearly to 12 d. Crop pH increased linearly from d 2 to 8 as ABC increased while at 10 d of age the relationship between diet ABC and crop pH was quadratic (Table 5.7).

5.4.3 Experiment 3

Feeding calcium citrate rather than limestone reduced dietary pH however the dietary ABC was not affected (Table 5.8). Reducing dietary calcium reduced the pH and ABC of the diet. Neither calcium source nor level of calcium affected gastrointestinal pH (Table 5.9), body weight or body weight gain (Table 5.10). Calcium source did not affect feed intake or feed conversion (Table 5.11). When calcium level was reduced, feed intake was increased numerically for 0-5 d (*P-value* = 0.1645) and significantly for 5-21 and 0-21 d (*P-value* $\leq$ 0.05). Feed to gain was increased with decreasing dietary calcium. Tibial bone ash was reduced at 5 d of age with reduced dietary calcium but no effect was observed at 21 d of age (Table 5.12). Calcium source had no effect on tibial bone ash.

Table 5.6. Effect of feeding diets formulated with increasing ABC and age on crop, proventriculus and gizzard pH1 (Experiment 2).

	Crop	Proventriculus	Gizzard
Diet (Calculated ABC[2])			
556	4.88[d]	3.86	3.17[c]
637	5.08[c]	3.81	3.26[c]
718	5.19[bc]	3.75	3.41[b]
799	5.33[ab]	3.84	3.59[a]
880	5.39[a]	3.84	3.55[a]
P-value	<0.0001	NS	<0.0001
Regression	Linear	NS	Linear
Age (d)			
2	5.50[a]	4.26[a]	3.57[a]
4	5.21[b]	3.83[bc]	3.34[b]
6	4.96[c]	3.78[bc]	3.42[ab]
8	5.06[bc]	3.56[c]	3.34[b]
10	5.13[bc]	3.85[b]	3.48[ab]
12	5.18[b]	3.63[bc]	3.35[b]
P-value	<0.0001	<0.0001	0.0153
SEM	0.029	0.039	0.025
Regression	Quadratic	Quadratic	NS
Interaction			
Diet x Age	0.0175	0.4288	0.1961

[1] Means of 5 replicates with 3 birds per replicate.

[2]ABC = volume of HCl (mEq) per kg of feed sample to lower the solution to pH 3.

[a,b,c,d] Means with no common superscript within a row and diet effect, differ significantly (*P-value* ≤ 0.05).

Table 5.7. Effect of increasing dietary ABC on crop pH[1] from 2 to 12 d (Experiment 2).

	Diet (Calculated ABC[2])					*P-value*	SEM
	556	637	718	799	880		
Age							
Crop							
2*	5.25^{b,x}	5.27^{b}	5.60^{ab,x}	5.57^{ab}	5.82^{a}	0.0134	0.062
4*	4.62^{b,z}	4.90^{b}	5.34^{a,xy}	5.57^{a}	5.61^{a}	<0.0001	0.083
6*	4.62^{c,z}	4.86^{bc}	4.70^{c,z}	5.25^{ab}	5.35^{a}	0.0039	0.078
8*	4.82^{yz}	5.05	5.12^{y}	5.24	5.12	NS	0.054
10**	4.89^{yz}	5.17	5.38^{xy}	5.13	5.08	NS	0.055
12	5.10^{xy}	5.24	5.02^{yz}	5.23	5.35	NS	0.072
P-value	<0.0001	NS	<0.0001	NS	NS		
SEM	0.047	0.053	0.059	0.064	0.081		
Regression	Quadratic	Quadratic	Quadratic	Linear	Linear		

[1] Means of 5 replicates with 3 birds per replicate.

[2] ABC = volume of HCl (mEq) per kg of feed sample to lower the solution to pH 3.

* Significant linear regression (*P-value* ≤ 0.05) for increasing dietary ABC.

** Significant quadratic regression (*P-value* ≤ 0.05) for increasing dietary ABC.

[a,b,c] Means with no common superscript within a row and diet effect, differ significantly (*P-value* ≤ 0.05).

[x,y,z] Means with no common superscript within a column and age effect, differ significantly (*P-value* ≤ 0.05).

Table 5.8. Effect of calcium level and source on diet pH and ABC[1] (Experiment 3).

	pH	ABC[2]
Calcium Level		
0.50	5.99[c]	598[b]
0.75	6.23[b]	757[ab]
1.00	6.43[a]	814[a]
P-value	<0.0001	0.0300
Source of Calcium		
Limestone	6.31[a]	761
Calcium Citrate	6.13[b]	684
P-value	<0.0001	NS
SEM	0.031	35.6
Interactions		
Level x Source	NS	NS

[1]Means of 2 replicates.

[2]ABC = volume of HCl (mEq) per kg of feed sample to lower the solution to pH 3.

[a,b,c] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Table 5.9. Effect of calcium level and source on gastrointestinal pH[1] at 5 d of age (Experiment 3).

	Gastrointestinal Section		
	Crop	Proventriculus	Gizzard
Calcium Level			
0.50	5.53	3.70	2.84
0.75	5.15	3.71	2.94
1.00	5.17	3.74	2.96
P-value	NS	NS	NS
Source of Calcium			
Limestone	5.27	3.61	2.86
Calcium Citrate	5.29	3.83	2.97
P-value	NS	NS	NS
SEM	0.097	0.097	0.044
Interactions			
Level x Source	NS	NS	NS

[1] Means of 5 replicates with 2 birds in each replicate.

Table 5.10. Effect of calcium level and source on broiler body weight and gain[1] (Experiment 3).

	BW (kg)		BW Gain (kg)		
	5d	21d	0-5d	5-21d	0-21d
Calcium Level					
0.50	0.104	0.695	0.059	0.591	0.649
0.75	0.108	0.720	0.063	0.612	0.675
1.00	0.103	0.692	0.058	0.589	0.647
P-value	NS	NS	NS	NS	NS
Source of Calcium					
Limestone	0.106	0.704	0.060	0.598	0.658
Calcium Citrate	0.105	0.701	0.059	0.596	0.656
P-value	NS	NS	NS	NS	NS
SEM	0.0010	0.0096	0.0011	0.0091	0.0097
Interactions					
Level x Source	NS	NS	NS	NS	NS

[1] Means of 5 replicates with 8 birds in each replicate.

Table 5.11. Effect of calcium level and source on broiler feed intake and the feed to gain ratio[1,2] (Experiment 3).

	Feed Intake (kg)			Feed:Gain[1]		
	0-5d	5-21d	0-21d	0-5d	5-21d	0-21d
Calcium Level						
0.50	0.074	0.994[a]	1.100[a]	1.259[a]	1.698[a]	1.644[a]
0.75	0.073	0.980[a]	1.081[a]	1.154[b]	1.603[ab]	1.546[ab]
1.00	0.069	0.910[b]	1.006[b]	1.200[ab]	1.536[b]	1.497[b]
P-value	NS	0.0225	0.0248	0.0370	0.0233	0.0194
Source of Calcium						
Limestone	0.074	0.957	1.061	1.216	1.601	1.552
Calcium Citrate	0.070	0.965	1.061	1.193	1.624	1.572
P-value	NS	NS	NS	NS	NS	NS
SEM	0.0013	0.0139	0.0151	0.0167	0.0255	0.0228
Interactions						
Level x Source	NS	NS	NS	NS	NS	NS

[1] Means of 5 replicates with 8 birds in each replicate.

[2] Feed:gain was mortality corrected.

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Table 5.12. Effect of calcium level and source on tibial bone ash[1] at 5 d and 21 d of age (Experiment 3).

	Age	
	5d	21d
Calcium Level		
0.50	37.8[c]	49.7
0.75	42.7[b]	49.9
1.00	44.2[a]	49.8
P-value	<0.0001	NS
Source of Calcium		
Limestone	41.6	49.6
Calcium Citrate	41.5	50.0
P-value	NS	NS
SEM	0.57	0.15
Interactions		
Level x Source	NS	NS

[1] Means of 5 replications with 2 birds in each replicate.

[a,b,c] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

5.5 Discussion

The ABC of feed ingredients were found to be different; increased ABC was associated with higher levels of calcium minerals or protein in the ingredient. Results were similar to those found by Lawlor et al. (2005). An exception was meat and bone meal, which was 920 mEq/kg compared to the current study which was 2873 mEq/kg. This can be expected since there are dramatic differences in the processing and calcium content of meat and bone meal.

Differences within ingredient types suggested that using a combination of ingredients with lower ABC could reduce diet ABC. This was generally found to be true, however the calculated ABC was not exactly the same as the actual dietary ABC. The differences between calculated and actual diet ABC indicate that the values for individual ingredients are not additive in a mixed diet and that there may be interactions between ingredients that influence diet ABC. Nonetheless, it was possible to formulate diets with different ABC based in the values for individual ingredients. Of note, protein source ingredients with low ABC tended to be those with low amino digestibility. This may be the effect of reduced solubility, which reduces nutrient digestibility by young birds and reduces the sites for HCl binding and as a consequence may reduce ABC. Therefore, caution must be taken to consider diet digestibility along with ABC when trying to formulate diets of lower ABC.

Birds fed diets formulated to have reduced ABC by using a combination of low ABC ingredients were found to have reduced crop and gizzard pH. This confirms that diet ABC can influence gastrointestinal pH. However, using calcium citrate instead of limestone in diets reduced diet pH but did not affect diet ABC, which is surprising based

on the ABC of the two Ca products. It would appear that the degree of interactions between ingredients in the diet and calcium citrate is different then that of limestone. Reducing dietary calcium during the first 5 d did not affect body weight or gain throughout the experiment however feed intake was increased from 5-21 d. As a consequence the feed to gain ratio was adversely affected. The relatively large effect is not easy to explain but may relate to birds increasing feed intake to compensate for the lower dietary calcium provided for the initial 5 d of life. Yan et al. (2005) found that reducing dietary calcium to 18 d decreased body weight and feed intake in contrast to the current study. It is possible that the more extended calcium deficiency resulted in rickets and a subsequent reduction in performance. Although bone ash was reduced at 5 d for birds fed diets with reduced calcium, this effect was not observed at 21 d indicating a strong ability to compensate for the early deficiency. Yan et al. (2005) similarly found that reducing calcium to 18 d reduced 18 d bone ash however by 32 d, bone ash was no different than birds fed control diets.

Comparing the effect of age on intestinal pH of this study to that reported in Chapter 3 shows some similar trends but also some differences. Chapter 3 reported a linear increase in crop pH, which was not seen in this study. The results of this study are comparable to that of Hinton et al. (1990), which found a decrease in crop pH with age. This data combined with the current research suggests a maturation of the acidogenic bacterial populations that are commonly found in the crop. The proventriculus and gizzard however, were shown to have a decrease in pH with age in both experiments.

In conclusion, results show ingredients vary in ABC and that diets can effectively be created to reduce dietary pH, ABC when using a combination of low ABC ingredients.

However, it is difficult to accurately predict the ABC of the diet indicating that the ABC of individual ingredients are not additive. Feeding diets of reduced ABC does not appear to affect intestinal pH or improve performance.

6 THE EFFECT OF HYDROCHLORIC ACID ON FEED AND INTESTINAL PH, AMINO ACID DIGESTIBILITY AND BROILER PERFORMANCE WHILE INITIATING LIGHTING PROGRAMS AT TWO AGES.

6.1 Abstract

Two experiments were conducted to examine the effect of hydrochloric acid (HCl) on feed and intestinal pH, amino acid digestibility and performance of Ross x Ross 308 broiler chickens. In experiment 1, birds were placed in battery cages and fed a diet supplemented with 0, 1, 2 or 4% 1 N HCl to 21 d. At 5 and 21 d of age, birds were sacrificed to collect intestinal contents from the crop, proventriculus and gizzard for pH measurement and the ileum for determining amino acid digestibilities. In experiment 2, birds were reared in floor pens and fed a diet supplemented with 0, 0.5, 1.0, 2.0 or 4.0% 1N HCl to either 7 or 35 d. These birds were also exposed to one of three lighting programs which included 23 h of light throughout the experiment (23L), 23 h of light to 7 d then 14 h of light to slaughter (14L at 7d) or 23 h of light to 21 d then 14 h of light to slaughter (14L at 21d). Birds and feed were weighed weekly and meat yield was performed after slaughter at 35 d. Adding acid reduced feed pH in experiment 1 but not in experiment 2. Intestinal pH was reduced in the proventriculus and gizzard with increasing diet acidification at 5 d but at 21 d increasing diet acidification increased proventricular and gizzard pH to a level of 3% HCl followed by a reduction for 4% HCl.

Amino acid digestibility was reduced with increasing acid concentration at 5 d while 21 d saw little effect of diet acidification on amino acid digestibility. In both experiments body weights and gain were increased with diet acidification. Acidification did not affect feed intake with the exception of a reduction from 0-7 d in experiment 1, while the feed to gain ratio was reduced in experiment 1 there was no effect in experiment 2. Early mortality was reduced with diet acidification in both experiment 1 (*P-value* ≤ 0.1) and experiment 2 (0-7 d infectious, *P-value* ≤ 0.05). Lighting program caused significant effects on performance with reduced body weight and feed intake at the initiation of the lighting program, however the feed to gain ratio and mortality were also reduced. Results show that both diet acidification and lighting program affect broiler performance. Diet acidification was found to improve broiler performance and reduce early infectious mortality, however the improvements are not the result of increased nutrient digestibility. Initiation of the lighting program reduced the feed to gain ratio and mortality while it also suppressed body weight, gain and feed intake. Subsequent improvements after lighting program initiation were observed regardless of the age that the program was initiated. Lighting programs started at a younger age provide the greatest overall improvements in feed conversion and reductions in mortality. Early initiation of lighting programs allow for more catch-up time for body weight gain and feed intake thus improving final performance when compared to birds on lighting programs that were initiated at an older age.

6.2 Introduction

Research shows that digestion is reduced in the young bird and that protein digestion may be the most limiting (Noy and Sklan, 1999; Sklan and Noy, 2000). In

addition to reduced proteolytic enzymes, acid secretion by the proventriculus is reduced in very young birds (Chapter 3). Low levels of gastric acid could compromise the first steps of protein digestion; denaturation of proteins and conversion of pepsinogen to pepsin, the first enzyme responsible for protein digestion. Consequently, since gastric acid secretion is the first step in protein digestion and secretion is limited in young birds, acidifying diets could improve intestinal function and therefore amino acid digestibility, growth and performance of broiler chickens. In recent years there has been significant interest in the effects of acidifying diets of pigs and poultry and numerous products have been developed with the hope of improving performance.

Most research on diet acidification has examined the effect of a single organic acid or combinations of organic acids, bactericides or supplements (Patten and Waldroup, 1988; Krause et al., 1994; Biggs and Parsons, 2008). Research on products using any combination of organic acids or other supplements, although beneficial in demonstrating effectiveness, may not demonstrate the basic action of diet acidification. Organic acids differ dramatically in composition, pH and pKa, which could result in different modes of action in the feed or animal. Little research has been done comparing organic and inorganic acids and establishing the exact mechanisms whereby organic acids affect the microflora of the gastrointestinal tract. However, it is suggested that organic acids are generally weak acids and therefore do not dissociate fully in water whereas inorganic acids are generally strong acids that fully dissociate in water (Dibner and Buttin, 2002). In the gut, organic acids (particularly short chain) do not fully dissociate allowing them to passively diffuse through the cell wall of bacteria. Once inside the bacteria, the pH within promotes the dissociation of the acid causing a drop in pH within the bacteria

preventing the normal functioning of the bacteria and possibly causing cell death (Dibner and Buttin, 2002). It has also been suggested that the type of bacteria may influence the effectiveness of an organic acid. On the other hand, since inorganic acids fully dissociate in water and therefore digesta they may act more directly on the intestinal digesta by lowering the pH of the digestive tract. This may in turn create an environment that is more conducive to protein digestion and less favourable to some bacteria. Additionally, the pH of the stomach or proventriculus must be between 2 and 3 for pepsinogen activation and protein denaturation, however the pKa of most organic acids is well above this and therefore acidification of the upper digestive tract with organic acids may be limited. Moreover, inorganic acids are generally more economical then organic acids; therefore if effective, they may be more desirable as diet acidifiers in poultry production. The current study used hydrochloric acid, which has a very low pH and pKa (pKa -8) compared to organic acids so that diet and intestinal acidification would not be limited by the acid. Additionally, since gastric acid is HCl, supplementing diets with this acid would be the most natural solution to remediate low gastric acid secretions.

Stress often reduces performance in broiler chickens. Stressors to broilers can include health, environmental, or nutritional factors. Research has suggested that these stressors often affect the intestinal environment. The period immediately after lighting program initiation may be a stressful period for broilers. If diet acidification can alleviate intestinal distress, then during times of stress diet acidification may prove to be beneficial to broiler health and performance. Therefore it is hypothesized that during the initial period after the commencement of a lighting program, broilers may benefit from diet acidification.

The objective of this study was to examine the effect of diet acidification with HCl on the pH of feed and broiler intestinal digesta as well as amino acid digestibility, performance, mortality and meat yield. A second objective was to examine the effect of diet acidification on performance after starting lighting programs at two ages.

6.3 Materials and Methods

6.3.1 Experiment 1. Effect of Diet Acidification on Intestinal pH, Ileal Amino Acid Digestibility and Performance: A Battery Experiment

6.3.1.1 Animals and Housing

Day of hatch, Ross x Ross 308 straight-run broilers (960) were used in this experiment. Birds were checked for gender to ensure that exactly half of each gender was placed in each replicate. A temperature curve (decreasing 0.5°C every day) starting at 35°C at d 0 was used. Birds were fed once and checked several times a day at different times each day as well as exposed to 24 hours of light. This was to reduce the development of circadian rhythms, which may influence the timing of feed intake and therefore acid production since acid is secreted in response to proventricular distention (Hersey and Sachs, 1995). Birds were also exposed to 24 hours of light for the same reason.

For ease of operation, this experiment was performed in two parts run concomitantly (A and B). Part A examined the effect of diet acidification on intestinal pH and ileal amino acid digestiblities at 5 d of age while Part B examined the effects of diet acidification on intestinal pH, ileal amino acid digestiblities and performance at 21 d

of age. In Part A, treatments were replicated 6 times with 22 broilers per replicate. In Part B each treatment was replicated 18 times with 6 broilers per replicate.

6.3.1.2 Dietary Treatments

For the length of the experiment birds were fed one of four dietary treatments with increasing levels of 1 N hydrochloric acid. Dietary treatments were 0, 1, 2 or 4% acid (Table 6.1). Water and/or 1 N HCl were added to the diet to make up a total of 4% liquid then pelleted using a cold pelleter and hand crumbled. To enhance the probability of identifying an acid effect on protein digestion, diets were formulated to be deficient in lysine (1.10%). Besides reduced lysine, diets were formulated to meet all other NRC nutrient requirements (NRC, 1994). Since an improvement in protein digestion was the hypothesized means of improving performance with diet acidification rather than microbial influence, all diets included antibiotic growth promoters to reduce the effect of acid on intestinal microbial populations.

6.3.1.3 Data Collection

Body weight and feed intake data were collected on the birds in Part B on d 0, 7, 14 and 21. From this data, the feed conversion (feed:gain ratio), corrected for mortality, was determined for each period. Mortality and culls were collected, weighed and necropsied.

On d 5 for Part A and d 21 for Part B, four birds (2 males and 2 females) from six cages per treatment were randomly selected, ensuring that they were healthy birds, for pH sampling. Birds were killed by cervical dislocation and then the contents from the entire crop, proventriculus and gizzard were extracted with 0.9 mL of double distilled water. Once the contents were extracted, the contents were weighed, taking into account the water added for rinsing. The contents were then diluted by 9 times with double distilled

Table 6.1. Ingredient composition and formulated nutrient profile of diets with increasing levels of HCl (Experiment 1).

	Diet (% HCl)			
	0	1	2	4
Ingredients (%)				
Wheat	60.14	60.14	60.14	60.14
Soybean meal	14.07	14.07	14.07	14.07
Canola meal	10.00	10.00	10.00	10.00
Canola oil	5.61	5.61	5.61	5.61
Dicalcium phosphate	1.23	1.23	1.23	1.23
Limestone	1.56	1.56	1.56	1.56
Sodium chloride	0.33	0.33	0.33	0.33
Vitamin/mineral premix [1]	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10
DL-methionine	0.37	0.37	0.37	0.37
L-threonine	0.17	0.17	0.17	0.17
L-lysine HCL	0.23	0.23	0.23	0.23
Enzyme[2]	0.05	0.05	0.05	0.05
Salinomycin sodium[3]	0.01	0.01	0.01	0.01
Virginiamycin[4]	0.02	0.02	0.02	0.02
Celite	1.50	1.50	1.50	1.50
Water	4.00	3.00	2.00	0.00
Hydrochloric acid	0.00	1.00	2.00	4.00
Formulated nutrient profile				
AME (kcal/kg)	3,000	3,000	3,000	3,000
Crude protein (%)	18.34	18.34	18.34	18.34
Arginine (%)	0.97	0.97	0.97	0.97
Lysine (%)	1.10	1.10	1.10	1.10
Methionine (%)	0.65	0.65	0.65	0.65
Met + Cys (%)	0.92	0.92	0.92	0.92
Threonine (%)	0.84	0.84	0.84	0.84
Tryptophan (%)	0.25	0.25	0.25	0.25
Calcium (%)	1.00	1.00	1.00	1.00
Chloride (%)	0.26	0.29	0.32	0.38
Non-phytate P (%)	0.45	0.45	0.45	0.45

[1] Supplied per kilogram of diet: 11,000 IU of vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU of vitamin D3 (cholecalciferol), 30 IU of vitamin E (DL-α-tocopheryl acetate), 2.0 mg of vitamin K3 (menadione), 1.5 mg of thiamine, 6.0 mg of riboflavin, 60 mg of niacin, 4 mg of pyridoxine, 0.02 mg of vitamin B12, 10.0 mg of pantothenic acid, 6.0 mg of folic acid, 0.15 mg of biotin, 0.625 mg of ethoxyquin, 500 mg of CaCO3, 80 mg of Fe, 80 mg of Zn, 80 mg of Mn, 10 mg of Cu, 0.8 mg of I, and 0.3 mg of Se.

[2] Avizyme 1302 (Danisco A/S, Copenhagen, DK distributed by PMT Inc., Regina, Canada).

[3] Coccistac (Phibro Animal Health, Ridgefield Park, NJ).

[4] Stafac-44 (Phibro Animal Health).

water minus the 0.9 mL water used to rinse the organs. After being weighed, diluted, and stirred for 1 minute using a stir plate, a pH probe[6] was inserted into the solution and a reading was recorded once the pH was stable to ± 0.001 for 1 minute. For ileal amino acid analysis, ileal samples were collected from the middle two-thirds of the ileum from 20 birds in Part A and 6 birds in Part B (including the birds used for pH sampling). The ileum was defined as the section of intestine from the Meckel's diverticulum to the ileocecal junction.

6.3.1.4 Chemical Analysis

Diets and ileal digesta were analyzed for crude protein, amino acids and acid insoluble ash. Bird ileal samples were pooled within a replicate. Crude protein and amino acids were analyzed by Evonik-Degussa Corporation (Hanau, Germany) using methods that conform to the Association of Official Analytical Chemists Official Method 994.12 (Llames and Fontaine, 1994). Crude protein was determined using a FP-2000 Nitrogen Analyzer (LECO Corp, St. Joseph, MI 49085-2396, USA) while amino acids were determined with high performance liquid chromatography using a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd, Cambridge, UK). Dry matter was determined using a Foss NIRS 5000/6500 Feed and Forage Analyzer (FOSS Analytical A/S, DK 3400 Hillerod, Denmark).

Acid insoluble ash was analyzed at the University of Saskatchewan using a modification of the method of Vogtmann, et al. (1975). First, 1-2 g of sample was

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[6] Futura™ Refillable Combination Electrode Epoxy, Calomel, 7 x 245mL. Distributed by Beckman (part #511084)

weighed into 125 mm disposable borosilicate tubes which were then placed into an ashing oven at 500°C for 24 h or until contents were reduced to white ash. Following ashing, 5 mL of 4N HCl was slowly added to the ash and vortexed. After vortexing, tubes were covered with glass marbles and placed in an oven at 120° for one hour. Samples were then centrifuged at 2500 × g for 10 minutes and the supernatant was then removed and samples were washed repeatedly with 5 ml water (using the vortex/centrifugation method as described above). Samples were then dried at 80ºC overnight, followed by ashing at 500°C overnight. The percent acid insoluble ash was calculated as (total ashed wt - tube wt) / (original - tube wt).

The pH of the feed was determined by suspending 0.5 grams of diet in 50 mL double distilled water by continuous stirring using a stir plate. The pH of the solution was recorded once the pH stabilized for 3 minutes (stable to 0.001). Feed acid binding capacity (ABC) was determined using the method of Jasaitis et al. (1987) as modified by Lawlor et al. (2005). ABC was expressed as the amount of hydrochloric acid in milliequivalents (mEq) required to lower the pH of 1 kilogram of sample to pH 3.0.

6.3.1.5 Design and Statistical Analysis

This experiment was designed to examine the relationship between increasing diet acidification and diet pH and ABC, gastrointestinal pH, ileal amino acid digestibility and broiler performance. Using the PROC GLM procedures of SAS (SAS Institute, 2002), data for intestinal pH, amino acid digestibility and performance were analyzed as a 2 (age) x 4 (dietary acid inclusion rate) factorial arrangement. The ANOVA analyzed both the individual effects as well as interactions that may have occurred between these factors. The dietary pH and ABC were analyzed using only one-way ANOVA to compare

dietary treatments. Diet data were also subjected to regression analysis using the Proc Reg and RSReg procedures of SAS (2002). Duncan's Multiple Range Test was used to separate means when the ANOVA was significant. Differences were considered significant when *P-values* were ≤ 0.05.

6.3.2 Experiment 2. Effect of Diet Acidification and Lighting Program on Broiler Performance and Meat Yield – A Production Experiment.

6.3.2.1 Animals and Housing

In this experiment 6,264 Ross x Ross 308 male and female broilers were reared using one of three lighting programs and fed 1 of 6 acidified diets. Feed and water was provided ad libitum throughout the experiment. The experiment used nine environmentally independent rooms each containing 12 pens; 29 male and 29 female day-of-hatch broilers were placed in each pen. The pens were bedded with straw and included a hanging feeder (0 to 28 d, 36 cm in diameter; 29 to 35 d, 44 cm in diameter) and one line of nipple drinkers (150 cm length; 6 nipples; 25 cm apart). A room temperature curve (decreasing 0.5°C every day) starting at 35°C at d 0 and decreasing to 22°C by d 26 was used.

6.3.2.2 Dietary Treatments

Six starter, grower and finisher diets were formulated to having increasing levels of hydrochloric acid (Table 6.2, 6.3 and 6.4). The starter diet (crumble) was fed from 0-14 d, the grower diet (crumble) was fed from 14-28 d and the finisher diet (pelleted) was fed from 28-35 d. Diets were formulated to meet or exceed NRC (1994) nutrient requirements with the exception of protein and amino acids. Since it was hypothesized that diet acidification would improve protein digestion, the level of lysine (1.20%) was

lowered in all diets to increase the probability of detecting an improvement in amino acid digestibility. Dietary treatments included 0.0, 0.5, 1.0, 2.0, 3.0, 4.0% 1N HCl, which was either included for 7 d (half the length of the starter diet) or throughout the entire experiment.

6.3.2.3 Lighting Programs

All birds received 23 Light (L):1 Dark (D) with a lighting intensity of 20 lux from 0-7 d. After 7 d the lights were dimmed to 10 lux and the treatments included one of the following lighting programs: 23L:1D from 7-35 d, 14L:10D from 7-35 d or 23L:1D from 0-21 d followed by 14L:10D from 21-35 d.

6.3.2.4 Data Collection

To determine the effect of diet acidification on broiler production, the parameters measured during this experiment included body weight, feed intake, mortality and meat yield. Body weight and feed intake data were collected on 0, 7, 14, 28 and 35 d of age. Feed:gain ratio, corrected for mortality, was determined for each period. Mortality and culls were collected and weighed daily and necropsied weekly.

Following slaughter, meat yield data were collected. Birds used for meat yield were only collected from the three rooms exposed to the 23L:1D from 0-35 d, therefore the effect of lighting treatment on meat yield was not examined. Meat yield data were collected for 6 dietary treatments only (0, 2 and 4% acid fed 0-7 d or 0-35 d) with 10 males and 10 females from each of 3 rooms dissected per treatment. Assessment of meat yield was done by weighing each of the following components: live weight (after 4 h pre-shipping feed restriction) and weights of whole eviscerated carcass (excluding neck),

Table 6.2. Ingredient composition and formulated nutrient profile of starter diets with increasing levels of HCl (Experiment 2).

	Diet (% HCl)					
	0.0	0.5	1.0	2.0	3.0	4.0
Ingredients (%)						
Wheat	69.41	69.41	69.41	69.41	69.41	69.41
Soybean meal	13.54	13.54	13.54	13.54	13.54	13.54
Canola meal	10.00	10.00	10.00	10.00	10.00	10.00
Canola oil	2.61	2.61	2.61	2.61	2.61	2.61
Dicalcium phosphate	1.14	1.14	1.14	1.14	1.14	1.14
Limestone	1.61	1.61	1.61	1.61	1.61	1.61
Sodium chloride	0.31	0.31	0.31	0.31	0.31	0.31
Vitamin/mineral premix [1]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20
L-threonine	0.09	0.09	0.09	0.09	0.09	0.09
L-lysine HCL	0.40	0.40	0.40	0.40	0.40	0.40
Enzyme[2]	0.05	0.05	0.05	0.05	0.05	0.05
Pellet binder[3]	0.50	0.50	0.50	0.50	0.50	0.50
Salinomycin sodium[4]	0.01	0.01	0.01	0.01	0.01	0.01
Virginiamycin[5]	0.02	0.02	0.02	0.02	0.02	0.02
Water	4.00	3.50	3.00	2.00	1.00	0.00
Hydrochloric acid	0.00	0.50	1.00	2.00	3.00	4.00
Formulated nutrient profile						
AME (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000
Crude protein (%)	21.70	21.70	21.70	21.70	21.70	21.70
Lysine (%)	1.20	1.20	1.20	1.20	1.20	1.20
Methionine (%)	0.52	0.52	0.52	0.52	0.52	0.52
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00
Non-phytate P (%)	0.45	0.45	0.45	0.45	0.45	0.45

[1] Supplied per kilogram of diet: 11,000 IU of vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU of vitamin D3 (cholecalciferol), 30 IU of vitamin E (DL-α-tocopheryl acetate), 2.0 mg of vitamin K3 (menadione), 1.5 mg of thiamine, 6.0 mg of riboflavin, 60 mg of niacin, 4 mg of pyridoxine, 0.02 mg of vitamin B12, 10.0 mg of pantothenic acid, 6.0 mg of folic acid, 0.15 mg of biotin, 0.625 mg of ethoxyquin, 500 mg of CaCO3, 80 mg of Fe, 80 mg of Zn, 80 mg of Mn, 10 mg of Cu, 0.8 mg of I, and 0.3 mg of Se.

[2] Avizyme 1302 (Danisco A/S, Copenhagen, DK distributed by PMT Inc., Regina, Canada).

[3] Pro-bond (pea starch) pellet binder (Parrheim Foods, Portage la Prairie, Manitoba, Canada).

[4] Coccistac (Phibro Animal Health, Ridgefield Park, NJ).

[5] Stafac-44 (Phibro Animal Health).

Table 6.3. Ingredient composition and formulated nutrient profile of grower diets with increasing levels of HCl (Experiment 2).

	Diet (% HCl)					
	0	0.5	1.0	2.0	3.0	4.0
Ingredients (%)						
Wheat	75.55	75.55	75.55	75.55	75.55	75.55
Soybean meal	8.09	8.09	8.09	8.09	8.09	8.09
Canola meal	10.00	10.00	10.00	10.00	10.00	10.00
Canola oil	2.11	2.11	2.11	2.11	2.11	2.11
Dicalcium phosphate	1.19	1.19	1.19	1.19	1.19	1.19
Limestone	1.61	1.61	1.61	1.61	1.61	1.61
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin/mineral premix [1]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.13	0.13	0.13	0.13	0.13	0.13
L-threonine	0.04	0.04	0.04	0.04	0.04	0.04
L-lysine HCL	0.31	0.31	0.31	0.31	0.31	0.31
Enzyme[2]	0.05	0.05	0.05	0.05	0.05	0.05
Pellet binder[3]	0.50	0.50	0.50	0.50	0.50	0.50
Salinomycin sodium[4]	0.01	0.01	0.01	0.01	0.01	0.01
Virginiamycin[5]	0.02	0.02	0.02	0.02	0.02	0.02
Water	4.00	3.50	3.00	2.00	1.00	0.00
Hydrochloric acid	0.00	0.50	1.00	2.00	3.00	4.00
Formulated nutrient profile						
AME (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000
Crude protein (%)	20.00	20.00	20.00	20.00	20.00	20.00
Lysine (%)	1.00	1.00	1.00	1.00	1.00	1.00
Methionine (%)	0.42	0.42	0.42	0.42	0.42	0.42
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00
Non-phytate phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45

[1] Supplied per kilogram of diet: 11,000 IU of vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU of vitamin D3 (cholecalciferol), 30 IU of vitamin E (DL-α-tocopheryl acetate), 2.0 mg of vitamin K3 (menadione), 1.5 mg of thiamine, 6.0 mg of riboflavin, 60 mg of niacin, 4 mg of pyridoxine, 0.02 mg of vitamin B12, 10.0 mg of pantothenic acid, 6.0 mg of folic acid, 0.15 mg of biotin, 0.625 mg of ethoxyquin, 500 mg of CaCO3, 80 mg of Fe, 80 mg of Zn, 80 mg of Mn, 10 mg of Cu, 0.8 mg of I, and 0.3 mg of Se.

[2] Avizyme 1302 (Danisco A/S, Copenhagen, DK distributed by PMT Inc., Regina, Canada).

[3] Pro-bond (pea starch) pellet binder (Parrheim Foods, Portage la Prairie, Manitoba, Canada).

[4] Coccistac (Phibro Animal Health, Ridgefield Park, NJ).

[5] Stafac-44 (Phibro Animal Health).

Table 6.4. Ingredient composition and formulated nutrient profile of finisher diets with increasing levels of HCl (Experiment 2).

	Diet (% HCl)					
	0.0	0.5	1.0	2.0	3.0	4.0
Ingredients (%)						
Wheat	75.16	75.16	75.16	75.16	75.16	75.16
Soybean meal	8.64	8.64	8.64	8.64	8.64	8.64
Canola meal	10.00	10.00	10.00	10.00	10.00	10.00
Canola oil	2.18	2.18	2.18	2.18	2.18	2.18
Dicalcium phosphate	1.18	1.18	1.18	1.18	1.18	1.18
Limestone	1.61	1.61	1.61	1.61	1.61	1.61
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin/mineral premix [1]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.08	0.08	0.08	0.08	0.08	0.08
L-lysine HCL	0.16	0.16	0.16	0.16	0.16	0.16
Enzyme[2]	0.05	0.05	0.05	0.05	0.05	0.05
Pellet binder[3]	0.50	0.50	0.50	0.50	0.50	0.50
Salinomycin sodium[4]	0.01	0.01	0.01	0.01	0.01	0.01
Virginiamycin[5]	0.02	0.02	0.02	0.02	0.02	0.02
Water	4.00	3.50	3.00	2.00	1.00	0.00
Hydrochloric acid	0.00	0.50	1.00	2.00	3.00	4.00
Formulated nutrient profile						
AME (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000
Crude protein (%)	20.00	20.00	20.00	20.00	20.00	20.00
Lysine (%)	0.90	0.90	0.90	0.90	0.90	0.90
Methionine (%)	0.38	0.38	0.38	0.38	0.38	0.38
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00
Non-phytate P (%)	0.45	0.45	0.45	0.45	0.45	0.45

[1] Supplied per kilogram of diet: 11,000 IU of vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU of vitamin D3 (cholecalciferol), 30 IU of vitamin E (DL-α-tocopheryl acetate), 2.0 mg of vitamin K3 (menadione), 1.5 mg of thiamine, 6.0 mg of riboflavin, 60 mg of niacin, 4 mg of pyridoxine, 0.02 mg of vitamin B12, 10.0 mg of pantothenic acid, 6.0 mg of folic acid, 0.15 mg of biotin, 0.625 mg of ethoxyquin, 500 mg of CaCO3, 80 mg of Fe, 80 mg of Zn, 80 mg of Mn, 10 mg of Cu, 0.8 mg of I, and 0.3 mg of Se.

[2] Avizyme 1302 (Danisco A/S, Copenhagen, DK distributed by PMT Inc., Regina, Canada).

[3] Pro-bond (pea starch) pellet binder (Parrheim Foods, Portage la Prairie, Manitoba, Canada).

[4] Coccistac (Phibro Animal Health, Ridgefield Park, NJ).

[5] Stafac-44 (Phibro Animal Health).

abdominal fat pad, breast skin, pectoralis major and minor, wings, drums, thighs and back (remains after the removal of previous components).

6.3.2.5 Chemical Analysis

Diets were analyzed for crude protein, pH and ABC. The pH of the feed was determined by suspending 0.5 grams of diet in 50 mL double distilled water by continuous stirring using a stir plate. The pH of the solution was recorded once the pH stabilized for 3 minutes (stable to 0.001). Feed acid binding capacity (ABC) was determined using the method of Jasaitis et al. (1987) as modified by Lawlor et al. (2005). ABC was expressed as the amount of hydrochloric acid in milliequivalents (mEq) required to lower the pH of 1 kilogram of sample to pH 3.0.

6.3.2.6 Design and Statistical Analysis

Using the PROC GLM procedure of SAS (SAS Institute, 2002), data (excluding dietary pH and ABC and meat yield data) were analyzed as a 2 (length of dietary acid inclusion) x 6 (dietary acid inclusion rate) factorial arrangement nested within 3 lighting programs. The ANOVA analyzed both the individual effects as well as interactions that may have occurred between these factors. Regression analysis was performed for the effect of diet acidification on diet pH, ABC and performance data. Since birds for meat yield were collected from only one lighting program, a 2 (length of dietary acid inclusion) x 3 (dietary acid inclusion rate) x 2 (gender) factorial arrangement was used. Duncan's Multiple Range Test was used to separate means when the ANOVA was significant. Differences were considered significant when $P\text{-}value \leq 0.05$.

6.4 Results

6.4.1 Dietary pH and ABC

Differences were found between experiments when comparing the relationship of diet pH and increasing dietary acid. Experiment 1 showed a decreasing linear relationship between increasing levels of dietary acid and diet pH but no relationship for ABC. Experiment 2 showed significant differences between the pH of the finisher diets however no linear or quadratic relationship was shown for pH or ABC in the starter, grower or finisher diets (Table 6.5).

6.4.2 Gastrointestinal pH

Experiment 1 showed that crop pH was not affected by diet or chick age (data not shown) however interactions between dietary treatment x age for proventriculus (*P-value* = 0.0275) and gizzard pH (*P-value* = 0.0029) were found. Therefore, regression analysis was performed for each digestive tract section x age subclass (Table 6.6). The interactive data for the proventriculus and gizzard at 5 d shows that as the percentage of acid included increased, the pH dropped. At 21 d the intestinal pH increased to 2% HCl in the diet then subsequently dropped for 4% HCl in a quadratic manner.

6.4.3 Nutrient Digestibility

For all amino acids except leucine and methionine, the interaction between age and dietary level of acid on nutrient digestibility was significant (Experiment 1). The *P-values* for leucine and methionine were 0.0591 and 0.0898 respectively, which

Table 6.5. Effect of diet acidification on broiler feed pH and ABC[1].

	Experiment 1		Experiment 2					
	Starter		Starter		Grower		Finisher	
% HCl in	pH[*]	ABC	pH	ABC	pH	ABC	pH	ABC
0.0	6.36	787	6.07	712	5.85	648	5.63[b]	636
0.5	-	-	6.15	712	6.13	593	5.95[a]	614
1.0	6.28	761	6.00	733	5.97	634	5.82[a]	603
2.0	6.23	755	6.19	775	5.95	606	5.62[b]	535
3.0	-	-	6.05	733	5.85	552	5.83[a]	567
4.0	6.16	724	6.10	668	5.75	616	5.62[b]	588
P-value	NS	NS	NS	NS	NS	NS	0.0026	NS
SEM	0.035	13.5	0.026	17.4	0.042	12.3	0.041	11.9

[1] Means of 2 replicates.

[*] Significant linear relationship (*P-value* ≤ 0.05).

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Table 6.6. Effect of diet acidification and age on gastrointestinal pH[1] (Experiment 1).

	Intestinal Section	
	Proventriculus	Gizzard
Age x Diet		
5d		
0 %	3.50[a]	3.51
1 %	3.12[ab]	3.44
2 %	3.14[ab]	3.39
4 %	2.90[b]	3.31
P-value	0.0280	NS
SEM	0.073	0.037
Regression analysis	Linear	Linear
21d		
0 %	3.25	3.41[b]
1 %	3.80	4.18[a]
2 %	4.14	4.31[a]
4 %	3.28	3.39[b]
P-value	NS	0.0160
SEM	0.157	0.136
Regression analysis	Quadratic	Quadratic

[1] Means of 6 replicates with 4 birds per replicate.

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

approached significance therefore only interactions are shown (Table 6.7). A decreasing linear relationship between increasing diet acidification and digestibility was found for crude protein and the amino acids alanine, asparagine, glutamine, glycine, histamine, isoleucine, proline, valine as well as total amino acids at 5 d of age while a quadratic relationship was found for arginine. In contrast, an increasing linear relationship was found for cysteine and Met + Cys at 21 d of age (Table 6.7) while there was no relationship for other AA. For all AA the 21 d digestibility values were greater then 5 d digestibility values.

6.4.4 Broiler Performance

6.4.4.1 Body Weight and Body Weight Gain

Average body weights at 0 d were 45g (Experiment 1) and 39g (Experiment 2). In experiment 1, a quadratic relationship was observed for an increase in dietary acid with improved body weight for 7, 14 and 21 d of age and gain between 0-7, 7-14 and 0-21 d to 2 or 3% acid followed by a subsequent decrease when dietary acid was increased to 4% (Table 6.8). In contrast, the quadratic effect of increasing diet acidification on body weight gain was lost from 14-21 d.

In experiment 2, interactions were observed between diet and period of acidification for 14 and 21d body weights and 7-14 and 14-21 d body weight gain; therefore both main effects (Table 6.9) and interactions (Table 6.10) are shown. A quadratic effect was observed for body weight at 7d with an increase to 1% acid followed by a decrease in body weight as acid levels were increased, however all acidified diets had either significantly or numerically improved body weights compared to the control. For 14 and 21 d significant differences were observed for body weight with all acidified

Table 6.7. Interactions between age and dietary acid inclusion rate on ileal amino acid digestibility coefficients[1] (Experiment 1).

	5 d							21d						
	Diet (% HCl)							Diet (% HCl)						
Age x Diet	0%	1%	2%	4%	*P-value*	SEM	Regression	0%	1%	2%	4%	*P-value*	SEM	Regression
Crude Protein	0.764	0.762	0.748	0.743	NS	0.0048	Linear	0.842	0.826	0.850	0.834	NS	0.0032	NS
Alanine	0.751	0.773	0.708	0.697	NS	0.0116	Linear	0.818^{ab}	0.798^{b}	0.826^{a}	0.803^{b}	0.0417	0.0041	NS
Arginine	0.827^{a}	0.791^{b}	0.797^{b}	0.798^{b}	0.0051	0.0043	Quadratic	0.880^{a}	0.854^{b}	0.885^{a}	0.876^{a}	0.0001	0.0030	NS
Asparagine	0.709	0.713	0.693	0.688	NS	0.0044	Linear	0.806^{ab}	0.794^{b}	0.823^{a}	0.805^{b}	0.0188	0.0035	NS
Cysteine	0.688	0.709	0.684	0.695	NS	0.0044	NS	0.788^{b}	0.787^{b}	0.815^{a}	0.807^{a}	0.0104	0.0038	Linear
Glutamine	0.864^{a}	0.856^{ab}	0.843^{b}	0.845^{b}	0.0314	0.0029	Linear	0.915^{ab}	0.906^{b}	0.921^{a}	0.912^{ab}	0.0359	0.0019	NS
Glycine	0.722^{a}	0.703^{ab}	0.679^{b}	0.685^{b}	0.0096	0.0054	Linear	0.814^{a}	0.795^{b}	0.823^{a}	0.806^{ab}	0.0176	0.0034	NS
Histamine	0.806^{a}	0.797^{ab}	0.779^{b}	0.776^{b}	0.0170	0.0043	Linear	0.872^{ab}	0.860^{c}	0.879^{a}	0.862^{bc}	0.0055	0.0024	NS
Isoleucine	0.767	0.760	0.751	0.741	NS	0.0044	Linear	0.853^{ab}	0.842^{b}	0.868^{a}	0.851^{b}	0.0261	0.0032	NS
Leucine[2]	0.788	0.784	0.772	0.767	NS	0.0041	-	0.865^{ab}	0.853^{b}	0.875^{a}	0.860^{b}	0.0234	0.0028	NS
Lysine	0.789	0.797	0.780	0.770	NS	0.0047	NS	0.879^{ab}	0.868^{b}	0.888^{a}	0.874^{b}	0.0185	0.0025	NS
Methionine[2]	0.882	0.897	0.890	0.884	NS	0.0025	-	0.930^{b}	0.932^{b}	0.944^{a}	0.935^{b}	0.0034	0.0015	NS
Met + Cys	0.809	0.826	0.811	0.811	NS	0.0030	NS	0.877^{b}	0.878^{b}	0.893^{a}	0.886^{ab}	0.0093	0.0022	Linear
Phenylalanine	0.780	0.774	0.770	0.767	NS	0.0036	NS	0.819^{a}	0.761^{b}	0.790^{b}	0.777^{b}	0.0032	0.0062	NS
Proline	0.833^{a}	0.805^{b}	0.791^{b}	0.789^{b}	0.0007	0.0048	Linear	0.920	0.907	0.913	0.909	NS	0.0021	NS
Serine	0.720	0.723	0.700	0.710	NS	0.0044	NS	0.814^{a}	0.798^{b}	0.824^{a}	0.815^{a}	0.0164	0.0032	NS
Threonine	0.703	0.719	0.697	0.692	NS	0.0050	NS	0.806^{b}	0.799^{b}	0.823^{a}	0.805^{b}	0.0427	0.0032	NS
Valine	0.743	0.738	0.729	0.713	NS	0.0046	Linear	0.838^{b}	0.832^{b}	0.858^{a}	0.837^{b}	0.0175	0.0034	NS
Total	0.793^{a}	0.787^{ab}	0.772^{b}	0.770^{b}	0.0505	0.0037	Linear	0.867^{a}	0.852^{b}	0.873^{a}	0.860^{ab}	0.0128	0.0025	NS

[1] Means of 6 replicates with 22 birds (5 d) or 18 birds (21 d) per replicate.

[2] Interactions between age and diet were not found therefore regression analysis was done on main effects only.

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Table 6.8. Effect of the level of diet acidification and on body weight and body weight gain[1] (Experiment 1).

	BW (g)			BW Gain (g)			
	7d**	14d**	21d**	0-7d**	7-14d**	14-21d	0-21d**
Diet (% HCl)							
0%	146.37	368.81	762.12	101.77	222.45	393.31	717.53
1%	151.70	386.79	808.66	106.94	235.09	421.88	763.90
2%	149.88	389.27	797.00	105.10	239.39	407.73	752.22
4%	144.15	370.82	782.75	99.47	226.68	411.93	738.07
P-value	NS	NS	NS	NS	NS	NS	NS
SEM	1.210	3.632	6.792	1.155	2.681	4.296	6.742

[1] Means of 18 replicates with 6 birds per replicate.

** Significant quadratic regression (*P-value* ≤ 0.05).

[a,b] Means with no common superscript within a column, differ significantly (*P-value* ≤ 0.05).

Table 6.9. Effect of lighting program and level and period of diet acidification and on body weight and body weight gain[1] (Experiment 2).

	BW (g)					BW Gain (g)					
	7d[**]	14d	21d	28d	35d	0-7d[**]	7-14d	14-21d	21-28d	28-35d	0-35d
Lighting											
14Lat7d	0.158	0.356[b]	0.734[b]	1.273[b]	1.927[b]	0.118[b]	0.198[b]	0.378[b]	0.539[a]	0.654	1.888[b]
14Lat21d	0.159	0.378[a]	0.779[a]	1.256[b]	1.879[c]	0.119[ab]	0.220[a]	0.401[a]	0.477[b]	0.623	1.839[c]
23L	0.162	0.378[a]	0.778[a]	1.337[a]	1.969[a]	0.113[a]	0.216[a]	0.400[a]	0.559[a]	0.632	1.929[a]
P-value	NS	0.0106	0.0063	0.0166	0.0004	0.0728	0.0133	0.0226	0.0052	NS	0.0004
Diet (% HCl)											
0.0	0.1560[c]	0.364[b]	0.757[b]	1.275	1.917	0.117[c]	0.208	0.392	0.518	0.642	1.878
0.5	0.1616[ab]	0.370[ab]	0.767[ab]	1.291	1.929	0.122[a]	0.208	0.397	0.524	0.639	1.890
1.0	0.1621[a]	0.375[a]	0.766[ab]	1.294	1.931	0.123[a]	0.213	0.391	0.529	0.636	1.891
2.0	0.1585[abc]	0.369[ab]	0.758[b]	1.282	1.914	0.119[abc]	0.211	0.389	0.524	0.632	1.875
3.0	0.1610[ab]	0.376[a]	0.770[a]	1.298	1.932	0.122[ab]	0.215	0.395	0.527	0.634	1.893
4.0	0.1580[bc]	0.370[ab]	0.764[ab]	1.292	1.926	0.118[bc]	0.212	0.394	0.528	0.634	1.886
P-value	0.0056	0.0432	0.0410	0.0675	NS	0.0073	NS	NS	NS	NS	NS
Period of Diet Acidification (d)											
0 to 7	0.159	0.371	0.770[a]	1.294[a]	1.931	0.120	0.211	0.399[a]	0.524	0.637	1.891
0 to 35	0.160	0.371	0.758[b]	1.283[b]	1.919	0.120	0.211	0.387[b]	0.526	0.636	1.880
P-value	NS	NS	<0.0001	0.0313	NS	NS	NS	<0.0001	NS	NS	NS
SEM	0.0006	0.0016	0.0028	0.0046	0.0052	0.0006	0.0013	0.0017	0.004	0.0027	0.0052
Interactions											
L x D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
L x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
D x P	NS	0.0129	0.0019	NS	NS	NS	0.0276	0.0268	NS	NS	NS
L x D x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Means of 3 replicates of lighting programs with 696 birds per replicate; means of 18 replicates of diet with 58 birds per replicate; means of 54 replicates of period of diet acidification with 58 birds per replicate.

[**] Significant quadratic regression for increasing dietary acidification (*P-value* ≤ 0.05).

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Lighting: 14Lat7d = Change from 23 L at 1d to 14 L:10 D at 7 days of age; 14Lat21d = Change from 23 L:1 D at 1d to 14 L:10 D at 21 days of age; 23L = 23 L:1 D from 0-35 days of age.

Interactions: L = lighting; D= diet; P = period of diet acidification.

Table 6.10. Interactions for body weight and body weight gain[1] (Experiment 2).

	BW		BW Gain
	14d	21d	14-21d
Diet x Period of Acidification			
0-7 d			
Diet (% HCl)			
0.0	0.368	0.762	0.394
0.5	0.373	0.779	0.406
1.0	0.372	0.773	0.401
2.0	0.373	0.770	0.397
3.0	0.376	0.777	0.402
4.0	0.362	0.757	0.394
Linear	NS	NS	NS
Quadratic	NS	NS	NS
0-35 d			
Diet (% HCl)			
0.0	0.361	0.751	0.391
0.5	0.366	0.754	0.388
1.0	0.379	0.759	0.380
2.0	0.365	0.746	0.381
3.0	0.376	0.765	0.389
4.0	0.378	0.771	0.394
Linear	0.025	NS	NS
Quadratic	NS	NS	0.030
P-value	0.0131	0.0022	0.0273

[1] Means of 18 replicates with 58 birds per diet replicate.

diets being either significantly or numerically improved over the control. The same effect was observed at 28 d however results only approached significance (*P-value* = 0.0675). Significant differences were no longer observed at 35 d. At 21 and 28 d, including acid in the diet to 7 d of age resulted in greater body weights and improved gain from 14-21 d compared to diets that had acid throughout the experiment. When examining the interactions between diet x period of acidification, results for body weight at 14 d show that when acid was included throughout production a linear increase in body weight was observed, however results for treatments including acid for only 7 d were generally higher.

Changing to 14L:10D at 7 or 21 d resulted in a reduction in body weight and gain. The reduction in gain for the 7-d change was eliminated by 21-28 d but for the 21-d lighting program the body weight was never regained.

6.4.4.2 Feed Intake and Feed Conversion Ratio

In experiment 1 there was a linear decrease in feed intake from 0-7 d as the level of dietary acid increased (Table 6.11) however this effect was not found from 7-14 d and from 14-21 d a quadratic relationship was observed with an increase in feed intake to 3% acid and a subsequent decrease for the 4% diet. Both 0-7 and 7-14 d saw a significant quadratic effect (*P-value* $\leq$ 0.05) on feed:gain ratio with increased diet acidification with a decrease in the feed:gain ratio to 3% acid and a subsequent increase when acid was increased to 4%. A quadratic relationship for overall results (0-21 d approached significance (*P-value* $\leq$ 0.10). This effect was not observed from 14-21 d.

Table 6.11. Effect of the level of diet acidification on feed intake and feed to gain ratio[1] (mortality corrected) (Experiment 1).

	Feed Intake (g)				Feed to Gain Ratio			
Diet (% HCl)	0-7d[*]	7-14d	14-21d[**]	0-21d	0-7d[**]	7-14d[**]	14-21d	0-21d
0	132.8[a]	335.2	572.5[b]	1052.6	1.30[a]	1.51[a]	1.46	1.45[a]
1	128.8[ab]	356.1	613.3[a]	1113.0	1.21[b]	1.49[ab]	1.45	1.43[ab]
2	126.5[ab]	343.6	592.0[ab]	1068.4	1.21[b]	1.44[b]	1.44	1.40[b]
4	124.1[b]	350.6	583.0[ab]	1069.6	1.26[a]	1.52[a]	1.42	1.43[ab]
P-value	0.0371	NS	0.0507	NS	0.0002	0.0420	NS	0.0750
SEM	1.11	3.12	5.44	9.26	0.010	0.012	0.008	0.007

[1] Means of 18 replicates with 6 birds per replicate.

[*] Significant linear regression for increasing dietary acidification (*P-value* ≤ 0.05).

[**] Significant quadratic regression for increasing dietary acidification (*P-value* ≤ 0.05).

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

In experiment 2, the level of dietary acid did not affect feed consumption or feed to gain ratio, however inclusion of acid for the entire production period reduced feed intake from 7-14 d of age and overall feed consumption when compared to diets that included acid for only 7 d (Table 6.12). Interactions were found for 14-21 d feed intake between light x period of diet acidification and feed:gain ratio for 7-14 d between diet x period of diet acidification however conclusive results were not found (data not shown).

Initiation of the 10 h dark period reduced body weight, body weight gain and feed intake at both 7 and 21 d. Feed to gain ratio was reduced for birds exposed to 10 h of darkness at 7 d and this effect remained throughout production. For birds exposed to 10 h of darkness at 21 d, feed to gain ratio was not improved from 21-28 d but was improved from 28-35 d. Overall, the birds exposed to 10 h of darkness at 7 d had the best feed to gain ratio followed by birds exposed to 10 h darkness at 21 d.

6.4.4.3 Mortality

A linear decrease in mortality from 0-7 d with increasing dietary acid was found in experiment 1; no relationship between dietary acid and mortality was found for any other periods (Table 6.13). In experiment 2, diet had no effect on overall mortality however including acid in the diet for the entire production period was found to reduce mortality caused by infection which in turn reduced total overall mortality for diets including acid throughout the production period when compared to diets that had acid for only 7 d (Table 6.14). Initiating the lighting program at 7 d of age significantly reduced overall metabolic mortalities and total mortalities when compared to the other programs.

When examining period mortality some interactions were observed between level and period of acidification however the interactive results failed to show any conclusive

Table 6.12. Effect of lighting program and level and period of diet acidification on feed intake and feed to gain ratio[1] (mortality corrected) (Experiment 2).

	Feed Intake (kg)						Feed to Gain Ratio					
	0-7d	7-14d	14-21d	21-28d	28-35d	0-35d	0-7d	7-14d	14-21d	21-28d	28-35d	0-35d
Lighting												
14Lat7d	0.157	0.308[b]	0.626[b]	0.947[b]	1.260[b]	3.324[c]	1.329	1.555[b]	1.655[b]	1.762[b]	1.924[b]	1.745[c]
14Lat21d	0.154	0.386[a]	0.723[a]	0.904[b]	1.200[c]	3.406[b]	1.295	1.754[a]	1.795[a]	1.901[a]	1.927[b]	1.826[b]
23L	0.154	0.387[a]	0.720[a]	1.020[a]	1.305[a]	3.650[a]	1.259	1.790[a]	1.795[a]	1.829[ab]	2.068[a]	1.854[a]
P-value	NS	<0.0001	0.0003	0.0025	0.0002	<0.0001	NS	0.0057	<0.0001	<0.0001	0.0040	<0.0001
Diet (% HCl)												
0.0	0.152	0.354	0.680	0.943	1.248	3.407	1.302	1.693	1.732	1.826	1.947	1.797
0.5	0.158	0.358	0.696	0.961	1.262	3.482	1.298	1.710	1.747	1.844	1.980	1.815
1.0	0.154	0.368	0.688	0.981	1.246	3.475	1.254	1.719	1.747	1.868	1.959	1.813
2.0	0.154	0.360	0.690	0.947	1.250	3.436	1.291	1.698	1.769	1.812	1.977	1.810
3.0	0.158	0.363	0.695	0.961	1.270	3.491	1.304	1.685	1.756	1.821	2.001	1.816
4.0	0.156	0.359	0.688	0.951	1.253	3.470	1.318	1.694	1.740	1.799	1.972	1.799
P-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Period of Diet Acidification (d)												
0 to 7	0.154	0.361	0.701[a]	0.962	1.258	3.481[a]	1.284	1.704	1.749	1.843	1.979	1.813
0 to 35	0.157	0.359	0.679[b]	0.952	1.252	3.439[b]	1.305	1.695	1.749	1.813	1.966	1.803
P-value	NS	NS	<0.0001	NS	NS	0.0435	NS	NS	NS	NS	NS	NS
SEM	0.0009	0.0039	0.0053	0.007	0.006	0.0171	0.0096	0.0135	0.0086	0.0114	0.0089	0.0057
Interactions												
L x D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
L x P	NS	NS	0.0173	NS	NS	NS	NS	NS	NS	NS	NS	NS
D x P	NS	NS	NS	NS	NS	NS	NS	0.0057	NS	NS	NS	NS
L x D x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Means of 3 replicates of lighting programs with 696 birds per replicate; means of 18 replicates of diet with 58 birds per replicate; means of 54 replicates of period of diet acidification with 58 birds per replicate.

[a,b,c] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Lighting: 14Lat7d = Change from 23 light at 1d to 14 light:10 dark at 7 days of age; 14Lat21d = Change from 23 light:1 dark at 1d to 14 light:10 dark at 21 days of age; 23L = 23 light:1 dark from 0-35 days of age.

Table 6.13. Effect of diet acidification on mortality[1] (% of total broilers placed) (Experiment 1).

	Period			
	0-7 d[*]	7-14 d	14-21 d	0-21 d
Diet (% HCl)				
0	3.33	1.11	2.22	6.66
1	1.11	3.61	2.22	6.94
2	0.00	1.11	2.22	3.33
4	0.00	5.56	1.11	6.67
P-value	0.0967	NS	NS	NS
SEM	0.544	0.844	0.703	0.958

[1] Means of 18 replicates with 6 birds per replicate.

[*] Significant linear regression for increasing dietary acidification (*P-value* ≤ 0.05).

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Table 6.14. Effect of lighting program and level and duration of acid on overall mortality[1] (% of total broilers placed) (Experiment 2).

	Causes of Mortality					
	Metabolic	Skeletal	Infectious	Unknown	Other	Total
Lighting						
14Lat7d	0.77[b]	0.53	1.39	0.43	0.19	3.31[b]
14Lat21d	1.68[a]	0.48	1.92	0.72	0.48	5.27[a]
23L	1.68[a]	0.77	2.25	0.72	0.38	5.80[a]
P-value	0.0074	NS	NS	NS	NS	0.0463
Diet (% HCl)						
0.0	1.05	0.38	2.40	0.48	0.58	4.89
0.5	1.25	0.58	1.92	0.38	0.29	4.41
1.0	1.72	0.38	1.72	0.77	0.19	4.79
2.0	1.05	0.86	1.34	0.58	0.10	3.93
3.0	1.15	0.58	2.11	0.77	0.67	5.27
4.0	2.01	0.77	1.63	0.77	0.29	5.46
P-value	NS	NS	NS	NS	NS	NS
Period of Diet Acidification (d)						
0 to 7	1.34	0.67	2.24[a]	0.67	0.35	5.27[a]
0 to 35	1.41	0.51	1.47[b]	0.58	0.35	4.31[b]
P-value	NS	NS	0.0184	NS	NS	0.0344
SEM	0.152	0.097	0.172	0.103	0.074	0.299
Interactions[2]						
L x D	NS	NS	NS	NS	NS	NS
L x P	NS	NS	NS	NS	NS	NS
D x P	NS	NS	NS	NS	NS	NS
L x D x P	NS	NS	NS	NS	NS	NS
Regression Analysis						
Linear	NS	NS	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS	NS

[1] Means of 3 replicates of lighting programs with 696 birds per replicate; means of 18 replicates of diet with 58 birds per replicate; means of 54 replicates of period of diet acidification with 58 birds per replicate.

[2] Interactions: L = lighting program, D = diet, P = period of diet acidification.

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Lighting: 14Lat7d = Change from 23 light at 1d to 14 light:10 dark at 7 days of age; 14Lat21d = Change from 23 light:1 dark at 1d to 14 light:10 dark at 21 days of age; 23L = 23 light:1 dark from 0-35 days of age.

Table 6.15. Effect of level of dietary acid and duration of acid inclusion in the diets on period mortality (% of total broilers placed) (Experiment 2).

	Period											
	0-7d						28-35d					
	Metabolic	Skeletal	Infectious	Unknown	Other	Total	Metabolic	Skeletal	Infectious	Unknown	Other	Total*
Diet (% HCl)												
0	0.10	0.00	1.15[a]	0.10	0.29	1.63	0.00	0.29	0.10	0.00	0.10	0.48[b]
0.5	0.19	0.00	0.38[b]	0.10	0.27	0.96	0.29	0.29	0.38	0.10	0.00	1.05[ab]
1	0.00	0.10	0.38[b]	0.19	0.19	0.86	0.29	0.00	0.19	0.00	0.00	0.48[b]
2	0.19	0.00	0.58[ab]	0.00	0.10	0.86	0.29	0.19	0.10	0.19	0.00	0.76[b]
3	0.10	0.00	0.86[ab]	0.19	0.38	1.53	0.29	0.29	0.29	0.19	0.10	1.15[ab]
4	0.19	0.10	0.19[b]	0.00	0.10	0.58	0.48	0.48	0.29	0.48	0.00	1.72[a]
P-value	NS	NS	0.0412	NS	NS	NS	NS	NS	NS	NS	NS	0.042
Period of Diet Acidification (d)												
0 to 7	0.10	0.06	0.64	0.16	0.19	1.15	0.19	0.26	0.26	0.13	0.03	0.86
0 to 35	0.16	0.00	0.54	0.03	0.26	0.99	0.35	0.26	0.19	0.19	0.03	1.02
P-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEM	0.044	0.023	0.091	0.038	0.060	0.117	0.061	0.067	0.056	0.048	0.023	0.13
Interactions[2]												
D x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Means of 18 replicates of diet with 54 birds per replicate; means of 54 replicates of period of diet acidification with 58 birds per replicate.

[2] Interactions: D = diet, P = period of diet acidification.

[*] Significant linear regression for increasing dietary acidification (*P-value* ≤ 0.05).

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

information therefore only the main effects are shown (Table 6.15). In most cases diet did not have a significant effect on mortality with the exception of reducing (non-linear) infectious morality from 0-7 d, linearly reducing 14-21 d mortality (*P-value* = 0.0733 – approaching significance – not shown) and linearly increasing total mortality from 28-35 d, however for most cases the mortality of birds on acidified diets was numerically reduced. Including acid for the entire production period significantly reduced total mortality from 14-21 d (data not shown).

Lighting program significantly reduced unknown and total mortality from 7-14 d, metabolic mortality from 14-21 d and infectious mortality from 21-28 d. These results are amplified when analyzed by the periods for lighting program initiation shown in Table 6.16. These results show that when the lighting program is initiated, mortality is reduced and this reduction continues for the rest of production.

6.4.4.4 Meat Yield

Results from meat yield failed to show an effect of level or length of diet acidification however an effect of gender was observed (data not shown). Male broilers were significantly larger, however when comparing section weights as a percentage of live weight, females had proportionately larger supracoracoideus (pectoralis minor), total breast and breast skin.

6.5 Discussion

The impact of adding HCl to diets on dietary pH and ABC was not consistent; a reduction in pH was associated with increasing dietary HCl in the first experiment, but this effect was not observed in the second experiment. The lack in consistency in response may relate to the different feed processing used in the experiments and the

Table 6.16. Effect of lighting program on broiler mortality during the period of lighting program initiation (% of total broilers placed) (Experiment 2).

	7-21 d						21-35 d					
	Metabolic	Skeletal	Infectious	Unknown	Other	Total	Metabolic	Skeletal	Infectious	Unknown	Other	Total
Lighting												
14Lat7d	0.07[b]	0.10	0.14	0.07	0.00	0.38	0.24	0.14	0.17[b]	0.07	0.00	0.62[b]
14Lat21d	0.53[a]	0.12	0.53	0.19	0.05	1.41	0.19	0.12	0.19[b]	0.14	0.05	0.69[b]
23L	0.38[a]	0.02	0.34	0.19	0.00	0.93	0.46	0.34	0.53[a]	0.12	0.10	1.53[a]
P-value	0.0326	NS	NS	NS	NS	0.0546	NS	NS	0.0103	NS	NS	0.0224

[1] Means of 3 replicates of lighting programs with 696 birds per replicate.

[a,b] Means with no common superscript within a column and a main effect, differ significantly (*P-value* ≤ 0.05).

Lighting: 14Lat7d = Change from 23 light at 1d to 14 light:10 dark at 7 days of age; 14Lat21d = Change from 23 light:1 dark at 1d to 14 light:10 dark at 21 days of age; 23L = 23 light:1 dark from 0-35 days of age.

volatility of HCl (Simonson and Palmer, 1993). In experiment 1 batch sizes were small and a cold pelleter was used for pelleting while in experiment 2 batch sizes were large and a commercial pelleter was used that steam conditions prior to pelleting. The large batch size in experiment 2 may also have decreased the accuracy of HCl delivery into the diets and the high temperatures of the commercial pelleter (75ºC compared to <50ºC in the cold pelleter) may have increased the evaporation of HCl therefore reducing the acid in the diet. If acid is highly volatile it may be more effective to provide acid in the water supply. Although many products have been developed for diet acidification, little research has been done to compare the effects of diet and water acidification on broiler performance.

In Experiment 1, adding acid to broiler diets linearly reduced proventricular and gizzard pH at 5 d of age while at 21 d of age an increasing quadratic relationship was observed. These results confirm that an effective delivery of acid to the gastrointestinal tract by diet acidification is possible. The results at 5 d of age would be expected and the change with age suggests that the increasing maturity of the digestive tract allows the bird to adapt to the dietary acid. The increase in pH with increasing acid supplementation to 2% acid inclusion in the older bird may indicate that the addition of acid triggers a compensatory reduction in gastric acid secretion by the proventriculus or an increase in bicarbonate production from the pancreas or intestinal mucosa; reverse peristalsis could bring digesta from the intestine back in to the gizzard and proventriculus (Noy et al., 1996). The reduction in pH observed for 4% acid inclusion may indicate a limited compensation capacity of 21 d broilers. Hughes et al. (2009) found that an increase in dietary calcium resulted in decreased duodenal pH and increased ileal pH, which, like our

results, suggests that birds are able to compensate for diet effects on the pH of the gastrointestinal tract.

Similar to other research, amino acid digestibility increased in older birds (Noy and Sklan, 1998; Sulistiyanto et al., 1999; Biggs and Parsons, 2008; Chapter 4). Based on the hypothesis that the digestive tract is not capable of producing adequate levels of HCl and that this affects amino acid digestibility, it might be expected that adding HCl to the diet would mitigate the reduced digestibility in young birds. Additionally, if the added HCl is denaturing dietary protein prior to ingestion, then digestibility should be improved. However, amino acid digestibility for most amino acids was reduced with increasing HCl levels at 5 d while at 21 d no effect was observed with the exception of an improvement in cysteine digestibility. Since diet acidification did not increase amino acid digestibility, it was expected that meat yield would also be unaffected. It seems improbable that adding acid to the diet would negatively impact the digestibility of the dietary protein and this is supported by the lack of effect at 21 d. More likely is that the negative effects on amino acid digestibility at a young age relate to intestinal distress and therefore increased endogenous secretions in the gut in an attempt to negate the impact of the additional acid, for example an increase in intestinal mucous production (Jamroz et al., 2006). The results of the current study are similar to Biggs and Parsons (2008) that found that adding gluconic acid reduced apparent amino acid digestibility. They theorized that gluconic acid increased the passage rate of digesta as diarrhea was observed in birds fed concentrations of 4% gluconic acid. This is in line with the results of the current study even though diarrhea was not observed. Conversely, Biggs and Parsons (2008) reported increased digestibility of diets supplemented with citric acid at 4

d of age, which suggests that the effect of acid on nutrient digestibility differs with the acid used. However, other factors such as ingredients in the diet, the use of growth promotant antibiotics, housing and bird age may also be responsible for the difference. The failure to improve digestion in the current study indicates that pH is not limiting protein digestion. It also implies that improvements in performance are due to another mechanism such as microbial population alteration (Paul et al., 2007).

Indications of improved performance were found in the present study hinting at the beneficial effects of diet acidification despite amino acid digestibility not being increased by diet acidification. Even though acid delivery could not be demonstrated in experiment 2, early body weight and body weight gain were quadratically increased in both experiments. The increase in body weight was maintained to week 3 in experiment 1 but only week 1 in experiment 2; the increase in body weight gain did not persist beyond week 2 in experiment 1 or week 1 in experiment 2. From 0 to 2 weeks in experiment 1, 4% HCl reduced gain, but from 2 to 3 weeks gain was equal to feeding other treatments, suggesting that the birds acclimatized to the higher acid levels with age. In experiment 1, feed intake was reduced from 0-7 d as dietary acid was increased, however overall consumption (0-21 d) was not affected. This suggests that in some cases acid may reduce the palatability of feed early in life. This early reduction in feed intake was not repeated in experiment 2. Feed to gain ratio was improved by diet acidification during week 1 in experiment 1 but not in experiment 2. The lack of effect on feed intake and feed to gain ratio in experiment 2 may indicate reduced delivery of acid into the diet as discussed earlier. Results suggest that slight increases in body weight, gain and feed

conversion can be obtained during the first weeks of the broiler's life with diet acidification if the delivery of acid into the diet is effective.

Roy et al. (2002) found that adding a product containing a combination of organic acids reduced mortality caused by poult enteritis and mortality syndrome. The results of the current study were similar in that mortality from 0-7 d, especially in the case of infectious causes, was reduced with diet acidification. A significant linear reduction in total mortality with increasing dietary acid was observed in experiment 1, while in experiment 2 the level of infectious mortality was reduced with acid supplementation. Necropsies were not performed in experiment 1, but high first week mortality in experiment 1 is suggestive of a high incidence of yolk sac infection. Diet acidification may positively affect the bacterial populations of the intestine thereby improving the health status of birds affected by intestinal infection (Roy et al., 2002). Since infectious mortalities early in life are usually yolk sac infections, reductions in these mortalities suggest that acid is reducing these infections. Nutrients have been found to move from the yolk sac to the blood, the blood into the yolk sac and from the yolk sac to the intestines (Noy et al., 1996). This movement of nutrients from the yolk sac and into the yolk sac suggests that there may be movement of digesta from the intestines to the yolk sac. If this is the case then adding acid to the diet may act as an antibacterial when a yolk sac infection is present. Moreover, if contaminated yolk is moving into the intestine, digesta with added acid may reduce the pathogen load of the incoming yolk thereby improving the health status of a bird with yolk sac infection.

Infectious mortality was reduced when acid was included from 0 to 35 d however the interactions indicate no relationship to diet acidification. This suggests that diet

acidification is more effective at reducing infectious morality early in life and may be an effective way of controlling early infectious mortality. Additionally, if the reduction in intestinal microbial load is the main benefit of diet acidification rather than an improvement in protein digestion, then as results by Dibner and Buttin (2002) indicate, using an organic acid such as formic acid rather than HCl may produce better results.

Introducing 10 hours of darkness reduced body weight gain at both 7 and 21 d of age. This was likely due to a reduction in feed intake. The impact of darkness on feed to gain ratio was not the same for the week after introduction at 7 and 21 d of age. Introduction at 7 d resulted in a reduction in feed to gain ratio, which is similar to the results found by Schwean-Lardner et al. (2006) while the 21 d introduction caused an increase in feed to gain. After one week the feed to gain of the 14L at 21 d birds were the same as the 14L at 7 d. The reason for the temporary negative effect on feed to gain ratio may relate to a disruption in gut microorganisms as a result of the 10 h without feed in birds accustomed to continuous feed access. The lack of interaction between lighting program and diet acidification suggests that diet acidification did not provide alleviation from intestinal distress caused by the introduction of the lighting program. To examine this further though, the effect of diet acidification and lighting program introduction on intestinal microbial populations must be examined.

Like other studies (Schwean-Lardner et al., 2006; Lien et al., 2007), the current study showed that shorter day length reduces mortality. The reduction in mortality was most likely the effect of improved immune response caused by exposure to darkness (Kliger et al., 2000; Moore and Siopes 2000; Ahmed et al., 2008). The reduction in mortality carried on throughout the production period. This reduction in overall mortality

is seen for both lighting programs but the 14L at 7 d has the lowest overall mortality because of the longer period of short day length. Age of lighting program introduction affects feed to gain ratio with a temporary increase when darkness is introduced later in life. Results suggest that providing a dark period is beneficial to production by improving the overall feed efficiency and reducing mortality. To maximize the beneficial effects of the lighting program it should be initiated at 7 rather than at 21 d of age.

In conclusion, low levels of diet acidification increase body weight gain and reduce the feed:gain ratio and early mortality in broilers. These improvements are likely not caused by improved nutrient digestion or utilization but rather another mechanism such as a beneficial effect on the intestinal microbial population of the bird. The improvements in performance were observed when HCl was supplemented in diets at 2 or 3% for the first week post-hatch. Reduced day length decreases mortality regardless of the time of lighting program application. By using diet acidification with 2 or 3 % HCl to 7 d of age and a lighting program providing 14L initiated at 7 d, improvements in broiler production and health may be possible, however research is required to determine if HCl is the best acid and if diet or water acidification is most practical.

7 DISCUSSION

The young broiler, although precocial at hatch, is digestively immature. Research has shown that development in the lower digestive tract continues post-hatch at a rapid rate. This leads to improved nutrient digestion and absorption, as the bird gets older. Although the digestion of most nutrients is reduced in the young bird compared to older birds, protein digestion appears to be most affected by the immaturity of the digestive tract (Noy and Sklan, 1995; 2001; Sulistiyanto et al., 1999). Little research has examined the initial steps of protein digestion in the young bird, which occur in the proventriculus and gizzard and are initiated by hydrochloric acid. Adequate protein nutrition during the initial period post-hatch is critical in the development of satellite cells and for subsequent muscle development. Therefore, an understanding of the factors limiting protein digestion could lead to methods of improving diet formulation for young broilers and therefore improve performance.

Based on the above premise, the research in this thesis focused on the initial stages of protein digestion and in particular the role of hydrochloric acid and the pH of the proventriculus and gizzard. The finding that the pH in the proventriculus and gizzard decreased during the early life of the broiler supports the idea that acid secretion may be limited to a point of reducing protein digestibility. It suggests that maturity of the digestive tract translates into proportionately increased acid production. As a

consequence of this research, methods were investigated to mitigate the effect of low acid production so as to improve broiler performance.

The initial method investigated was the provision of pre-starter and starter diets with low ABC that require less acid to reach pH levels appropriate for protein digestion. The ABC of a range of feed ingredients was determined. Mineral ingredients such as limestone were found to have the highest ABC followed by protein ingredients and then energy sources. Within the ingredient types, differences in ABC were also found. For instance, calcium citrate had a much lower ABC compared to limestone. These results to a large degree confirmed similar work done with swine dietary ingredients (Jasaitis et al., 1987; Lawlor et al., 2005). Because of the differences between and within ingredient classes, it was judged to be possible to formulate diets with varying ABC.

Two approaches were taken to reduce diet ABC; producing diets with a combination of low ABC ingredients, and reducing the influence of dietary calcium by reducing the level in the diet and using calcium citrate rather then limestone. Including ABC as a formulation factor was successful in producing diets with a wide range of ABC by using a combination of ingredients. However, the range for analyzed diet ABC values was less than for the predicted values. This suggests that the ABC of ingredients is not additive and/or that interactions between ingredients may occur. Despite the lower range in analyzed diet ABC, when the diets were fed to broilers they were capable of affecting gastrointestinal pH. Of interest, low ABC diets tended to have more ingredients with low amino acid digestibility suggesting that a characteristic of low digestibility may be low ABC. In contrast to the results with ABC and use of multiple ingredients, diets formulated with different sources of calcium did not affect diet ABC, intestinal pH or

performance. No obvious explanation for the discrepancy was established. Another strategy used was to reduce dietary calcium for 5 d post-hatch so as to reduce the ABC during early life when acid secretion is more likely to be limited. The strategy reduced ABC as expected but there again was no effect on gastrointestinal pH. In addition, the short-term calcium deficiency produced a long-term reduction in the efficiency of feed utilization. Taken together, these results demonstrate that the strategy of reducing diet calcium levels to reduce ABC is not an appropriate method of affecting gastrointestinal pH in young birds. In conclusion, formulating diets based on ABC may have relevance in situations where alteration of intestinal pH has value, but further investigation is required to more fully understand the inconsistencies noted above.

The second method of affecting gastrointestinal pH and thereby improving performance in young birds was diet acidification. Adding acid decreased the pH in the proventriculus and gizzard in a linear fashion. In addition, acidifying diets using HCl improved performance and reduced early mortality. Specifically, the results indicate that diets supplemented with 2 or 3% HCl provided the most dramatic improvements to performance and reductions to mortality. An important finding in this research was that acid addition did not improve amino acid digestibility despite the impact on intestinal pH. The importance of this finding relates to the hypothesis that reduced acid secretion in young birds was negatively impacting protein digestion. This finding does not support this hypothesis. Since the improvement in performance with acid addition was not the result of improved AA digestibility, as hypothesized, alternative explanations are required. The finding that infectious mortality was reduced by acid addition suggests that an alternative hypothesis might involve the gastrointestinal tract microbiota. Others have

suggested a role for acids in altering gastrointestinal microbiota (Paul et al., 2007) and therefore this again is an area that requires further study.

In addition to anatomical and physiological changes that occur in the digestive tract post-hatch, the microbiota of the broiler is also being established during this period of time (Forder et al., 2007). It is likely that gastrointestinal pH can influence the development of the microbial community in all sections of the digestive tract. With a full understanding of the influence of bird age, diet ABC and the use of acids on intestinal pH, it may be possible to stabilize the development of the microbial population and thereby improve bird health. The use of other acids or methods of acid delivery (e.g. water) is also of interest in this regard and may provide improved results and be more practical for use in industry than diet acidification using HCl.

Ileal amino acid digestibility studies were completed to gain an understanding of the effect of age on protein digestion by the broiler. Digestibility values from older birds are commonly applied to young birds for diet formulation of pre-starter and starter diets. Results from the current studies indicated that amino acid digestibility increases with age (Chapter 4 and 6). Moreover, the degree of increase differs between ingredients with some ingredients such as CM and PEA showing larger increases, while other ingredients such as FM and SBM showing a smaller increase with age (Chapter 4). Also of importance is that some ingredients are low in essential AA, which coupled with low digestibility would be detrimental to production when used in the diets of young birds. Therefore, differences in AA content of feed ingredients combined with the variation in improvement of AA digestibility with age demonstrates the importance of using age

specific AA coefficients for young birds or being selective when choosing ingredients with formulated pre-starter and starter diets.

Reducing daylength at 21 d rather then 7 d caused an unexpected temporary increase in feed:gain ratio. This suggests that initiation of a lighting program provides the greatest improvements when initiated early in life. It also raises an important question as to why the efficiency of feed utilization decreased in contrast to the expected increase. In birds that have acclimatized to a long daylength with continuous feed access, the sudden change to 10 hours of darkness may have had an adverse effect on an aspect(s) of digestive function or caused a disruption of the intestinal microbial community. Providing diet acidification during this hypothesized period of stress (the start of a lighting program) was not found to alleviate the temporary increase in the feed:gain ratio. More research to determine the effect of lighting program and diet acidification on the intestinal microbial environment is required. Results of the lighting program research suggest that providing a dark period reduces mortality regardless of the age of initiation. Initiation of a dark period early in life would provide the greatest reduction to mortality and feed:gain ratio.

As the market age of the broiler chicken decreases, the initial period post-hatch is making up an increasingly large proportion of the broiler's life. Much of the development occurring immediately post-hatch, plays a role in the end performance of the broiler. Hence diet formulation during this period is critically important for overall broiler performance. An improved understanding of the digestive development and the discovery of methods of diet formulation may improve the early digestive and muscular development of the broiler and therefore overall performance.

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