The response of broilers to acidification of drinking water and feed

By

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Declaration

I, the undersigned, declare that this thesis, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me or another individual for a degree at this or any other tertiary institution.

K. J. Westergaard

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Abstract

The response of broilers to acidification of drinking water and feed

Alternatives to antibiotics are constantly being studied and one such alternative is organic acids. Organic acids can lower the pH in the gastrointestinal tract (GIT) and the lowered pH renders the environment unfavourable to pathogenic bacteria, thus resulting in a healthier GIT. This can enhance nutrient digestion, absorption and utilisation, as well as enhanced growth and efficiency of the bird.

The main objective of this study was to assess the optimal drinking water pH for broilers and what effects it would exhibit on the GIT. The second part of this study was to compare water acidification and feed acidification, as well as a combination thereof.

Two different feeds and five different water pH levels were fed to 7200 Ross 308 broilers, randomly allocated to 120 pens, with 12 replicates per treatment and 60 birds per pen. Feed 1 was considered as 'standard' and feed 2 was considered as 'acidified', containing 0.3% FORMI® (ADDCON 40% formic acid product). The five water pH levels tested were 3.0, 3.8, 5.5, 6.5 and tap water (pH of 7.9). Broiler performance and pH in various GIT segments were measured weekly.

The standard feed performed better than the acidified feed, irrespective of water pH. Standard feed resulted in significantly greater bodyweight (BW) and European performance efficiency factor (PEF) at weekly weighing intervals from 7-35 days, as well as significantly lower feed intakes (FI) and feed conversion ratio (FCR).The different water pH levels used throughout the trial showed clear trends and significant differences amongst the various treatments, irrespective of the feed used. Any level of drinking water acidification proved better than no acidification, with significantly higher BW and PEF on majority of the recordings, as well as significantly lower FCR and FI. Water intake was significantly higher for a water pH of 3.8 when compared to a pH of 7.9. When comparing the different drinking water pH levels across the two feeds, broiler performance always favoured the standard feed. Mortality was not significantly different and GIT pH was highly variable, showing no clear trends.

This study suggests that feed acidification is not as effective as water acidification and that a lower drinking water pH can significantly improve economically important measurements, such as BW and FCR. It can also be concluded that the effects exhibited on the pH of various GIT segments cannot be predicted. Based on this study, there is no clear benefit to combining feed and water acidification and a drinking water pH of 3.0 – 3.8 is recommended.

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List of abbreviations

PS	Pre-starter
S	Starter
G	Grower
F	Finisher
PF	Post-finisher
BW	Bodyweight
FCR	Cumulative feed conversion ratio
PEF	European performance efficiency factor
WI	Water intake
H0	Null hypothesis
HA	Alternate hypothesis
FI	Cumulative feed intake
GLM	General linear model
SEM	Standard error of the mean
LSM	Least square means

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Chapter 1

General Introduction

It has been predicted that by 2050, 70% of the world's population will live in cities and there will be a 70% increase in demand for animal source foods (Robinson *et al.*, 2015). High levels of efficient production are necessary to meet these demands. Global meat production has increased substantially and much of this growth has been focused around poultry and pork production, particularly in developing countries (Thornton, 2010). This rapid growth in production can be attributed to the controlled environment and rapid genetic progress seen in these species. Enzymes and other feed additives, particularly antibiotic growth promoters (AGP) are key ingredients to increase efficiency (Hassan *et al.*, 2010). However, antibiotic resistance has become an ever-present concern amongst researchers and consumers alike (Hamid *et al.*, 2018), thus alternative means of improving health and consequently efficiency, is sought-after in the industry today. In many countries, the use of AGP has been banned entirely and consequently, poultry health problems, such as necrotic enteritis, have become prevalent issues (Khan & Iqbal, 2016). This has pushed the exploration of alternative means to improving health, including non-therapeutic options such as enzymes, probiotics, prebiotics, herbs, essential oils, immunostimulants and organic acids.

Organic acid products are usually a blend of several organic acids and have been successfully used as antimicrobial alternatives, showing similar activity to antibiotics (Khan & Iqbal, 2016). Organic acids are available as liquids or as sodium-, potassium- or calcium salts, that are mixed into either the drinking water or the feed of the birds (Huyghebaert *et al.*, 2011). The mode of action is centred around the decrease in pH caused by the organic acids. The lowered pH renders the gastro-intestinal tract (GIT) environment unfavourable to pathogenic bacteria and promotes the growth of 'healthy' bacteria, resulting in competitive exclusion within the GIT (Mansoub *et al.*, 2011). This healthier GIT results in enhanced nutrient digestion, absorption and utilisation, as well as enhanced growth and efficiency of the bird (Lückstädt & Mellor, 2011). This is due to the beneficial effects of lower pH in the crop and alterations to the physiology of the proventriculus (Van Immerseel *et al.*, 2006). Other significant benefits accompanying acidification include improved bone mineralisation and phytate-P utilisation (Boling-Frankenbach *et al.*, 2001), increased cellular turnover and improved pancreas function (Dibner & Buttin, 2002), decreased *Escherichia coli* counts (Moharrery & Mahzonieh, 2005) and enhanced proteolysis and protein and amino acid digestibility (Symeon *et al.*, 2010). The acidification is particularly beneficial during periods of fasting, such as pre-slaughter fasting, when the GIT is most susceptible to bacterial infection, particularly by *Salmonella spp.* and *Escherichia coli* (Corrier *et al.*, 1999), resulting in decreased microbe counts on carcasses post-slaughter. Corrier *et al.* (1999) also found that acidified water promotes feed intake, particularly during heat stress. It is also important to note that the water quality itself is improved through acidification. *E. coli* is a major concern in all ages of poultry

and for consumers alike, and the acidification of the water disinfects the water resulting in fewer external bacteria being introduced into the gut (Krug *et al.*, 2012), thus further promoting the GIT integrity of the bird.

The aim of this project was to assess the effects that water and feed acidification would have on broilers under commercial conditions. Broiler performance was assessed by comparing BW, FI, FCR, PEF and mortality after a 35-day rearing period. The effects on GIT pH were also assessed on a weekly basis by measuring the pH in each segment along the GIT.

Hypothesis of the study

The first null hypothesis (H_0) of this study is that acidification will have no effect on performance in Ross 308 broilers.

The first alternative hypothesis (H_A) is that acidification will improve performance in Ross 308 broilers.

The second null hypothesis (H_0) of this study is that acidification will have no effect on GIT pH in Ross 308 broilers.

The second alternative hypothesis (H_A) is that acidification will lower GIT pH in Ross 308 broilers.

Chapter 2

Literature Review

Organic acid supplementation is a cost-effective method of improving broiler performance without the use of AGP in the feed. Various benefits have been observed when adding organic acids to the diet and this review serves to provide a broad overview on the reasons and consequent effects of using organic acids in broiler production.

2.1 Organic acids effects on broiler performance

Many organic acid products are available on the market and several studies have been conducted to justify its wide application in broiler production (Chowdhury *et al.*, 2009; Haque *et al.*, 2010; Nourmohammadi *et al.*, 2010; Salgado-Tránsito *et al.*, 2011). However, although there is a general understanding of the functions/benefits of organic acid supplementation (Table 2.1), their mode of action remains unclear and this poses limitations. The most common theories revolve around (1) pH decline within diets and the gastrointestinal tract, (2) improved nutrient retention and utilisation and (3) the inhibition of pathogenic bacterial growth (Afsharmanesh & Pourreza, 2005; Mroz, 2005). Commonly tested acids include citric acid, formic acid and its various salts, and butyric acid. A multitude of organic acid blends have also been widely tested. Due to many factors influencing organic acid efficacy, varying results have been observed, but for the most part, the results were beneficial (Table 2.2).

Citric acid ($C_6H_8O_7$) is a weak organic acid that is commonly used as a natural preservative and has also been widely used as an organic acid supplement for monogastric livestock (Kim *et al.*, 2015). Various experiments have been conducted to quantify the effects of citric acid on poultry, but varying, and sometimes contradictory, results have been observed. Data indicates that it generally results in bodyweight and feed conversion improvements, whilst decreasing feed intake (Chowdhury *et al.*, 2009; Haque *et al.*, 2010; Nourmohammadi *et al.*, 2010; Salgado-Tránsito *et al.*, 2011). Haque *et al.* (2010) and Nourmohammadi *et al.* (2010) obtained significant improvements in bodyweight gain, whilst Chowdhury *et al.* (2009) and Salgado-Tránsito *et al.* (2011) reported significant improvements in efficiency.

Formic acid (CH_2O_2) is the simplest carboxylic acid, yet it is highly volatile and emits a pungent odour (Kim *et al.*, 2015). As such, it is not commonly used in its free form but rather as formates (formic acid salts), and these formates have been regularly added to broiler diets. Upon supplementing some of these formates, Al-Kassi & Mohssen (2009) reported significant increases in bodyweight and feed intake, whilst Paul *et al.* (2007) reported a significant decrease in feed intake and a significant increase in feed efficiency. Hernández *et al.* (2006) and Panda *et al.* (2009) reported a dose-dependent response to formic acid. Contrary to the other papers, Patten & Waldroup (1988) and Garcia *et al.* (2007) found decreased feed efficiency and decreased bodyweight gain respectively, when supplementing broilers with formates. Contrasting results may be as a

result of the type of formic acid salt used, which can be observed when comparing Garcia *et al.* (2007) and Paul *et al.* (2007), who supplemented calcium- and ammonium formate respectively. Calcium-formate resulted in decreased bodyweight gain, whilst ammonium-formate resulted in improved efficiency and bodyweight gain. Thus, it is reasonable to assume that results are dependent on the type and quantity of salts added to the diet. Ragaa & Korany (2016) supplemented formic acid as both a liquid and a salt, potassium diformate, and concluded that formic acid in both forms significantly improved bodyweight, dressing percentage and efficiency of broilers compared to the control groups. The highest breast and thigh weights were also observed in the potassium diformate groups when compared to the rest of the treatments.

Butyric acid ($C_4H_8O_2$) has been extensively studied and is considered an essential nutrient for normal epithelial cell development, as it can be used as a direct energy source and it also has strong bactericidal properties in the GIT (Pryde *et al.*, 2003). Comparing results from multiple experiments, a general improvement in bodyweight and feed efficiency can be noted, 19% and 2.5% respectively. The increased efficiency is however linked to decreased intake with minimal effects on growth, rather than improved growth itself. An explanation for this decrease in intake is yet to be postulated. Butyric acid seems to have the most positive effects when supplemented at low levels, whereas on the contrary, high levels have been shown to decrease feed efficiency (Aghazadeh & TahaYazdi, 2011).

Other organic acids and blends thereof have also been tested. Paul *et al.* (2007) reported that adding 3 g/kg calcium propionate to the diet significantly improved feed efficiency and Al-Kassi & Mohssen (2009) observed significant improvements in feed efficiency, feed intake and bodyweight gain, when adding 2 g/kg propionic acid to broiler diets. Recently, advances in organic acid blend formulations, based on the assumption of synergistic effects of the individual organic acids (Kil *et al.*, 2011), has led to a variety of blended products being available. Upon reviewing various experiments, it can be concluded that blends have greater benefits than individual acids. Although not statistically significant, Alçiçek *et al.* (2004) found that 2.5 g/kg of lactic acid, formic acid, and citric acid blends improved growth performance. Gunal *et al.* (2006) also found that a 2 g/kg blend of propionate- and formate-salts, led to improved bodyweights and feed intake. Likewise, Samanta *et al.* (2008) reached the same conclusions when adding 1 g/kg of calcium propionate, formic-, propionic- and ortho-phosphoric-acid blends. Kim *et al.* (2009) also reported increased bodyweights and improved feed efficiency of 1.8-3.2% and 4%, respectively. On the contrary, two experiments resulted in negative effects when blending organic acids. Świątkiewicz & Arczewska-Wlosek (2012) fed a diet containing 4 g/kg organic acid blends, and Smulikowska *et al.* (2010) who fed a diet containing 6 g/kg organic acid blends, reported decreases in feed efficiency and feed intake, respectively, whilst both reporting decreases in bodyweight. Yang *et al.* (2018) reported that a combination of sorbic- and formic acids supplemented with thymol resulted in significantly improved efficiency, despite not showing notable changes in bodyweight. Wen *et al.* (2018) supplemented broilers with sodium diacetate and reported significant increases in bodyweight, carcass weight and breast muscle weight, compared to the organic acid-free control group.

Table 2.1 Organic Acids and their functions/benefits, (adapted from Anjum & Chaudhry, 2010)

Type of Organic Acid	Chemical Formula	Inclusion (g/kg of feed)	Functions/Benefits
Tartaric Acid	$COOHCH(OH)CH(OH)COOH$	3	Gut morphology & growth
Propionic Acid	CH_3CH_2COOH		Gut microbes
Mixed Organic Acid		1-2	Improved bodyweight & carcass
Acids and salts	Genex (a feed additive containing plant extracts, essential oils and organic acids as ammonium salts)	2	Low intestinal & gram-negative bacteria
Malic Acid	$COOHCH_2CH(OH)COOH$	3	Intestinal morphology
		0 – 1.5	Low *E. coli* & pH High pancreatic fluid & digestion
Lactic Acid	$CH_3CH(OH)COOH$	3	Gut morphology Low bacteria, fungi, pathogens & ammonia
Lactic Acid + Organic Acids			
Fumaric Acid	$HO_2CCH=CHC0_2H$	3	Gut morphology, bodyweight & carcass
Formic Acid	$HCOOH$	3	Gut morphology, bodyweight & carcass
Euroguard mixture (humic acid product)	$C_9H_9NO_6$	Unclear	2% Gain & FCR down 6%
Organic Acids	Mixed (propionic, formic, lactic)	0.08-0.25	Low *Campylobacter*
Organic Acid combinations	Lactic Acid	3	Low microbe load, no poisoning or meat spoilage, improved bodyweight & FCR and Low caecal pH
Organic Acid + β-glucanase			
Citric Acid	$CHO_2CH_2C(OH)(COOH)CH_2COOH$	3	Intestinal Morphology
Butyric Acid	$CH_3CH_2CH_2COOH$		Low pH & high digestion
Ascorbic Acid	$C_6H_8O_6$		Improved efficiency
Acetic Acid	CH_3C00H	3	Gut morphology, low pH & high digestibility

The variability in positive, negative or neutral responses to organic acids may be influenced by several factors. One obvious and well-understood factor is the buffering capacity of the diet (Mroz *et al.*, 1997; Partanen, 2001). The degree of acidification incurred when the organic acid is included may be affected by the source and amount of dietary proteins in the feed (Partanen & Mroz, 1999). This is not evident in broilers, but it has however been reported in pigs. Ravindran & Kornegay (1993) found that the efficacy of organic acids and thus positive effects, were greater in diets with a low buffering capacity when compared to a high buffering capacity diet. Thus, one can conclude that the dietary constituents may have a large influence on the observed results when testing organic acids. As with any experiment, the environment plays a crucial role and thus, results from trials testing organic acids may depend on the environment in which the trial was conducted. It

can be postulated that when birds are exposed to less sanitary conditions, the effects of the organic acids will be more pronounced, as organic acids are known to strongly influence the microbial population in the GIT, notably the reduction in pathogenic bacteria (Kim *et al.*, 2005). Another possible factor is the palatability of the feed. Some organic acids, such as citric acid, have strong smells and tastes that may deter birds. Partanen & Mroz (1999) concluded that the type of organic acid had the strongest effect on intake, with formic acid resulting in increased intake and citric acid resulting in decreased intake. On the contrary, Oh (2004) and Kim *et al.* (2005) reported that organic acids had no influence on feed intake in weaner piglets, provided the correct carrier was used. Lastly, the inclusion levels of the organic acid may also contribute to the varying positive and negative results. Hernández *et al.* (2006) and Panda *et al.* (2009) showed a strongly positive dose-dependent response when testing varying levels of formic acid in the diet, whilst on the contrary, Garcia *et al.* (2007) showed a negative dose-dependent response. The differences in results may be attributed to the above-mentioned factors, amongst others, or a combination thereof.

The effects of organic acids on performance can be explained by the various effects that organic acids exhibit within the GIT, both physically and physiologically. The effects on the GIT directly influence the digestive and absorptive capabilities of the birds. Moreover, organic acids exhibit strong antibiotic qualities within the GIT that translate to both a healthier bird and a healthier environment. A combination of these factors leads to improved efficiency and health within the entire flock.

Table 2.2 Effects of organic acids and their salts on broiler performance (adapted from Kim *et al.*, 2015)

Organic Acid	No. Exp.[1]	Inclusion g/kg	BWG, % changes [2]				FI, %changes [2]				Gain:Feed, %changes[2]			
			Mean	Range	No. Sig. (P <0.05)[3]	+/-[4]	Mean	Range	No. Sig. (P <0.05)[3]	+/-[4]	Mean	Range	No. Sig. (P <0.05)[3]	+/-[4]
Citric	8	5~60	4.7	-16.7~25.2	9/14	8/6	-1.3	-24.9~13.1	4/14	6/7	6.0	-4.2~25.2	5/14	11/3
Fumaric	3	1.25~45	1.3	-2.3~4.0	1/6	5/1	1.9	-1.0~5.0	2/6	4/1	0.2	-2.2~3.1	0/9	4/5
Formic	5	1~10	2.8	-3.8~10.3	1/10	8/2	0.4	-1.0~4.1	1/4	1/3	5.3	0.5~18.2	2/11	11/0
Formate Salt	2	3~28.9	2.6		0/1	1/0	-0.5		1/1	0/1	-11.5	-25.0~3.1	1/5	1/4
Butyric	5	1~25	1.9	0.3~4.0	2/10	10/0	-0.6	-4.5~2.1	0/10	4/6	2.5	-1.0~5.9	3/10	9/1
Propionic	1	2	11.2		1/1	1/0	5.1		1/1	1/0	6.1		1/1	1/0
Propionate salt	1	3	0.5		0/1	1/0	-6		1/1	0/1	6.5		1/1	1/0
Blend	6	1~6	0.3	-5.8~3.2	1/7	5/2	-1.7	-9.9~1.3	0/6	3/3	3.2	-2.3~12.4	2/7	5/2

[1] Total number of experiments conducted to test each organic acid
[2] The increase or decrease (%) in the bodyweight gain (BWG) and feed intake (FI) in the organic acid-supplemented groups compared to the control group.
[3] Number of organic acid-supplemented groups showing significant changes (P <0.05) compared to the total number of organic acid-supplemented groups.
[4] Number of organic acid-supplemented groups showing the positive impact compared to organic acid-supplemented groups showing the negative impact.

2.2 Effect of organic acids on the gastrointestinal tract

It is well established that a healthy gut is essential in achieving growth and efficiency targets in the poultry industry. Without a healthy gut, digestion and absorption of nutrients are greatly inhibited and this may lead to suboptimal results (Khan & Iqbal, 2016). Many researchers have documented the positive effects that organic acid supplementation has on the GIT. Villi height and width in the small intestine, have been shown to improve when organic acid supplements are used, thus increasing the surface area of the intestine. Leeson *et al.* (2005) and Panda *et al.* (2009) reported increased villus height and crypt depth in the duodenum of birds fed butyrate, irrespective of the concentration. Garcia *et al.* (2007) reported deeper crypts in the jejunum of birds fed organic acids compared to that of birds fed antibiotics. Pelicano *et al.* (2005) reported 43% higher villus in the ileum of chicks fed organic acid salts compared to those fed an organic acid-free diet. Eftekhari *et al.* (2015) fed diets with graded levels of threonine and two levels of acidified drinking water and reported that the acidified drinking water had significant effect on the villus width, crypt depth and villus height:crypt depth, irrespective of the threonine level supplemented. In three separate experiments, Paul *et al.* (2007) who fed diets containing ammonium formate and calcium propionate, Kum *et al.* (2010) who fed a diet supplemented with an array of organic acids and Rodríguez Lecompte *et al.* (2012) who fed a diet supplemented with a blend of sorbic- and citric-acid, reported significantly increased villus height and area in the duodenum, jejunum and ileum when compared to the birds fed a conventional diet. Adil *et al.* (2011) concluded that diets containing 3% fumaric acid, 3% butyric acid and 2% fumaric acid, led to the greatest villus height in the jejunum, duodenum and ileum respectively. Ragaa & Korany (2016) found that supplementation of either 0.5% potassium diformate or 0.5% formic acid, resulted in significantly increased villus height and villus:crypt ratio in the ileum. The potassium diformate yielded slightly better results, as the salts can act further down the GIT than unbound organic acids, which are readily utilised in the upper GIT. Hamid *et al.* (2018), who used a liquid acidifier to supplement the birds for varying periods of time throughout the experiment, reported significantly higher villi in the jejunum and significantly higher crypt depths in all segments for at least one of the treatments when compared to the birds fed no organic acids. They also found a significantly higher crypt depth in the duodenum and jejunum. Yang *et al.* (2018) fed organic acids to broilers at different phases, namely the grower and finisher phases, and found significant increases in duodenal and jejunal villus heights and greater crypt depth of the jejunum and ileum. The birds supplemented during the grower phase also had significantly thicker muscular layers of the jejunum and ileum.

Increases in villi height and crypt depth may be attributed to the reduction in mucous secreted by the epithelium, thus resulting in a thinner mucosal layer (Khan, 2013). The reduction is a result of the organic acids' ability to inhibit growth of pathogenic bacteria and thus reduces the need for a thicker protective layer in the GIT (Khan, 2013). The thinner mucosal layer is also beneficial in increasing digestion and absorption of nutrients, as a thicker mucosal layer has been shown to inhibit these processes (Teirlynck *et al.*, 2009).

Table 2.3 Effects of organic acids on the pH of GIT segments (Kim *et al.*, 2015)

Organic acid	Inclusion g/kg	Changes in pH, unit							
		Diet pH	**Crop**	**Proventriculus**	**Gizzard**	**Duodenum**	**Jejunum**	**Ileum**	**Ceacum**
Citric Acid	20		-0.15*		0.02	0.26	-0.11	0.13	-0.13
	40		-0.31*		0.12	0.28	0.19	-0.15	-0.05
Citric Acid	6.25	-0.60*		0.10	-0.20	-0.10	-0.10	0.10	
	12.5	-1.10*		0.00	0.00	-0.20	0.00	0.00	
	25	-1.70*		-0.10	0.30	-0.20	-0.10	-0.10	
	50	-2.30*		-0.10	-0.10	0.10	0.00	0.10	
Citric Acid	10	-0.64*	-0.30		-0.40			-0.06	-0.20
	20	-1.14*	-1.10		0.20			-0.26	-0.23
Citric Acid	30		-0.17*		-0.02	-0.01	-0.14*	-0.01	
	60		-0.28*		-0.12*	-0.09*	-0.40*	-0.06*	
Citric Acid	30							-0.08	
Butyric Acid	2		-0.57*	-0.30*	-0.24*	-0.12*	-0.13	-0.18	
	4		-0.82*	-0.44*	-0.40*	-0.24*	-0.22	-0.22	
	6		-0.83*	-0.46*	-0.42*	-0.27*	-0.18	-0.08	
Blend[3]	6		-0.28				0.12	0.24	0.46
Blend[4]	1		0.15	-0.08	-0.64*	-0.32*		-0.03	
Ammonium Formate	3		0.00	0.00	-0.21	0.30	-0.05	0.00	
Calcium Propionate	3		-0.10	0.20	-0.15	0.10	0.00	0.00	
Mean		-1.25	-0.37	-0.12	-0.14	-0.04	-0.08	-0.04	-0.03
SEM[5]		0.27	0.10	0.07	0.06	0.06	0.04	0.03	0.13

[1]An asterisk mark (*) represents significant difference compared to the control group (P <0.05).
[2]Changes in pH (unit) = pH measured in broiler chickens fed diets containing organic acids minus the pH measured in broiler chickens fed diets containing no organic acids.
[3]Blend = lactic-, formic- and citric-acid and their salts.
[4]Blend = formic-, propionic- and ortho-phosphoric-acid + calcium propionate.
[5]SEM: Standard error of the mean.

Organic acids also have a marked effect on the pH in the various GIT compartments. These effects may vary according the pK_a values of the diet and the organic acid, as well as the 'pre-acidified' pH conditions within the GIT (Kim *et al.*, 2015). The pH of the diet decreases with an increasing amount of organic acid until an equilibrium point is reached. However, the pH recorded in the various GIT compartment varies greatly (Table 2.3). The general degree of pH reduction is usually greater in the upper GIT (crop, proventriculus, and

gizzard) than in the low GIT (duodenum, jejunum, ileum, and caecum). Multiple previous studies have shown that the majority of organic acids lead to a crop pH reduction (Atapattu & Nelligaswatta, 2005; Paul *et al.*, 2007; Samanta *et al.*, 2008; Panda *et al.*, 2009; Aydin *et al.*, 2010; Nourmohammadi *et al.*, 2010; Smulikowska *et al.*, 2010; Esmaeilipour *et al.*, 2011; Salgado-Tránsito *et al.*, 2011; Hamid *et al.*, 2018), with more than half showing significant results. Some of the studies even showed a dose dependent response when feeding a diet containing citric- and butyric-acids (Panda *et al.*, 2009; Nourmohammadi *et al.*, 2010; Esmaeilipour *et al.*, 2011). The crop is likely to be the most influenced GIT compartment due to the short transit time to the crop (Kim *et al.*, 2015).

Moving past the crop, the effect become less pronounced and fewer researchers have found significant changes in pH. Far fewer organic acids showed changes in the pH of the proventriculus (Paul *et al.*, 2007; Samanta *et al.*, 2008; Panda *et al.*, 2009; Salgado-Tránsito *et al.*, 2011; Hamid *et al.*, 2018), with only two yielding significant results (Panda *et al.*, 2009; Hamid *et al.*, 2018). The degree of pH reduction was also notably smaller than in the crop. In the lower GIT, the effects become rather insignificant, as it is speculated that marginal amounts of organic acid actually reach these parts of the GIT (Kim *et al.*, 2015). However, Samanta *et al.* (2008) and Nourmahammadi *et al.* (2010) reported significant pH reductions in the duodenum and further down the GIT, Hamid *et al.* (2018) reported significant changes in the ileum. However, Ragaa & Korany (2016) found significantly decreased pH values in the crop, gizzard, duodenum, jejunum and ileum of birds supplemented with either formic acid or potassium diformate at 5 kg/ton inclusion, when comparing it to the pHs of the control groups. Moreover, the potassium diformate showed a numerical decrease in the pH of both the caecum and colon when compared to the formic acid and control groups. Wen *et al.* (2018) also reported that a sodium diacetate salt showed a tendency toward lower pH values in duodenum, jejunum, ileum and caecum of the supplemented birds compared to the organic acid-free control groups. This may be due to the fact that organic acid complexes/salt, can travel further down the GIT than free organic acids, as demonstrated by Sugiharto (2016), who found that coating organic acids with a fat-complex resulted in greater epithelial cell proliferation in the distal GIT. Salts and other organic acid complexes offer the organic acid a degree of protection and consequently, is not readily digested in the upper GIT and can thus exhibit effects more distally, such as its ability to modulate mucosal morphology and microbiota (Hu & Guo, 2007). Considering this, it can be concluded that organic acids have the most notable effects in the crop and lose efficacy as they move down the GIT and mix with dietary constituents and buffers produced by the bird. As previously mentioned, the developmental benefits of the organic acids in the GIT, such as increased mucosal surface area, will have a direct influence on the digestive and absorptive capabilities of the bird. The correct combination of these effects is key to using organic acids to improve performance and efficiency of the birds.

2.3 Effect of organic acids on nutrient digestibility and utilisation

Any changes in the GIT will have a consequential effect on digestibility and nutrient utilisation. To maximise returns in any livestock production systems, one must increase efficiency, with feed conversion ratio

(FCR) being the most obvious measurement of this. Organic acid supplementation is an important step in moving towards improved efficiency without use of medications that may leave residues in the meat and risk development of antimicrobial resistance (Kim *et al.*, 2015). The pH decrease in the GIT stimulates various enzymes, such as pepsinogen and other zymogens, by bringing the extracellular pH closer to that required for optimal activity (Afsharmanesh & Pourreza, 2005). The increased pepsinogen activity leads to increased proteolysis, which in turn produces various peptides that stimulate cholecystokinin and gastrin release (Adil *et al.*, 2010; Samanta *et al.*, 2010). These increased secretions further enhance digestibility as the concentration of various other enzymes involved in proteolysis increase, including procarboxy-peptidase, chymotrypsinogen and trypsinogen. The increased acidity has also been reported to decrease the rate of gastric emptying and this has been reported in pigs as early as 1978 (Kidder & Manners, 1978) and later by Mayer (1994). This increased retention time is the reason for improved digestion (Van der Sluis, 2002). This mechanism has been explained in human gut physiology as a result of the lower pH causing gastric reflux until such time that the duodenal chyme has been neutralised by various secretions (Cheng, 2016).

Many studies have been conducted with organic acids to quantify the effects on digestion in broilers. In a review by Kim *et al.* (2015), it was reported that both dry matter (DM) and protein retention were improved by an average of 1.0% and 1.7%, respectively, when compared to the control diets. However, it is not only DM and protein that were affected; improvements in ether extract (EE), nitrogen-free extract (NFE), neutral detergent fibre (NDF), crude fibre (CF), apparent metabolisable energy (AME) and apparent ileal digestibility (AID) have also been reported. This is particularly important in poultry fed diets containing soybean meal, as the high levels of galacto-oligosaccharides require α-galactosidase enzyme for it to be digested (Lee *et al.*, 2014). Ao (2005) supplemented 2% citric acid and found that α-galactosidase activity was increased. This is a direct result of the lowered pH in the crop as the enzyme is most active at a pH of $\pm$ 4.5. Ao *et al.* (2009) further concluded that NDF, DM and CP retention were all improved in broilers supplemented 2% citric acid in the diet when compared to the control groups. Studies supplementing other organic acids found similar results; Ghazala *et al.* (2011) reported improvements in CP, EE, CF and NFE digestibility compared to control groups when various levels of fumaric, formic, acetic and citric acids were used. Nourmohammadi *et al.* (2012) reported that 3% citrate, in combination with microbial phytases, led to improved AME and CP digestibility. Lohakare *et al.* (2005) found that supplementation of ascorbic acid led to improved digestibility of CP, EE and gross energy by 3.45%, 5.20% and 1.81% respectively, when compared to the control groups. Similarly, Emami *et al.* (2013) reported improvements in digestibility of CP and EE of 11.07% and 6.11%, respectively, when supplemented with a combination of organic acids and microbial phytases. Smulikowkska *et al.* (2009) reported improved nitrogen retention compared to the control group when supplementing the diet with a fat-coated organic acid. The AID of DM and CP have also been reported to improve by 11.4% and 11.8%, respectively, when the birds were supplemented formic acid in their finisher diet, (Hernández *et al.*, 2006; Garcia *et al.*, 2007).

Organic acids have also been found to improve mineral digestibility and improve phytate-phosphorus utilisation, especially when combined with microbial phytases, both directly and indirectly (Vieira *et al.*, 2018). The indirect effect is related to the ability of the organic acid to negate the inhibitory effects that calcium cations (Ca^{2+}) have on phytases, namely phytic acid hydrolysis inhibition (Maenz *et al.*, 1999). Nourmohammadi *et al.* (2012) found that a combination of microbial phytase and citric acid improved ileal digestibility of calcium (Ca) and phosphorus (P), only when phytase was absent from the diet. P retention significantly increased by 23.9%, 32.5% and 34.2% when Liem *et al.* (2008) supplemented malic-, fumaric- and citric acid, respectively in broiler diets. Phytate P retention also significantly improved by 105.1% and 98.1% with citric- and malic acids, respectively. Esmaelipour *et al.* (2011) found similar results, with significant improvements in P retention by 16.3% when supplementing 4% citric acid, a strong quadratic response. Adding 2% citric acid to broiler diets significantly improved the retention of Ca and P by 7.7% and 4.7%, respectively (Brenes *et al.*, 2003). A combination citric acid and phytase increased P digestibility by 44.3% (Afsharmanesh & Pourreza, 2005), whilst a combination of an organic acid blend (formic- and propionic acids) and phytase, significantly increased P digestibility by 18.2% (Emami *et al.*, 2013).

A common method of quantifying mineral retention lies in the analysis of tibia ash and the composition thereof. Many researchers have studied the effects that organic acids have on tibia ash levels. Upon reviewing numerous experiments, only one reported decreased tibia ash when organic acids were supplemented; Boling-Frankenbach *et al.* (2001) found an average decrease in tibia ash of 4%, when supplementing citric acid and Ca. However, in the same paper, a second experiment concluded that 6% citric acid in combination with 0.2% available phosphorus (aP), resulted in an average increase of 26.9% in tibia ash. Boling *et al.* (2000) concluded that 6% citric acid reached up to 43.5% improvements in tibia ash and Brenes *et al.* (2003), observed a significant increase when supplementing 2% citric acid. Afsharmanesh & Pourreza, (2005) found that whilst phytase and citric acid did not improve tibia ash, it did prevent the 14.3% decrease in tibia ash observed in the negative control group that had neither supplement added. Significant improvement in tibia ash were reported by Rafacz-Livingston *et al.* (2005b), for all tested organic acids and in a subsequent paper, (Rafacz-Livingston *et al.* (2005a), significant increases in tibia ash of 10.5% were observed when 6% citric acid was combined with 0.23% aP. Similar significant increases in tibia ash of 11% were also found by Chowdhurry *et al.* (2009), when supplementing 0.5% citric acid. Tibia ash magnesium increased by 13.9% with citric acid alone, and 22.2% when phytase and citric acid were combined (Hariharan & Gangadevi, 2015). A similar combination used by Vieira *et al.* (2017) resulted in a significant improvement of 3.27% in overall tibia ash.

These results can be explained by the effects exhibited on the GIT by the organic acids. Han *et al.* (1998) postulated two theories; organic acids increase P solubility in digesta, consequently prolonging the time that P spends in the small intestine and secondly, the organic acids may provide a more suitable environment in which the phytase can work. Edwards & Baker (1999) found that the acidic anion forms complexes with cations, such as Ca^{2+}, P, magnesium and zinc, resulting in improved digestion. Ziaie *et al.* (2011) found that supplementing a mixture of sodium bentonite and propionic acid, resulted in an improvement in nutrient digestibility and

availability, namely Ca and P, which resulted in improved mineral retention and bone mineralisation. This was found to be a direct result of the increased numbers of desirable microbiota, such as *Lactobacillus* spp.

The improved digestive and absorptive capabilities of the broilers that receive diets supplemented with organic acids has been well documented and thus it can be concluded that organic acids have a positive effect ton nutrient utilisation of the birds, improving overall flock performance and efficiency.

2.4 Effect of organic acids on health

Organic acids have many well documented benefits on the health of the birds. Organic acids have strong antibacterial properties, particularly exhibited on gram negative bacteria. This is due to the ability of undissociated organic acids to diffuse through the lipid membrane and decrease cytoplasmic pH after dissociating – ultimately disrupting normal enzymatic activity and causing cell leakage (Ricke, 2003). Other possible mechanisms include alterations to purine structure that disrupts DNA synthesis, as well as RNA and protein synthesis disruption, and the interference of the cell membrane structure when the change in extracellular pH disrupts the normal proton gradient (Mani-Lopez *et al.*, 2012). These disruptive effects have been recorded by numerous studies on a variety of bacteria, including *E. coli, Campylobacter* spp., *Salmonella* spp. and *Listeria monocytogenes* (Suresh *et al.*, 2018). Kil *et al.* (2011) also reported that organic acids may reduce the transfer of bacteria from the environment, both on/within the feed and in the water. This is especially important in promoting gut health. As previously stated, a healthy gut is essential for optimum productivity and any disruptions or damage may interfere with overall flock productivity. Pathogenic bacteria in the GIT may damage villi and thicken the mucosal membrane, thus interfering with normal digestive and absorptive function (Haq *et al.*, 2017). Various studies have reported higher reductions in numbers of bacteria such as *E. coli* and *Salmonella*, than that of *Lactobacilli* spp. One such study by Yousaf *et al.* (2017) showed alterations to total *Lactobacilli* populations in birds supplemented with encapsulated benzoic acid. Numerical increases were observed in the crop, whilst significantly larger numbers were observed in the jejunum and ileum. Different species of *Lactobacillus* responded in slightly different ways, but all except for *Lactobacillus salivarus*, which did not show any response, followed a similar pattern to the total *Lactobacilli* response. The positive responses of *Lactobacilli* to pH changes compared to other bacteria, is a result of their acidophilic nature (Kim *et al.*, 2009). This is also beneficial, however, as the increased numbers of *Lactobacilli* help maintain an acidic environment within the GIT and promotes competitive exclusion of other undesirable bacteria such as coliforms. This is especially important in chicks, as it promotes a healthy GIT platform on which to grow (Haq *et al.*, 2017).

2.4.1 Changes to the gut microbiota

E. coli, Campylobacter spp., *Salmonella* spp. and *Clostridium* spp. are of particular importance in poultry and pork industries. Poultry have been linked to increased resistance to antibiotics against these bacteria, particularly *Salmonella* and *Campylobacter* (Dittoe *et al.*, 2018). As such, numerous studies have

been conducted in an attempt to reduce their prevalence in the final marketed products and the environment in which the animals are reared. These bacteria, especially *E. coli*, can be detrimental to optimal production in any broiler industry. Organic acids have been successfully used in multiple experiments to not only reduce *E. coli* counts, but also to maximise performance under challenged conditions. Allam *et al.* (2016) challenged 20 day-old broilers with 0.3 mL *E. coli* solution and monitored the results at day 35. The groups supplemented with formic acid in combination with a probiotic, had a 20% lower mortality rate and performed better in all aspects. They also had lower *E. coli* counts in their intestines. Similarly, Ramigani *et al.* (2015) reported significantly lower *E. coli* counts of intestinal contents in birds supplemented with a combination of citric-, formic- and propionic acid compared to the supplemented control group. Ragaa & Korany (2016) tested 0.5% potassium diformate and found a numerical decrease in total *E.* coli isolated from the caecum. More recently, Emami *et al.* (2017) challenged birds with *E. coli* K88 and found that organic acid supplemented groups had significantly reduced caecal *E. coli*. Most organic acid supplemented groups exhibited responses greater to or equivalent to the unchallenged birds. Similarly, Wen *et al.* (2018) supplemented diets with sodium diacetate and found significant reductions in *E. coli* counts in both the large- and small intestine.

Campylobacter is another bacterial group that causes concern in the poultry meat industry. Attempts to reduce its transmission and prevalence in the gut have been explored in an attempt to reduce its prevalence in the final products. Organic acids have been tested in numerous studies and successfully reduced *Campylobacter* GIT counts as well as counts in the meat itself, both directly and indirectly. Wagle *et al.* (2017) inoculated birds at seven days-of-age with four *Campylobacter jejuni* strains and supplemented test groups β-resorcyclic acid from the first day. At day 14, they concluded that supplemented groups had significantly lower *Campylobacter* populations with just 0.5% and 1% organic acid supplementation. They also found that β-resorcyclic acid significantly inhibited the *Campylobacter* motility, attachment to and invasion of Caco-2 cells, reducing prevalence in the final products. Further analysis also found that the *Campylobacter* genes regulating motility were significantly down-regulated. Another study conducted by Zeiger *et al.* (2017) found that supplementation of lauric acid resulted in significantly decreased *Campylobacter* counts in broiler carcasses by 0.8 $\log_{10}$ cfu/g, possibly due to the increased lauric acid levels in the fatty acid profile of the birds inhibiting *Campylobacter* growth post slaughter. Guyard-Nicodème *et al.* (2017) reported a significant reduction of *Campylobacter* counts by 0.82 $\log_{10} \pm 0.25 \log_{10}$ cfu/g in free range chickens supplemented with a combination of a dietary cation exchange clay-based product and an organic acid water additive comprised of formic-, lactic, and propionic acids, as well as sodium formate.

Salmonella is a well-known bacterial group involved in cases of GIT ailments in people that eat raw or undercooked poultry products. A major concern over the past two decades, is the antibiotic resistance of these bacteria, linked to the poultry and pork industries. Thus, extensive studies to reduce the use of these antibiotics have been conducted and organic acids have proved a successful alternative, both on their own and in combination with other nutraceuticals. Ragaa & Korany (2016) supplemented broilers with either 0.5% of formic acid or potassium diformate and gut microbes were counted. They found a significant decrease in total

Salmonella spp. isolated from the caecum. Abudabos *et al.* (2017) challenged birds with *Salmonella typhimurium* in the starter phase and found that a diet containing organic acids and *Bacillus subtilis* triggered immunological responses similar to that of antibiotics, namely increased blood albumin and aspartate aminotransferase, as well as increased protein and triglyceride concentrations in the blood. Similarly, Bourassa *et al.* (2017) performed three different experiments, also challenging the birds with *S. typhimurium* and examined the results after 6 weeks. In the first experiment, 1 kg/ton formic- and 5 kg/ton propionic acids were used, and no differences between test and control groups were reported. However, in experiment two, the organic acid fed groups, propionic acid in feed at 2 kg/ton and formic acid in the water at 1.0 mL/L, had 25% lower caecal recovery of *S. typhimurium* than the control groups. Moreover, in experiment three, the test groups were supplemented with formic acid at 4 or 6 kg/ton or with propionic acid at 5 or 10 kg/ton in the feed. All test levels were conducted for the entire period, or just the last week, and broilers fed 4 kg/ton formic acid for the entire period, had no *Salmonella*-positive caeca amongst 30 different pens. Wolfenden *et al.* (2007) ran three separate experiments, using an organic acid mixture and probiotics, either alone or in combination, to test their efficacy against *Salmonella enteritidis*. They found that organic acid supplementation significantly reduced *Salmonella* recovery from the caecal tonsils (45% less) and from the crop (25% less) when compared to the unsupplemented control. Skřivanová *et al.* (2015) contaminated the feed of individually housed broilers with 10^7 cfu *S. enteritidis* per 100 g of feed. They supplemented test groups with caprylic acid and reported significant decreases in *S. enteritidis* counts in the crop and caecum. These effects, as expected, were much more pronounced in the crop than in the caecum.

Clostridium bacteria are a group of spore-forming, anaerobic, Gram-positive bacteria that are commonly found in the GIT of poultry and are consequently classified as part of a normal gut microbiome (Timbermont *et al.*, 2010). *Clostridium perfringens* is an important member of this family and its strains can be classified into five types, namely A through E. The type is dependent on the major toxin produced by these bacteria; alpha, beta, epsilon and iota toxins specifically (Petit *et al.*, 1999). Although *C. perfringens*, specifically type A, is ubiquitously present in the poultry GIT and forms part of the microbiome, it is also strongly associated with the incidence of necrotic enteritis (NE), one of the most costly diseases in the poultry industry (Jayaraman *et al.*, 2013). NE is characterised by the sudden onset of diarrhoea and mucosal necrosis, particularly in the small intestine, and can result in mortalities of up to 1% per day or 30% in total (Dahiya *et al.*, 2006). Various antibiotics are available to prevent NE in flocks but as previously mentioned, an effort to move away from antibiotics is being made. Thus alternative feed additives should be explored in an attempt to reduce NE cases and maintian growth parameters under challenging conditions, because *C. perfringens* is a Gram positive bacteria and organic acids have no influence on their populations. As such, very few studies tested the effects of organic acids on NE. Nonetheless, a few studies have been conducted; Skřivanová *et al.* (2006) reproted that use of lauric acid monoglycerides at 3 mg/mL and citric acid at 4 mg/mL inhibited growth of *C. perfringens*. Wu *et al.* (2010) performed an experiment with varying levels of fishmeal, either at 500 g/kg or 250 g/kg. Interestingly, the 250 g/kg group had a higher incidence of NE than the 500 g/kg group, but had a

2.67% lower mortality rate. This may be associated with the higher levels of formic acid detected in the ileum of those birds. Thus, this study might have indirectly proved the efficacy of organic acids in reducing mortality during NE infection. Timbermont *et al.* (2010) tested encapsulated butyric acid with other fatty acids (mainly lauric acid) and essential oils. At minimum inhibitory concentrations, lauric acid was shown to be very effective in inhibiting *C. perfringens* growth. Combining butyric acid, fatty acids and essential oils proved highly effective in reducing the amount of birds exhibiting necrotic lesions in their intestines, indicating that *Clostridia* populations were controlled by the combination. Abudabos *et al.* (2017) infected test groups with *C. perfringens* and found that a diet supplemented with an organic acid blend or a mixture of organic acids and *Bacillus subtilis*, showed no significant differences in feed intake, feed conversion ratio or performance efficiency factor compared to the uninfected control groups. Although the control group had heavier bodyweights and was more efficient, the organic acid blend group had heavier bodyweights than the antibiotic group. Although few, these studies indicate that organic acids and blends of organic acids and other neutraceuticals, have the potential to replace antibiotics in an effort to control NE in poultry.

In almost all studies, improved growth and immune response was noted for broilers supplemented with various forms of organic acids in either water or feed, whether challenged or not, compared to control groups. Therefore, one can conclude that the antimicrobial properties of organic acids directly influence the performance of the birds.

2.4.2 Immunological effects of organic acids

In any production system, prevention is better than cure, and this is no different for the poultry industry, especially since most disease outbreaks affect the entire house or site and it is not financially or practically feasible to treat an entire flock. As such, many preventative measures are available, such as vaccines. However, vaccines are expensive and cheaper alternatives are continuously explored. Organic acids are one such alternative and it has been proven to enhance the immune function of the birds by stimulating their immune systems (Chowdhury *et al.*, 2009; Haque *et al.*, 2010). Birds have a complex immune system, centred around their lymphoid organs, and this system is comprised of multiple cells and soluble factors that work synergistically together to initiate immune response (Khan & Iqbal, 2016). Organic acids have been proven to stimulate many of these cells and organs, resulting in improved immunity and overall productivity of the bird. Good management practices, such as strict biosecurity, coupled with the correct feeding scheme of additives such as organic acids can have invaluable benefits.

Various measures of the efficacy of organic acids in immune stimulation can be recorded and, as such, these effects have been well documented. In a review by Khan & Iqbal (2016), it was concluded that organic acids increase CD4 cell counts and T-Cell Receptors (TCR), which is directly correlated to faster immune responses as more TCR lymphocytes ultimately leads to increased stimulation on Interleukin-2 synthesis, which activates CD8-, B- and natural killer cells (Khan & Iqbal, 2016). Heavier immune organs, such as the bursa of Fabricius and the thymus gland, as well as higher serum globulin levels have also been reported

(Ghazalah *et al*., 2011). Similarly, Park *et al.* (2009), who used a blend or organic acids, Ca and P, and Emami *et al.* (2013), who used a combination of phytases and organic acids, reported significantly higher levels of immunoglobulin-Y and total immunoglobulin levels, most notably immunoglobulin G. Globulin levels are a strong indicator of immune response, as immunoglobulins are important transporter proteins produced by lymphocytes (Calder, 2007). Thus, it can be inferred that increased globulin levels are a direct result of increased lymphocyte activity and, as previously mentioned, bird immune systems revolve around their lymphoid organs, indicting a stimulation to these organs by the organic acids. Infectious bursal disease (IBD) antibodies have been shown to positively respond to ascorbic acid supplementation, resulting in stronger immune response when the disease is prevalent (Lohakare *et al.*, 2005). These increases were explained as a result of the faster lymphoid organ differentiation due to enhanced activity of the hexose monophosphate pathway. The same study found that CD4 and TCR-II cells significantly increased. Even Newcastle Disease Virus, a serious threat in the poultry industry, is preventable with organic acids, as demonstrated by Houshmand *et al.* (2012), who reported that supplementing a commercial organic acid blend resulted in significant increases of antibodies against the virus in birds at 21 days-of-age. However, these responses were no longer present at 42 days-of-age. Similarly, Abbas *et al.* (2013) reported similar antibody increases in laying hens supplemented with formic acid in their water. Haque *et al.* (2010) reported enhanced density of lymphocytes when supplementing citric acid, thus improving non-specific immunity. Phenyllactic acid has also been reported to increase short-term lymphocyte percentages, causing hyperthyroidism and stimulating peripheral T4-T3 conversion in layer hens (Wang *et al.*, 2009), which is indicative of enhanced immunocompetence and bursa growth (Khan & Iqbal, 2016). Similarly, Rodríguez-Lecompte *et al.* (2012) reported that a combination of organic acids and probiotics were able to alter TLR-II and cytokine profiles. These alterations included the up-regulation of caecal tonsil IFN-y and ileal IL-6 and IL-10 at 22 days-of-age, whilst down-regulating caecal tonsil TLR-II, ileal IL-12p35 and IFN-y at 11 days-of-age. These alterations, specifically down-regulation of cytokines, are indicative of an anti-inflammatory response via the Th-II associated pathways.

Some of these responses were observed after just 7 days of supplementation, indicating that short term supplementation may be enough to elicit the immunological benefits of organic acids. This strongly suggests that organic acids may be able to completely replace antibiotics and even minimise, perhaps eliminate, the need to vaccinate. However, although the above data is promising, further studies on immunity for a greater variety of organic acids are necessary to fully understand their individual effects.

2.5 Optimum organic acid inclusion

The precise dosage of organic acids is unclear, and the optimum level is likely dependent on many factors, including, but not limited to, organic acid type, environment and breed of bird. Optimal levels may be a specific dose or a specific target-response, such as a target feed or drinking water pH. For the purpose of this review, inclusion levels and drinking water pH will be discussed, however, as many inclusion levels of organic

acids have already been mentioned, drinking water pH will be emphasised. There has been much debate about the ideal drinking water pH for broilers and results can vary greatly. There is no clear answer as to whether lower pH is better or not; according Philipsen (2006), a pH of 4 is desirable. On the contrary, Jacob (2015) stated that poultry prefer drinking water with a pH of 6.0 to 6.8 and that water with a pH less than 6 has been shown to negatively affect broiler performance. Table 2.4 summarises the findings of various studies where different levels of organic acids and drinking water pH were investigated. Many studies do not account for the pH of the drinking water, but only specify the type of organic acid.

Many studies have shown no significantly positive effects on performance with acidification of water (De Avila *et al.*, 2003; Mohyla *et al.*, 2007; Aclkgoz *et al.*, 2011). De Avila *et al.* (2003) and Mohyla *et al.* (2007) reported significantly reduced *Salmonella* counts when water was acidified (pH <2.7), compared to the control groups. Van Bunnik (2014) tested the efficacy of acidified water on *Campylobacter* transmission and reported that a water pH of 4, obtained using a Selko® BV product, Forticoat ®, significantly reduced indirect transmission between pens. This is significant in the poultry industry as the reduced transmission of *Campylobacter* between birds results in safer and higher quality end-products. Chaveerach *et al.* (2004) also reported decreases in *Eneterobacteriaceae* and zero *Campylobacter* in the drinking water when acidifiers were added, thus reducing the external transfer of bacteria from bird to bird through the drinking water. Perhaps the most positive results were that of Hamid *et al.* (2018) who found significant improvements in bodyweights and efficiency of birds fed acidified water with a pH of 4.2.

An important property of any acid is its pK_a value (Table 2.5). The pK_a value may be defined as the pH at which 50% of the acid will be dissociated (Philipsen, 2006). The lower the actual pH compared to the pK_a value of the acid, the more undissociated acid there will be. As previously mentioned, the undissociated organic acid in the GIT elicits more pronounced antimicrobial effects as it can diffuse through the bacterial cell membrane. Thus, it can be inferred that a drinking water pH lower than the pK_a of the organic acid used should be optimal for antimicrobial properties and the difference between the drinking water pH and the pK_a value of the organic acid used may explain the variability in results, but this is not always the case. pK_a values are determined using water, a 'single-phase' system (De Maria *et al.*, 2009). The GIT is an intricate mixture of multiple fluids, enzymes and other substrates. These different media with which the organic acid interact, affect its 'true' pK_a and result in a more aptly referred to, 'apparent' pK_a, (De Maria *et al.*, 2009). One must consider the partition coefficients of the organic acids in the different media to fully quantify the effects of the organic acids' pK_a. Thus, although pK_a may provide a platform on which to select an organic acid, it does not provide a certain answer as to how the organic acid will act.

Although results are variable and very few studies recorded the drinking water pH, there are some clear trends. Acidified drinking water appears to decrease water intake (De Avila *et al.*, 2003; Mohyla *et al.*, 2007) as with most organic acids, it clearly has a beneficial effect on health (Byrd *et al.*, 2001; De Avila *et al.*, 2003; Mohyla *et al.*, 2007). Most pathogenic bacterial numbers are reduced by organic acids, with a trend for greater decrease in the more acidic waters, as can be seen in table 2.4. More research is required to quantify the effects

of pH alone, rather than the effects of the organic acids, in order to fully understand the role that drinking water could play in the future. Furthermore, it is essential to understand how the organic acids will act within the GIT fluids and media, thus research is required to better understand the interaction between the feed and the organic acid, as well as the bird and the organic acid.

Table 2.4 Effects of drinking water pH on broilers

Organic Acid	Inclusion levels (%)	Drinking Water pH	Effect [1]						Reference
			Bodyweight	Efficiency	Feed Intake	Gut Microbes[2]	GIT pH[3]	Water Intake	
Acetic ACid	0.50					0			Byrd *et al.* (2001)
CitroMix[4]	0.40	2.69				- S	- C	-*	De Avila *et al.* (2003)
	0.80	2.73				- S	- C	-*	
Citric Acid	0.80	2.52				- S*	- C	-*	
		2.60				- S + Ca		+	Mohyla *et al.* (2007)
Formic Acid	0.50					- S *			Byrd *et al.* (2001)
LactoMix[5]	0.35	3.11				+ S	- C	-	De Avila *et al.* (2003)
	0.51	2.88				- S	- C	-	
Lactic Acid	0.47	2.71				- S	- C	-	
	0.44					- S*			Byrd *et al.* (2001)
	0.50					- Ca* - S*			
Forticoat®[6]		4.50				- Ca transfer (indirect)			Van Bunnik (2014)
Lupro-Mix®[7]		4.20	+ *	+ *	+	- E	- (C, P, G I) *		Hamid *et al.* (2018)
							- (J, Ce)		
Sodium Acid Sulphate		2.60				- S + Ca - Ca*		-	Mohyla *et al.* (2007)
Selko®-DWB[8]		4.00	0		0	- En (in water)	+ Ce		Chaveerach *et al.* (2004)

[1] Increase (+), Decrease (-) or No effect (0) compared to control groups. Results in table quantified from average responses observed. * represents a significant response (P <0.05).
[2] Effect on microbe counts. S – *Salmonella* Ca – *Campylobacter* E – *Escheria.* En – *Enyterobacteriacae.*
[3] Effect on GIT pH in Crop (C), Proventriculus (P), Gizzard (G), Duodenum (D), Jejunum (J), Ileum (I), Caeca (Ce), Colon (Co).
[4] Blend consisting of 95.5% of citric acid, 1.0% of soluble d-Limonene and 3.5% of cupric sulphate.
[5] Blend consisting of 92.9% of lactic acid, 1.6% soluble d-Limonene and 5.5% of cupric sulphate.
[6] Selko® BV product consisting of sorbic-, formic-, acetic-, lactic-, propionic-, citric- and L-ascorbic acids as well as ammonium formate.
[7] BASF Co., Ltd. product, consisting of propionic- and formic acids as well as ammonium- propionate and formate.
[8] Selko® BV product. Discontinued product, thus unknown composition.

Table 2.5 True pK_a of various organic acids

Organic acid		True pK_a		Reference
Acetic Acid		4.756		De Maria *et al.* (2009)
Ascorbic Acid [1]		4.170		Martin (1961)
Butyric Acid		4.720		Martin *et al.* (1983)
Caproic Acid		4.849		De Maria *et al.* (2009)
Caprylic Acid		4.895		De Maria *et al.* (2009)
Capric Acid		4.900		Whittle *et al.* (1996)
Citric Acid		3.140		Whittle *et al.* (1996)
Formic Acid		3.750		Gildberg & Raa (1977)
Lactic Acid		3.080		Whittle *et al.* (1996)
Lauric Acid		4.900		De Maria *et al.* (2009)
Malic Acid [2]		1: 3.400	2: 5.200	Elliott *et al.* (1959)
Propionic Acid		4.874		De Maria *et al.* (2009)
Sorbic Acid		4.760		Lingwood & Simons (2010)
Tartaric Acid	L (+)	1: 2.890	2: 4.400	Elliott *et al.* (1959)
	Meso	1: 3.220	2: 4.850	

[1] Ascorbic acid as Vitamin C
[2] 1 and 2 denote the pK_a1 and pK_a2 respectively, i.e. after first and second deprotonation.

Conclusion

Organic acids and feed acidifiers have been considered as alternatives to antibiotics for decades. Recent studies have solidified this, as the positive results observed as a result of organic acid supplementation and a better understanding of their mode of action, has led to an increase in available supplements and subsequent use. pH reduction and pathogen reduction in the GIT is one of the most positive effects that can be expected from organic acid supplementation. The decreased pH not only increases nutrient utilisation due to increased retention time, but it also inhibits the growth of pathogenic bacteria. The undissociated organic acids freely diffuse into Gram-negative cells and disrupt cellular function, ultimately leading to cell death. This reduction in pathogens consequently promotes the growth of beneficial bacteria, resulting in competitive exclusion of other pathogens. Organic acids may also be a means of reducing incidence of many diseases, such as IBD and Newcastle Disease Virus, as it has been shown that organic acids increase antibody counts for both diseases. They also have been shown to greatly improve immunoglobulin and lymphocyte counts in the serum of broilers and layers alike, indicating a stimulatory effect on the lymphatic system of the birds; the organs around which their immune system is centred. Organic acid complexes have greater effects in the distal GIT, as they do not

readily undergo absorption in the upper GIT as free organic acids do. Nutrient utilisation as a result of organic acid supplementation has been reported to be variable, but a trend towards increased utilisation can inferred. The combination of improved health, due to the immunostimulatory and antimicrobial properties of acids, and nutrient utilisation results in the most economically important benefit of acids; improved broiler performance. The improved performance, for a relatively small cost, greatly benefits producers and consumers alike, increasing turnover for producers and consequently increasing product flow into the market, which may reduce consumer prices. However, more research is still required to fully understand acidification, such as inclusion levels and other possible modes of action. Many factors may influence the results one may obtain, such as dietary constituents, proximity to feeding and/or drinking, environmental influences, other management practices and even the individual bird itself; breed, physiological age and health status. Thus, it is difficult to conclude the overall effects of organic acids but a general trend towards positive results is strongly indicative of their ability to replace AGPs. Further studies to solidify this opinion are required.

Chapter 3

Materials and Methods

This project was approved by the Faculty of Natural and Agricultural Sciences Ethics Committee of the University of Pretoria (NAS 146/2019).

3.1 Birds and housing

The trial was run at the test facilities at Daybreak Farms, Sundra, South Africa. Broilers were housed in a standard open-sided broiler house, fitted with tunnel ventilation. This house was divided into two separately controlled sides. Seven thousand two hundred (7200) vaccinated day-old Ross 308 chicks were purchased from the Daybreak Merinovlakte hatchery. On arrival at the trial house, a total of 60 chicks were randomly selected and allocated to one of 120 pens (60 pens per side). Each pen had an area of 3 m^2; however, the bell drinker used occupied an area of 0.407 m^2, resulting in a total usable area for the chicks of 2.593 m^2. Thus, the chicks were placed at a stocking density of 23 birds per m^2. The temperature profile that was followed from 2 days pre-placement to Day 35 is shown in appendix A; the lighting profile is shown in appendix B and the vaccination program is shown in appendix C. The bedding consisted of pine shavings, approximately 10 cm deep and extra pine shavings were added to the pen where needed throughout the trial due to spillages caused by the birds and water change. Each pen contained two tube feeders and a bell drinker of 12 L capacity.

The birds were monitored daily by the principal investigator and trial farm staff. There was farm personnel on the premises at all times throughout the trial to monitor the birds' comfort regarding heat, ventilation, feed and water supply, as well as general health. Temperature and humidity loggers were installed in both sides at the beginning of the trial to ensure maximum comfort was maintained for the birds throughout the trial. The birds had *ad libitum* access to feed and water at all times.

3.2 Experimental design and treatments

The experiment was a 5x2 design consisting of two feed treatments (F_1 and F_2) and five water treatments (W_{pH3}, $W_{pH3.8}$, $W_{pH5.5}$, $W_{pH6.5}$, and $W_{ph7.9}$) resulting in 10 treatments, with 12 replicates per treatment. The 10 treatments used were therefore: Treatment 1 (F_1W_{pH3}); Treatment 2 ($F_1W_{pH3.8}$); Treatment 3 ($F_1W_{pH5.5}$); Treatment 4 ($F_1W_{pH6.5}$); Treatment 5 ($F_1W_{pH7.9}$); Treatment 6 (F_2W_{pH3}); Treatment 7 ($F_2W_{pH3.8}$); Treatment 8 ($F_2W_{pH5.5}$); Treatment 9 ($F_2W_{pH6.5}$) and Treatment 10 ($F_2W_{pH7.9}$). Appendix D depicts the layout of the pens and blocks within the house. However, due to human error, treatment 3 ($F_1W_{pH5.5}$) had only 11 replicates, whilst treatment 10 ($F_2W_{pH7.9}$) had 13 replicates, resulting in an unbalanced design. F_1 was considered as 'standard' and F_2 was considered as 'acidified', containing 0.3% FORMI® (ADDCON 40% formic acid product). The five water pH levels tested were 3.0, 3.8, 5.5, 6.5 and facility water (pH of 7.9 ± 21% uncertainty)

as reported by WaterLab (Pty) Ltd. For this dissertation, the facility water will be referred to as pH 7.9. All treatments were applied continuously throughout the rearing period.

Drinking water was supplied via a 12 L bell drinker in each pen. The drinking water was mixed in a 225 L drum, from which the bell drinkers were filled. The desired water pH was obtained by adding Selko®-pH (Trouw Nutrition, a Nutreco company, organic acid blend of E4 copper, 3b601 zinc acetate, dihydrate, E236 formic acid, E260 acetic acid, E280 propionic acid, E295 ammonium formate, benzoic acid , 1,2-propanediol and lactulose) to the drinking water in order to obtain a pH of 6.5, 5.5 and 3.8. In order to achieve a drinking water pH of 3.0, a solution of 30% hydrochloric acid (HCl) was added to the mixture of water and Selko®-pH. The inclusion levels of Selko®-pH and HCl can be seen in Table 3.1. The pH of the drinking water was measured upon refill of each 225 L drum to ensure the desired drinking water pH was supplied to the birds.

Both feeds were a maize-soybean based diets, formulated according to Ross 308 guidelines and were blended for AFGRI Animal Feeds at the Daybreak Poultry Kinross feed mill (Evander, 2280, South Africa). The birds received *ad libitum* feed and water and were monitored daily to ensure that water and feed was refilled as needed. A five-phase feeding program was used, consisting of a pre-starter (PS) fed as an expanded crumble from placement of the day-old chick to 10 days of age; a starter (S) also fed as an expanded crumble from 11 to 17 days of age; a grower (G) fed as a 4 mm pellet from 18 to 27 days of age; a finisher (F) fed as a 4 mm pellet from 28 to 30 days of age and a post-finisher (PF) fed as a 4 mm pellet from 31 to 35 days of age, after which the birds were caught and taken to the abattoir. The expected feed intakes and feed allocation per pen, per phase for the two treatment diets are shown in appendix E. Diet constituents, inclusions and calculated specifications, of the two feeds used, for each phase are shown in Tables 3.2, 3.3, 3.4, 3.5 and 3.6. Both diets contained similar raw material inclusions and were formulated on the same specifications, the only difference was in the acidified diet, which contained 0.3% FORMI® in each phase. The trial feeds were formulated separately on a least cost basis using Format (© Format International Limited, Woking, England. Version 1-May-1998/23.4).

Table 3.1 Selko®-pH and HCl inclusions per 225 L drum

Drinking Water pH	Selko®-pH (mL)	HCl (mL)	Total Acid Inclusion (%)
3.0	150	92	0.134
3.8	150	0	0.083
5.5	45	0	0.025
6.5	20	0	0.011
7.9	0	0	0.000

Table 3.2 Raw material inclusion (%) and calculated nutrient composition for pre-starter treatments

Raw material	Standard (Treatments 1 - 5)	Acidified (Treatments 6 - 10)
Yellow Maize	57.88	57.35
Soya Oilcake Meal 46	30.63	30.67
Sunflower Oilcake CF 20-24 CP ≥ 38	3.000	3.000
Carcass Meal	1.967	2.000
Synthetic Valine	0.063	0.064
Synthetic Lysine	0.238	0.238
Synthetic Methionine	0.267	0.268
Synthetic Tryptophan	.	.
Synthetic Threonine	0.094	0.095
Soya Oil (Mixer)	1.833	2.000
Soya Oil (Coater)	.	.
Limestone (Savanna)	1.407	1.403
Fine Salt	0.517	0.517
FORMI® (ADDCON 40% Formic Acid product)	.	0.300
Olaquindox (10%)	0.040	0.040
Choline Chloride Liquid LM (75%)	0.067	0.067
Cycostat® (Zoetis robenidine hydrochloride product)	0.050	0.050
Mycofix® Select (BIOMIN mycotoxin binder)	0.100	0.100
Hemicell® HT (Elanco mannanase product)	0.033	0.033
Monocalcium Phostphate	1.175	1.175
CreAMINO® (Philagro guanidinoacetate product)	0.060	0.060
Digestarom® (BIOMIN phytogenic supplement)	0.037	0.037
Lysine sulphate 70% (55% true lysine)	0.230	0.230
Axtra® XAP/Axtra® Phy2000FTU (Chemuniqué xylanase, amylase and protease/phytase blend)	0.050	0.050
Natuphos® 1000 FTU broiler (BASF phytase product)	0.010	0.010
Calculated Nutrient Composition (%)		
Dry Matter	88.08	88.02
AME ($MJ.kg^{-1}$)[1]	12.44	12.44
Moisture	11.65	11.70
Crude Protein	22.97	22.994
Crude Fat	4.300	4.149
Crude Fibre	3.670	3.683
Ash	5.760	5.763
Calcium	0.950	0.951
Phosphorus	0.650	0.650
Available Phosphorus	0.350	0.348
Lysine (Total)	1.380	1.380
Methionine (Total)	0.600	0.600

[1]AME for broiler chicks (CVB)

Table 3.3 Raw material inclusion (%) and calculated nutrient composition for starter trial feed

Raw material	Standard (Treatments 1 - 5)	Acidified (Treatments 6 - 10)
Yellow Maize	58.89	58.42
Soya Oilcake Meal 46	27.40	27.40
Sunflower Oilcake CF 20-24 CP ≥ 38	3.000	3.000
Carcass Meal	4.433	4.433
Synthetic Valine	0.012	0.012
Synthetic Lysine	0.185	0.186
Synthetic Methionine	0.220	0.2220
Synthetic Tryptophan	.	.
Synthetic Threonine	0.050	0.052
Soya Oil (Mixer)	2.167	2.333
Soya Oil (Coater)	.	.
Limestone (Savanna)	1.177	1.177
Fine Salt	0.433	0.433
FORMI® (ADDCON 40% formic acid product)	.	0.300
Olaquindox (10%)	0.040	0.040
Choline Chloride Liquid LM (75%)	0.067	0.067
Cycostat® (Zoetis robenidine hydrochloride product)	0.050	0.050
Mycofix® Select (BIOMIN mycotoxin binder)	0.100	0.100
Hemicell® HT (Elanco mannanase product)	0.033	0.033
Monocalcium Phostphate	1.093	1.095
CreAMINO® (Philagro guanidinoacetate product)	0.060	0.060
Digestarom® (BIOMIN phytogenic supplement)	0.037	0.037
Lysine sulphate 70% (55% true lysine)	0.240	0.240
Axtra® XAP/Axtra® Phy2000FTU (Chemuniqué xylanase, amylase and protease/phytase blend)	0.050	0.050
Natuphos® 1000 FTU broiler (BASF phytase product)	0.010	0.010
Calculated Nutrient Composition (%)		
Dry Matter	88.03	88.08
AME ($MJ.kg^{-1}$)[1]	12.75	12.75
Moisture	11.69	11.64
Crude Protein	22.34	22.30
Crude Fat	4.650	4.800
Crude Fibre	3.610	3.600
Ash	5.250	5.241
Calcium	0.860	0.860
Phosphorus	0.620	0.619
Available Phosphorus	0.330	0.328
Lysine (Total)	1.280	1.280
Methionine (Total)	0.540	0.540

[1]AME for broiler chicks (CVB)

Table 3.4 Raw material inclusion (%) and calculated nutrient composition for grower trial feed

Raw material	Standard (Treatments 1 - 5)	Acidified (Treatments 6 - 10)
Yellow Maize	59.54	58.73
Soya Oilcake Meal 46	20.50	21.57
Sunflower Oilcake CF 20-24 CP ≥ 38	4.500	5.000
Carcass meal	8.900	8.900
Synthetic Valine	0.015	0.015
Synthetic Lysine	0.285	0.289
Synthetic Methionine	0.205	0.189
Synthetic Tryptophan	0.015	0.010
Synthetic Threonine	0.044	0.024
Soya Oil (Mixer)	1.300	0.500
Soya Oil (Coater)	1.500	1.500
Limestone (Savanna)	0.990	1.007
Fine Salt	0.350	0.373
FORMI® (ADDCON 40% formic acid product)	.	0.300
Olaquindox (10%)	0.040	0.040
Choline Chloride Liquid LM (75%)	0.067	.
Cycostat® (Zoetis robenidine hydrochloride product)	0.050	0.050
Mycofix® Select (BIOMIN mycotoxin binder)	0.100	0.100
Hemicell® HT (Elanco mannanase product)	0.033	0.033
Monocalcium Phostphate	0.921	0.850
CreAMINO® (Philagro guanidinoacetate product)	0.060	.
Digestarom® (BIOMIN phytogenic supplement)	0.037	0.037
Lysine sulphate 70% (55% true lysine)	0.240	0.180
Axtra® XAP/Axtra® Phy2000FTU (Chemuniqué xylanase, amylase and protease/phytase blend)	0.050	0.050
Natuphos® 1000 FTU broiler (BASF phytase product)	0.010	0.010
Calculated Nutrient Composition (%)		
Dry Matter	88.18	88.10
AME ($MJ.kg^{-1}$)[1]	13.17	12.85
Moisture	11.55	11.62
Crude Protein	21.47	21.86
Crude Fat	5.560	4.780
Crude Fibre	3.750	3.882
Ash	4.630	4.685
Calcium	0.770	0.770
Phosphorus	0.570	0.563
Available Phosphorus	0.290	0.276
Lysine (Total)	1.220	1.220
Methionine (Total)	0.510	0.510

[1]AME for broiler chicks (CVB)

Table 3.5 Raw material inclusion (%) and calculated nutrient composition for finisher trial feed

Raw material	Standard (Treatments 1 - 5)	Acidified (Treatments 6 - 10)
Yellow Maize	59.49	57.96
Soya Oilcake Meal 46	21.57	21.63
Sunflower Oilcake CF 20-24 CP ≥ 38	5.000	5.000
Carcass meal	6.667	7.667
Synthetic Valine	0.010	0.010
Synthetic Lysine	0.237	0.230
Synthetic Methionine	0.194	0.190
Synthetic Tryptophan	.	.
Synthetic Threonine	0.037	0.030
Soya Oil (Mixer)	1.900	2.100
Soya Oil (Coater)	2.167	2.167
Limestone (Savanna)	0.900	0.890
Fine Salt	0.327	0.327
FORMI® (ADDCON 40% formic acid product)	.	0.300
Olaquindox (10%)	.	.
Choline Chloride Liquid LM (75%)	0.067	0.067
Cycostat® (Zoetis robenidine hydrochloride product)	.	.
Mycofix® Select (BIOMIN mycotoxin binder)	0.100	0.100
Hemicell® HT (Elanco mannanase product)	0.033	0.033
Monocalcium Phostphate	0.704	0.697
CreAMINO® (Philagro guanidinoacetate product)	0.060	0.060
Digestarom® (BIOMIN phytogenic supplement)	0.037	0.037
Lysine sulphate 70% (55% true lysine)	0.240	0.240
Axtra® XAP/Axtra® Phy2000FTU (Chemuniqué xylanase, amylase and protease/phytase blend)	0.050	0.050
Natuphos® 1000 FTU broiler (BASF phytase product)	0.010	0.010
Calculated Nutrient Composition (%)		
Dry Matter	88.30	88.38
AME ($MJ.kg^{-1}$)[1]	13.50	13.52
Moisture	11.48	11.40
Crude Protein	21.29	21.51
Crude Fat	6.630	6.872
Crude Fibre	3.870	3.844
Ash	4.350	4.336
Calcium	0.700	0.700
Phosphorus	0.520	0.523
Available Phosphorus	0.240	0.242
Lysine (Total)	1.200	1.200
Methionine (Total)	0.500	0.500

[1]AME for broiler chicks (CVB)

Table 3.6 Raw material inclusion (%) and calculated nutrient composition for post-finisher trial feed

Raw material	**Standard (Treatments 1 - 5)**	**Acidified (Treatments 6 - 10)**
Yellow Maize	58.52	57.71
Soya Oilcake Meal 46	18.37	18.30
Sunflower Oilcake CF 20-24 CP ≥ 38	6.500	6.000
Carcass meal	10.00	11.10
Synthetic Valine	.	.
Synthetic Lysine	0.263	0.280
Synthetic Methionine	0.165	0.166
Synthetic Tryptophan	0.010	0.010
Synthetic Threonine	0.023	0.020
Soya Oil (Mixer)	1.500	1.500
Soya Oil (Coater)	2.300	2.300
Limestone (Savanna)	0.810	0.800
Fine Salt	0.300	0.297
FORMI (ADDCON 40% formic acid product)	.	0.300
Olaquindox (10%)	.	.
Choline Chloride Liquid LM (75%)	0.067	0.067
Cycostat® (Zoetis robenidine hydrochloride product)	.	.
Mycofix® Select (BIOMIN mycotoxin binder)	.	.
Hemicell® HT (Elanco mannanase product)	0.033	0.033
Monocalcium Phostphate	0.594	0.595
CreAMINO® (Philagro guanidinoacetate product)	.	.
Digestarom® (BIOMIN phytogenic supplement)	0.037	0.037
Lysine sulphate 70% (55% true lysine)	0.250	0.227
Axtra® XAP/Axtra® Phy2000FTU (Chemuniqué xylanase, amylase and protease/phytase blend)	0.050	0.050
Natuphos® 1000 FTU broiler (BASF phytase product)	0.010	0.010
Calculated Nutrient Composition (%)		
Dry Matter	88.32	88.36
AME ($MJ.kg^{-1}$)[1]	13.51	13.51
Moisture	11.46	11.41
Crude Protein	21.32	21.41
Crude Fat	6.580	6.651
Crude Fibre	4.100	3.975
Ash	4.100	4.069
Calcium	0.660	0.660
Phosphorus	0.500	0.500
Available Phosphorus	0.220	0.220
Lysine (Total)	1.190	1.190
Methionine (Total)	0.470	0.470

[1]AME for broiler chicks (CVB)

3.4 Measurements

3.4.1. Chemical analysis of feed samples

Analyses of feed samples for each phase was conducted using near infrared spectroscopy (NIRS) before the feed was delivered to ensure that the two feeds within each phase contained the same nutrient composition. Table 3.7 shows the NIR results that were obtained via analysis at the quality control laboratory on site for AFGRI Animal Feeds at the Daybreak Poultry feed mill in Kinross (Evander, 2280, South Africa).

Table 3.7 NIR results obtained per phase analysed

Feed	Phase	Protein	Moisture	Fat
Standard	Pre-starter	21.8	10.9	4.36
	Starter	20.7	10.8	4.71
	Grower	20.8	10.3	4.55
	Finisher	19.9	11.1	6.44
	Post-finisher	19.4	11.9	5.57
Acidified	Pre-starter	21.6	11.2	3.93
	Starter	20.5	10.8	4.90
	Grower	19.6	10.2	6.20
	Finisher	19.9	11.1	6.44
	Post-finisher	20.5	11.2	6.03

3.4.2 Performance measurements

3.4.2.1 Bodyweight (BW)

Broilers were weighed weekly to obtain average BW for each individual pen. All the birds in a pen were weighed collectively in a crate, which was tared before every weighing, and the average bodyweight was then calculated by dividing the recorded value by the number of birds in the pen. The day-old chicks were weighed at placement and then again at 7, 14, 21, 28 and 35 days of age.

3.4.2.2 Feed intake (FI)

Weekly feed intake was measured by weighing out a specific amount at the beginning of the phase and weighing the feed that was left over at the end of the week. The weekly weighing of feed intake occurred

at the same time as the weighing of the birds. Cumulative feed intake was calculated by the summation of the weekly feed intakes.

3.4.2.3 Feed conversion ratio (FCR)

The cumulative FCR was calculated by dividing the cumulative FI of the pen by the total BW gained per pen, over the experimental period and was corrected for mortality by adding the bodyweight gain of the mortalities during the week to the bodyweight gain of the pen during the week.

3.4.2.4 European performance efficiency factor (PEF)

The PEF value is a calculated value incorporating all of the performance factors and is regarded as a good measure of overall performance, for commercial purposes.

PEF = (Liveability % x Mass (kg)/ Age in Days x FCR) x100

3.4.2.5 Mortalities

The trial house was inspected twice daily; any mortalities were removed, weighed and recorded.

3.4.2.6 Water intake (WI)

Water intake was measured daily by monitoring water level relative to indicators on the bell drinkers, spaced 1 L apart on the clear plastic 'bell'. Water was refilled daily, at which time the water levels were also recorded. Four bell drinkers were distributed evenly throughout the house to measure evaporation over the trial period.

3.5 Measurements of water pH

A Boeco ™ BT-675 pH Meter was used to measure the pH of the drinking water. It was calibrated daily using a two-point calibration, with 4.01 and 7.00 buffer solutions. The drinking water pH was measured upon refilling of the 225 L water drums, after the organic acid had been thoroughly mixed into the water, to ensure that the pH was always within 0.1 of the desired pH.

3.6 Measurements of GIT pH

A Boeco ™ BT-675 pH Meter was used to measure the pH of the GIT segments. The pH in the segments of the GIT was measured weekly. Three pens were randomly selected per treatment and one bird was randomly selected from each pen. The birds were kept in a crate and humanely euthanised by cervical dislocation. The pH was measured as soon after death as possible; starting with the crop and working down the GIT, segment by segment, to the colon. Three pH recordings per segment were recorded and the average of the three used as the result. Three recordings were taken in each caecum, resulting in an average deduced from six recordings

for the caeca. Day-0 pH measurements were obtained by humanely euthanising 10 randomly selected day-old chicks and measuring the pH in the various GIT segments.

3.7 Statistical analysis

Data was analysed statistically as a randomized block design with the general linear model (GLM) (Statistical Analysis Systems, 2019) for the average effects over time. Repeated Measures Analysis of Variance with the GLM was used for repeated week or period measures. Least squares means (LSM) and standard error (SE) were calculated and significance of difference ($P < 0.05$) between means was determined by Fischer's test (Samuels, 1989).

The linear model used is described by the following equation:

$$Yij = \mu + Ti + Lj + TLij + eij$$

Where Y = variable studied during the period

μ = overall mean of the population

T = effect of the ith treatment

L = effect of the jth level

TL = effect of the ijth interaction between treatment and level

e = error associated with each Y

Linear and quadratic relationships between the pH of the water and the two different feeds were determined with the dependent variables, BW, FI, FCR, PEF, WI, mortality, crop-, proventriculus-, gizzard-, duodenum-, jejunum-, ileum-, caeca- and colon-pH, in a multivariate analysis with the GLM (Statistical Analysis System, 2019).

Chapter 4

Results

4.1 Performance data

4.1.1 Bodyweight (BW)

The day 0 BW (g) of broilers at the start of the trial is shown in Table 4.1 whilst Table 4.2 illustrates the weekly BW (g) of the birds as a result of drinking water pH, irrespective of feed received. The average weekly BW (g) of the broilers that received different combinations of feed and drinking water during the 35-day trial period is shown in Table 4.3.

Table 4.1 Day-old BW(g) (± standard error of the mean) of Ross 308 broilers that received different feed and drinking water pH combinations

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age**		
		Feed		
		Standard	Acidified	$\bar{x}$ (± SEM)
3.0	0.134	44.8 (± 0.14)	44.9 (± 0.14)	44.9 (± 0.10)
3.8	0.083	44.7 (± 0.14)	45.1 (± 0.14)	44.9 (± 0.10)
5.5	0.025	45.0 (± 0.15)	44.9 (± 0.144)	44.9 (± 0.10)
6.5	0.011	44.7 (± 0.14)	44.8 (± 0.14)	44.8 (± 0.10)
7.9	0.000	45.00 (± 0.14)	44.7 (± 0.14)	44.8 (± 0.10)
$\bar{X}_{Feed}$		44.8 (± 0.07)	44.9 (± 0.06)	

Day old

There were no significant differences (P >0.05) in BW between treatments on day 0.

Day 7

The broilers that received standard feed were significantly heavier (P <0.05) than the birds that received the acidified feed, irrespective of water pH. Irrespective of the feed used, a water pH of 6.5 resulted in significantly higher (P <0.05) BW when compared to a drinking water pH of 7.9. Between feeds, broilers that received a drinking water pH of 3.0 along with the standard feed, were significantly heavier (P <0.05) than

those that received the acidified feed. Broilers that received the standard feed and a drinking water pH of 6.5, had a significantly heavier (P <0.05) BW than those receiving the acidified feed. Within the standard feed, broilers that received water pH levels of 3.0 and 6.5 were both significantly heavier (P <0.05) than those that received water pH levels of 3.8, 5.5 and 7.9. No significant differences (P >0.05) were observed within the acidified feed.

Day 14

The broilers that received standard feed were significantly heavier (P <0.05) than those that received the acidified feed, irrespective of water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in BW. Between feeds, broilers that received a drinking water pH of 3.0 with the standard feed were significantly heavier (P <0.05) than those receiving the acidified feed. Between feeds, a drinking water pH of 6.5 combined with the standard feed, resulted in significantly heavier (P <0.05) broilers than those receiving the acidified feed. When a drinking water pH of 7.9 was compared across the two feeds, broilers receiving the standard feed were significantly heavier (P <0.05) than the birds receiving the acidified feed. Within the two feeds, no significant differences (P >0.05) were observed.

Day 21

The broilers that received standard feed were significantly heavier (P <0.05) than those that received the acidified feed, irrespective of water pH. Irrespective of feed, broilers that received water pH levels of 3.0, 3.8, 5.5 and 6.5 were all significantly heavier (P <0.05) than those that received a drinking water pH of 7.9. Between feeds, a drinking water pH of 6.5 combined with the standard feed, resulted in significantly heavier (P <0.05) broilers than those receiving the acidified feed. Within the standard feed, broilers that received a water pH of 6.5 were in significantly heavier (P <0.05) than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

Day 28

No significant differences (P >0.05) were observed between the two feeds, irrespective of water pH. Irrespective of feed, broilers that received water pH levels of 3.0, 3.8 and 6.5 were all significantly heavier (P <0.05) than those that received a drinking water pH of 7.9. Between feeds, a drinking water pH of 6.5 combined with the standard feed, resulted in significantly heavier (P <0.05) broilers than those receiving the acidified feed. Within the standard feed, a water pH of 6.5 resulted in significantly heavier (P <0.05) broilers than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

Day 35

The broilers that received standard feed were significantly heavier (P <0.05) than those that received the acidified feed, irrespective of water pH. Irrespective of feed, broilers that received water pH levels of 3.0, 3.8 and 6.5 were all significantly heavier (P <0.05) than those that received a drinking water pH of 7.9. When comparing the drinking water pH of 6.5 and 7.9 across the two feeds, the broilers fed the standard feed were significantly heavier (P <0.05) than those fed the acidified feed. Within the standard feed, broilers that received a water pH of 6.5 were in significantly heavier (P <0.05) than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

In summary, the birds receiving standard feed tended to be numerically or significantly (P <0.05) heavier than the birds receiving the acidified feed, with a few exceptions throughout the rearing period. The birds receiving a more acidic drinking water pH were always numerically or significantly (P <0.05) heavier than the birds receiving no acidification. However, no significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.2 Weekly BW (g) (± standard error of the mean) of Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**					
		0	7	14	21	28	35
3.0	0.134	44.9 (± 0.10)	188.5 (± 1.60)[12]	459.00 (± 2.84)	945.7 (± 4.89)[1]	1570.1 (± 7.09)[1]	2255.00 (± 10.03)[1]
3.8	0.083	44.9 (± 0.10)	186.9 (± 1.60)[12]	452.8 (± 2.84)	947.8 (± 4.89)[1]	1571.4 (± 7.09)[1]	2246.4 (± 10.03)[1]
5.5	0.025	44.9 (± 0.10)	187.2 (± 1.64)[12]	451.8 (± 2.91)	946.19 (± 5.01)[1]	1564.6 (± 7.27)[12]	2241.2 (± 10.28)[12]
6.5	0.011	44.8 (± 0.10)	190.70 (± 1.60)[1]	451.2 (± 2.84)	948.78 (± 4.89)[1]	1577.2 (± 7.09)[1]	2246.2 (± 10.03)[1]
7.9	0.000	44.8 (± 0.10)	186.22 (± 1.57)[2]	448.8 (± 2.79)	927.00 (± 4.81)[2]	1551.00 (± 6.97)[2]	2214.4 (± 9.86)[2]

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

Table 4.3 Weekly BW (g) (± standard error of the mean) of Ross 308 broilers that received different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)									
		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	193.2 (± 2.26)^{13a}	183.7 (± 2.26)^{b}	462.7 (± 4.02)^{a}	441.3 (± 4.02)^{b}	953.5 (± 6.92)^{1}	937.9 (± 6.92)	1574.8 (± 10.03)^{12}	1565.4 (± 10.03)	2265.7 (± 14.19)^{12}	2244.2 (± 14.19)
3.8	0.083	187.4 (± 2.26)^{1}	186.3 (± 2.26)	454.0 (± 4.02)	451.6 (± 4.02)	946.4 (± 6.92)^{12}	949.2 (± 6.92)	1571.1 (± 10.03)^{12}	1571.7 (± 10.03)	2256.4 (± 14.19)^{12}	2236.4 (± 14.19)
5.5	0.025	186.4 (± 2.37)^{2}	187.9 (± 2.26)	453.8 (± 4.21)	449.8 (± 4.02)	943.9 (± 7.26)^{12}	947.2 (± 6.92)	1554.6 (± 10.52)^{12}	1573.6 (± 10.03)	2233.1 (± 14.88)^{12}	2245.7 (± 14.19)
6.5	0.011	195.3 (± 2.26)^{3a}	186.1 (± 2.26)^{b}	460.1 (± 4.02)^{a}	442.3 (± 4.02)^{b}	963.9 (± 6.92)^{1a}	933.7 (± 6.92)^{b}	1592.6 (± 10.03)^{1a}	1561.8 (± 10.03)^{b}	2275.3 (± 14.19)^{1a}	2217.1 (± 14.19)^{b}
7.9	0.000	188.1 (± 2.26)^{1}	184.2 (± 2.18)	455.8 (± 4.02)^{a}	441.7 (± 3.87)^{b}	930.4 (± 6.92)^{2}	923.8 (± 6.67)	1555.5 (± 10.03)^{2}	1545.8 (± 9.67)	2235.1 (± 14.19)^{2a}	2195.8 (± 13.68)^{b}
$\bar{x}$ FEED		190.1 (± 1.02)^{a}	185.7 (± 1.00)^{b}	457.3 (± 1.81)^{a}	445.3 (± 1.78)^{b}	947.9 (± 3.13)^{a}	938.3 (± 3.07)^{a}	1569.9 (± 4.53)	1563.8 (± 4.45)	2253.8 (± 6.41)^{a}	2227.4 (± 6.30)^{b}

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.1.2 Cumulative feed intake (FI)

The FI (g/bird/day) was relatively similar between the treatments, though some significant results (P <0.05) were obtained. Table 4.4 illustrates the FI of the birds as a response to drinking water pH irrespective of feed received, whilst Table 4.5 illustrates the FI of the birds across the treatments for each week during the rearing period.

Day 0 - 7

No significant differences (P >0.05) were observed between feeds, irrespective of water pH. Irrespective of feed, no significant differences (P >0.05) were observed between drinking water pH levels. Between feeds, broilers fed the standard feed combined with a drinking water pH of 5.5, had significantly lower (P <0.05) FI than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 5.5 had a significantly lower (P <0.05) FI than those that received a drinking water pH of 6.5. No significant differences (P >0.05) were observed within the acidified feed.

Day 0 - 14

No significant differences (P >0.05) were observed between feeds, irrespective of water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in FI. Between feeds, broilers that received a drinking water pH of 5.5 and the standard feed had a significantly lower (P <0.05) FI than those receiving the acidified feed. Within the two feeds, no significant differences (P >0.05) were observed.

Day 0 - 21

No significant differences (P >0.05) were observed between feeds, irrespective of water pH. Irrespective of feed, broilers receiving a water pH of 5.5 had a significantly lower (P <0.05) FI than the birds receiving a water pH of 6.5. Similarly, broilers receiving a drinking water pH of 7.9 had a significantly lower (P <0.05) FI than the birds receiving a water pH of 6.5. Between feeds, broilers fed the standard feed combined with a drinking water pH of 5.5, had significantly lower (P <0.05) FI than those receiving the acidified feed. Within the standard feed, broilers receiving a water pH of 5.5 had a significantly lower (P <0.05) FI than the those receiving a water pH of 6.5. Similarly, broilers receiving a drinking water pH of 7.9 had a significantly lower (P <0.05) FI than those receiving a water pH of 6.5. No significant differences (P >0.05) were observed within the acidified feed.

Day 0 - 28

FI between the two feeds, irrespective of drinking water pH, was significantly lower (P <0.05) in the broilers receiving standard feed than those receiving acidified feed. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in FI. Between feeds, broilers fed the standard feed combined with a drinking water pH of 3.0, had significantly lower (P <0.05) FI than those receiving acidified feed. Similarly, the broilers receiving standard feed combined with a water pH of 5.5, had a significantly lower (P <0.05) FI than those receiving the acidified feed. Within the standard feed, broilers receiving a water pH of 5.5 had a significantly lower (P <0.05) FI than those receiving a water pH of 6.5. No significant differences (P >0.05) were observed within the acidified feed.

Day 0 - 35

Between the two feeds used, irrespective of drinking water pH, no significant differences (P >0.05) were observed. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in FI. Between feeds, broilers fed the standard feed combined with a drinking water pH of 3.0, had significantly lower (P <0.05) FI than those receiving acidified feed. Within the standard feed, no significant differences (P >0.05) were observed. Within the acidified feed, broilers that received a water pH of 5.5 had a significantly lower (P <0.05) FI than the broilers receiving a water pH of 3.0.

In summary, the cumulative feed intake remained consistent across the treatments and was most notably different ($P \leq 0.05$) from day 14 to 21 throughout the rearing period, for both drinking water pH irrespective of feed and feed irrespective of drinking water pH. No significant (P >0.05) relationships, linear or quadratic, were observed.

Table 4.4 Cumulative feed intakes (± standard error of the mean) (g/bird) of Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**				
		0 - 7	0 - 14	0 - 21	0 - 28	0 - 35
3.0	0.0134	164.1 (± 1.21)	519.9 (± 2.79)	1213.8 (± 7.04)[123]	2176.4 (± 11.48)	3252.4 (± 16.95)
3.8	0.083	164.3 (± 1.21)	522.0 (± 2.79)	1216.4 (± 7.04)[123]	2172.6 (± 11.48)	3239.3 (± 16.95)
5.5	0.025	164.2 (± 1.24)	517.8 (± 2.86)	1197.2 (± 7.21)[13]	2157.0 (± 11.76)	3236.8 (± 17.37)
6.5	0.011	166.4 (± 1.21)	519.7 (± 2.79)	1221.7 (± 7.04)[2]	2179.7 (± 11.48)	3250.2 (± 16.95)
7.9	0.000	164.4 (± 1.19)	517.2 (± 2.74)	1202.0 (± 6.91)[3]	2166.5 (± 11.27)	3235.1 (± 16.65)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

Table 4.5 Cumulative FI (± standard error of the mean) (g/bird) of Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)									
		0 - 7		0 - 14		0 - 21		0 - 28		0 - 35	
		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	165.5 (± 1.71)[12]	162.8 (± 1.71)	520.1 (± 3.94)	518.9 (± 3.94)	1206.8 (± 9.95)[123]	1220.9 (± 9.95)	2147.6 (± 16.23)[12a]	2205.3 (± 16.23)[b]	3206.6 (± 23.98)[a]	3298.2 (± 23.98)[1b]
3.8	0.083	162.1 (± 1.71)[12]	166.5 (± 1.71)	519.7 (± 3.94)	524.4 (± 3.94)	1209.6 (± 9.95)[123]	1223.2 (± 9.95)	2158.1 (± 16.23)[12]	2187.1 (± 16.23)	3228.3 (± 23.98)	3250.3 (± 23.98)[12]
5.5	0.025	161.1 (± 1.80)[1a]	167.2 (± 1.71)[b]	511.3 (± 4.13)[a]	524.3 (± 3.94)[b]	1177.0 (± 10.44)[13a]	1217.5 (± 9.95)[b]	2133.0 (± 17.02)[1a]	2181.1 (± 16.23)[b]	3216.4 (± 25.15)	3257.2 (± 23.98)[12]
6.5	0.011	166.4 (± 1.71)[2]	166.5 (± 1.71)	521.4 (± 3.94)	517.9 (± 3.94)	1230.1 (± 9.95)[2]	1213.4 (± 9.95)	2190.3 (± 16.23)[2]	2169.2 (± 16.23)	3269.9 (± 23.98)	3230.4 (± 23.98)[2]
7.9	0.000	163.7 (± 1.71)[12]	165.1 (± 1.65)	520.0 (± 3.94)	514.3 (± 3.80)	1196.9 (± 9.95)[3]	1207.2 (± 9.60)	2159.2 (± 16.23)[12]	2165.4 (± 15.65)	3232.7 (± 23.98)	3237.6 (± 23.12)[12]
$\bar{x}_{FEED}$		163.8 (± 0.77)	165.6 (± 0.76)	518.7 (± 1.78)	520.0 (± 1.75)	1204.1 (± 4.50)	1216.4 (± 4.42)	2157.6 (± 7.33)[a]	2173.9 (± 7.21)[b]	3230.8 (± 10.83)	3254.7 (± 10.65)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.1.3 Cumulative feed conversion ratio (FCR)

The FCR (g feed/g BW) was low throughout the trial, with some significant differences (P <0.05) obtained, favouring the lower drinking water pH and/or the standard feed. Table 4.6 illustrates the FCR of the birds as a response to drinking water pH irrespective of feed received, whilst Table 4.7 illustrates the FCR of the birds across the treatments for each week during the rearing period.

Day 0 - 7

FCR between the two feeds, irrespective of drinking water pH, was significantly lower (P <0.05) in the broilers receiving standard feed than those receiving acidified feed. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in FCR. Between feeds, broilers fed the standard feed combined with a drinking water pH of 3.0, had significantly lower (P <0.05) FCR those receiving the acidified feed. Similarly, broilers receiving a drinking water pH of 6.5 combined with the standard feed had a significantly lower (P <0.05) FCR than those receiving the acidified feed. Within the two feeds, no significant differences (P >0.05) were observed.

Day 0 - 14

FCR between the two feeds, irrespective of drinking water pH, was significantly lower (P <0.05) in the broilers receiving standard feed than those receiving acidified feed. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in FCR. Between feeds, broilers fed the standard feed combined with a drinking water pH of 3.0, had significantly lower (P <0.05) FCR than those receiving the acidified feed. Similarly, broilers that received a water pH of 5.5 combined with the standard feed had a significantly lower (P <0.05) FCR than those that received the acidified feed. Broilers that received a water pH of 6.5 combined with the standard feed, also had a significantly lower (P <0.05) FCR. No significant differences (P >0.05) were observed within the two feeds.

Day 0 - 21

Irrespective of drinking water pH, the broilers that received the standard feed had significantly lower (P <0.05) FCR than those that received the acidified feed. Irrespective of feed, broilers that received a drinking water pH of 5.5 had a significantly lower (P <0.05) FCR than those that received a drinking water pH of 6.5 and 7.9. Between feeds, broilers fed the standard feed combined with a drinking water pH of 3.0, had significantly lower (P <0.05) FCR than those fed the acidified feed. Similarly, broilers that received a water pH of 5.5 combined with the standard feed had a significantly lower (P <0.05) FCR than those that received the acidified feed. Within the standard feed, broilers that received a water pH of 5.5 had a significantly lower

(P <0.05) FCR than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

Day 0 - 28

FCR of broilers receiving the standard feed, irrespective of drinking water pH, was significantly lower (P <0.05) than those that received the acidified feed. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in FCR. Between feeds, broilers fed the standard feed combined with a drinking water pH of 3.0, had significantly lower (P <0.05) FCR than those fed the acidified feed. Similarly, broilers that received a drinking water pH of 7.9 combined with the standard feed had a significantly lower (P <0.05) FCR than those that received the acidified feed. Within the two feeds, no significant differences (P >0.05) were observed.

Day 0 - 35

Broilers that received the standard feed had significantly lower (P <0.05) FCR than those that received the acidified feed, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 3.0 and 3.8 had a significantly lower (P <0.05) FCR than those that received a drinking water pH of 7.9. Between feeds, broiler fed the standard feed combined with a drinking water pH of 3.0, had significantly lower (P <0.05) FCR than those fed the acidified feed. Similarly, broilers that received a drinking water pH of 6.5 combined with the standard feed had a significantly lower (P <0.05) FCR than those that received the acidified feed. Broilers that received a drinking water pH of 7.9 combined with the standard feed had a significantly lower (P <0.05) FCR than those fed the acidified feed. Within the standard feed, broilers that received a water pH of 3.0 had a significantly lower (P <0.05) FCR than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

In summary, significant (P <0.05) differences were most notable on days 21 and 35 between drinking water pH, regardless of feed received. The more acidic drinking water always proved to be numerically lower than no acidification, except on day 14. Between two feeds, the standard feed resulted in a significantly lower (P <0.05) FCR than the acidified feed on all recordings. Likewise, when comparing drinking water pH across the two feeds, the standard feed always resulted in a numerically or significantly (P <0.05) lower FCR than the acidified feed. However, no significant (P >0.05) relationships, linear or quadratic, were observed.

Table 4.6 FCR (± standard error of the mean) (g feed/g BW) of broilers receiving different drinking water pH levels

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)				
		0 - 7	0 - 14	0 - 21	0 - 28	0 - 35
3.0	0.0134	1.14 (± 0.013)	1.28 (± 0.009)	1.35 (± 0.007)[12]	1.43 (± 0.008)	1.47 (± 0.007)[1]
3.8	0.083	1.16 (± 0.013)	1.28 (± 0.009)	1.35 (± 0.007)[12]	1.42 (± 0.008)	1.47 (± 0.007)[1]
5.5	0.025	1.16 (± 0.013)	1.27 (± 0.009)	1.33 (± 0.008)[1]	1.42 (± 0.008)	1.48 (± 0.007)[12]
6.5	0.011	1.104 (± 0.013)	1.28 (± 0.009)	1.35 (± 0.007)[2]	1.42 (± 0.008)	1.48 (± 0.007)[12]
7.9	0.000	1.17 (± 0.012)	1.28 (± 0.008)	1.36 (± 0.007)[2]	1.44 (± 0.007)	1.49 (± 0.007)[2]

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

Table 4.7 FCR (g feed/g BW) (± standard error of the mean) of Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**									
		0 -7		0 - 14		0 - 21		0 - 28		0 - 35	
		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	1.11 (± 0.018)[a]	1.17 (± 0.018)[b]	1.25 (± 0.012)[a]	1.31 (± 0.012)[b]	1.33 (± 0.011)[12a]	1.37 (± 0.011)[b]	1.40 (± 0.011)[a]	1.45 (± 0.011)[b]	1.44 (± 0.010)[1a]	1.50 (± 0.010)[b]
3.8	0.083	1.14 (± 0.018)	1.18 (± 0.018)	1.27 (± 0.012)	1.29 (± 0.012)	1.34 (± 0.011)[12]	1.35 (± 0.011)	1.41 (± 0.011)	1.43 (± 0.011)	1.46 (± 0.010)	1.48 (± 0.010)
5.5	0.025	1.14 (± 0.019)	1.17 (± 0.018)	1.25 (± 0.013)[a]	1.30 (± 0.013)[b]	1.31 (± 0.011)[1a]	1.35 (± 0.011)b	1.41 (± 0.011)	1.43 (± 0.011)	1.47 (± 0.010)	1.48 (± 0.010)
6.5	0.011	1.11 (± 0.018)[a]	1.18 (± 0.018)[b]	1.26 (± 0.012)[a]	1.30 (± 0.012)[b]	1.34 (± 0.011)[12]	1.37 (± 0.011)	1.42 (± 0.011)	1.43 (± 0.011)	1.47 (± 0.010)	1.49 (± 0.010)
7.9	0.000	1.15 (± 0.018)	1.19 (± 0.017)	1.27 (± 0.012)	1.30 (± 0.012)	1.35 (± 0.011)[2]	1.37 (± 0.010)	1.43 (± 0.011)	1.45 (± 0.010)	1.48 (± 0.010)[2a]	1.51 (± 0.010)[b]
$\bar{x}_{FEED}$		1.13 (± 0.008)[a]	1.18 (± 0.007)[b]	1.26 (± 0.005)[a]	1.30 (± 0.005)[b]	1.33 (± 0.005)[a]	1.36 (± 0.005)[b]	1.42 (± 0.005)[a]	1.44 (± 0.005)[b]	1.46 (± 0.004)[a]	1.49 (± 0.004)[b]

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.1.4 European performance efficiency factor (PEF)

Table 4.8 illustrates the weekly PEF (g) of the birds as a result of drinking water pH, irrespective of feed received. The average weekly PEF (g) of the broilers during the 35-day trial period is shown in Table 4.9.

Day 7

The broilers that received standard feed had a significantly higher (P <0.05) PEF than those that received the acidified feed irrespective of the drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in PEF. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed had a significantly higher (P <0.05) PEF than those that received the acidified feed. Between feeds, the PEF for the broilers receiving standard feed combined with a drinking water pH of 6.5, was significantly higher (P <0.05) than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 6.5 had a significantly higher (P <0.05) PEF than the broilers receiving a drinking water pH of 3.8, 5.5 and 7.9. No significant differences (P >0.05) were observed within the acidified feed.

Day 14

Comparison of the two feeds used, irrespective of drinking water pH, shows that the broilers that received standard feed had a significantly higher (P <0.05) PEF than those that received the acidified feed. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in PEF. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed, had a significantly higher (P <0.05) PEF than those that received the acidified feed. The PEF for the broilers receiving standard feed combined with a drinking water pH of 6.5, was significantly higher (P <0.05) than those receiving acidified feed. Similarly, broilers receiving a drinking water pH of 7.9 with standard feed had a significantly higher (P <0.05) PEF than those receiving acidified feed. Within the feeds, no significant differences (P >0.05) were observed.

Day 21

Between the two feeds used, irrespective of drinking water pH, the broilers that received standard feed had a significantly higher (P <0.05) PEF than those that received the acidified feed. Irrespective of feed, broilers that received drinking water pH levels of 3.0, 3.8 and 5.5 resulted in significantly higher (P <0.05) PEF than those receiving a drinking water pH of 7.9. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed had a significantly higher (P <0.05) PEF than those that received the acidified feed. Between feeds, a drinking water pH of 6.5 combined with the standard feed, resulted in significantly higher (P <0.05) PEF than those receiving acidified feed. Within the standard feed, broilers that

received a drinking water pH of 3.0, 5.5 and 6.5 all had significantly higher (P <0.05) PEF than the broilers that received a drinking water pH of 7.9. Within the acidified feed, broilers that received drinking water pH of 3.8 and 5.5 had significantly higher (P <0.05) PEF when compared to the broilers receiving a drinking water pH of 7.9.

Day 28

The broilers that received standard feed had a significantly higher (P <0.05) PEF than those that received the acidified feed, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in PEF. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed had a significantly higher (P <0.05) PEF than the birds that received the acidified feed. Within the feeds, no significant differences (P >0.05) were observed.

Day 35

Irrespective of drinking water pH, the broilers that received standard feed had a significantly higher (P <0.05) PEF than those that received the acidified feed. Irrespective of feed, broilers that received drinking water pH levels of 3.0 and 3.8 had significantly higher (P <0.05) PEF than those that received a drinking water pH of 7.9. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed had a significantly higher (P <0.05) PEF than those that received the acidified feed. PEF for the broilers receiving standard feed and a drinking water pH of 6.5, was significantly higher (P <0.05) than those receiving acidified feed. Within the standard feed, broilers that received a drinking water pH of 3.0 had a significantly higher (P <0.05) PEF than those receiving a drinking water pH of 5.5 and 7.9. Within the acidified feed, no significant differences (P >0.05) were observed.

In summary, PEF of the broilers at the end of the rearing period, favoured the more acidic drinking water pH levels, resulting in both numerically and significantly (P <0.05) higher PEF compared to the groups that received a drinking water pH of 7.9. As with the other performance parameters, the PEF also favoured the standard feed, having significantly higher (P <0.05) PEF throughout the rearing period. When comparing the water pH levels across the two feeds, a similar trend was seen. The standard feed combined with any drinking water pH, at any stage, resulted in significantly higher (P <0.05) PEF than the birds receiving the acidified feed. This suggests that PEF favours a more acidic drinking water pH and that feed acidification has no effect on PEF. The only exception was a drinking water pH of 5.5 on day 28, which when combined with the acidic feed, had a numerically higher PEF than the birds receiving the standard feed. No significant (P >0.05) relationships, linear or quadratic, were observed.

Table 4.8 PEF (± standard error of the mean) of Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)				
		7	14	21	28	35
3.0	0.0134	233 (4.0)	249 (3.1)	327 (3.2)[1]	379 (± 3.6)	411 (± 3.8)[1]
3.8	0.083	229 (4.0)	249 (3.1)	328 (3.2)[1]	382 (± 3.6)	412 (± 3.8)[1]
5.5	0.025	230 (4.1)	250 (3.2)	332 (3.3)[1]	381 (± 3.7)	407 (± 3.9)[12]
6.5	0.011	237 (4.0)	248 (3.1)	327 (3.2)[12]	383 (± 3.6)	407 (± 3.8)[12]
7.9	0.000	228 (3.9)	248 (3.1)	318 (3.2)[2]	375 (± 3.6)	401 (± 3.7)[2]

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

Table 4.9 PEF of Ross 308 (± standard error of the mean) broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)									
		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	246 (± 5.7)^{12a}	221 (± 5.7)^{b}	262 (± 4.4)^{a}	236 (± 4.4)^{b}	335 (± 4.6)^{1a}	318 (± 4.6)^{12b}	387 (± 5.1)^{a}	371 (± 5.1)^{b}	425 (± 5.4)^{1a}	398 (± 5.4)^{b}
3.8	0.083	233 (± 5.7)^{2}	224 (± 5.7)	251 (± 4.4)	247 (± 4.4)	328 (± 4.6)^{12}	327 (± 4.6)^{1}	384 (± 5.1)	380 (± 5.1)	416 (± 5.4)^{12}	409 (± 5.4)
5.5	0.025	233 (± 5.9)^{2}	228 (± 5.7)	256 (± 4.6)	244 (± 4.4)	337 (± 4.8)^{1}	328 (± 4.6)^{1}	381 (± 5.4)	382 (± 5.1)	409 (± 5.6)^{2}	404 (± 5.4)
6.5	0.011	250 (± 5.7)^{1a}	225 (± 5.7)^{b}	257 (± 4.4)^{a}	239 (± 4.4)^{b}	335 (± 4.6)^{1a}	318 (± 4.6)^{12b}	390 (± 5.1)	377 (± 5.1)	416 (± 5.4)^{12a}	398 (± 5.4)^{b}
7.9	0.000	234 (± 5.7)^{2}	222 (± 5.5)	255 (± 4.4)^{a}	241 (± 4.2)^{b}	322 (± 4.6)^{2}	315 (± 4.4)^{2}	377 (± 5.1)	372 (± 5.0)	407 (± 5.4)^{2}	395 (± 5.2)
$\bar{x}$ FEED		239 (± 2.6)^{a}	224 (± 2.5)^{b}	256 (2.0)^{a}	241 (2.0)^{b}	331 (2.1)^{a}	321 (2.0)^{b}	384 (2.3)^{a}	376 (2.3)^{b}	415 (2.4)^{a}	401 (2.4)^{b}

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.1.5 Water intake (WI)

The water intake was consistent throughout the rearing period and showed little differences between the treatments. Significantly greater (P <0.05) WI was observed in the birds that received a drinking water pH of 3.8, regardless of feed received, when compared to a drinking water pH of 7.9. Within the acidified feed, a drinking water pH of 3.8 resulted in significantly higher (P <0.05) WI than the birds that received a drinking water pH of 7.9. No other significant differences (P >0.05) were observed. This is illustrated in Table 4.10. However, no significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.10 Total water intake (L/pen) (± standard error of the mean) of Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Feed**		
		Standard	Acidified	$\overline{x}$ (± SEM)
3.0	0.0134	484.85 (± 4.167)	482.23 (± 4.167)[12]	483.54 (± 2.947)[12]
3.8	0.083	487.81 (± 4.167)	493.18 (± 4.167)[1]	490.49 (± 2.947)[1]
5.5	0.025	484.43 (± 4.371)	491.40 (± 4.167)[12]	487.92 (± 3.020)[12]
6.5	0.011	486.17 (± 4.167)	482.15 (± 4.167)[12]	484.16 (± 2.947)[12]
7.9	0.000	479.78 (± 4.167)	481.29 (± 4.018)[2]	480.53 (± 2.895)[2]
$\overline{x}$ FEED		484.61 (± 1.882)	486.05 (± 1.882)	

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

4.1.6 Cumulative mortality

No significant differences (P >0.05) were observed in mortality at any stage throughout the rearing period. This can be seen in Table 4.11 and 4.12 that illustrate the effects that water pH has, irrespective of feed received, and the effect that water pH and feed combinations have on mortality respectively. However, no significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.11 Cumulative mortality (%) (± standard error of the mean) of Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**				
		0 - 7	7 - 14	14 - 21	21 - 28	28 - 35
3.0	0.0134	1.11 (± 0.217)	1.67 (± 0.279)	2.29 (± 0.310)	3.68 (± 0.439)	6.04 (± 0.552)
3.8	0.083	0.97 (± 0.217)	1.53 (± 0.279)	2.22 (± 0.310)	3.20 (± 0.439)	5.49 (± 0.552)
5.5	0.025	1.01 (± 0.223)	1.55 (± 0.285)	2.19 (± 0.318)	3.20 (± 0.450)	6.33 (± 0.565)
6.5	0.011	0.97 (± 0.217)	1.81 (± 0.279)	2.36 (± 0.310)	3.26 (± 0.439)	6.46 (± 0.552)
7.9	0.000	0.87 (± 0.214)	1.17 (± 0.273	1.86 (± 0.305)	2.80 (± 0.431)	5.64 (± 0.542)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

Table 4.12 Cumulative mortality (%) (± standard error of the mean) of Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)									
		0 -7		0 - 14		0 - 21		0 - 28		0 - 35	
		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	0.70 (± 0.307)	1.53 (± 0.307)	1.25 (± 0.394)	2.08 (± 0.394)	1.95 (± 0.439)	2.64 (± 0.439)	3.33 (± 0.621)	4.03 (± 0.621)	5.14 (± 0.780)	6.94 (± 0.780)
3.8	0.083	1.11 (± 0.307)	0.84 (± 0.307)	1.81 (± 0.394)	1.25 (± 0.394)	2.37 (± 0.439)	2.08 (± 0.439)	3.48 (± 0.621)	2.92 (± 0.621)	5.84 (± 0.780)	5.14 (± 0.780)
5.5	0.025	0.91 (± 0.322)	1.11 (± 0.307)	1.44 (± 0.413)	1.67 (± 0.394)	2.29 (± 0.460)	2.08 (± 0.439)	3.20 (± 0.651)	3.20 (± 0.621)	6.01 (± 0.818)	6.68 (± 0.780)
6.5	0.011	1.11 (± 0.307)	0.83 (± 0.307)	1.94 (± 0.394)	1.67 (± 0.394)	2.36 (± 0.439)	2.36 (± 0.439)	3.20 (± 0.621)	3.33 (± 0.621)	6.25 (± 0.780)	6.67 (± 0.780)
7.9	0.000	0.97 (± 0.307)	0.77 (± 0.296)	1.11 (± 0.394)	1.22 (± 0.380)	1.95 (± 0.439)	1.78 (± 0.423)	3.06 (± 0.621)	2.55 (± 0.598)	6.11 (± 0.780)	5.17 (± 0.752)
$\bar{x}_{FEED}$		0.96 (± 0.139)	1.02 (± 0.137)	1.51 (± 0.178)	1.58 (± 0.175)	2.18 (± 0.198)	2.19 (± 0.195)	3.25 (± 0.280)	3.20 (± 0.276)	5.87 (± 0.352)	6.12 (± 0.347)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.2 Gastrointestinal tract segment pH

The gastrointestinal tract (GIT) pH proved to be highly variable throughout the trial. Tables 4.13 to 4.28 illustrate the pH recording in the various GIT segments throughout the rearing period.

4.2.1 Crop pH

Tables 4.13 and 4.14 illustrate the effects observed in the crop pH as a result of the water pH irrespective of feed and as a result of the different feed and drinking water pH combinations respectively.

Day 7

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 3.8 and 6.5 had a significantly lower (P <0.05) crop pH than those receiving a drinking water pH of 7.9. Between feeds, no significant differences (P >0.05) were observed. Within the standard feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) crop pH than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

Day 14

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) crop pH than those receiving a drinking water pH of 3.0. Between feeds, no significant differences (P >0.05) were observed. Within the acidified feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) crop pH than those that received a drinking water pH of 3.8. No significant differences (P >0.05) were observed within the standard feed.

Day 21

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 7.9 had a significantly higher (P <0.05) crop pH than those receiving a drinking water pH of 3.0, 3.8, 5.5 and 6.5. Between feeds, broilers that received a drinking water pH of 5.5 combined with the standard feed, had a significantly lower (P <0.05) crop pH than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 5.5 and 6.5 had a significantly lower (P <0.05) crop pH than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

Day 28

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 5.5, 6.5 and 7.9 all had a

significantly lower (P <0.05) crop pH than those receiving a drinking water pH of 3.0, 3.8. Between feeds, broilers that received a drinking water pH of 6.5 combined with the standard feed, had a significantly lower (P <0.05) crop pH than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) crop pH than those that received a drinking water pH of 3.0, 3.8, and 5.5. Broilers that received a drinking water pH of 7.9 had a significantly lower (P <0.05) crop pH than those a drinking water pH of 3.0 and 3.8. Within the acidified feed, broilers that received a drinking water pH of 3.8 had a significantly higher (P <0.05) crop pH than those that received a drinking water pH of 5.5.

Day 35

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 6.5 had a significantly higher (P <0.05) crop pH than those receiving a drinking water pH of 3.0 and 3.8. Between feeds, no significant differences (P >0.05) were observed. Within the acidified feed, broilers that received a drinking water pH of 6.5 had a significantly higher (P <0.05) crop pH than those that received a drinking water pH of 3.0. No significant differences (P >0.05) were observed within the standard feed.

In summary, crop pH was most notably different on days 21 and 28, however no trends were observed. The pH was highly variable, both within and between treatments. However, a significant (P <0.05) linear relationship between water pH and crop pH (y = 0.098x + 4.623) was observed.

Table 4.13 pH of the crop in Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**					
		0	7	14	21	28	35
3.0	0.134	5.72	5.21[12]	4.94[1]	4.81[2]	5.11[1]	4.75[2]
3.8	0.083	5.72	5.02[2]	4.84[12]	4.64[2]	5.07[1]	5.12[2]
5.5	0.025	5.72	5.14[12]	4.83[12]	4.72[2]	4.83[2]	5.10[12]
6.5	0.011	5.72	4.94[2]	4.61[2]	4.83[2]	4.83[2]	5.59[1]
7.9	0.000	5.72	5.42[1]	4.68[12]	5.11[1]	4.82[2]	5.19[12]
SEM		0.000	0.127	0.093	0.094	0.054	0.157

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
SEM: standard error of the mean.

Table 4.14 pH of the crop (± standard error of the mean) in Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)											
		0		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	5.72 (± 0.000)	5.72 (± 0.000)	5.26 (± 0.179)^{12}	5.16 (± 0.179)	5.00 (± 0.131)	4.87 (± 0.131)^{12}	4.79 (± 0.133)^{12}	4.82(± 0.133)	5.15 (± 0.133)^{1}	5.08 (± 0.133)^{1}	4.86 (± 0.133)	4.64 (± 0.133)^{2}
3.8	0.083	5.72 (± 0.000)	5.72 (± 0.000)	5.04 (± 0.179)^{12}	5.00 (± 0.179)	4.71 (± 0.131)	4.97 (± 0.131)^{1}	4.65 (± 0.133)^{12}	4.64 (± 0.133)	5.06 (± 0.133)^{1}	5.07 (± 0.133)^{1}	5.10 (± 0.133)	5.13 (± 0.133)^{12}
5.5	0.025	5.72 (± 0.000)	5.72 (± 0.000)	5.19 (± 0.179)^{12}	5.09 (± 0.179)	4.84 (± 0.131)	4.83 (± 0.131)^{12}	4.52 (± 0.133)^{2a}	4.91 (± 0.133)^{b}	4.84 (± 0.133)^{13}	4.82 (± 0.133)^{2}	5.13 (± 0.133)	5.06 (± 0.133)^{12}
6.5	0.011	5.72 (± 0.000)	5.72 (± 0.000)	5.00 (± 0.179)^{2}	4.89 (± 0.179)	4.69 (± 0.131)	4.53 (± 0.131)^{2}	4.93 (± 0.133)^{1}	4.74 (± 0.133)	4.62 (± 0.133)^{2a}	5.04 (±0.133)^{12b}	5.47 (± 0.133)	5.70 (± 0.133)^{1}
7.9	0.000	5.72 (± 0.000)	5.72 (± 0.000)	5.57 (± 0.179)^{1}	5.27 (± 0.179)	4.70 (± 0.131)	4.66 (± 0.131)^{12}	5.11 (± 0.133)^{1}	5.12 (± 0.133)	4.74 (± 0.133)^{23}	4.89 (± 0.133)^{12}	5.24 (± 0.133)	5.14 (± 0.133)^{12}
$\bar{x}$ FEED		5.72 (± 0.000)	5.72 (± 0.000)	5.21 (± 0.080)	5.08 (± 0.080)	4.79 (± 0.059)	4.77 (± 0.059)	4.80 (± 0.060)	4.84 (± 0.060)	4.88 (± 0.034)	4.98 (± 0.034)	5.16 (± 0.099)	5.14 (± 0.099)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.2.2 Proventriculus pH

Tables 4.15 and 4.16 illustrate the effects observed in the proventriculus pH as a result of the water pH irrespective of feed and as a result of the different feed and drinking water pH combinations respectively.

Day 7

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the proventriculus. Between feeds, broilers that received a drinking water pH of 5.5 combined with the standard feed, had a significantly lower (P <0.05) proventriculus pH than those that received the acidified feed. Within the acidified feed, broilers that received a drinking water pH of 5.5 had a significantly higher (P <0.05) proventriculus pH than those that received a drinking water pH of 3.0, 3.8, 6.5 and 7.9. No significant differences (P >0.05) were observed within the standard feed.

Day 14

There were no significant differences (P >0.05) observed at any level.

Day 21

There were no significant differences (P >0.05) observed at any level.

Day 28

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 5.5 had a significantly lower (P <0.05) proventriculus pH than those receiving a drinking water pH of 6.5. Between feeds, broilers that received a drinking water pH of 3.8 combined with the standard feed, had a significantly lower (P <0.05) proventriculus pH than those receiving the acidified feed. Within the acidified feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) proventriculus pH than those that received a drinking water pH of 3.8. Broilers that received a drinking water pH of 7.9 had a significantly lower (P <0.05) proventriculus pH than those a drinking water pH of 3.0 and 3.8. No significant differences (P >0.05) were observed within the standard feed.

Day 35

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the proventriculus. Between feeds, broilers that received a drinking water pH of 7.9

combined with the standard feed, had a significantly higher (P <0.05) proventriculus pH than those that received the acidified feed. No significant differences (P >0.05) were observed within the two feeds.

In summary, proventriculus pH was not greatly affected by the feed and water combinations. The pH was variable, both within and between treatments. No significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.15 pH of the proventriculus in Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**					
		0	7	14	21	28	35
3.0	0.134	2.14	1.91	1.84	1.72	1.94[12]	1.52
3.8	0.083	2.14	1.93	2.04	1.52	2.00[12]	1.63
5.5	0.025	2.14	2.09	1.76	1.62	2.07[1]	1.74
6.5	0.011	2.14	2.06	1.94	1.67	1.87[2]	1.86
7.9	0.000	2.14	1.94	2.19	1.96	1.98[12]	1.63
SEM		0.000	0.106	0.176	0.201	0.056	0.134

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
SEM: standard error of the mean.

Table 4.16 pH of the proventriculus (± standard error of the mean) in Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)											
		0		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	2.14 (± 0.000)	2.14 (± 0.000)	2.08 (± 0.150)	1.74 (± 0.150)^{2}	1.78 (± 0.249)	1.89 (± 0.249)	1.58 (± 0.133)	1.87 (± 0.133)	1.88 (± 0.079)	1.9 (± 0.079)^{12}	1.46 (± 0.189)	1.57 (± 0.189)
3.8	0.083	2.14 (± 0.000)	2.14 (± 0.000)	1.9 (± 0.150)	1.96 (± 0.150)^{2}	2.00 (± 0.249)	2.08 (± 0.249)	1.47 (± 0.133)	1.56 (± 0.133)	1.86 (± 0.079)^{a}	2.13 (± 0.079)^{1b}	1.65 (± 0.189)	1.60 (± 0.189)
5.5	0.025	2.14 (± 0.000)	2.14 (± 0.000)	1.76 (± 0.150)^{a}	2.42 (± 0.150)^{1b}	1.83 (± 0.249)	1.69 (± 0.249)	1.45 (± 0.133)	1.78 (± 0.133)	2.06 (± 0.079)	2.09 (± 0.079)^{12}	1.60 (± 0.189)	1.88 (± 0.189)
6.5	0.011	2.14 (± 0.000)	2.14 (± 0.000)	2.17 (± 0.150)	1.95 (± 0.150)^{2}	1.71 (± 0.249)	2.17 (± 0.249)	1.30 (± 0.133)	2.03 (± 0.133)	1.86 (± 0.079)	1.87 (± 0.079)^{2}	1.92 (± 0.189)	1.79 (± 0.189)
7.9	0.000	2.14 (± 0.000)	2.14 (± 0.000)	2.02 (± 0.150)	1.85 (± 0.150)^{2}	2.31 (± 0.249)	2.07 (± 0.249)	1.87 (± 0.133)	2.08 (± 0.133)	2.00 (± 0.079)	1.95 (± 0.079)^{12}	1.92 (± 0.189)^{a}	1.34 (± 0.189)^{b}
$\bar{x}$ FEED		2.14 (± 0.000)	2.14 (± 0.000)	1.99 (± 0.0067)	1.98 (± 0.067)	1.93 (± 0.111)	1.98 (± 0.111)	1.53 (± 0.111)	1.86 (± 0.000)	1.93 (± 0.036)	2.01 (± 0.036)	1.71 (± 0.085)	1.64 (± 0.085)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.2.3 Gizzard pH

Tables 4.17 and 4.18 illustrate the effects observed in the gizzard pH as a result of the water pH irrespective of feed and as a result of the different feed and drinking water pH combinations respectively.

Day 7

The broilers that received the standard feed had significantly higher (P <0.05) gizzard pH than those that received the acidified feed, irrespective of drinking water. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the gizzard. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed, had a significantly higher (P <0.05) gizzard pH than those receiving the acidified feed. No significant differences (P >0.05) were observed within the two feeds.

Day 14

There were no significant differences (P >0.05) observed at any level.

Day 21

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 5.5 had a significantly lower (P <0.05) gizzard pH than those that received a drinking water pH of 7.9. Between feeds, no significant differences (P >0.05) were observed. Within the standard feed, gizzard pH of broilers that received a drinking water pH of 3.0, 3.8 and 5.5 was significantly lower (P <0.05) gizzard pH than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

Day 28

There were no significant differences (P >0.05) observed at any level.

Day 35

The gizzard pH of broilers that received standard feed was significantly higher (P <0.05) than those that received the acidified feed, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 3.0, 3.8 and 6.5 had a significantly lower (P <0.05) pH than those that received a drinking water pH of 7.9. were observed. Between feeds, broilers that received a drinking water pH of 5.5 combined with standard feed, had a significantly higher (P <0.05) gizzard pH than those that received the acidified feed. When comparing the gizzard pH within the standard feed, broilers that received a drinking water pH of 3.0 and 3.8 had significantly lower (P <0.05) gizzard pH than those that received a drinking water pH of 7.9. No significant differences (P >0.05) were observed within the acidified feed.

In summary, gizzard pH was also not greatly affected by the feed and water combinations. The pH was variable, both within and between treatments. No significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.17 pH of the gizzard in Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**					
		0	7	14	21	28	35
3.0	0.134	3.53	2.83	2.85	2.75[12]	2.98	2.44[2]
3.8	0.083	3.54	2.61	2.84	2.62[12]	3.08	2.33[2]
5.5	0.025	3.55	2.80	2.80	2.56[2]	2.99	2.54[12]
6.5	0.011	3.56	2.78	2.95	2.85[12]	3.01	2.40[2]
7.9	0.000	3.56	2.78	2.88	3.05[1]	2.96	2.75[1]
SEM		0.000	0.101	0.077	0.127	0.06	0.092

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
SEM: standard error of the mean.

Table 4.18 pH of the gizzard (± standard error of the mean) in Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)											
		0		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	3.53 (± 0.000)	3.53 (± 0.000)	3.09 (± 0.142)^{a}	2.57 (± 0.142)^{b}	2.86 (± 0.109)	2.83 (± 0.109)	2.57 (± 0.180)^{2}	2.93 (± 0.180)	2.87 (± 0.084)	3.07 (± 0.084)	2.47 (± 0.131)^{2}	2.42 (± 0.131)
3.8	0.083	3.53 (± 0.000)	3.53 (± 0.000)	2.75 (± 0.142)	2.47 (± 0.142)	2.72 (± 0.109)	2.97 (± 0.109)	2.59 (± 0.180)^{2}	2.64 (± 0.180)	3.06 (± 0.084)	3.09 (± 0.084)	2.46 (± 0.131)^{2}	2.2 (± 0.131)
5.5	0.025	3.53 (± 0.000)	3.53 (± 0.000)	2.86 (± 0.142)	2.74 (± 0.142)	2.78 (± 0.109)	2.82 (± 0.109)	2.46 (± 0.180)^{2}	2.67 (± 0.180)	3.04 (± 0.084)	2.94 (± 0.084)	2.75 (±0.131)^{12a}	2.32 (± 0.131)^{b}
6.5	0.011	3.53 (± 0.000)	3.53 (± 0.000)	2.86 (± 0.142)	2.71 (± 0.142)	2.86 (± 0.109)	3.04 (± 0.109)	2.93 (±0.180)^{12}	2.76 (± 0.180)	3.05 (± 0.084)	2.97 (± 0.084)	2.55 (±0.131)^{12}	2.26 (± 0.131)
7.9	0.000	3.53 (± 0.000)	3.53 (± 0.000)	2.86 (± 0.142)	2.70 (± 0.142)	2.82 (± 0.109)	2.93 (± 0.109)	3.18 (± 0.180)^{1}	2.91 (± 0.180)	2.98 (± 0.084)	2.93 (± 0.084)	2.86 (± 0.131)^{1}	2.64 (± 0.131)
$\bar{x}$ FEED		3.53 (± 0.000)	3.53 (± 0.000)	2.89 (± 0.064)^{a}	2.64 (± 0.064)^{b}	2.81 (± 0.049)	2.92 (± 0.049)	2.74 (± 0.081)	2.78 (± 0.081)	3.00 (± 0.038)	3.00 (± 0.038)	2.62 (± 0.058)^{a}	2.37 (± 0.058)^{b}

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.2.4 Duodenum pH

Duodenal pH is illustrated in Tables 4.19 and 4.20, as a result of the water pH irrespective of feed and as a result of the different feed and drinking water pH combinations respectively.

Day 7

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the duodenum. Between feeds, broilers that received a drinking water pH of 3.8 combined with the standard feed, had a significantly higher (P <0.05) duodenal pH than those receiving the acidified feed. Similarly, broilers that received a drinking water pH of 6.5 combined with the standard feed, had a significantly higher (P <0.05) duodenal pH than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 3.8 and 6.5 had significantly higher (P <0.05) duodenal pH than those that received a drinking water pH of 5.5. Within the acidified feed, broilers that received a drinking water pH of 3.8 had a significantly lower (P <0.05) duodenal pH than those that received a drinking water pH of 5.5.

Day 14

There were no significant differences (P >0.05) observed at any level.

Day 21

There were no significant differences (P >0.05) observed at any level.

Day 28

There were no significant differences (P >0.05) observed at any level.

Day 35

There were no significant differences (P >0.05) observed at any level.

In summary, duodenal pH was only affected by the feed and water combinations on day 7, with no significant differences (P >0.05) seen thereafter. The pH was variable, both within and between treatments. No significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.19 pH of the duodenum in Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)					
		0	7	14	21	28	35
3.0	0.134	6.42	5.90	5.99	6.05	6.05	5.98
3.8	0.083	6.42	5.90	5.93	5.99	6.00	6.11
5.5	0.025	6.42	5.91	6.09	6.13	6.00	6.03
6.5	0.011	6.42	5.98	5.86	6.06	6.01	6.14
7.9	0.000	6.42	6.00	5.91	6.03	6.09	6.01
SEM		0.000	0.081	0.084	0.085	0.056	0.068

[12] Column means with the same super script are not significantly (P >0.05) different from each other.
SEM: standard error of the mean.

Table 4.20 pH of the duodenum (± standard error of the mean) in Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)											
		0		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	6.42 (± 0.000)	6.42 (± 0.000)	5.89 (± 0.114)^{12}	5.91 (± 0.114)^{12}	6.06 (± 0.119)	5.92 (± 0.119)	6.07 (± 0.121)	6.03 (± 0.121)	6.02 (± 0.079)	6.07 (± 0.079)	5.93 (± 0.096)	6.04 (± 0.096)
3.8	0.083	6.42 (± 0.000)	6.42 (± 0.000)	6.12 (± 0.114) $_{1a}$	5.69 (± 0.114)^{2b}	5.88 (± 0.119)	5.97 (± 0.119)	5.99 (± 0.121)	5.90 (± 0.121)	6.10 (± 0.079)	5.9 (± 0.079)	5.98 (± 0.096)	6.25 (± 0.096)
5.5	0.025	6.42 (± 0.000)	6.42 (± 0.000)	5.77 (± 0.114)^{2}	6.04 (± 0.114)^{1}	6.17 (± 0.119)	6.01 (± 0.119)	6.22 (± 0.121)	6.14 (± 0.121)	5.98 (± 0.079)	6.02 (± 0.079)	6.04 (± 0.096)	6.02 (± 0.096)
6.5	0.011	6.42 (± 0.000)	6.42 (± 0.000)	6.15 (± 0.114)^{1a}	5.80 (±0.114)^{12b}	5.99 (± 0.119)	5.72 (± 0.119)	6.12 (± 0.121)	6.10 (± 0.121)	6.07 (± 0.079)	5.94 (± 0.079)	6.07 (± 0.096)	6.21 (± 0.096)
7.9	0.000	6.42 (± 0.000)	6.42 (± 0.000)	6.00 (± 0.114)^{12}	6.00 (± 0.114)^{12}	5.84 (± 0.119)	5.98 (± 0.119)	5.99 (± 0.121)	6.07 (± 0.121)	6.09 (± 0.079)	6.09 (± 0.079)	5.96 (± 0.096)	6.05 (± 0.096)
$\bar{X}$ FEED		6.42 (± 0.000)	6.42 (± 0.000)	5.99 (± 0.051)	5.89 (± 0.051)	5.99 (± 0.053)	5.92 (± 0.053)	6.06 (± 0.054)	6.05 (± 0.054)	6.05 (± 0.036)	6.00 (± 0.036)	6.00 (± 0.043)	6.12 (± 0.043)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.2.5 Jejunum pH

Jejunal pH is illustrated in Tables 4.21 and 4.22, as a result of the water pH irrespective of feed and as a result of the different feed and drinking water pH combinations respectively.

Day 7

Broilers that received the standard feed had a significantly higher (P <0.05) jejunal pH than those that received the acidified feed, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the jejunum. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed, had a significantly higher (P <0.05) jejunal pH than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 3.0 had significantly higher (P <0.05) jejunal pH than those that received a drinking water pH of 6.5. Within the acidified feed, broilers that received a drinking water pH of 3.0 had a significantly lower (P <0.05) jejunal pH than those that received a drinking water pH of 5.5, 6.5 and 7.9.

Day 14

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) jejunal pH than those that received a drinking water pH of 5.5. Between feeds, broilers that received a drinking water pH of 3.8 combined with the standard feed, had a significantly lower (P <0.05) jejunal pH than those receiving the acidified feed. Within the standard feed, no significant differences (P >0.05) were observed. Within the acidified feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) jejunal pH than those that received a drinking water pH of 5.5 and 3.8.

Day 21

There were no significant differences (P >0.05) observed at any level.

Day 28

Irrespective of drinking water pH, the broilers that received the standard feed had a significantly higher (P <0.05) jejunal pH than those that received the acidified feed. Irrespective of feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) jejunal pH than those that received a drinking water pH of 3.0 and 5.5. Between feeds, broilers that received a drinking water pH of 3.8 combined with the standard feed, had a significantly higher (P <0.05) jejunal pH than those receiving the acidified feed. Similarly, broilers that received a drinking water pH of 5.5 combined with the standard feed, had a significantly lower (P <0.05) jejunal pH than those receiving the acidified feed. Within the standard feed, broilers that received a

drinking water pH of 3.8 and 5.5 had a significantly higher (P <0.05) jejunal pH than those that received a drinking water pH of 6.5 and 7.9. Within the acidified feed, no significant differences (P >0.05) were observed.

Day 35

Significantly lower (P <0.05) jejunal pH was observed in broilers that received the standard feed, when compared to those that received the acidified feed, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 5.5 had a significantly lower (P <0.05) jejunal pH than those that received a drinking water pH of 3.8. Between feeds, broilers that received a drinking water pH of 3.8 combined with the standard feed, had a significantly lower (P <0.05) jejunal pH than those receiving the acidified feed. No significant differences (P >0.05) were observed within the two feeds.

In summary, jejunal pH varied greatly from week to week as a result of the feed and water combinations, with no observable patterns or trends. The pH was variable, both within and between treatments. No significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.21 pH of the jejunum in Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**					
		0	7	14	21	28	35
3.0	0.134	6.80	6.18	5.90[12]	6.03	6.06[1]	6.11[12]
3.8	0.083	6.80	6.19	5.90[12]	5.98	6.04[12]	6.17[1]
5.5	0.025	6.80	6.22	5.99[1]	6.06	6.12[1]	5.96[2]
6.5	0.011	6.80	6.20	5.79[2]	6.04	5.92[2]	6.09[12]
7.9	0.000	6.80	6.24	5.94[12]	5.94	6.01[12]	6.08[12]
SEM		0.000	0.054	0.093	0.056	0.042	0.047

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
SEM: standard error of the mean.

Table 4.22 pH of the jejunum (± standard error of the mean) in Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)											
		0		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	6.80 (± 0.000)	6.80 (± 0.000)	6.44 (± 0.077)^{1a}	5.91 (± 0.077)^{2b}	5.96 (± 0.073)	5.84 (± 0.073)^{12}	6.11 (± 0.079)	5.95 (± 0.079)	6.11 (± 0.059)^{12}	6.02 (± 0.059)	6.04 (± 0.067)	6.19 (± 0.067)
3.8	0.083	6.80 (± 0.000)	6.80 (± 0.000)	6.26 (± 0.077)^{12}	6.12 (± 0.077)^{12}	5.78 (± 0.073)^{b}	6.03 (± 0.073)^{1a}	6.01 (± 0.079)	5.94 (± 0.079)	6.19 (± 0.059)^{1a}	5.90 (± 0.059)^{b}	6.07 (± 0.067)^{b}	6.27 (± 0.067)^{a}
5.5	0.025	6.80 (± 0.000)	6.80 (± 0.000)	6.26 (± 0.077)^{12}	6.18 (± 0.077)^{1}	5.94 (± 0.073)	6.04 (± 0.073)^{1}	6.00 (± 0.079)	6.12 (± 0.079)	6.20 (± 0.059)^{1a}	6.03(± 0.059)^{b}	5.91 (± 0.067)	6.01 (± 0.067)
6.5	0.011	6.80 (± 0.000)	6.80 (± 0.000)	6.20 (± 0.077)^{2}	6.20 (± 0.077)^{1}	5.81 (± 0.073)	5.78 (± 0.073)^{2}	6.14 (± 0.079)	5.93 (± 0.079)	5.98 (± 0.059)^{2}	5.87 (± 0.059)	6.03 (± 0.067)	6.15 (± 0.067)
7.9	0.000	6.80 (± 0.000)	6.80 (± 0.000)	6.23 (± 0.077)^{12}	6.25 (± 0.077)^{1}	5.93 (± 0.073)	5.95 (± 0.073)^{12}	5.93 (± 0.079)	5.95 (± 0.079)	6.00 (± 0.059)^{2}	6.01 (± 0.059)	6.07 (± 0.067)	6.10 (± 0.067)
$\bar{x}$ FEED		6.80 (± 0.000)	6.80 (± 0.000)	6.28 (± 0.034)^{a}	6.13 (± 0.034)^{b}	5.88 (± 0.033)	5.92 (± 0.033)	6.04 (± 0.036)	5.98 (± 0.036)	6.10 (± 0.027)^{a}	5.96 (± 0.027)^{b}	6.02 (± 0.030)^{b}	6.14 (± 0.030)^{a}

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.2.6 Ileum pH

Ileal pH is illustrated in Tables 4.23 and 4.24, as a result of the water pH irrespective of feed and as a result of the different feed and drinking water pH combinations respectively.

Day 7

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 3.8 had a significantly lower (P <0.05) ileal pH than those that received a drinking water pH of 6.5. Similarly, broilers that received a drinking water pH of 5.5 had a significantly lower (P <0.05) ileal pH than those that received a drinking water pH of 6.5 and 7.9. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed, had a significantly higher (P <0.05) ileal pH than those receiving the acidified feed. Broilers that received a drinking water pH of 6.5 combined with the standard feed, had a significantly lower (P <0.05) ileal pH than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 3.0 had significantly higher (P <0.05) ileal pH than those that received a drinking water pH of 3.8 and 5.5. Within the acidified feed, broilers that received a drinking water pH of 6.5 had a significantly lower (P <0.05) ileal pH than those that received a drinking water pH of 3.0, 3.8, 5.5 and 7.9.

Day 14

There were no significant differences (P >0.05) observed at any level.

Day 21

Irrespective of drinking water pH, broilers that received the standard feed had a significantly higher (P <0.05) ileal pH than those that received the acidified feed. No other significant differences (P >0.05) were observed.

Day 28

There were no significant differences (P >0.05) observed at any level.

Day 35

Broilers that received the standard feed had a significantly lower (P <0.05) ileal pH than those that received the acidified feed, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 5.5 had a significantly lower (P <0.05) ileal pH than those that received a drinking water pH of 7.9. Between feeds, broilers that received a drinking water pH of 6.5 combined with the standard feed, had a significantly lower (P <0.05) ileal pH than those receiving the acidified feed. Similarly, broilers that received a drinking water pH of 7.9 combined with the standard feed, had a significantly lower (P <0.05) ileal

pH than those receiving the acidified feed. Within the standard feed, no significant differences (P >0.05) were observed. Within the acidified feed, broilers that received a drinking water pH of 3.0 had a significantly lower (P <0.05) ileal pH than those that received a drinking water pH of 7.9.

In summary, ileal pH varied greatly from week to week as a result of the feed and water combinations, with no observable patterns or trends. Day 7 and day 35 showed the greatest responses. The pH was variable, both within and between treatments. No significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.23 pH of the ileum in Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**					
		0	7	14	21	28	35
3.0	0.134	7.11	6.55[123]	5.97	6.02	6.14	6.10[12]
3.8	0.083	7.11	6.38[13]	6.20	6.07	6.02	6.23[12]
5.5	0.025	7.11	6.35[1]	5.97	6.09	6.14	6.08[2]
6.5	0.011	7.11	6.69[2]	5.74	6.07	6.02	6.13[12]
7.9	0.000	7.11	6.59[23]	5.88	5.91	6.08	6.28[1]
SEM		0.000	0.078	0.142	0.118	0.052	0.066

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
SEM: standard error of the mean.

Table 4.24 pH of the ileum (± standard error of the mean) in Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)											
		0		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	7.11 (± 0.000)	7.11 (± 0.000)	6.79 (± 0.110)^{1a}	6.32 (± 0.110)^{2b}	5.91 (± 0.200)	6.03 (± 0.200)	6.19 (± 0.167)	5.85 (± 0.167)	6.10 (± 0.074)	6.18 (± 0.074)	6.10 (± 0.094)	6.09 (± 0.094)^{2}
3.8	0.083	7.11 (± 0.000)	7.11 (± 0.000)	6.39 (± 0.110)^{2}	6.37 (± 0.110)^{2}	6.26 (± 0.200)	6.13 (± 0.200)	6.29 (± 0.167)	5.85 (± 0.167)	6.02 (± 0.074)	6.02 (± 0.074)	6.18 (± 0.094)	6.27 (± 0.094)^{12}
5.5	0.025	7.11 (± 0.000)	7.11 (± 0.000)	6.35 (± 0.110)^{2}	6.35 (± 0.110)^{2}	5.80 (± 0.200)	6.15 (± 0.200)	6.25 (± 0.167)	5.92 (± 0.167)	6.09 (± 0.074)	6.19 (± 0.074)	5.99 (± 0.094)	6.16 (± 0.094)^{12}
6.5	0.011	7.11 (± 0.000)	7.11 (± 0.000)	6.47 (± 0.110)^{12b}	6.91 (± 0.110)^{1a}	5.81 (± 0.200)	5.66 (± 0.200)	6.22 (± 0.167)	5.95 (± 0.167)	5.97 (± 0.074)	6.08 (± 0.074)	5.98 (± 0.094)^{b}	6.28 (± 0.094)^{12a}
7.9	0.000	7.11 (± 0.000)	7.11 (± 0.000)	6.60 (± 0.110)	6.58 (± 0.110)^{2}	5.83 (± 0.200)	5.94 (± 0.200)	5.94 (± 0.167)	5.89 (± 0.167)	6.11 (± 0.074)	6.05 (± 0.074)	6.10 (± 0.094)^{b}	6.45 (± 0.094)^{1a}
$\bar{x}$ FEED		7.11 (± 0.000)	7.11 (± 0.000)	6.52 (± 0.049)	6.51 (± 0.049)	5.92 (± 0.090)	5.98 (± 0.090)	6.07 (± 0.075)^{a}	5.89 (± 0.033)^{b}	6.06 (± 0.033)	6.10 (± 0.033)	6.07 (± 0.0042^{b}	6.25 (± 0.042)^{a}

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.

[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.2.7 Caeca pH

Caecal pH is illustrated in Tables 4.25 and 4.26, as a result of the water pH irrespective of feed and as a result of the different feed and drinking water pH combinations respectively.

Day 7

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the caeca. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed, had a significantly higher (P <0.05) caecal pH than those receiving the acidified feed. Broilers that received a drinking water pH of 6.5 combined with the standard feed, had a significantly lower (P <0.05) caecal pH than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 3.0 and 5.5 had significantly higher (P <0.05) caecal pH than those that received a drinking water pH of 6.5. Within the acidified feed, broilers that received a drinking water pH of 6.5 had a significantly higher (P <0.05) caecal pH than those that received a drinking water pH of 7.9.

Day 14

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the caeca. Between feeds, broilers that received a drinking water pH of 3.0 combined with the standard feed, had a significantly lower (P <0.05) caecal pH than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 3.8, 5.5 and 6.5 had significantly higher (P <0.05) caecal pH than those that received a drinking water pH of 3.0. Within the acidified feed, no significant differences (P >0.05) were observed.

Day 21

There were no significant differences (P >0.05) observed at any level.

Day 28

Irrespective of drinking water pH, broilers that received standard feed had a significantly lower (P <0.05) caecal pH than those that received the acidified feed. Irrespective of feed, broilers that received a drinking water pH of 6.5 had a significantly higher (P <0.05) caecal pH than those that received a drinking water pH of 7.9. Between feeds, broilers that received a drinking water pH of 5.5 combined with the standard feed, had a significantly higher (P <0.05) caecal pH than those receiving the acidified feed. Within the standard feed, no significant differences (P >0.05) were observed. Within the acidified feed, broilers that received a

drinking water pH of 3.0, 3.8, 5.5 and 6.5 had a significantly higher (P <0.05) caecal pH than those that received a drinking water pH of 7.9.

Day 35

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the caeca. Between feeds, no significant differences (P >0.05) were observed. Within the standard feed, broilers that received a drinking water pH of 5.5 had a significantly higher (P <0.05) caecal pH than those that received a drinking water pH of 3.0 and 7.9. Within the acidified feed, no significant differences (P >0.05) were observed.

In summary, caecal pH varied greatly from week to week as a result of the feed and water combinations, with no observable patterns or trends. pH was variable, both within and between treatments. No significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.25 Average pH of the caeca in Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**					
		0	7	14	21	28	35
3.0	0.134	6.77	6.75	6.50	6.78	6.88[12]	6.33
3.8	0.083	6.77	6.71	6.72	6.85	6.85[12]	6.50
5.5	0.025	6.77	6.92	6.73	6.76	6.83[12]	6.52
6.5	0.011	6.77	6.83	6.63	6.90	6.89[1]	6.44
7.9	0.000	6.77	6.64	6.65	6.64	6.72[2]	6.41
SEM		0.000	0.108	0.102	0.132	0.052	0.110

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
SEM: standard error of the mean.

Table 4.26 Average pH of the caeca (± standard error of the mean) in Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)											
		0		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	6.77 (± 0.000)	6.77 (± 0.000)	7.03 (± 0.153)^{1a}	6.46 (±0.153)^{12b}	6.29 (± 0.144)^{2b}	6.72 (± 0.144)^{a}	6.96 (± 0.187)	6.60 (± 0.187)	6.81 (± 0.074)	6.97 (± 0.074)^{1}	6.18 (± 0.156)^{2}	6.47 (± 0.156)
3.8	0.083	6.77 (± 0.000)	6.77 (± 0.000)	6.71 (± 0.153)^{12}	6.71 (± 0.153)^{12}	6.72 (± 0.144)^{1}	6.72 (± 0.144)	6.84 (± 0.187)	6.87 (± 0.187)	6.74 (± 0.074)	6.95 (± 0.074)^{1}	6.43 (± 0.156)^{12}	6.57 (± 0.156)
5.5	0.025	6.77 (± 0.000)	6.77 (± 0.000)	7.02 (± 0.153)^{1}	6.82 (± 0.153)^{12}	6.92 (± 0.144)^{1}	6.54 (± 0.144)	6.87 (± 0.187)	6.66 (± 0.187)	6.66 (± 0.074)^{b}	6.99 (± 0.074)^{1a}	6.72 (± 0.156)^{1}	6.31 (± 0.156)
6.5	0.011	6.77 (± 0.000)	6.77 (± 0.000)	6.54 (± 0.153)^{2b}	7.12 (± 0.153)^{1a}	6.72 (± 0.144)^{1}	6.53 (± 0.144)	6.90 (± 0.187)	6.90 (± 0.187)	6.83 (± 0.074)	6.94 (± 0.074)^{1}	6.29 (± 0.156)^{12}	6.59 (± 0.156)
7.9	0.000	6.77 (± 0.000)	6.77 (± 0.000)	6.74 (± 0.153)^{12}	6.55 (± 0.153)^{2}	6.62 (± 0.144)^{12}	6.68 (± 0.144)	6.78 (± 0.187)	6.49 (± 0.187)	6.79 (± 0.074)	6.64 (± 0.074)^{2}	6.25 (± 0.156)^{2}	6.57 (± 0.156)
$\bar{x}$ FEED		6.77 (± 0.000)	6.77 (± 0.000)	6.81 (± 0.068)	6.73 (± 0.068)	6.65 (± 0.065)	6.64 (± 0.065)	6.87 (± 0.084)	6.70 (± 0.084)	6.77 (± 0.033)^{b}	6.90 (± 0.033)^{a}	6.38 (± 0.070)	6.50 (± 0.070)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

4.2.8 Colon pH

Colonic pH is illustrated in Tables 4.27 and 4.28, as a result of the water pH irrespective of feed and as a result of the different feed and drinking water pH combinations respectively.

Day 7

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. The different water pH levels used, irrespective of feed, resulted in no significant differences (P >0.05) in the pH of the colon. Between feeds, no significant differences (P >0.05) were observed. Within the acidified feed, the colonic pH of broilers that received a drinking water pH of 3.8 was significantly higher (P <0.05) than those that received a drinking water pH of 3.0. Within the standard feed, no significant differences (P >0.05) were observed.

Day 14

There were no significant differences (P >0.05) observed at any level.

Day 21

Irrespective of drinking water pH, broilers that received standard feed had a significantly higher (P <0.05) colonic pH than those that received the acidified feed. Irrespective of feed, broilers that received a drinking water pH of 6.5 had a significantly higher (P <0.05) colonic pH than those that received a drinking water pH of 7.9. Between feeds, broilers that received a drinking water pH of 6.5 combined with the standard feed, had a significantly higher (P <0.05) colonic pH than those receiving the acidified feed. Within the standard feed, broilers that received a drinking water pH of 6.5 had a significantly higher (P <0.05) colonic pH than those that received a drinking water pH of 7.9. Within the acidified feed, no significant differences (P >0.05) were observed.

Day 28

No significant differences (P >0.05) were observed between the two feeds used, irrespective of drinking water pH. Irrespective of feed, broilers that received a drinking water pH of 3.0 had a significantly higher (P <0.05) colonic pH than those that received a drinking water pH of 5.5. Between feed, no significant differences (P >0.05) were observed. Within the standard feed, broilers that received a drinking water pH of 3.0 and 3.8 had a significantly higher (P <0.05) colonic pH than those that received a drinking water pH of 5.5. Within the acidified feed, broilers that received a drinking water pH of 3.0 had a significantly higher (P <0.05) colonic pH than those that received a drinking water pH of 3.8.

Day 35

There were no significant differences (P >0.05) observed at any level.

In summary, colonic pH varied greatly from week to week as a result of the feed and water combinations, with no observable patterns or trends. pH was variable, both within and between treatments. No significant (P >0.05) dose responses, linear or quadratic, were observed.

Table 4.27 pH of the colon in Ross 308 broilers that received different drinking water pH levels

Drinking Water pH	**Organic Acid Inclusion (%)**	**Age (Days)**					
		0	7	14	21	28	35
3.0	0.134	6.22	6.63	6.46	6.30	6.79[1]	6.26
3.8	0.083	6.22	6.87	6.77	6.58	6.55[12]	6.36
5.5	0.025	6.22	6.87	6.80	6.51	6.37[2]	6.48
6.5	0.011	6.22	6.79	6.32	6.61[1]	6.38[12]	6.35
7.9	0.000	6.22	6.81	6.59	6.16[2]	6.57[12]	6.45
SEM		0.000	0.162	0.184	0.150	0.115	0.123

[12] Column means with the same superscript are not significantly (P >0.05) different from each other
SEM: standard error of the mean.

Table 4.28 pH of the colon (± standard error of the mean) in Ross 308 broilers receiving different feed and drinking water pH combinations

Drinking Water pH	Organic Acid Inclusion (%)	Age (Days)											
		0		7		14		21		28		35	
		Feed		Feed		Feed		Feed		Feed		Feed	
		Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified	Standard	Acidified
3.0	0.0134	6.22 (± 0.000)	6.22 (± 0.000)	6.84 (± 0.229)	6.41 (± 0.229)^{2}	6.76 (± 0.260)	6.16 (± 0.260)	6.45 (± 0.212)^{12}	6.14 (± 0.212)	6.74 (± 0.162)^{1}	6.84 (± 0.162)^{1}	6.27 (± 0.174)	6.25 (± 0.174)
3.8	0.083	6.22 (± 0.000)	6.22 (± 0.000)	6.58 (± 0.229)	7.17 (± 0.229)^{1}	6.65 (± 0.260)	6.90 (± 0.260)	6.51 (± 0.212)^{12}	6.64 (± 0.212)	6.75 (± 0.162)^{1}	6.36 (± 0.162)^{2}	6.37 (± 0.174)	6.35 (± 0.174)
5.5	0.025	6.22 (± 0.000)	6.22 (± 0.000)	7.00 (± 0.229)	6.74 (± 0.229)^{12}	6.72 (± 0.260)	6.87 (± 0.260)	6.61 (± 0.212)^{12}	6.41 (± 0.212)	6.15 (± 0.162)^{2}	6.59 (± 0.162)^{12}	6.68 (± 0.174)	6.28 (± 0.174)
6.5	0.011	6.22 (± 0.000)	6.22 (± 0.000)	6.54 (± 0.229)	7.04 (± 0.229)^{12}	6.40 (± 0.260)	6.25 (± 0.260)	7.03 (± 0.212)^{1a}	6.18 (± 0.212)^{b}	6.40 (± 0.162)^{12}	6.35 (± 0.162)^{12}	6.23 (± 0.174)	6.47 (± 0.174)
7.9	0.000	6.22 (± 0.000)	6.22 (± 0.000)	6.93 (± 0.229)	6.69 (± 0.229)^{12}	6.61 (± 0.260)	6.56 (± 0.260)	6.26 (± 0.212)^{2}	6.06 (± 0.212)	6.52 (± 0.162)^{12}	6.62 (± 0.162)^{12}	6.38 (± 0.174)	6.52 (± 0.174)
$\bar{x}$ FEED		6.22 (± 0.000)	6.22 (± 0.000)	6.78 (± 0.102)	6.81 (± 0.102)	6.63 (± 0.116)	6.55 (± 0.116)	6.57 (± 0.095)^{a}	6.29 (± 0.095)^{b}	6.51 (± 0.073)	6.55 (± 0.073)	6.39 (± 0.078)	6.37 (± 0.078)

[12] Column means with the same superscript are not significantly (P >0.05) different from each other.
[ab] Row means within the same time period, with the same superscript are not significantly (P >0.05) different from each other.

Chapter 5
Discussion

5.1 Performance of broilers

Organic acids have been extensively explored as AGP alternatives and as such, a variety of effects have been observed. As with various studies many conflicting results were obtained in this study. Although the results agree with many previous studies, it also disagree with others. This study examined three aspects of acidification: feed acidification, drinking water acidification and a combination thereof, an aspect that has not been extensively studied previously.

Feed acidification

In this study, the broilers that received standard feed showed the greatest performance results, with heavier (P <0.05) BW at days 7, 14, 21 and 35. This contradicts the findings of various studies, in which the addition of organic acids to feed resulted in heavier BW (Afsharmanesh & Pourreza, 2005; Al-Kassi & Mohssen, 2009; Chowdhury *et al.*, 2009; Kim *et al.*, 2009; Haque *et al.*, 2010). In previous studies, various organic acids were used and the form in which it was supplemented, as well as inclusion levels, have been shown to yield different results. Garcia *et al.* (2007), Paul *et al.* (2007) and Ragaa & Korany (2016), for example, all supplemented formic acid but as calcium formate, ammonium formate and potassium formate respectively, with different results. In this study, the use of formic acid in the feed as a salt in small quantities, may explain the contradictory outcomes.

In general, the acidification of the feed had no significant effects on FI, except on day 28 where lower (P <0.05) FI was observed in the broilers fed the standard feed. Chowdhury *et al.* (2009) and Haque *et al.* (2010) reported that addition of citric acid to the diet increased (P <0.05) FI at 35 days, whereas Alçiçek *et al.* (2004) reported that a combination of formic-, lactic-, and citric acid had no effects (P >0.05) at any age. This suggests that acidification of the feed did not affect FI. As with BW, the type and inclusion level of organic acid used may explain the differences observed.

FCR at days 7, 14, 21, 28 and 35 was lower (P <0.05) for the broilers fed the standard feed, contrary to the findings of several other studies. Lower FCR has been reported in various studies, for various organic acids, such as citric- and ascorbic acid (Afsharmanesh & Pourreza, 2005), butyric acid (Aghazadeh & TahaYazdi, 2011), formic acid (Hernández *et al.*, 2006; Garcia *et al.*, 2007) and a combination of lactic-, citric- and formic acid (Alçiçek *et al.*, 2004). Several studies have also reported no effects (P >0.05) when supplementing organic acids at any level (Gunal *et al.*, 2006; Chowdhury *et al.*, 2009; Haque *et al.*, 2010). The high levels of variability of results observed in the above-mentioned studies, suggest that FCR may again be influenced by the type, form and inclusion levels of the various organic acids.

No differences (P >0.05) were observed in mortality or WI throughout the growing period, similar to the findings of Chowdhury *et al.* (2009) and Haque *et al.* (2010). However, the results of this study were contrary to the results of previous studies that were carried out to 42 days-of-age, where significant decreases in mortality were observed (Gunal *et al.*, 2006; Al-Kassi & Mohssen, 2009) This study was carried out to 35 days-of-age and as such, agreed with the findings of studies carried out to the same age. This suggests that WI and mortality may be influenced by feed acidification in older birds that are reared to 42 days-of-age.

Water acidification

The different water pH levels used affected some of the growth parameters throughout the trial. Broilers that received a drinking water pH of 6.5 resulted in higher (P <0.05) bodyweight at 7 days than those that received a drinking water pH of 7.9. On days 21, 28 and 35, drinking water pH levels of 3.0, 3.8, 5.5 and 6.5 all resulted in higher (P <0.05) BW than a water pH of 7.9. The present study suggests that any level of water acidification will increase BW, however different organic acids have delivered different results, and various other studies have found no effects (P >0.05) on BW when acidifying the water (Garcia *et al.*, 2007; Mohyla *et al.*, 2007; Paul *et al.*, 2007; Ragaa & Korany, 2016), whilst Hamid *et al.* (2018) found that a drinking water pH of 4.2 improved (P <0.05) BW at days 21 and 42. This study was conducted using Selko®-pH, a blend of various organic acids, which may explain the differences in the results obtained.

At 21 days, broilers that received a drinking water pH of 5.5 had a lower (P <0.05) FI than those that received a drinking water pH of 6.5, whilst broilers receiving a water pH of 6.5 had higher (P <0.05) FI than those that received a drinking water pH of 7.9. Mohyla *et al.* (2007) reported no significant effects on FI when supplementing citric acid. However, this disagrees with Hamid *et al.* (2018), who reported that FI was lower (P <0.05) from 0 to 21 days in broilers that received a drinking water pH of 4.2. They also reported that from day 21 to 42, the FI was higher (P <0.05) in the broilers that received a drinking water pH of 4.2 than those that received normal drinking water. The type of organic acids used may influence the feed intake (Partanen & Mroz, 1999), which is variable between the studies.

A water pH of 5.5 resulted in a lower (P <0.05) FCR at 21 days than both water pH of 6.5 and 7.9. At 35 days, water pH of 3.0 and 3.8 resulted in lower (P <0.05) FCR than a drinking water pH of 7.9. This agrees with the findings of Hamid *et al.* (2018), who reported that acidification of drinking water to a pH of 4.2, improved (P <0.05) FCR at day 21 and 42. This suggests that acidification may improve FCR in broilers.

WI was higher (P <0.05) for a water pH of 3.8 than a drinking water pH of 7.9, whilst no significant differences were observed for mortality throughout the rearing period. Increased WI with higher levels of acidification have also been reported by Mohyla *et al.* (2007), who found that acidification of water to a pH of 2.6, numerically increased water intake. This increase in WI may be explained by the findings of Feddes *et al.* (2002), who reported that increased bodyweights resulted in increased WI and the birds receiving a drinking water pH of 3.8, were heavier (P >0.05) than the birds receiving a drinking water pH of 7.9. However, De

Avila *et al.* (2003) reported that drinking water pH levels of 2.69, 2.73 and 2.52, resulted in decreased (P <0.05) WI in broilers when compared to the non-treated groups. The results observed by De Avelia *et al.* (2003) may be explained by Partanen & Mroz (1999), who found that citric acid had a negative effect on palatability of water, which reduced WI.

Interaction between feed and water acidification

No reports could be found on studies that investigated feed and water acidification as was done in this study. As such, no comparisons can be drawn but this study indicates that any level of water acidification combined with the standard feed resulted in various improvements (P <0.05) when compared to the same level of acidification with the acidified feed.

The results obtained in this study indicate that any level of water acidification may be beneficial to broiler production. However, the combination of feed and water acidification is antagonistic and thus is not a feasible option in the broiler industry. This may be due to 'over-acidifying' the diet, resulting in the bird possibly using more energy to maintain a constant GIT pH rather than using that energy for growth. The variability in responses of broilers to organic acid may be due to several factors including, as suggested by Dibner & Buttin, (2002), buffering capacity of the test diet, presence of antimicrobial and/or anticoccidial compounds, microbiota of the gut and the environment itself.

5.2 pH within different segments of the gastrointestinal tract

GIT pH was not influenced to a great extent by feed or water acidification, however some significant results were obtained. The variability in these responses may be due to several reasons, from the bird itself, to human error in sampling and measuring. The results of this study and others before it, illustrate the variable responses that can be observed in the GIT.

Feed acidification

The GIT pH in various segments showed little variation as a response to the feed received, however the acidic feed resulted in lower (P <0.05) pH recordings in the gizzard and jejunum at day 7, in the colon at day 21, in the jejunum at day 28 and in the gizzard at day 35. The standard feed resulted in lower pH recordings in the caeca at day 21, in the caeca at day 28 and in the jejunum and ileum at day 35. Addition of an organic acid blend (Smulikowska *et al.*, 2010) or citric acid (Salgado-Tránsito *et al.*, 2011) resulted in no differences (P >0.05) in GIT segment pH. Samanta *et al.* (2008) found lower (P <0.05) pH in the duodenum and the gizzard. The inconsistency in organic acid responses may be due to several factors including, as suggested by Dibner & Buttin (2002), buffering capacity of the test diet, presence of antimicrobial and/or anticoccidial compounds, microbiota of the gut and the environment itself. The high variability within and between these studies further

suggests that GIT segment pH is difficult to manipulate via feed acidification due to the numerous factors that influence it.

Water acidification

As with feed acidification, high variability in results was observed with water acidification. Lower pH levels were recorded in various GIT segments throughout the trial, with the crop being most influenced by the water acidification. A linear relationship ($P < 0.05$) was observed between water pH and crop pH at day 35. De Avila *et al.* (2003) and Hamid *et al.* (2018) found similar results in the crop, whilst Chaveerach *et al.* (2004) reported higher pH recordings in the crop. Further down the GIT, the pH proved to become more variable and few consistent observations were made, although some differences ($P < 0.05$) were observed throughout the GIT on various days. As suggested by Dibner & Buttin (2002), the greatest influence of organic acids is in the foregut, namely the crop and the gizzard, as was observed in this study when acidifying via water. However, other variable results were observed throughout the rest of the GIT and this variation in results is likely due to variable factors, such as buffering capacity of the diet and environment (Dibner & Buttin, 2002) that are difficult to quantify and control.

Interaction between feed and water acidification

No reports could be found on studies that investigated feed and water acidification as was done in this study. This study provides some insight on this topic, but as seen with feed and water acidification alone, variability in responses were high and unpredictable.

The results indicated that water acidification was a more effective method of improving broiler performance and the combination of feed and water acidification was antagonistic. Therefore, it appears that this combination is not a feasible option in the broiler industry. Denli *et al.* (2003) and Hernández *et al.* (2006) suggested that the birds have a strong buffering action within the GIT and the reduced performance may be due to 'over-acidifying' the diet, resulting in the bird using more energy to maintain a constant GIT pH rather than using that energy for growth.

When examining the effects that organic acids exhibit on the GIT pH, variable results were obtained at all levels and forms of acidification. This variation may be attributed to many factors, including but not limited to diet, broiler health, gut microbiota, broiler physiological age, proximity of recordings to eating and/or drinking and human error (Dibner & Buttin, 2002; Denli *et al.*, 2003; Rahmani & Speer, 2005; Hernández *et al.*, 2006; Salgado-Tránsito *et al.*, 2011). As proven in previous studies, the diet constituents can affect the buffering capacity of the diet and this has a direct influence on the efficacy of the acidification (Dibner & Buttin, 2002). High buffering capacities have proven to reduce the effects of acidification. The broiler itself can cause variation of the results (Denli *et al.*, 2003); an unhealthy broiler may have an inhibited ability to control the pH within its GIT and thus an invalid recording may be observed. The proximity of the recording

to the broilers last meal and/or drink, may have a large influence on the recordings observed. A broiler that ingested acidified water close to the recording, will likely have a more acidic crop pH than one that has not ingested water for a while before the recording. This would be due to the lack of time available for the bird to buffer the acidified water (Denli *et al.*, 2003; Hernández *et al.*, 2006). The same principle is applicable to ingestion of feed. Human error may have also resulted in variation; the recordings are time-consuming process and although steps were taken to record results in the same manner each time, slight differences in location within the segment, for example, may influence the observed result.

Chapter 6
Conclusion and Recommendations

6.1 Conclusion

The effects of feed and water acidification have been widely explored. This study furthers this field of research and provided valuable insight on the interaction effects of feed and water acidification, alone and in combination. Organic acid supplementation in the feed has previously been used successfully to improve broiler production, however this study concluded that it is not the most effective method of organic acid supplementation. Water acidification proved to be the more profitable form of organic acid supplementation, resulting in broilers that were significantly heavier than the broilers that received tap water with a pH of 7.9. The water acidification also resulted in heavier broilers when receiving standard feed, than those that received acidified feed. This indicates that water acidification may be a more effective method of providing organic acids.

Due to the ever-present issue of antibiotic resistance, it is crucial that effective AGP alternatives are found. Organic acids have proven to be one such alternative, which have been widely explored. The results of this study have shown that water acidification may have positive effects on broiler production and the inclusion of organic acids in water may therefore increase profitability for the broiler producer. The consumer can also benefit when organic acids are used, instead of AGPs, to promote broiler growth – as this may lower the incidence of antibiotic resistance.

6.2 Critical Review and Recommendations

1. The acidified grower diet in this trial contained 1.7% more fat and 1.2% less protein than the standard diet. This difference in nutrients may explain some of the differences observed in the trial, such as the poorer BW and FCR observed at 35 days-of-age when comparing the results in the acidified groups to the standard groups.

2. Extended research is required to accurately quantify the effects that feed- and water acidification have on the GIT. The results observed in this study suggest that there are several factors, other than the acidification of the diet, that influence the pH within the GIT. The small sample size of the GIT pH measurements may have affected the results and therefore, it may be worthwhile to conduct a similar study with a larger sample size to accurately assess the effects.

3. The sample size from which the GIT pH recordings were taken was relatively small and this small sample may have influenced the overall results observed. A larger sample size may have provided more consistent results.

4. Future research is required to quantify the optimal drinking water pH for broilers. This study suggests that water acidification is a more effective form of organic acid supplementation compared to feed acidification, and that broiler performance increases as drinking water pH decreases. Thus, a more acidic water pH is desirable to improve broiler performance.

5. The inclusion levels of organic acid in this trial was lower than those suggested by various additive companies and lower than those seen in previous studies. A desirable pH was the target, rather than organic acid inclusion and as such, this may indicate that pH may have a greater influence than organic acid inclusion as such.

6. Based on the results of this trial, a drinking water pH between 3.0 and 3.8 is recommended for best results.

7. This trial was conducted in Sundra, Mpumalanga, an area with 'hard' water. Water varies from area to area and farm to farm, thus effective water treatment for one farm, may not be as effective for another. A drinking water pH of 3.0 may be beneficial at one farm, but harmful on another. It is recommended that the drinking water that the broilers receive, be tested regularly and the acidification levels adjusted accordingly.

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Appendix

Appendix A Temperature profile of the trial house from 2 days before placement to slaughtering at 35 days

Day	Target floor temperature (°C, 50 % rH[1])
1 day before placement to 2	35.5
3 to 5	34.5
6 to 8	33.5
9 to 11	29.7
12 to 14	27.2
15 to 17	26.2
18 to 20	25.0
21 to 23	24.0
24 to 35	23.0

[1]rH=Relative Humidity

Appendix B Lighting program of the trial house from placement of the Ross broiler chicks to slaughter at 35 days-of-age

Day	Controller's set point			
	Lights on	Lights off	Hours of Daylight	Hours of Darkness
1 to 3	00:00	23:00	23	1
4 to 8	00:00	21:00	21	3
9 to 11	05:00	22:00	17	7
12 to 15	05:00	20:00	15	9
16 to 33	05:00	19:00	14	10
34 to 35	02:00	22:00	20	4

Appendix C Vaccination program (New Castle Disease and Infectious Bronchitis) of the Ross 308 broilers during the trial

Age (days)	Vaccination	Method	Trade name	Supplier
Hatchery	NCB[1]	Spray	Avinew	Merial South Africa (Pty) Ltd
Hatchery	IB[2]	Spray	Bioral H120	Merial South Africa (Pty) Ltd
10-12 days	NCB	Water	TAbic VH	Phibro Animal Health
10-12 days	IB	Water	TAbic MB	Phibro Animal Health
16-18 days	NCB	Water	Avinew	Merial South Africa (Pty) Ltd

[1]NCB = New Castle Disease
[2]IB = Infectious Bronchitis

Appendix D Layout of the pens and blocks in the trial house with the random treatment allocations to each pen

HOUSE A				HOUSE B			
BLOCK 1	T[1]1[2]	T3	BLOCK 6	BLOCK 1	T3	T6	BLOCK 6
	T9	T2			T5	T9	
	T10	T4			T1	T4	
	T8	T1			T8	T2	
	T7	T10			T4	T1	
	T5	T6			T9	T8	
	T2	T9			T2	T7	
	T3	T7			T7	T10	
	T4	T8			T6	T3	
	T6	T5			T10	T5	
BLOCK 2	T10	T10	BLOCK 5	BLOCK 2	T10	T2	BLOCK 5
	T7	T9			T5	T9	
	T6	T8			T7	T6	
	T4	T2			T3	T1	
	T8	T5			T6	T3	
	T10	T1			T8	T8	
	T9	T6			T1	T4	
	T5	T3			T2	T10	
	T1	T7			T9	T7	
	T2	T4			T4	T5	
BLOCK 3	T3	T2	BLOCK 4	BLOCK 3	T6	T8	BLOCK 4
	T8	T1			T2	T2	
	T10	T4			T10	T10	
	T9	T5			T8	T3	
	T2	T3			T1	T7	
	T7	T7			T3	T4	
	T1	T6			T4	T6	
	T5	T8			T7	T9	
	T4	T9			T9	T5	
	T6	T10			T5	T1	

[1]T=Treatment

[2]Number 1-10 = treatment number

Appendix E The feeding phases with feeding periods and expected intakes per bird.

Feed (5 phases)	Feeding period (days)	Feed intake (g/bird)	Feed allocation/pen (kg)
Pre-starter	10	450	27
Starter	7	580	35
Grower	10	1417	85
Finisher	3	663	38
Post-finisher	5	1000	60