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Effect of Diet Structure, Conformation and Acidification on Performance and Gastrointestinal Tract Development of Broilers

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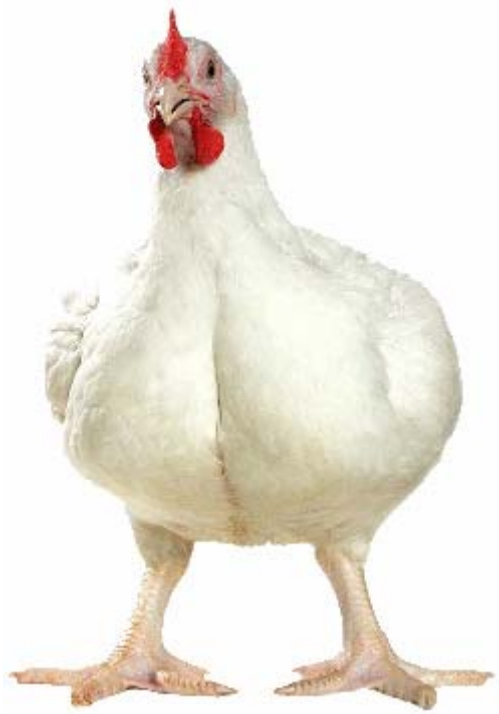

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ABBREVIATIONS

AOAC	Association of Official Analytical Chemist
ASAE	American Association of Agricultural Engineers
BWG	Body weight gain
CP	Crude protein
DM	Dry matter
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FI	Feed intake
g	Gram
GIT	Gastrointestinal tract
GMD	Geometric mean diameter
GSD	Geometric standard deviation
MF	Modulus of fineness
MU	Modulus of uniformity
N	Nitrogen
NRC	National Research Council
SD	Standard deviation
WI	Water intake
µm	Micro meter

ABSTRACT

The effect of diet structure (coarse *vs* fine), conformation (wet *vs* dry) and acidification (acidified *vs* nonacidified) fed during starter phase (0-21 days) and their carry over effect on dry and wet fed growers (22-42 days) were studied using 308 male broilers on floor pen. Birds were given *ad libitum* meal feeding 3 times during starter phase and 2 times during grower phase, with photoperiod of 18L: 6D and16L: 8D respectively.

Wet feeding increased feed intake by about 48 % and body weight gain by 85 % during starter phase, while 39% and 86 % respectively during grower phase, with carry over effect as well. Diet structure and acidification did not show any significant effect on feed intake and body weight gain during either phase, but carry over effect of starter diet structure was observed. However, there was a significant interaction found between diet conformation and structure on feed intake and body weight gain during starter phase.

Although water intake from water bottle was significantly ($P < 0.05$) reduced in wet fed birds during whole study period (0-42 days), total water intake was significantly higher (approximately 67 %) than dry fed birds during the starter phase but no significant difference was found during grower phase. There was approximately 15% lower feed water intake ratio in wet fed birds during starter phase. Diet structure and acidification did not show any effect on water intake during either stage of this experiment.

Fresh weight of the gastrointestinal organs was influenced by wet feeding during whole study period. Relative weight of gastrointestinal organs was significantly ($P < 0.05$) higher in dry fed birds by approximately 21% during the starter phase and 11 % during the grower phase. There was carry over effect of diet conformation found during grower phase as well. Similarly, bigger gastrointestinal organs (approximately 10%) were found in coarse diet fed birds during starter phase but it had no significant carry over effect during grower phase.

Wet feeding significantly improved the performance of broiler during whole study period, while diet structure showed only carry over effect during grower period of the study.

1. INTRODUCTION

1.1 BACKGROUND

Broilers have undergone enormous changes in growth characteristics during the past decades due to the progress in different aspects. 80 years ago, broiler chicken slaughter weight was reached in 16 weeks of age. But now, they will approach their slaughter weight during 6-8 weeks (Pezeshkian, 2002). A similar trend has been noted by different workers like Havenstein *et al*. (1994) and Nicholson (1998). This remarkable improvement in growth rate can be mainly attributed to improved genetics. However, tremendous improvements in feed and nutrition have made it possible to take advantage of the genetic changes. Several investigations have shown that chicken weight at six and seven weeks of age had a linear relationship with their weight in the first week of rearing. This was not due to the breeder age and day old chickens weight (Pezeshkian, 2002). This improvement in growth potential of commercial broilers was paralleled by improvements in poultry nutrition. Since feed represents above 70 percent of overall production cost in broiler, improving the efficiency of feed utilization will have a tremendous impact on cost of production. Thus, feed and feeding strategy could affect broiler performance and overall broiler economics in the whole production chain.

Several methods of feed manipulation and feeding strategy have been tried to get better performances and health from the present day broilers. Results from literature showed that the inclusion of whole wheat or the use of a coarse mash (larger particle sizes) were associated with larger gizzards. This was a result of variation in feed structure (Nir *et al.*, 1995; Hetland and Svihus, 2001). A larger and more muscular gizzard may increase the grinding and absorptive capacities of the gastrointestinal tract (Nir *et al.*, 1995), thus contributing to a better performance of broilers. Similarly, wet feeding for broilers has been reported to have promising effects on feed intake and feed utilization efficiency, due to the improvement of a better nutrient retention (Yalda and Forbes, 1995). On the other-hand, acidified fermented liquid feeding has a favorable effect on the development of the foregut, especially the development of the duodenum, as well as on feed intake and body weight gain (Bosch *et al.*, 2006, submitted).

There are three phases in feed intake behaviour in poultry: identification, pecking (the physical characteristics of feed particles and beak touch sensitivity) and intake follow stepwise adaptation levels to the environment and remains basically energy regulated at medium term (Picard, 1997). Different type of feed form (mash, pellet, grains) thus has different effects on diet selection of broilers (Rose *et al.*, 1985; Nir and Ptichi, 2001). The fact that the modern broiler responds so well in terms of growth performance and slaughter quality, is much more a feature of the adaptability of the broiler itself and a result of including highly digestible ingredients than a confirmation that we feed our birds so adequately (Cumming, 1994).

In contrast to the extensive works on the performance of broiler fed with different type of feeds (coarse *vs* fine feed, acidified *vs* nonacidified feed and wet *vs* dry feed) separately, comparative studies on effect of these factors in combination are relatively limited. Even

among the few works accomplished, the majority of them are limited to early few weeks (mostly up to 3 weeks of age) of broilers life. Thus, a study on the carry over effects in the later days of broilers due to the different feed structure / conformation / acidification fed on early days is missing. This situation has created the need of this research work.

1.2 SCOPE OF THE STUDY

The literature was reviewed to get a picture of the need for development of new feeding strategy of broilers. There are limited literatures available explaining the combined effect of diet structure, conformation and acidification on broilers performance. Moreover, majority of the works done were limited to specific time period of broilers life. It would be nice to have detailed information about the whole broiler production cycle (0-42 days). It was thought that there is still a lot of information missing with regards to different types of diet and their effect on the broiler performance.

The objective of this experimental study was to investigate the effect of (1) Particle size distribution of feed (2) Acidification of feed; and (3) Wet feeding on performance and gastrointestinal tract development of broilers in the starter phase (0-21 days) and carry over effects to dry and wet fed growers (22-42 days).

The ultimate scope of a larger study over years is to investigate possibilities to enhance functional gastrointestinal tract (GIT) development and health during the starter period of broiler life by the above mentioned dietary modifications. Contrasts from this trial may be used in future trails to study broiler GIT pathology and GIT development.

1.3 OUTLINE OF THE THESIS

This thesis describes the details about the experiment itself and its findings. The first part of this thesis presents the introduction and previous work done in related field, followed by the materials used and methodology of this study. The experimental research was executed in two phase: starter phase and grower phase. In the starter phase, the birds were reared in 8 different treatments with three different feed variables; “coarse *vs* fine feed”, “wet *vs* dry feed” and “acidified *vs* nonacidified feed”. While only the “wet *vs* dry feeding” was applied during the grower period to study carry-over effects. It was analyzed if the different feed type given on starter phase had influence on the performances of broilers during the grower phase as well. The interaction of different treatments in starter phase was studied with relation to the feed offered in grower phase.

The data collected and analyzed were focused on feed intake (FI), water intake (WI), body weight gain (BWG) and development of different parts of GIT; in relation to the feed offered to them. Measurement of FI was taken on every times feed offered, while water intake and body weight was measured on the weekly basis. Sample birds were dissected at the end of 3 and 6 weeks to get the required information about GIT.

The results and discussion on performance of birds and GIT development is divided into two sections: starter and grower phase, so that a clear idea can be developed during both phases.

Within these two separate sections, data and interpretation on FI, BWG, FCR and GIT organs development along with feed water intake ratio are presented. In addition, chemical and physical analysis of the diets has been presented at the start of results and discussion to relate its effect on the performances and GIT development. It has been tried to relate the present findings with the previous reports, so that new recommendations can be made.

The basic data of interest to the researcher are presented in the main content of the thesis, while related other data are presented in the appendixes. It might be helpful for further information, if anywhere required.

Effect of different diets on performance and GIT development of broilers is presented in this thesis. It is expected that the findings of the research might be useful to develop feeding strategy for broilers in future. In addition, it will open the way for alternative feeding system to the existing common practice of commercial broiler farming. Furthermore, this work may be helpful for other researchers in this field for further research.

2. LITERATURE REVIEW

2.1 FEED STRUCTURE

An important nutritional factor, in addition to the composition of the diet and its caloric value, is the structure of the food. It induces marked changes in behavioural and metabolic parameters (Nir and Ptichi, 2001). Particle size distribution has long been an area of interest, in both the feed and poultry industries (Kleyn, 2005). It has become more crucial again as we continue to fine-tune our feeding strategy.

In this study, we were concerned to know the effect of feed structure as particle size distribution in relation to coarseness and fineness of feed.

2.1.1 Feed particle size distribution

The definition of particle size distribution is very broad, especially where irregular shapes are concerned. The precise definition depends on the method of measurement (Allen, 1974, cited by Waterhouse, 1995). The most common used is the volume diameter which is the diameter of a sphere with the equivalent volume to the particle. Particle size distribution is a profile of all the different sized particles in a sample, giving a normal or cumulative frequency of different size categories in a sample (Waterhouse, 1995).

Terms such as “fine” “medium” and / or “coarse” are often used to describe particle sizes of grains in the literature. These are relative terms and are of little use in evaluating research on particle size. Standardized procedures for particle size determination have been developed (ASAE, 1973) and determination of particle size of grain is fairly simple procedure (Behnke, 1985).

Particle size measurement is established by calculating the geometric mean diameter (GMD). However, the complete information on particle size must include a measure of dispersion. This measure is the geometric standard deviation (GSD), which establishes the range of variation among the different particle sizes (Nir *et al.*, 1994). Both of these measures are described by the ASEA (1973), but they are seldom reported in the literatures as they independently affect broiler growth and performance as shall be seen.

Similarly, the modulus of uniformity (MU) gives the distribution of the different sieve fractions (arbitrary classified as coarse: medium: fine) in terms of percentage, whereas the modulus of fineness (MF) gives an indication of the fineness of the diet (Anonymus, 1961).

The average particle size of the sample can also be determined by standard formulas and given as the GMD, expressed as microns or µ. Particle size uniformity is described by GSD; a small GSD is representing a higher uniformity. The size uniformity of the various ingredients that compromise the finished feed can directly impact final ingredient dispersion. Finally, from these values (GMD and GSD) the number of particles per gram and amount surface area can be calculated (Baker and Herrman, 1995; Pfost and Headly, 1976). The

GMD in combination with the GSD gives more information about the size of the particles in a batch. Pfost and Headly (1976) have set a ranking to evaluate the GSD, shown below:

GSD ranking	Value
Excellent	1.0 – 2.0
Good	2.0 – 2.3
Fair	2.3 – 2.6
Poor	>2.6

The use of different methods for particle size analysis depends on the upper or bottom limits of the particle size of the material. The most commonly used method for determination of particle size distribution for feedstuffs and complete feeds are sieving analysis. This can be performed by both dry and wet sieving. The wet sieve analysis may give an indication of the particle size distribution which enters the (wet) gastrointestinal tract of the animal. Thus it gives more realistic value for particle size of a diet entering in the GIT of animal. Moreover, this method can also be used for particle size determination of both crumble and pelleted diets (Goelema, 1996).

It is important to note that the particle size distribution of the feed is determined by its calculation from the weight measurement of the sieve fractions in this method. However, it can be directly measured by some other methods like laser diffraction technique and coulter counter techniques.

There are thus other methods available to measure the real particle size like the laser diffraction or coulter counter technique. In laser diffraction particle size analysis, a representative cloud or 'ensemble' of particles passes through a broadened beam of laser light this scatters the incident light onto a Fourier lens. This lens focuses the scattered light onto a detector array and, using an inversion algorithm, a particle size distribution is inferred from the collected diffracted light data. Sizing particles using this technique depends upon accurate, reproducible, high resolution light scatter measurements to ensure full characterization of the sample. These days, laser diffraction is the most widely used technique for particle size analysis. However, there are some limitations of this technique, making it not suitable for all purposes. Major drawbacks of this technique noted were observed as this technique exhibited poor inter-instrument reproducibility, offered limited resolution (often missing shoulders, tails and sub-populations in particle size distributions) and gave information of limited value for submicrometre particles (Cooper, 1998).

Particle size distribution of the feed can be altered in different ways. In several investigations (Hamilton and Proudfoot, 1995; Nir *et al*., 1995; Kwakkel *et al*., 1997) grains in the diet were ground with different clearances by use of a hammer mill and subsequent a sieve or a roller mill, the two most common techniques to reduce particle sizes in feed manufacturing practices. Both types of equipment are capable to produce a satisfactory particle size for poultry feeds; however they differ in many respects like initial cost, operation cost and ability to produce uniform particle size (Audet, 1995; Waldroup, 1997).

2.1.2 Effect of particle size distribution on performance and GIT development of broilers

The GIT is a very complex organ and is obliged passage of the nutrients that support basic metabolism, growth and maintenance, supplying the resources to support the immune, skeletal and nervous systems (Ferket, 2000). The GIT development and health is the key to productivity in all farm animals and poultry. A multitude of factors can influence the performance of the GIT; intestinal health, immune stimulation, environment, nutrition, feed ingredient choice and quality, toxins, microflora equilibrium, endogenous secretions, motility, additives etc. Among these, the digestive function could be considered the most limiting factors in performances (Gauthier, 2002).

The physical and functional development of the GIT of the broiler is related to diet structure (Nir and Ptichi, 2001). A positive correlation between feed particle size and broiler growth has been shown by different authors. Results from different literature showed that the inclusion of whole wheat in diet, use of a coarse ground mash or bulky diet (larger particle sizes) was associated with larger gizzards as a result of variation in feed structure (Nir *et al.*, 1995). Findings of several other workers (Reece *et al.*, 1985; Rogel *et al.*, 1987; Munt *et al.*, 1995; Preston *et al.*, 2000; Hetland and Svihus, 2001; Engberg *et al.*, 2002; Gabriel *et al.*, 2003) were also in the same line, showing positive correlation of feed particle size and development of gizzard in broiler. However, it was not clear whether the technological treatments of the diets have effect on the changes of functional development in the foregut segment during the first 10 days post-hatched chicks caused by pelleting versus mash and / or coarse versus fine ground diets. (Nir *et al.*, 1994; Nir *et al.*, 1995). Later, Kwakkel *et al.* (1997) found that a coarse diet (i.e. with on average, large feed particles) lead to a better development of the GIT possibly related to enhanced GIT motility and reverse peristalsis throughout the colon.

The manner in which ingredients are ground and the coarseness of that grind has a direct impact on the digestive physiology of the birds. Nir *et al.* (1994) asserted that the nutrient digestibility decreases when small particles are used because they cause gizzard atrophy and discrete intestinal hypertrophy caused by bacterial fermentation. In contrast, larger and more muscular gizzard may increase the grinding and absorptive capacities of the GIT. It was further suggested that particle breakdown in the proximal small intestine is slower when particles are larger. This causes an increase in peristalsis, leading to a better nutrient utilization (Nir *et al.*, 1995).

Moreover, Cumming (1994) suggested that when fine diets are fed to broilers or laying hens, the gizzard acts as "transit" rather than a grinding organ. As a result of this the feed is not retained in the gizzard for any significant period and is therefore not exposed to the digestive enzymes of the proventriculus at a low pH. The role of these poorly digested feed particles in the upper intestinal tract is unknown; however they may play a role in aberrant bacterial populations such as *E. coli*. There is also evidence to suggest that an active, normal gizzard plays a role in the chicken's resistance to coccidiosis (Cumming, 1992).

Magro & Penz (1998), working with diets containing different particle size dimensions, found the best production results with the highest mean geometric diameter feeds. They were also able to illustrate the impact that particle size has on the bird's digestive system. Carre (2000) makes the point that coarse grinding should be positive for reducing water losses, and also in some cases for improved protein digestibility. The latter effects would be explained by a better control of the intestine transit time by the gizzard emptying rate when using coarse ground feeds. Krabbe (2000) verified this by showing that finely ground diets (561µ) compromised nutrient metabolism, with particle size affecting metabolisable energy, nitrogen retention and dry matter retention (Cited by Kleyn, 2005).

The physical attributes of the diet seem to affect FI and performance of broilers (Nir *et al.*, 1994). Broiler chicks perform better with a mash containing coarse rather than fine particles (Reece *et al.*, 1985). Similar finding was shown by Wilson (2001) while compared to growth performance and feed efficiency of broilers fed with varying particle sizes (cited by Behnke and Beyer, 2002). In contrast, Deaton (1995) found no difference in weight gain and feed utilization in male broilers when they were fed pellets produced from different particle size.

On the other hand, some workers have advocated for the fine particle size feeding to broilers as well. Goodband *et al.* (2002) noted that particle size reduction increases the surface area of the grain, thus allowing for greater interaction with digestive enzymes. Cabrera (1994) found no effect of diet particle size (1,000 to 400 microns) on growth performance of broiler chicks fed a complex diet (added tallow, meat and bone meal and feather meal) in crumblized form. However, in his second trial, he found that feed efficiency was improved 3 percent by reducing particle size from 1000 to 500 microns in simple diets fed as a meal form but not in crumblized diets. Dietary processing had no significant effect on bird basic performance parameters (Cramer *et al.*, 2003). Similar finding was shown by Nir *et al.* (1995), in which 21-d-old male birds fed crumbles showed similar weight gain but consumed significantly more feed than the male birds fed mash treatment. In contrast, Douglas *et al.* (1990) found that chicks fed crumbled diets had higher weight gains and improved feed conversion than chicks fed mash based diet for 21days.

2.2 WET FEEDING

The theory behind wet feeding is that by adding water to the diet before feeding, the diet is then already hydrated and digestion can begin immediately. This faster rate of digestion enables the bird to eat more and grow faster. Dietary manipulations such as these are expected to alter some aspects of the digestive tract response of the birds.

Wet-mash feeding has been practiced for many decades in back-yard poultry keeping using, for example, waste food scraps, potatoes and their peelings and many other available materials mixed up to give a sloppy mash. But it has not been in the common practice for the commercial poultry farming yet (Forbes, 2003).

Relatively few studies have been made of wet feeding for poultry, compared to other species. Early research into complete wet synthetic diets for chickens reported a major osmotic pressure disturbance resulting in a critical loss of tissue water indicated by dehydration and

mortality (Kopfler and Wilkinson, 1963; cited by Forbes, 2003). In a series of short-term (2-7 days) studies with chickens to 1 to 2 weeks of age it was found that weight gain was significantly less with wet food than with the dry diet (Waibel *et al.* 1966; cited by Forbes, 2003). On both practical and experimental grounds, therefore, wet feeding was contra-indicated. But, Pittard (1969) found that wet feeding of poultry improves feed intake and efficiency.

Water treatments and subsequent drying of cereal grains has been shown to improve the nutritional value of grains of broilers (Fry et al., 1958; Lepkovsky and Furuta, 1960; cited by Forbes, 2003) but this was not found to be economical as the savings made from the increase in food utilization did not meet the costs of the energy required to dry the food after wetting. However, later Yasar and Forbes (1995) showed that it is not necessary to re-dry wetted food in order to obtain these benefits. They found that dry food mixed with water significantly increased weight gain and feed conversion efficiency in broiler chickens.

Yalda and Forbes (1995) mentioned that wet feeding for broilers has promising effects on feed intake and feed utilization efficiency, due to the better nutrient retention. Thus, the wet feeding stimulates growth directly. In their next study, Yalda and Forbes (1996) reported that broiler chickens fed on commercial pelleted food mixed with water had increased weights of liver, crop, proventriculus and small intestine, compared to those fed on the same food without water addition. Similarly, Yasar *et al.* (1997) also claimed that wet feeding might be a promising strategy, particularly during certain age-intervals of broilers life. Wet feeding (in a diet with 80% wheat and a commercial wheat enzyme) increased feed intake and weight gain up to 17 days of age by almost 20% (Scott, 2002). Scott indicated that wet feeding increased growth rate, but had a varied effect on feed conversion ratio when different sources of wheat were used. These studies indicate that broilers cannot eat enough dry diet to attain their genetic potential for growth. Slade and Forbes (1997) found that chicks fed in wet form gained significantly more efficiently during the first 10 days of life and still had significantly heavier carcass weights at 21 days.

Later, Yasar and Forbes (1999) confirmed that wet feeding significantly increases feed intake, total water intake and body weight gain of broiler chickens, without improvements in food conversion efficiency. They also reported that the fresh empty weight of the gut was increased by wet feeding while its relative weight to body weight and length of the gut was not affected by the dietary treatments. In addition, they mentioned that the mechanism of the beneficial effects of wet feeding could be attributed to the decreased viscosity of gut contents; the greater the development of the layer of the villi in the digestive segments and the reduced crypt cell proliferation rate in the crypts of the epithelium. Adding water has a positive effect on solubilisation of dietary components (Yasar and Forbes, 2001). Given the very rapid transit of feed particularly in broilers, this early solubilisation gives more time for absorption to take place. This allows the actual digestibility of the feed to approach more closely to the potential digestibility that would be achieved if the feed stayed longer in the GI tract (Forbes, 2003).

Another important aspect of wet feeding is the feed water mixing ratio. There are very limited literatures reporting about comparative performance of broiler when fed wet fed with

different feed water mixing ratio. Robinson (1948), writing about "consistency of wet mash" stated that "Although it is customary to speak of wet mash, the mash should be fed in a crumbly-moist condition. Only a small quantity of water should be added to the dry meal – just sufficient to make it hold together when thrown to the ground" (Cited by Forbes, 2003). It was meant to give wet feed the 'porridgy' consistency, supported by Yasar and Forbes (1999) as well. They recommended that the upper limit of water addition should be that which results in a layer of free water on the top of the food discouraging feeding. They added 1.3 kg of water with 1 kg air dry mash diet and found significant effect on performance of broilers. Similarly, Scott and silversides (2003) found that mixing 1.2g water per g air dry feed significantly increased BWG of broilers. The appropriate consistency is described as 'porridgy' and this is achieved by different amounts of added water for foods with different ingredients and in different physical forms (Forbes, 2003).

2.3 ACIDIFICATION OF FEED

Acidification is a method of eliminating the high occurrence of pathogens in the poultry environments (Andrys *et al*., 2003). A very important objective of the dietary acidification is the inhibition of intestinal bacteria competing with the host for available nutrients, and a reduction of possibly toxic bacterial metabolites, e.g. ammonia and amines, thus improving weight gain of the host animal. Furthermore, the growth inhibition of potential pathogen bacteria and zoonotic bacteria (e.g. *E. coli* and *Salmonella sps.)* in the feed and in the GI-tract are of benefit with respect to animal health (Canibe *et al*., 2002).

Very high animal densities are used in poultry production, which increases the susceptibility to diseases (Andrys *et al*., 2003). Similarly, disturbed FI or total feed withdrawal results in decrease of the fermentation of feed in the crop. This lack of fermentation automatically results in a higher pH and a lower lactic acid level in the crop. The decreased lactic acid and increased pH may provide an important environment for the proliferation of pathogenic bacteria. On the other hand, a disturbance of the balance of the microflora can result in digestion problems, leading to proliferation of pathogenic bacteria. This finally results in a decreased technical performance expressed in lower feed efficiency and an increased mortality of birds. In such condition, acidification of feed and /or water is of prime importance to reduce the pH of the crop (Anonymus, 2001). Smith (1965) reported a favorable effect of a decreased pH value caused by feed acidification on the alimentary tract microflora.

Several studies about acidified feed for broiler chicken have shown that the *Salmonella* numbers decreased faster in the crops and the gizzards of fermented liquid feed fed chickens in comparison with conventionally fed chickens (Heres *et al*., 2003). However, in the later study of Heres *et al*. (2004) it was shown that the acidified fed chickens were less susceptible to infection with *Campylobacter*, but not with *Salmonella*. Different bacteria will show different levels of sensitivity to different organic acids, under specific circumstances. Contrary to antibiotics, week-acids appear to share a common mode of action, despite their various chemical structures. All become more potent as antimicrobial agent at more acidic pH (Lambert & Stratford, 1999), which is in fact incompatible with normal physiological functions in the animal and even incompatible with life.

There are several reports of using organic and inorganic acids for acidification of feed. Organic acids have been used for decades in feed preservatives. This is aimed to protect feed from microbial and fungal destruction or to increase the preservation effect of fermented feed. In poultry production, organic acids have mainly been used to sanitize the feed considering problems with salmonella infections (Iba & Berchieri, 1995; Berchieri and Barrrow, 1996; Thompson and Hinton, 1997). The key basic principle on the mode of action of organic acids on bacteria is that non-dissociated (non-ionized, more lipophilic) organic acids can penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria. Organic acids show an enormous bactericidal effect and they are readily absorbed through the bacterial cell wall (Langhout, 2000). On the other hand, inorganic acids such as phosphoric acid can exert a bactericidal effect due to a dramatic decrease in pH value. In many studies, acidifiers showed the strongest effect in the first four weeks of chick's life (Versteegh and Jongbloed, 1999).

The efficacy of organic acids in swine nutrition has been proven time after time (Partanen, 1999) but in poultry this innovative approach is still in infancy. Organic acids have not gained attention in poultry production as much as in pig production. One reason for this may be that the results regarding weight gain and feed conversion ratio following dietary addition of organic acids are not as convincing as the results from the pig production (Langhout, 2000). However, a positive influence on either feed conversion ratio or growth performance has been reported for fumaric acid, propionic acid, sorbic acid and tartaric acid (Vogt *et al.*, 1981, Vogt *et al.*, 1982, cited by Mujdat *et al.*, 1999). Similarly, acidified fermented liquid feeding had a favorable effect on the development of the foregut, especially the development of the duodenum, as well as on feed intake and body weight gain (Bosch *et al.*, 2006, submitted). In contrast, Gentle (1971) found no differences in feed intake while feeding chicken with an addition of 6% citric acid.

Supplementation of organic acids to animal feed can also lower the buffering capacity of the feed. This is important because a low buffering capacity of the feed helps to create an acidic gastric environment, which is essential for activation and secretion of certain gastric and pancreatic enzymes. As a result, digestibility and utilization of nutrients are enhanced by supplementation of organic acids (Anonymus, 2005a).

The lactic acid supports acidification of the fore-gut of the animals. Unlike other acids, it does not suppress the positive crop fermentation. It survives the stomach or crop to function at the small intestine of pig and poultry. By stimulating the secretion of pancreatic enzymes, lactic acid stimulates digestibility and FI (Anonymus, 2004).

The combination of acetic acid, lactic acid and phosphoric acid has synergistic effect. In a trail conducted by Research Institute for Animal Husbandry, the Netherlands (Anonymus, 2005b), it was found that the use of Calprona AL® (a mixture of acetic acid, lactic acid and phosphoric acid) gave better growth and feed conversion ratio with little mortality both in starter and grower period. In another field trial in Brazil (Anonymus, 2003), it was found that Calprona AL® has by far outperformed a probiotic in growth and feed conversion ratio.

3. MATERIALS AND METHODS

3.1 ANIMAL ETHICS

All the procedures involving animals in this experiment were done in accordance with the Dutch Law on experimental animals and had been approved by the Animal Ethical Commission of the Netherlands and Animal Experimental Committee of Wageningen University, the Netherlands.

3.2 EXPERIMENTAL SITE AND PERIOD

The experiment was conducted at the experimental farm "De Haar" of Wageningen University, the Netherlands, from 28 October to 8 December 2005. It was followed by laboratory works for physical and chemical analysis of diets in the Animal Nutrition Laboratory of the same University.

3.3 BIRDS, HOUSING AND CARE

320 day-old male broiler chickens (Ross-308) were obtained from a commercial hatchery (Morren Breeders B.V., Lunteren, The Netherlands). Upon arrival, the chickens were wing tagged for identification. After that all the chickens were assigned randomly to 32 pens, of each pen 10 chickens were housed. Chickens in pen were weighted for initial weight and on weekly interval to get the growth performance.

All the birds had access to feed and water *ad libitum*, however the feed was made available on meal basis. The experimental diets were offered 3 times a day during the starter period and 2 times a day during the grower period. There were 3 nipple drinkers and 1 feed trough placed in each pen to have easy and equal access to feed and water for all the chickens. In first day, the birds were offered feed on a flat trough to have easy access to feed.

Full wood shavings covered the floor pens, each having a solid wall with dimension of 1m x 1.5m and 0.6m height was used. The temperature inside the room was initially maintained at 32^0C from day 0 to 7 and gradually decreased by 3^0C per week until it reached 21^0C at day 28. This temperature was set and maintained until day 42.

Artificial light was provided at schedule of 18 hours light and 6 hours dark in the starter phase. Light was provided considering the equal 6 hours feeding time interval: 8:00 h, 14:00 h and 20:00 h followed by dark period from 2:00 h till 8:00 h. In first 3 days, light was provided 23 hours to enable the young chicken having enough time to eat and drink. It was done to prevent birds from dehydration during the critical period of early life. However, light schedule was rearranged as 16 hours light and 8 hours dark during the grower phase considering the equal 8 hours feeding time interval: 8:00 h and 16:00 h followed by dark period from 24:00 h to 8:00 h. Light intensity was maintained above 20 lux at the bird's level during all lighting period throughout the experiment.

3.4 EXPERIMENTAL DESIGN

The experiment was covered in two distinct stages: starter and grower phase.

3.4.1 Starter phase (0-21 days)

The experiment was of a 2 x 2 x 2 factorial design; "wet *vs* dry feed", "coarse *vs* fine feed" and "acidified *vs* nonacidified feed" diet structure as experimental factors. The pens were randomized within the four replicates, each having all the eight treatments. The detail of the experimental design and pen allocation is presented in Appendix 1.

Eight experimental diets were prepared to meet this 2 x 2 x 2 factorial design (presented in the table below) during the starter period from 0-21days of age. All the experimental diets were fed 3 times daily at 8:00, 14:00 and 20:00 hrs to the birds during this period.

Dry diet				Wet diet			
Acidified (pH≤5)		Nonacidified (pH≤ 7)		Acidified (pH≤5)		Nonacidified (pH≤ 7)	
Coarse	Fine	Coarse	Fine	Coarse	Fine	Coarse	Fine

3.4.2 Grower phase (22-42 days)

At day 22, all the 32 pens were split up to make 64 pens with 4 chickens in each pen. The pen allocation of the birds in this phase is presented in Appendix 2. The birds were offered feed twice daily at 8:00 h and 16:00 h. Only two meals a day was done assuming that when broilers become older they are better capable to ingest sufficient wet diet; therefore no third meal is necessary during the grower period.

The grower diet was fed in either wet or dry form until 42 days of age to study carry-over effects of the starter phase diets. This means that all diets in this period were of fine grind and were non-acidified. An interaction between the diet form in the grower period and the nutritional history was assessed in the result.

The feed preparation and mixing was done outside the experimental shed to prevent any disturbance to the chickens. All feed troughs were taken out every time while offering feed in similar way to maintain uniformity. Wet diets were always offered completely new to have fresh feed at every meal. In the dry feeders, new feed was just added to fulfill the requirement and given new feed at the beginning of every day. Every precaution was taken to prevent the contamination of different types of feeds to each other during the whole process of feed preparation and distribution.

3.5 EXPERIMENTAL DIETS

All diets were formulated to meet the nutrients and energy requirements for broilers (NRC, 1994) in both starter and grower period, which is presented in Table 1. Details of the nutrients requirement are presented in Appendix 3.

Table 1: Selected nutrient requirements for broilers* (NRC, 1994)

Nutrient (g / kg of diet; 90% dry matter)	Age	
	0-3 weeks	3-6 weeks
Crude protein	230.00	200.00
Calcium	10.00	9.00
Non-phytate phosphorus	4.50	3.50
Potassium	3.00	3.00
Copper, mg/kg	8.00	8.00
Zinc, mg/kg	40.00	40.00
Sodium	2.00	1.50
Lysine	11.00	10.00
Methionine	5.00	3.80

*The requirements are based on the dietary metabolizable energy concentration of approximately 13 MJ/ kg.

To fulfill the nutrient requirements of the birds, a diet was formulated with the composition as is shown in Table 2, along with the calculated nutritive value. The more detailed nutrient content of the diet used is presented in Appendix 4.

Although, it is common practice to use two different diet formulations for starter and grower phase, only one diet formulation was used in this experiment. It was decided considering the objective of this experiment to study carry over effects of starter phase diets during the grower phase of broilers. So, no difference in feed composition was desirable to avoid the effect of feed composition itself. Similarly, both in starter and grower phase, all the diets were in mash form irrespective of their structure and conformation. It was also done with the intention to get better comparison of carry over effects.

Table 2: Diet ingredient composition and its calculated nutritive value (0-6 weeks)

Ingredient	Proportion	Nutrient (g/ kg of feed)	
Wheat	34.58	Metabolisable energy (MJ/kg)	12.00
Corn	27.36	Dry matter	880.00
Toasted full fat soybeans	20.07	Crude protein	208.21
Soybean meal 44/7	9.40	Crude fat	60.35
Fishmeal 66% CP	5.19	Crude fiber	31.17
Ca CO_3	1.33	Starch	380.69
L-Threonine	0.03	Ca	8.00
Vitamin & mineral premixture	0.50	P	5.82
Monocalciumphosphate	0.54	Ileal digestible lysine	10.00
Phytase	0.30	Ileal digestible methionine	4.61
Salt	0.19	Ileal digestible cystine	2.69
DL-Methionine	0.16		
L-Lysine HC l	0.06		

The diet defined as 'coarse', was processed by using a roller-mill with one roller pair and rollers distance of 1.6 mm (roll 1, 480 rpm and roll 2, 1022 rpm) for wheat and two roller pairs for corn (roller pair 1: roll 1, 480 rpm and roll 2, 1214 rpm; roller pair 2: roll 1, 480 rpm and roll 2, 1214 rpm). The ingredients 'toasted full fat soybeans' and 'soybean meal 44/7' were added without hammer milling. Diets defined as 'fine' were processed by using hammer mill with opening screen of 3.0 mm (modified after Hamilton and Proudfoot, 1995).

The acidified diets were made by mixing 990g dry feed + 10g Calprona AL® (Verdugt B.V., The Netherlands). Calprona AL® is the solution of lactic acid (80g/kg), Phosphoric acid (610 g/kg) and acetic acid (85 g/kg) with total acids 785 g/kg.

The wet diets were made by mixing 1 kg of dry feed with 1 kg of extra tap water 20 minutes prior to the every feeding time (modified after Yasar and Forbes, 1999). However, in the early 3 days of first week it was in the ratio of 1:1.3 respectively. This was done to make wet feed moister thus facilitate the young birds easy to pick and adapt on wet feeds while shifting from yolk nutrition to 'solid diet'.

3.6 OBSERVATIONS AND ANALYSIS

3.6.1 Chemical and physical analysis of feed

Feed samples from offered and refusal were collected once a week. These samples were subjected to measure pH level and dry matter (DM) contents, and particle size distribution of the feed before and after feeding.

DM content of the coarse and fine diets were determined after sample preparation by milling at 1 mm (ZM- 100, Retsch, Germany) following Standard Operational Procedure (AOAC, 1985). Likewise, pH level of the both acidified and nonacidified offered diets was measured in the laboratory by using electronic pH meter (Model- pH300, Hanan Instrument, Portugal).

The samples were analyzed for the particle size distribution using the sieving technique as recommended by ASAE (1973). The diet offered to the birds were subjected to both wet and dry sieving, while the refusal samples were analyzed by wet sieving only.

Wet sieving of duplicate samples of 25 grams was subjected at amplitude of 2 mm with 6 sec interval through a set of 6 steel sieves (internal diameter, 20 cm, height, 5 cm; AS200 Control Retsch, Germany) using a water sieve (AS200 Control, Retsch, Germany). The mesh size were 2.5, 1.25, 0.630, 0.315, 0.160 and 0.071 mm. Prior to sieving, the samples were soaked in tap water (25 g sample and 500 ml water) for 45 minutes with gentle stirring the suspension with glass rod (± 5 times during the soaking period).

Dry sieving was done by passing known weights of the diet through a series of sieves (2.83; 2.00; 1.41; 1.00; 0.71 ad 0.50 mm screens) and weighing the amount of material collected on each screen and the tray under the 0.50 mm screen (Hamilton and Proudfoot, 1995).

Weights on the different sieves were used to calculate the particle size distribution of the different diets. For both dry and wet sieving, geometric mean diameter (GMD), geometric standard deviation (GSD), modulus of fineness (MF) and modulus of uniformity (MU) was calculated according to Pfost and Headley (1976).

$$GMD = \log^{-1} [\Sigma (Wi \log Di) / \Sigma Wi]$$

$$GSD = \log^{-1} \sqrt{} [(\Sigma \log Di - \log GMD)^2 / \Sigma Wi]$$

Where,

Wi = weight fraction on the sieve *i*, or volume fraction of class *i*

Di = diameter of sieve *i*, or diameter of particle in class *i*

$$Di = \sqrt{} (Du * Do)$$

Where,

Du = diameter opening which particles will pass, or upper limit class

Do = diameter opening which particles will not, or bottom limit class

$$MF = \Sigma (i * Wi) / 10$$

$$MU = (\Sigma (Wj) /10 : \Sigma (Wk) /10 : \Sigma (Wj) /10)$$

Where,

Wi = weight fraction on the i^{th} sieve

Wjkl = weight fraction on the j^{th}, k_{th} or i^{th} sieve

(In this experiment: j = sieve 1, 2; k = sieve 3, 4; and i = sieve 5, 6, 7 (pan))

3.6.2 Feed and water intake

To measure feed intake, the given quantity of feed was subtracted with the weight of the refusals in the feeder after each meal. The quantity of wet feed was calculated on the basis of dry feed quantity offered to the birds. Two feeders filled with wet feed (one from each coarse and fine feed) in starter phase and one in the grower phase were placed in an empty cage to know the loss of water as evaporation. This value was used to adjust the daily intakes of the birds. Spoilage of feed was ignored as it was negligible amount due to the structure of feeders used. All the measurement and calculations of feed were based on dry feed basis. Similarly, all the data were considered on per pen bird-day basis.

Water consumption per pen was determined in similar manner throughout the starter phase from all the 32 pens. However, water intake was measured only once a week. In grower phase, water intake was measured only from 32 pens (16 each from dry and wet feeding groups) out of 64 pens as sample representative. However, we were unable to measure WI in week 4 due to problem in fixing the drinking water bottles in the pens separately in due time.

3.6.3 Growth performance

The experiment was conducted for 6 weeks starting from day-one age of the birds. Initial weight and the weekly growth (on every weekend morning before feeding) of birds were recorded. The birds were weighted in group as a whole (per pen).

3.6.4 Weights of gastrointestinal organs

Sample chickens were killed and dissected at the end of 3 and 6 weeks to investigate the effect of different feeds on the structural and functional development of the GIT segment. At the end of starter period, altogether 62 birds were killed which were randomly selected having close to the mean weight of each group. It represented 2 birds from each pen except in 2 pens, where 1 bird from each pen had died in the 1st week of experiment. Similarly, 1 bird from all the 64 pens were killed and studied at the end of grower phase.

All the birds were killed by using euthanasia T 61 (Intervet Nederland B.V.). It is a solution containing Embutramide 200 mg, Mebezoniumjodide 50 mg and Tetracainehydrochloride 5mg per ml. For euthanasia purpose, 0.5 ml of T 61 was given intravenously in the wing vein of each bird. Immediately thereafter, the birds were weighted and dissected to separate the whole gastrointestinal tract from the body. The crop, proventriculus, gizzard, duodenum with intact pancreas (whole pancreatic loop) and jejunum (from pancreatic loop to Meckel's diverticulum) were separated from the GIT very carefully. These organs were cleaned and excess water was removed with tissue before weighting. The fresh weight of these organs were recorded and expressed as per 100 g body weight of the bird.

3.7 STATISTICAL ANALYSIS

Analysis of data was performed using SPSS® software (SPSS Inc., 2003) with pen as experimental unit. Similarly, carry over effects from 3 weeks onwards were analyzed.

The data of starter phase were analyzed using full factorial model with F test with 3 factors: diet conformation, structure and acidification. Totally, there were 8 combined treatments. The data of grower phase were analyzed using full factorial model as well, but a little bit different. There were 4 factors: conformation, structure and acidification of starter phase, and conformation of grower phase diet. Totally there were 16 combined treatments. Significance, if not stated otherwise, is based on the 0.05 level of probability.

The model used for analysis was $Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha_i\beta_j + \alpha_i\gamma_k + \beta_j\gamma_k + \alpha_i\beta_j\gamma_k + e_{ijkl}$
Where,

Y_{ijkl} = Individual performance of bird with
α_i = Effect of conformation (wet / dry);
β_j = Effect of structure (coarse / fine);
γ_k = Effect of acidification (acidified / nonacidified);
$\alpha_i\beta_j$ = Interaction between conformation and structure;
$\alpha_i\gamma_k$ = Interaction between conformation and acidification;
$\beta_j\gamma_k$ = Interaction between structure and acidification;
$\alpha_i\beta_j\gamma_k$ = Interaction among conformation, structure and acidification; and
e_{ijkl} = Random error.

4. RESULTS

4.1. CHEMICAL AND PHYSICAL ANALYSIS OF FEED

4.1.1 pH level and dry matter content of the diets

The pH value of acidified and nonacidified feed was 5.0 and 5.8, respectively. It was assumed that the PH value of the nonacidified diet will be around 7.0, but we did not found that.

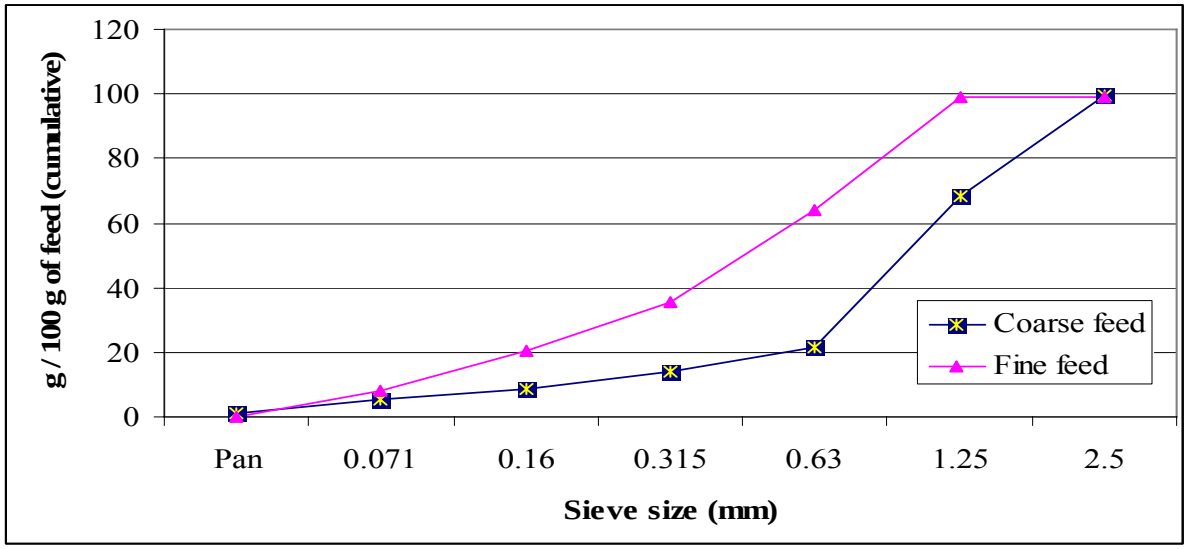
Similarly, DM of the coarse and fine feed offered was 87.47 % and 87.57%, respectively.

4.1.2 Particle size distribution of the different diets

Data of particle size distribution of was taken for starter phase only, as structure of diet was considered only during this phase.

Particle size distribution of diets offered over different size of sieves on dry sieving is presented in Figure 1. The MU of coarse and fine diet offered was 8:1:0 and 6:3:1, while MF of was 5.79 and 4.67 respectively. Similarly, the GMD and GSD of the coarse feed offered were 1393.22 µm and 2.29 µm, and that of fine feed was 722.88µm and 2.45µm respectively.

Figure 1: Particle size distribution of offered diets (dry sieving)



The GSD and GMD of the different diets are presented in Table 3. The values give a good insight in the mean particle size and the distribution of the particle size. The GSD can be used to calculate the limits of particle size of the middle 68% of the particles in the sample, which is the GMD $\pm 1 * \sigma$. The lower limit is the GMD minus one times the GSD, and the upper limit is the GMD plus one times GSD. This gives us information about the majority of the particles in the sample and is a contribution to the GMD.

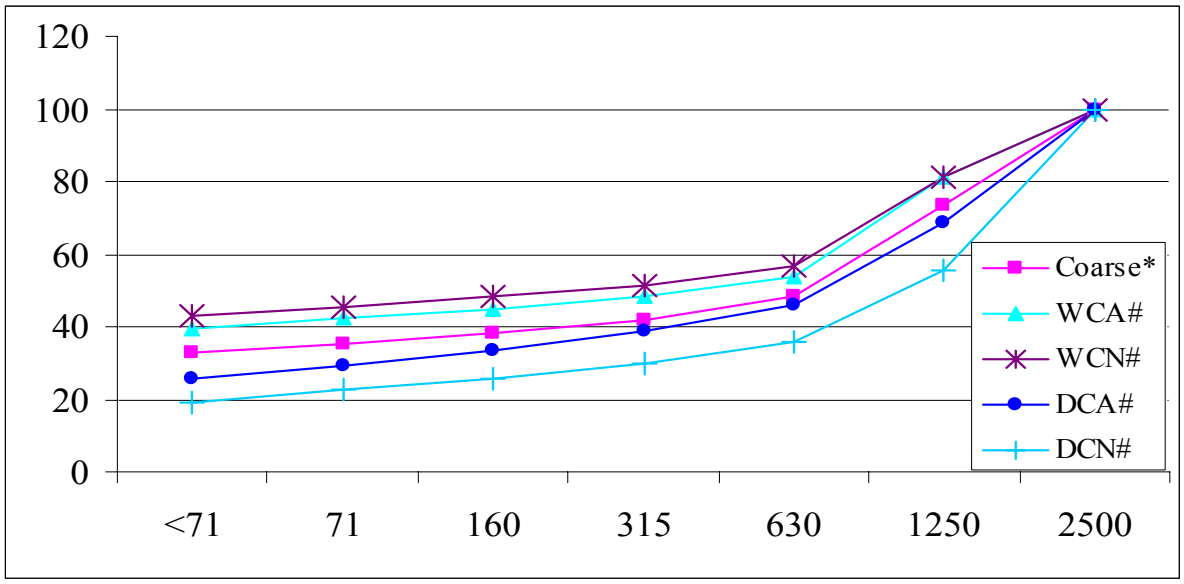
Table 3: Particle size distribution of different feeds during starter phase, week wise

Feed type / Treatments		Week 1		Week 2		Week 3	
		GMD	GSD	GMD	GSD	GMD	GSD
Dry sieving							
Offered	Coarse	1393.22	2.29	1393.22	2.29	1393.22	2.29
	Fine	722.88	2.45	722.88	2.45	722.88	2.45
Wet sieving							
Offered	Coarse	840.78	4.20	840.78	4.20	840.78	4.20
	Fine	336.34	4.11	336.34	4.11	336.34	4.11
Leftover	Dry acidified coarse	NA	NA	349.53	4.27	629.51	4.46
	Fine	237.66	3.58	214.37	3.34	227.38	3.49
	Dry nonacidified coarse	756.20	4.44	578.89	4.54	NA	NA
	Fine	232.80	3.50	229.13	3.45	243.10	3.57
	Wet acidified coarse	326.84	4.96	383.56	4.95	425.99	4.87
	Fine	181.48	3.65	202.92	3.75	192.78	3.76
	Wet nonacidified coarse	291.72	4.89	408.47	5.06	385.16	4.94
	Fine	178.44	3.68	194.19	3.72	189.03	3.72

*NA – not available

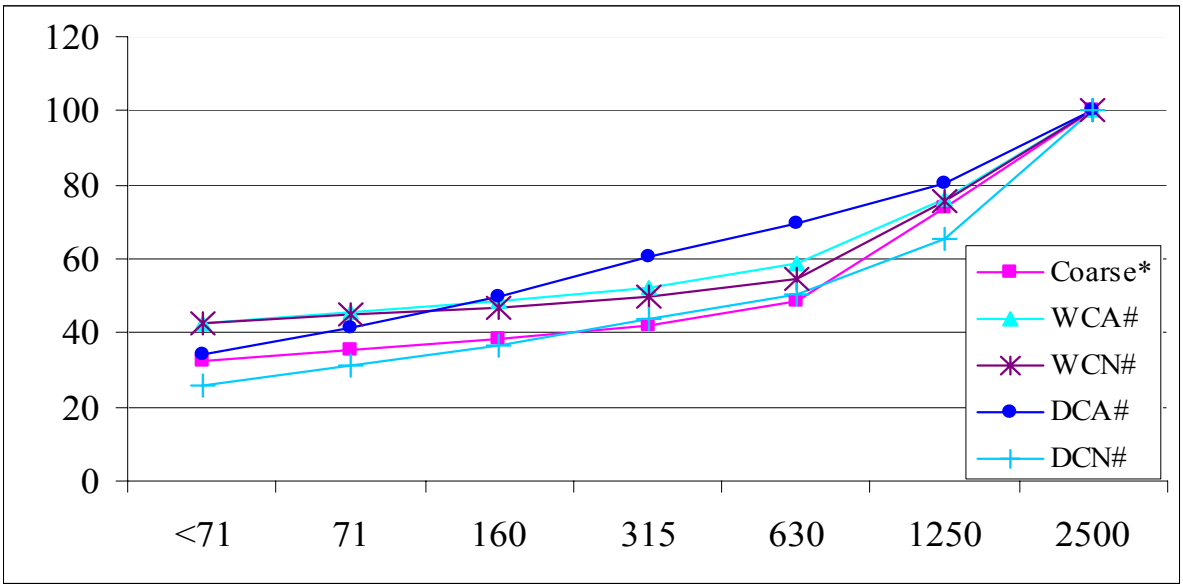
Wet sieving analysis of the leftover coarse diets was carried out to compare the particle size distribution of the coarse diets in wet and dry form before and after feeding. The results are presented in Figure 2, 3 and 4 for week 1, 2 and 3 respectively.

Figure 2: Particle size distribution of offered and leftover coarse diets in week 1 (wet sieving)



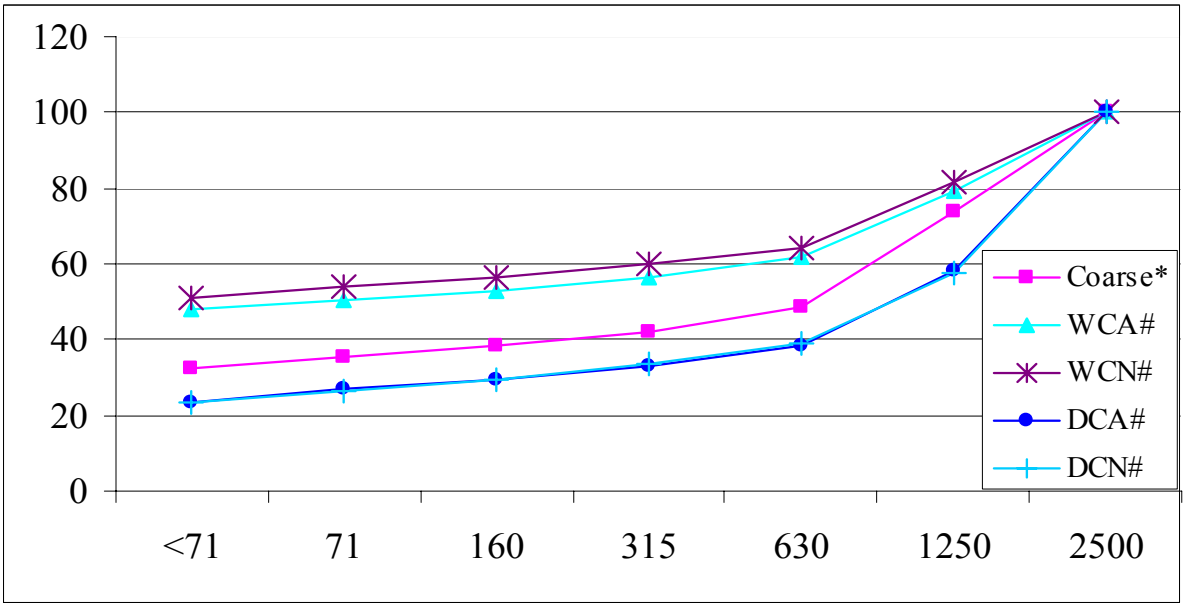
*Offered feed # Leftover feed

Figure 3: Particle size distribution of offered and leftover coarse diets in week 2 (wet sieving)



*Offered feed # Leftover feed

Figure 4: Particle size distribution of offered and leftover coarse diets in week 3 (wet sieving)



* Offered feed # Leftover feed

Starting at week 1, the birds tended to select the larger particles from wet feeds than from the dry feeds. It resulted in a lager amount of particles in wet feed which are in the range from 71 micro millimeters to 630 micro millimeters found in leftover feed. The same trend was found during week 2 and week 3 as well. Compared to the offered coarse feed, leftover dry coarse feeds were found to have the same particle size distribution.

4.2 PERFORMANCE AND GASTROINTESTINAL TRACT DEVELOPMENT OF BIRDS

Considering the different factors (conformation, structure and acidification) involved in the treatments (8 different combinations of these factors), data has been presented based on their treatment and their effects on the broilers performance. However, analysis has been centered towards the effect of different diet factors involved in the experiment as per the objective of this study. Similarly, results on starter and grower phase have been presented separately to have a clear idea on the effects of the diets and their carry over effects on the later stage of the broilers life. All diets were calculated on dry feed basis to give uniformity in comparison of wet and dry feed. The weights of the gastrointestinal organs are expressed as relative weight to body weight of the birds (g/100 g; as is) to have better comparative information.

Results of grower phase have been presented with the central idea if the performance of birds was affected by diet offered during that period only, or if there was carry over effects of diet fed during starter phase as well.

It is worth to mention here that different diets are presented with their initials (D- dry, W- wet, A- acidified, N- nonacidified, C- coarse and F- fine) and their combinations (i.e. DAF- Dry Acidified Fine) in the text of this thesis. Similarly, combination of three initials followed by dry or wet represents for the dry or wet diet offered in grower phase to the birds of particular initials diets fed bird during starter phase (i.e. DAFdry – dry feed offered during grower phase to the birds which were fed DAF diet during starter phase).

4.2.1 Starter phase

Overall performance of broiler was better in all the wet diet groups than the dry diet groups. However, the effect of diet structure and acidification had effect on some specific performances which is mentioned below in details.

4.2.1.1 Feed intake, body weight gain and feed conversion ratio

The effects of different diet factors on the Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers during starter phase of the experiment are presented in Table 4.

It can be seen from the results that the diet's conformation had effect on feed intake of broilers as wet-fed birds consumed significantly more feed (about 48%) than dry-fed birds. Among the wet feeding groups, highest FI was found in WAF diet group, while birds of DAF group consumed least feed during the whole starter phase. The diet structure and acidification did not affect FI of broilers significantly. However, there was significant interaction found between conformation and structure of diet, but not between other factors.

Table 4: Effect of different treatments on feed intake (g), body weight gain (g) and feed conversion ratio of birds during 0- 21 days (Mean ± SD).

Conformation	Acidification	Structure	FI	BWG	FCR
Dry	Acidified	Coarse	902 ± 57	560 ± 61	1.62 ± 0.09
		Fine	720 ± 74	401 ± 71	1.82 ± 0.16
	Non acidified	Coarse	847 ± 75	468 ± 99	1.85 ± 0.35
		Fine	737 ± 117	409 ± 112	1.85 ± 0.21
Wet	Acidified	Coarse	1139 ± 89	766 ± 109	1.50 ± 0.16
		Fine	1241 ± 51	925 ± 48	1.34 ± 0.02
	Non acidified	Coarse	1174 ± 45	830 ± 37	1.41 ± 0.02
		Fine	1192 ± 56	890 ± 124	1.50 ± 0.32
Probability level of contrast					
Conformation			< 0.001	<0.001	<0.001
Structure			0.133	0.997	0.668
Acidification			0.623	0.617	0.242
Conformation x Structure			0.001	0.001	0.357
Structure x Acidification			0.912	0.990	0.904
Acidification x Conformation			0.804	0.309	0.515

Weekly FI of birds in different diet treatments is presented in Figure 5. It can be seen from the Figure 5 that there was high increase in FI rate during week 3 among the wet-fed birds, while increment of FI of dry-fed birds remained almost the same throughout the whole period. On the other hand, FI was following similar trend during whole starter period, when structure and acidification factors were taken as a basis for analysis. It showed that there was no effect of these factors on FI of birds.

During the whole starter phase, body weight gain was highest in birds on WAF diet, followed by WNF diet groups. Nevertheless, it was not significantly different within wet feeding groups. Compared to birds fed dry diets, wet-fed birds showed the positive improvement of BWG by almost double, accounting 85 %. It was varying on different treatments, but it was not significantly different within dry or wet feeding groups. These findings gave a strong evidence of the effect of conformation of the diets on BWG. However, it was not significantly different due to structure and acidification of diet.

Similar to FI, there was highly significant interaction found between structure and conformation on BWG. However, it was not significant between other diet factors.

When BWG was analyzed on weekly basis, effect of diet conformation was quite distinct. There was high increase in the BWG of birds on wet diets while that was lower in the dry-fed birds. On the other hand, diet structure and acidification did not show any notable effect.

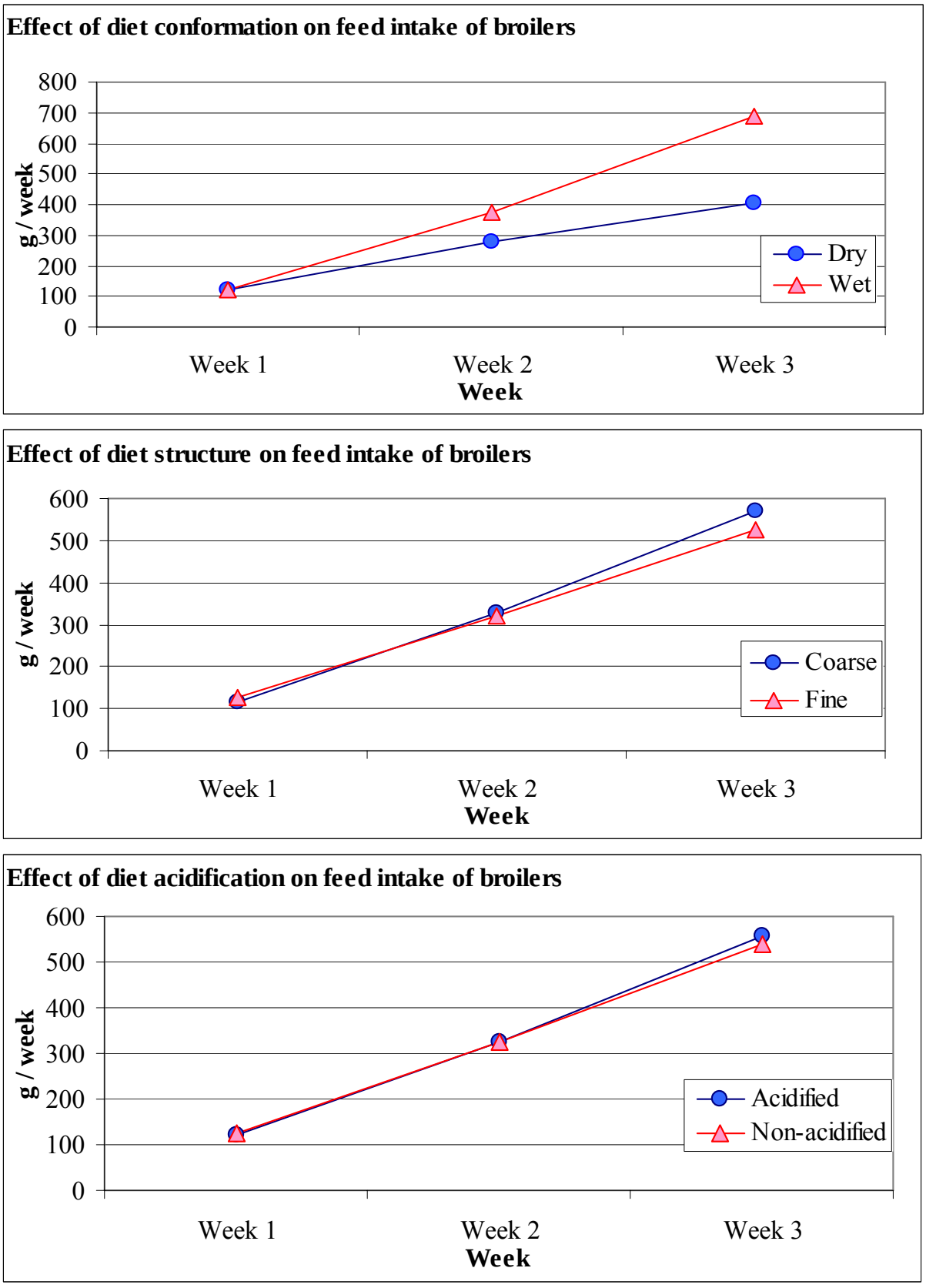


Figure 5 : Effect of main diet factors on feed intake in the starter phase, week wise

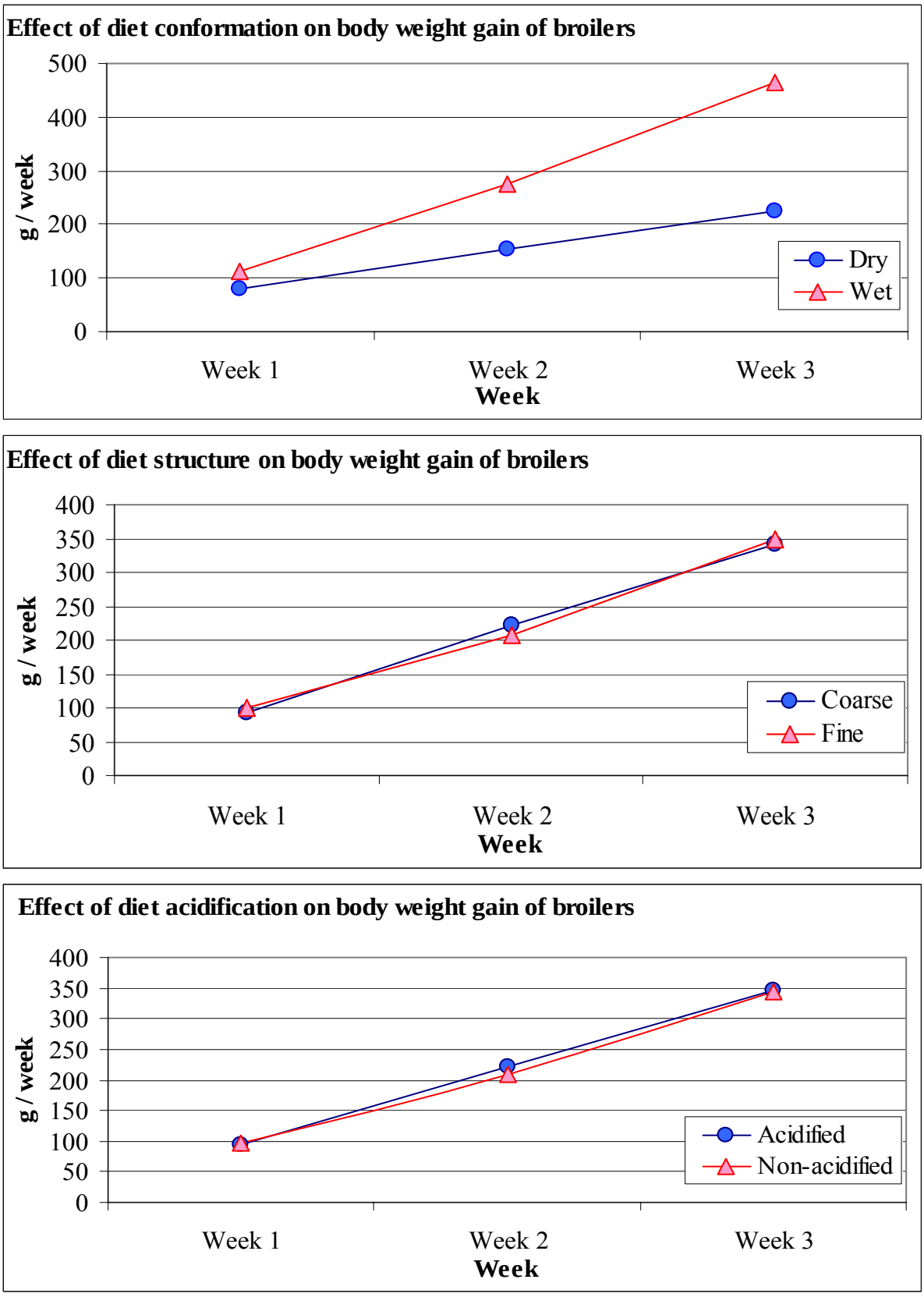


Figure 6 : Effect of main diet factors on body weight gain in the starter phase, week wise

The analysis of feed conversion ratio showed a similar trend compared to FI and BWG in different treatments. Conformation of diet had a significant effect on FCR; this trait was not found to have an effect due to diet structure and acidification. Similarly, there were no significant interaction found between any factors, unlike in FI and BWG.

WAF-fed birds showed the best performance by having the lowest FCR, followed by WNC-fed birds. This trend was little bit different compared to the FI and BWG of birds, where WAF-fed birds ranked second. The worst performance was noted in birds on DNC diet and DNF diet, which was also not similar to FI and BWG.

4.2.1.2 Water intake

There was a big difference found in feed intake and body weight of birds between different treatments. Thus, it was not logical to compare the water consumption of individual bird of different treatment. So, Water intake (WI) was interpreted from feed water intake ratio. Feed water intake ratio implies for water intake (ml) per gram of feed intake.

Feed water intake ratio of birds from two different ways is presented in Table 5. First, the WI from the nipple only (fresh water) was considered. Secondly, water mixed in the feed in addition to WI from nipple (total water) was also calculated as birds of wet feeding group consumed water from this route as well.

Table 5: Feed water intake ratio of birds in different treatments during starter phase (Mean ± SD)

Conformation	Acidification	Structure	F/W intake ratio (WI from nipple only)	F/W intake ratio (WI from nipple and wet feed)
Dry	Acidified	Coarse	0.61 ± 0.06	0.61 ± 0.06
		Fine	0.66 ± 0.09	0.66 ± 0.09
	Non acidified	Coarse	0.60 ± 0.04	0.60 ± 0.04
		Fine	0.62 ± 0.07	0.62 ± 0.07
Wet	Acidified	Coarse	1.13 ± 0.12	0.53 ± 0.03
		Fine	1.15 ± 0.12	0.53 ± 0.03
	Non acidified	Coarse	1.31 ± 0.07	0.56 ± 0.01
		Fine	1.25 ± 0.15	0.55 ± 0.03
Probability level of contrast				
Conformation			<0.001	<0.001
Structure			0.773	0.412
Acidification			0.110	1.000
Conformation x Structure			0.406	0.222
Structure x Acidification			0.434	0.537
Acidification x Conformation			0.021	0.139

There was almost half feed water intake ratio in the all dry groups, when only fresh water consumption was taken into account. It was as per our expectation that dry feeding groups

should consume more water than wet feeding birds. However, it was lower by approximately 21% in wet feeding groups when total water consumption was considered. The less WI was compensated from the water mixed with feed. Feed water intake ratio was not significantly different within dry and wet feeding groups. But, there was significantly higher WI in wet-fed birds, as affected by diet's conformation. However, no significant effect of diet structure and acidification was found on WI of birds.

4.2.1.3 Weights of gastrointestinal organs

For better comparison, fresh weights of different parts of GIT are presented in relative weight as g/100g body weight of birds, which is shown in Table 6. It can be seen from the table that the growth of all parts of the GIT had more or less similar trend as it was affected by diet treatments. Birds from treatment DNC had the biggest all GIT parts while WAF had the smallest among all the 8 treatments. When the effect of different diet factors were taken into account, there was varying results for different parts of GIT development. As a whole, relative weight of total GIT was significantly higher in dry fed birds by approximately 21% than wet-fed birds. Similarly, bigger GIT (about 10 %, as a whole) was found in the coarse-fed birds as compared to fine-fed birds.

Relative weight of the crop was influenced by the conformation of diet and was significantly higher in dry-fed birds compared to wet-fed birds. But there was no effect of structure and acidification found on development of these organs. Among the different diets, birds from DNC treatment had the highest crop weight, while WAF-fed birds had the lowest crop weight. Similarly, there was no interaction found between any of the three diet factors used in the experiment.

The diet's conformation and acidification had significant effect on the growth of the proventriculus, whereas the diet's structure showed no effect on this trait. Unlike in the crop, there was significant interaction between conformation and structure of the diets. However, there was no interaction found between any other factors.

As per our expectation, there was a significant difference in the gizzard weight of different treatments. It is interesting to note that there was effect of all the three diet factors; conformation, structure and acidification. Wet-fed birds had almost 37% bigger gizzard than dry-fed birds, the figure was 14 % higher in coarse-fed birds were compared to fine-fed birds. In contrast, there were no interactions among any dietary factors.

As combined effect of different diet factors, the gizzard was found to have biggest in DNC-fed birds, following similar trend of other parts of the GIT. The lowest gizzard weight was found in WAF-fed birds, which was almost half weight compared to DNC-fed birds. There were significant differences in the gizzard weight of different treatment groups of birds.

Similar to the other parts of the GIT, DNC-fed birds had the biggest duodenum followed by WNC and the lowest duodenum weight was found in WAF-fed birds. The diet factors, conformation and structure had significant effect on the development of the duodenum. However, no significant effect of acidification was found on the development of this part.

Table 6: Relative fresh organ weights of different parts of GIT (g/100g BW of birds) during starter phase (Mean ± SD)

Conformation	Acidification	Structure	Crop	Proventriculus	Gizzard	Duodenum*	Jejunum
Dry	Acidified	Coarse	0.32 ± 0.06	0.55 ± 0.04	2.89 ± 0.69	1.53 ± 0.19	1.76 ± 0.25
		Fine	0.32 ± 0.10	0.58 ± 0.06	3.01 ± 0.62	1.51 ± 0.24	1.79 ± 0.22
	Non acidified	Coarse	0.37 ± 0.09	0.58 ± 0.06	3.39 ± 0.56	1.61 ± 0.20	1.80 ± 0.17
		Fine	0.34 ± 0.05	0.57 ± 0.06	2.97 ± 0.47	1.53 ± 0.22	1.82 ± 0.10
Wet	Acidified	Coarse	0.28 ± 0.05	0.50 ± 0.07	2.46 ± 0.32	1.37 ± 0.20	1.77 ± 0.17
		Fine	0.27 ± 0.03	0.46 ± 0.03	1.77 ± 0.09	1.28 ± 0.09	1.50 ± 0.13
	Non acidified	Coarse	0.28 ± 0.05	0.57 ± 0.08	2.56 ± 0.28	1.54 ± 0.11	1.79 ± 0.12
		Fine	0.31 ± 0.06	0.48 ± 0.05	2.19 ± 0.17	1.30 ± 0.16	1.50 ± 0.19
Probability level of contrast							
Conformation			0.003	<0.001	<0.001	<0.001	0.001
Structure			0.698	0.083	0.002	0.030	0.011
Acidification			0.055	0.041	0.031	0.072	0.350
Conformation x Structure			0.497	0.018	0.127	0.208	0.001
Structure x Acidification			0.852	0.162	0.799	0.254	0.654
Acidification x Conformation			0.636	0.279	0.946	0.677	0.521

*Including pancreas

In contrast to the other parts, jejunum was found to have the heaviest in DNF-fed birds followed by DNC and DAF-fed birds. There was significant effect of conformation and structure on the growth of the jejunum, while acidification had no significant effect on development of this organ. There was an interaction found between diet conformation and structure but no interaction was found between other factors.

4.2.2 Grower Phase

In general, there was better performance of the wet-fed birds during the whole grower phase. It is interesting to note that the variations in the results during grower phase were affected by the different diets given during the starter phase. But it was found significantly different for BWG only. There was also significant interaction found between the factorial treatment (conformation) of both starter and grower phase on BWG but not on FI and FCR.

4.2.2.1 Feed intake, body weight gain and feed conversion ratio

The overall performance during the grower phase (Table 7) showed that birds on WNCwet diet achieved best performance compared to all other groups. It was associated with higher FI. However, FCR was found lowest in DNCwet, DAFwet and DNFwet-fed birds. On the other hand, dry-fed birds during grower phase, which previously received dry diets during starter phase performed very poor.

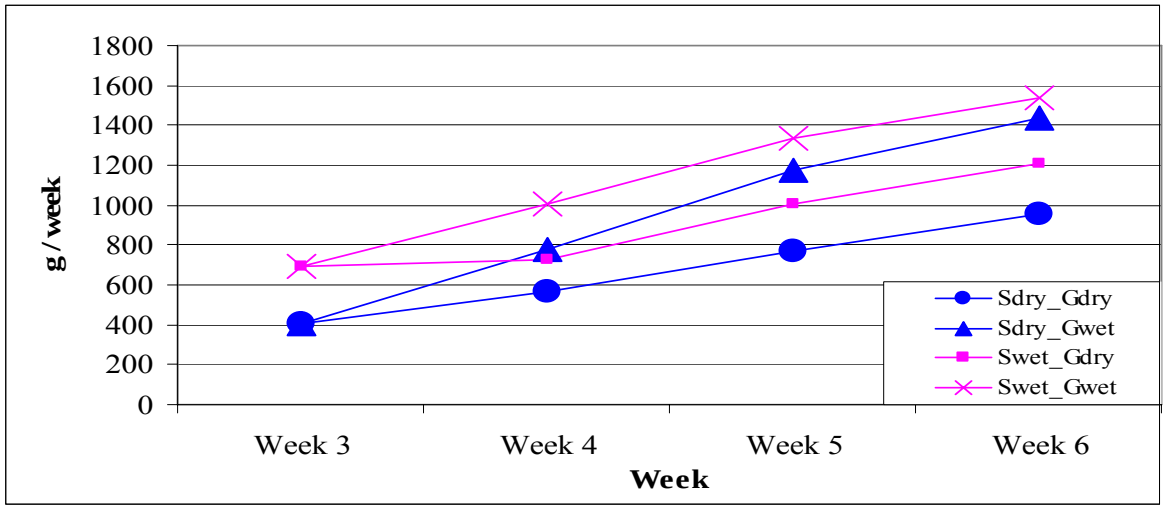
Feed intake was higher by about 39% in wet-fed birds as a whole during grower phase. Similar result was found on BWG (higher by about 86 %) and with better FCR. There was a significant influence of treatments in starter phase on FI, BWG and FCR during grower phase. It was mainly due to the carry over effect of diet conformation and structure. Nevertheless, starter phase structure had no carry over effect on the FCR.

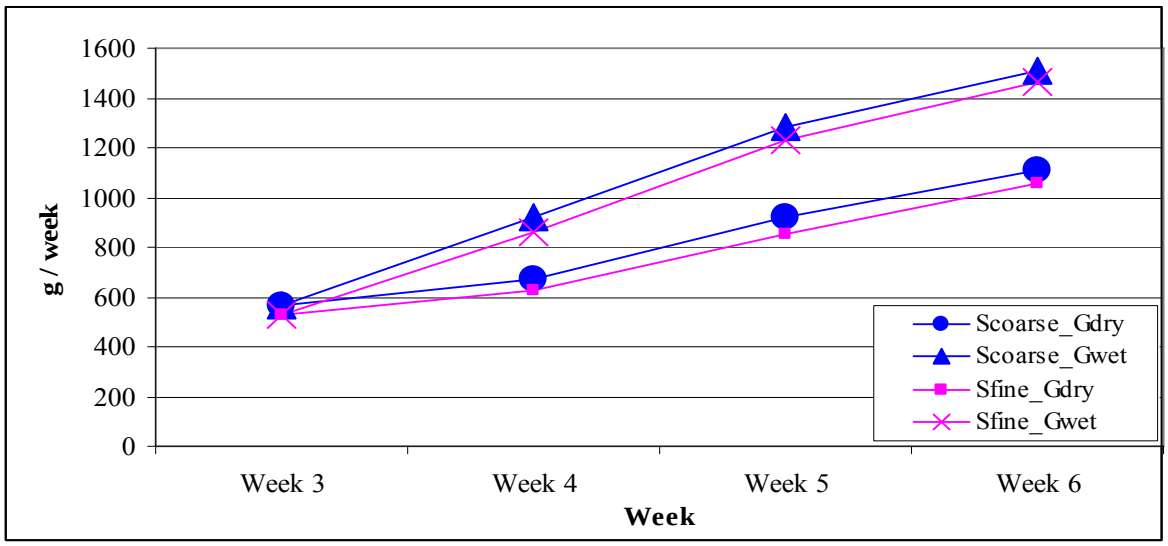
There was no significant interaction found between starter phase treatment and diet conformation of grower phase as a whole. But, there was interaction found between starter phase conformation and grower phase diets on BWG of birds.

When we analyze week wise FI and BWG of the birds in different groups (Figure 6), the trend of FI and growth were significantly different between diet factors. The wet-fed birds during starter phase, those remained receiving wet feed during grower phase, had decreased the growth trend whereas those switched to dry feed showed an increased growth rate during grower phase. However, birds with wet feed in grower phase and previously on dry feed in the starter phase had the highest BWG during the last week. That was significantly showing the carry over effect of the diet fed during starter phase. There was significant effect of the diet structure fed during starter phase on FI and BWG in the grower phase, unlike the starter phase. In contrast, no carry over effect of diet acidification was found on either performance during grower phase.

Table 7: Effect of different treatments on feed intake (g), bodyweight gain (g) and feed conversion ratio of birds during grower phase (Mean ± SD)

Starter diet			Grower diet	FI	BWG	FCR
Dry	Acidified	Coarse	Dry	2418 ± 194	1367 ± 65	1.77 ± 0.11
			Wet	3652 ± 169	2272 ± 130	1.61 ± 0.02
		Fine	Dry	2189 ± 287	1307 ± 152	1.67 ± 0.10
			Wet	3201 ± 177	2061 ± 66	1.55 ± 0.06
	Nonacidified	Coarse	Dry	2571 ± 189	1602 ± 122	1.61 ± 0.13
			Wet	3422 ± 263	2220 ± 123	1.54 ± 0.10
		Fine	Dry	2000 ± 431	1091 ± 320	1.87 ± 0.16
			Wet	3299 ± 497	2120 ± 223	1.55 ± 0.10
Wet	Acidified	Coarse	Dry	2808 ± 158	1523 ± 104	1.84 ± 0.03
			Wet	3857 ± 177	2187 ± 91	1.76 ± 0.04
		Fine	Dry	3050 ± 101	1598 ± 153	1.92 ± 0.04
			Wet	3842 ± 74	2191 ± 149	1.76 ± 0.14
	Nonacidified	Coarse	Dry	2991 ± 179	1661 ± 108	1.80 ± 0.12
			Wet	3939 ± 376	2305 ± 148	1.71 ± 0.03
		Fine	Dry	2905 ± 84	1603 ± 48	1.81 ± 0.06
			Wet	3859 ± 140	2145 ± 189	1.81 ± 0.10
Probability level of contrast						
Conformation starter				<0.001	<0.001	<0.001
Acidification starter				0.951	0.433	0.312
Structure starter				0.011	0.001	0.118
Conformation grower				<0.001	<0.001	<0.001
Conformation starter x Conformation grower				0.195	0.006	0.095
Acidification starter x Conformation grower				0.943	0.786	0.851
Structure starter x Conformation grower				0.961	0.776	0.290





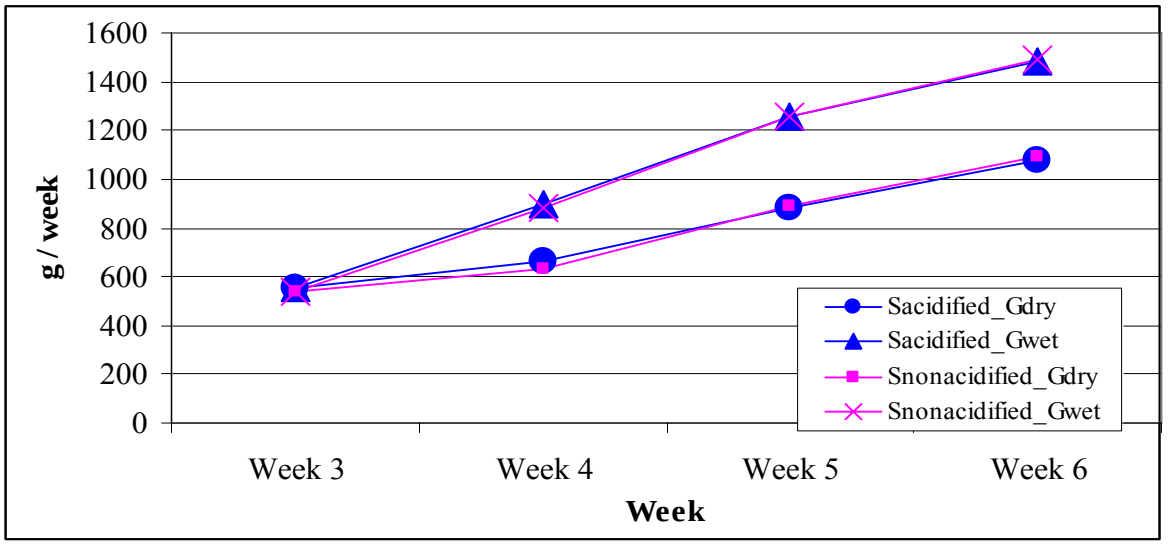
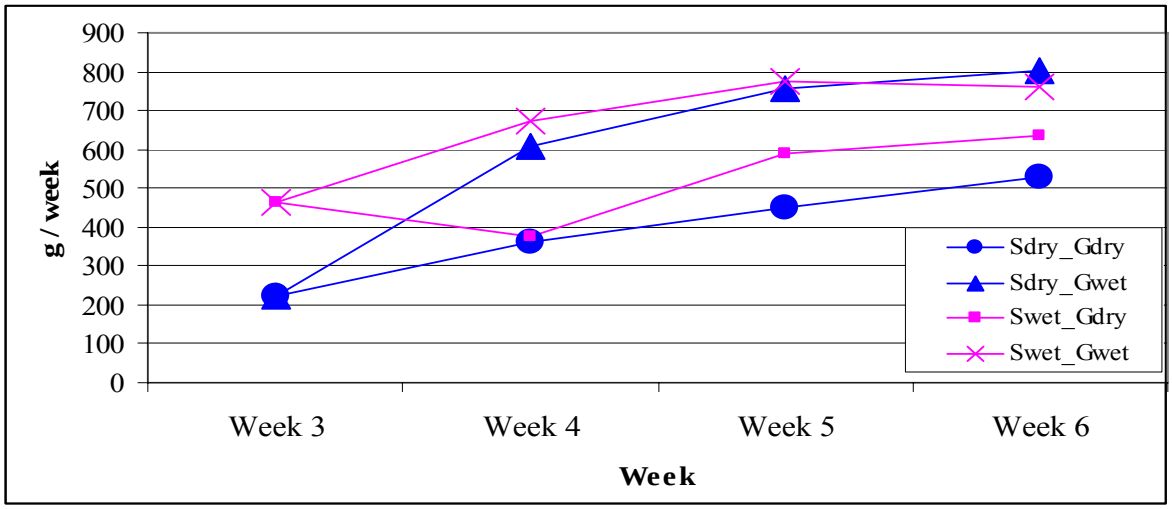
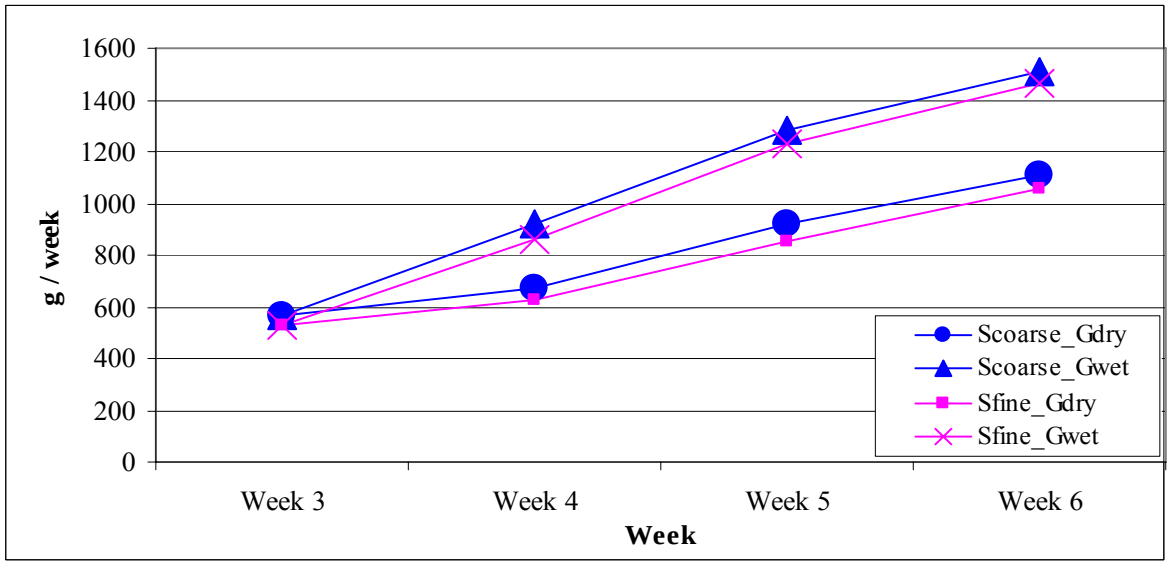


Figure 7 : Effect of main diet factors on feed intake in the grower phase, week wise





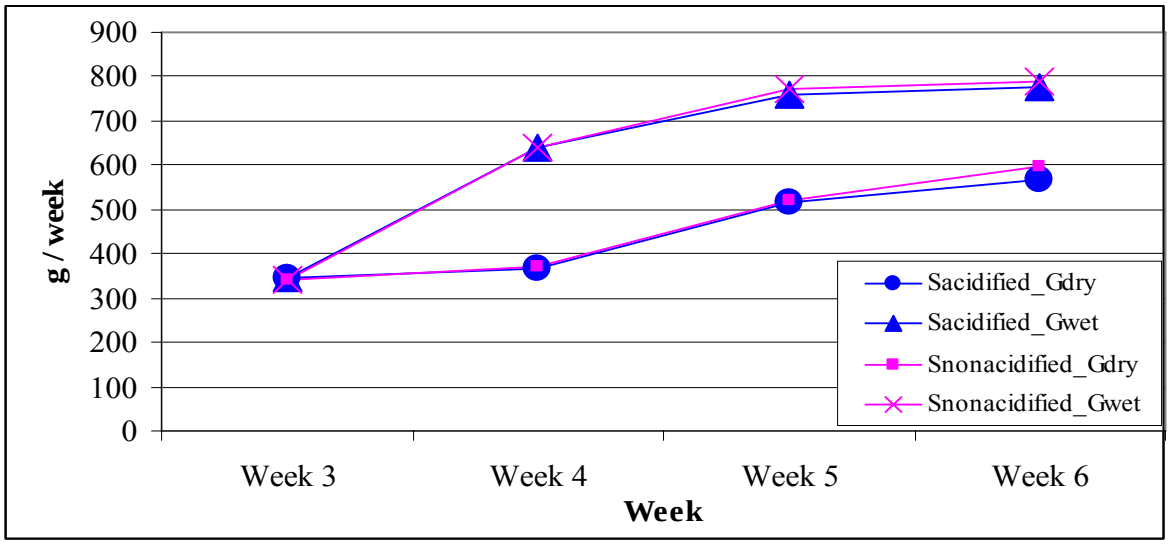


Figure 8 : Effect of main diet factors on body weight gain in the grower phase, week wise

4.2.2.2 Water intake

It can be seen from the Table 8 below that feed water intake ratio was significantly lower in the dry feed group when only fresh WI was considered. But figure of result was in reverse order when total WI was taken into account. However, it was not significantly different. Carry over effects of starter phase diet conformation was found on the Feed water intake ratio during grower phase. Similarly, no interaction was found between any factors of starter phase treatments and grower phase diets.

Table 8: Feed water intake ratio of birds in different treatments during grower phase* (Mean ± SD)

Starter diet			Grower diet	F/W intake ratio (WI from nipple only)	F/W intake ratio (WI from nipple and wet feed)
Dry	Acidified	Coarse	Dry	0.56 ± 0.04	0.56 ± 0.04
			Wet	1.24 ± 0.02	0.55 ± 0.00
		Fine	Dry	0.59 ± 0.03	0.59 ± 0.03
			Wet	1.38 ± 0.04	0.58 ± 0.01
	Nonacidified	Coarse	Dry	0.53 ± 0.04	0.53 ± 0.04
			Wet	1.31 ± NA	0.57 ± NA
		Fine	Dry	0.50 ± 0.08	0.50 ± 0.08
			Wet	1.31 ± 0.01	0.57 ± 0.00
Wet	Acidified	Coarse	Dry	0.59 ± 0.02	0.59 ± 0.02
			Wet	1.28 ± 0.04	0.56 ± 0.01
		Fine	Dry	0.57 ± 0.04	0.57 ± 0.04
			Wet	1.15 ± NA	0.54 ± NA
	Nonacidified	Coarse	Dry	0.55 ± 0.09	0.55 ± 0.09
			Wet	1.37 ± 0.01	0.58 ± 0.00
		Fine	Dry	0.58 ± 0.00	0.58 ± 0.00
			Wet	1.01 ± 0.12	0.50 ± 0.03
Probability level of contrast					
Conformation starter				0.009	0.042
Acidification starter				0.388	0.396
Structure starter				0.363	0.840
Conformation grower				<0.001	0.310
Conformation starter x Conformation grower				0.473	0.107
Acidification starter x Conformation grower				0.486	0.432
Structure starter x Conformation grower				0.353	0.823

*week 5 and 6 only, NA - not available

4.2.2.3 Weights of gastrointestinal organs

Growth of the different parts of GIT had mixed effect of the diet conformation during the grower phase and the carry over effect of the starter phase diets. It can be seen from Table 9 that growth of some parts was affected by starter phase diets, while other did not have such effect. Fresh weight of the GIT organs as a whole was influenced by wet feeding, while relative weight of GIT as a whole was higher by approximately 11 % in dry-fed birds.

There was significant effect grower diet conformation on growth of gizzard, duodenum and jejunum. These organs were 14%, 11% and 8 % bigger in dry-fed birds respectively. In contract, there was no significant effect of diet conformation on the development of crop and proventriculus. On the other hand, there was significant carry over effect of starter phase conformation found on the development of all organs of GIT taken in to account during this study. However, acidification showed carry over effect only on proventriculus growth. In contrast, structure of starter phase diet did not show effect on growth of any GIT organs. There was no interaction found between any factor of starter phase treatments and grower diet on development of GIT, except gizzard where interaction was found between diet conformation of both starter and grower phase.

Table 9: Relative fresh weights of different organs of GIT (g/100g BW) during grower phase (Mean ± SD)

Starter phase			Grower diet	Crop	Proventriculus	Gizzard	Duodenum*	Jejunum
Dry	Acidified	Coarse	Dry	0.25 ± 0.04	0.28 ± 0.04	1.76 ± 0.35	0.97 ± 0.15	1.32 ± 0.22
			Wet	0.24 ± 0.04	0.26 ± 0.05	1.35 ± 0.09	0.91 ± 0.21	1.11 ± 0.18
		Fine	Dry	0.25 ± 0.03	0.32 ± 0.07	2.14 ± 0.66	1.07 ± 0.10	1.42 ± 0.10
			Wet	0.25 ± 0.05	0.26 ± 0.05	1.41 ± 0.23	0.97 ± 0.06	1.43 ± 0.12
	Nonacidified	Coarse	Dry	0.23 ± 0.05	0.33 ± 0.01	1.93 ± 0.26	0.92 ± 0.02	1.31 ± 0.16
			Wet	0.24 ± 0.05	0.30 ± 0.02	1.36 ± 0.13	0.85 ± 0.08	1.23 ± 0.17
		Fine	Dry	0.27 ± 0.04	0.34 ± 0.07	2.02 ± 0.63	1.01 ± 0.17	1.43 ± 0.29
			Wet	0.26 ± 0.06	0.29 ± 0.05	1.25 ± 0.22	0.83 ± 0.04	1.28 ± 0.17
Wet	Acidified	Coarse	Dry	0.21 ± 0.03	0.25 ± 0.02	1.51 ± 0.19	0.93 ± 0.05	1.39 ± 0.04
			Wet	0.23 ± 0.04	0.28 ± 0.03	1.25 ± 0.08	0.83 ± 0.07	1.13 ± 0.11
		Fine	Dry	0.24 ± 0.04	0.26 ± 0.02	1.36 ± 0.10	0.78 ± 0.10	1.10 ± 0.06
			Wet	0.25 ± 0.03	0.24 ± 0.03	1.28 ± 0.19	0.80 ± 0.07	1.17 ± 0.13
	Nonacidified	Coarse	Dry	0.26 ± 0.04	0.26 ± 0.02	1.60 ± 0.19	0.99 ± 0.08	1.29 ± 0.07
			Wet	0.20 ± 0.01	0.24 ± 0.03	1.27 ± 0.22	0.81 ± 0.11	1.17 ± 0.06
		Fine	Dry	0.22 ± 0.02	0.26 ± 0.02	1.46 ± 0.07	0.79 ± 0.06	1.13 ± 0.09
			Wet	0.22 ± 0.07	0.31 ± 0.06	1.18 ± 0.14	0.75 ± 0.06	1.07 ± 0.08

Probability level of contrast					
Conformation starter	0.020	0.001	<0.001	<0.001	<0.001
Acidification starter	0.951	0.029	0.784	0.313	0.722
Structure starter	0.200	0.390	0.656	0.459	0.539
Conformation grower	0.390	0.204	<0.001	<0.001	0.005
Conformation starter x Conformation grower	0.853	0.051	0.005	0.337	0.684
Acidification starter x Conformation grower	0.425	0.882	0.298	0.156	0.762
Structure starter x Conformation grower	0.758	0.495	0.384	0.824	0.086

***** including pancreas

5. DISCUSSION

Numerous studies (Reece *et al.*, 1985; Rogel *et al.*, 1987; Cumming, 1994; Munt *et al.*, 1995; Kwakkel *et al.*, 1997; Hetland and Svihus, 2001; Engberg *et al.*, 2002; Gabriel *et al.*, 2003; Dahike *et al.*, 2003 Nir et al, 1990; Nir et al., 1994; Nir *et al.*, 1995; Proudfoot and Hulan, 1989; Yalda and Forbes, 1995; Yalda and Forbes, 1996; Slade and Forbes, 1997; Yalda and Forbes, 1999; Preston et al. 2000; Versteegh and Jongbloed, 1999) have been published for optimization of broiler performances through different diets. Among them, several have made concluding remarks with the benefits of coarse diets, acidified diets and wet diets feeding respectively. Moreover, these studies have shown some variation in results, mainly due to differences in experimental conditions. But there are no studies reported which simultaneously compare combination of all these three factors (coarse *vs* fine feed, acidified *vs* nonacidified feed and wet *vs* dry feed), which is presented in this study.

There was no control group in this experiment which exactly followed the commercial management recommended for the broiler strain used in this experiment. Considering the need for future broiler feeding strategy, lighting hours and other management factors were arranged as per the interest and objectives of this study, which has given some interesting findings.

Generally, the influence of feed conformation (wet versus dry feeding) on different parameters was found to be much greater than that of diet structure and acidification. The effect of particle size was varying on different parameters, showing small or no effect on some parameters and showing large effects on the gizzard development. Similarly, effect of acidification of the diet was non visible in almost all parameters of this study, except on the development of the gizzard and proventriculus.

5.1 FEED INTAKE, BODY WEIGHT GAIN AND FEED CONVERSION RATIO

Wet feeding improved broiler growth rate during both starter and grower phase, which was associated with increased FI and better feed conversion ratio. It was in accordance with the other authors (Pittard, 1996; Yalda and Forbes, 1995; Yalda and Forbes, 1996; Slade and Forbes, 1997), which might be due to better nutrient retention (Yalda and Forbes, 1995). The advantages of increased retention of DM and protein with wet feeding can either be exploited by feeding conventional diets and expecting to see improved conversion efficiency, or by reducing the cost of the diet by reducing its content of digestible nutrients (Forbes, 2001). In this study, wetting of diet significantly increased body weight gain and lowered FCR in relation with the FI. This was in accordance with the finding of Yasar and Forbes (1999).

Yasar and Forbes (1999) reported that wet feeding significantly increased feed intake, total water intake, body weight gain of broiler chickens, without improvements in food conversion efficiency. However, this study had some different results. In addition to FI, total WI, BWG and FCR was also found better in the wet feeding birds, in both starter and grower phase. Later, they (Yasar and Forbes, 2000) interpreted this by correcting feed conversion efficiency (FCE) for difference in body weight and suggested that wet feeding increased FCE,

compared with dry feeding, when equivalent period of growth was compared, which supports the finding of this study as well.

The mechanism of the beneficial effects of wet feeding could be attributed to the decreased viscosity of gut contents; the greater the development of the layer of the villi in the digestive segments and the reduced crypt cell proliferation rate in the crypts of the epithelium (Yasar and Forbes, 1999). Adding water has a positive effect on solubilisation of dietary components. Given the very rapid transit of feed particularly in broilers, this early solubilisation gives more time for absorption to take place. Moreover, wetting feed increases a more rapid penetration of digestive juices (Yasar and Forbes, 2001), rendering the feed more digestible. This allows the actual digestibility of the feed to approach more closely to the potential digestibility that would be achieved if the feed stayed longer in the GI tract (Forbes, 2003).

Similarly, Preston *et al.,* (2000) working with dry and wet mash feed found that birds with wet mash has higher FI and FCR, with almost 50% higher BWG compared to birds fed with dry mash. Outperforming growth (about 85%) was found in this study. However, the rate of growth was in changing trend, when broilers became older (Figure 6 and 8). It was mainly due to the carry over effect of the diet fed during starter phase of the experiment. Moreover, wet-fed birds were attaining their genetic potential of weight gain.

One of the striking aspects of this study was the poor performance of birds fed on dry mash diet, which was similar with the finding of Preston *et al*. (2000). Poor performance of the birds fed with dry-mass diet was mainly due to low FI. It was supported by the study of Scott (2002), who found that broilers cannot eat enough dry diet to attain their genetic potential for growth. There are several factors influencing FI like breed, management, metabolic needs of the broilers and the diet itself. The large effect in the present study compared with other studies mentioned elsewhere may be due to the lighting schedule of this experiment. Feeds offered in this trail were based on *ad libitum* meal feeding with 18 hr light and 6 hours dark during the starter phase, making less time available for the birds to access the feeders. However, birds on wet feed can eat faster and more in limited time than the birds on dry feed could do. This finding was in line with Nielsen (2004) who mentioned an increased FI when offered bulky food containing non-nutritive materials or due to time restricted feeding. Although, he used sand as nonnutritive material and increasing the bulkiness of feed, while water was used in this study.

In this study, the birds with wet diets could consume more feed compared to dry feed groups. One reason may be due to the bulky nature of feed. On the other hand, swallowing of wet diet is faster in crop, thus higher FI can be expected in given limited time (light) for eating. It was supported by Savory (1976), who found that broilers kept on a lighting schedule that includes periods of dusk and darkness will increase their feeding activity during the dusk in order to fill their crop with feed, which can then be digested during the period of darkness. As, less time and energy are required in eating and digestion process of wet feed. This will lead to a better growth and FCR. In contrast, dry-fed birds requires more time and energy for feed intake and digestion, thus devote less food energy to growth leading to poor growth and FCR. It seems more likely that extra time was required for the dry feed to become hydrated

in the gut and facilitate digestion and passage through the gut, resulting to less FI of feed among the dry-fed birds.

The wet-fed birds gained body weight significantly more efficiently during the whole study period (0-42 days), which was similar with the report of Yalda and Forbes (1999). Carry over effect of diet fed during starter phase was found during the grower phase in this study, which was similar with the finding of Yalda and Forbes (1995). They found that the beneficial effects of wet feeding from 28 to 42 days persisted for two weeks after return to dry feeding, *i.e.* 56 days. The increased crop and intestine weight tended to persist and presumably allowed the improvement in feed utilization to continue after dry feeding was resumed.

There was effect of selective feeding behavior of birds seen, affecting the feed intake and performance of birds as well. Starting at week 1, the birds tended to select the larger particles from wet feeds than from the dry feeds. It resulted in a lager amount of particles in wet feed which are in the range from 71 micro millimeters to 630 micro millimeters found in leftover feed. The same trend was found during week 2 and week 3 as well. Compared to the offered coarse feed, leftover dry coarse feeds were found to have the same particle size distribution. This indicated that there was no selection of feeds by birds when fed dry coarse diets. In wet diets, the selection of diets might be due to the different physical characteristic of the particles in different sizes when soaked in water; this phenomenon resulted in the segregation of particles during the feeding time and it made the larger particles easily accessible to the birds, leading to the insufficient nutrient balance intake. It might be one of the reasons of better performance found in wet fine fed birds during the starter period compared to the wet fine fed birds.

However, the results were not that much difference due to the effect of particle size distribution and acidification of the diet, as reported by some previous workers.

There was no significant effect of diet structure found on the FI and growth of the birds, which contradicts with the finding of several workers (Nir *et al.*, 1990; Nir *et al.*, 1994; Proudfoot and Hulan, 1989). These workers found that FI and BWG improved positively with particle size. It may be due to the effect of other dominant factor (conformation of diet) involved in this experiment. There was significant interaction in between conformation and structure of diet as well, which might affect this finding. On the other hand, some of these studies were done in different circumstance and feed were in the pellet form, which uses very fine particles making big difference between coarse and fine particle size distribution. However, mash feed was used in this study. Moreover, selected feeding was found in birds (Table 3), which reduced the difference of particle size at feed intake level. It might be due to the selection behavior of birds that birds have difficulty for eating particles that are bigger or much smaller than the size of the beak (Moran, 1982, cited by Dahike, 2003).

We were not able to get the positive result from the acidification of diet, which was against our expectation. The information sheet of Calprona AL® (Anonymus, 2004), the acidifier used in this experiment, suggests that lactic acid stimulates digestibility and FI by stimulating the secretion of pancreatic enzymes. This claim was supported by trail conducted by Research Institute for Animal Husbandry, the Netherlands (Anonymus, 2005b), where better

growth and feed conversion ratio with little mortality both in starter and grower period was found in the broiler by using dry feed acidified with Calprona AL®. In another field trial in Brazil (Anonymus, 2003), it was found that Calprona AL® has by far outperformed the probiotic in growth and feed conversion ratio. In contrast, we did not find any significant effect of the acidification on FI of birds, which is in the line with the finding of Gentle (1971).

Studies showed that acidification of diets may have favorable effect on poultry production by creating healthy gut environment (Heres *et al.*, 2003; Heres *et al.*, 2004; Iba & Berchieri, 1995; Berchieri and Barrrow, 1996; Thompson and Hinton, 1997), rather than directly increasing the FI. Thus, this study remained inconclusive on the effect of acidification of feed, as GIT microbiology and pathology was beyond the scope of this study.

5.2 WATER INTAKE

Although fresh WI (water intake from nipple only) was reduced in birds given wet feed, total WI (from the nipple plus that from the wet feed) were significantly higher in wet-fed birds than dry-fed birds. Similarly, DM intake was higher in wet-fed birds. These were in accordance with findings of Yasar and Forbes (1999). However, ratio of total water to dry feed was also significantly different in both feeding regimens during starter phase, which was not in line with the finding of Yasar and Forbes (1999). In contrast, ratio of total water to dry feed was not significantly different during grower phase, supporting the report of Yasar and Forbes (1999). It was related with the FI and BWG. Water consumption is influenced by several factors, including size and age of birds and type and amount of feed consumed. Higher FI leading to higher body growth requires more water to maintain the normal physiology (Duke, 1986). Water is essential for the digestion and metabolism of food and the voluntary intake of water are usually closely related to dry matter in many species, including poultry (Patrick and Ferrise, 1962). It is supported by the data of present study, when we interpret from the feed water intake ratio, which was lower in the entire wet feeding group. There was no effect of diet structure and acidification found on the WI of either group of birds.

5.3 WEIGHTS OF GASTROINTESTINAL TRACT

The GIT development had mixed result due to the wet feeding of birds, which is not in line with the finding of Yalda and Forbes (1996). They reported that broiler chickens fed on commercial pelleted food mixed with water had increased weights of liver, crop, proventriculus and small intestine, compared to those fed on the same food without water addition. It was almost reverse in this study. It may be due to the effect of diet type as they used pelleted feed which uses very fine particles, while mash feed was used in this study. Empty fresh weight of individual digestive segments tended to increase with wet feeding, but no significant difference with dry fed birds was found in the study of Yasar and Forbes (2000). However, there was significant effect of diet conformation found in this study. It might be due to the interaction effect of diet conformation and structure, which was significant for growth of different organs (Tables 5 and 8).

On the other hand, there was a significant effect of diet structure and acidification found on the development of the GIT organs, which was in accordance with other workers. There was bigger gizzard in birds fed with coarse diet compared to birds on fine diet. Findings of several other workers (Reece *et al.*, 1985; Rogel *et al.*, 1987; Cumming, 1994; Munt *et al.*, 1995; Nir *et al.*, 1995; Kwakkel *et al*., 1997; Preston *et al.*, 2000; Hetland and Svihus, 2001; Engberg *et al*., 2002; Gabriel *et al*., 2003; Dahike *et al*., 2003) were also in the same line, showing positive correlation of feed particle size and development of the gizzard in broilers.

Size of duodenum and jejunum in birds fed with fine diet were bigger compared to coarse diet groups, which is similar with the finding of other workers like Gabriel *et al*., 2003; Dahike *et al*., 2003. However, effect of diet structure was not prominent in the development of proventriculus, unlike reported by Gabriel *et al*. (2003). Similarly, acidification did not show significant effect on growth of duodenum, which is in contrast with the finding of Bosch *et al*. (2006). In addition, weight gain of these organs was highly affected by the conformation of diet. Birds with dry diet had bigger proventriculus, duodenum and jejunum during both starter and grower phase. However it was influenced by the diet of starter phase as well.

The differences observed in the GIT development imply that texture had an effect on the rate of the feed's passage through it. In other studies (Nir and Ptichi, 2001), it was shown that the passage rate of large particles through the gizzard of young chicken is slower than that of small particles. The slower passage of the feed, resulting from coarse-mash feeding, is not accompanied by a reduction in FI. It was proposed (Nir and Ptichi, 2001) that when fed a fine mash, feed flows through the stomachs to the duodenum and small intestine. This phenomenon was accompanied by marked atrophy of the gizzard, mild hypertrophy of the small intestine. However, this study does not fully support this argument, as there was not much differences in some fine fed birds as well. It seems that the development in the some fine-diet-fed birds were also big. It may be due to the effect of the wet feeding, which has a visible effect on the growth of the GIT.

6. CONCLUSIONS AND RECOMMENDATIONS

Overall performance of broiler was better in all the wet diet groups than the dry diet groups. There was better growth in wet fed birds associated with higher FI and lower FCR during whole broiler production cycle, even in limited time of feeding. Structure and acidification of diet has mixed effect on performance and GIT development of broilers, making inconclusive from this study.

There was carry over effect of starter phase diet conformation on overall performance and GIT development of broilers during grower phase, while that of diet structure was limited to FI and BWG, with highest bodyweight gain in wet and coarse diet fed birds. Thus, coarse diet in wet form during the starter phase and wet fine diets during grower phase may be better feeding strategy.

Wet feeding of diet has promising effect on the performance and GIT development of broilers. However, other aspects of wet feeding like water mixing labor and hygiene should be taken into account. Further studies need to be carried out to investigate the mechanism of wet feeding to the improvement of feed intake and functional development of GIT. Similarly, the interaction effect between the diet structure and conformation needs to be studied.

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8. APPENDIXES

Appendix 1: Plan of the experimental design in starter phase

Replicate 1	Replicate 2	Replicate 3	Replicate 4
Pen No. 1 **Wet-acidified-coarse**	Pen No. 9 **Dry-nonacidified-coarse**	Pen No. 17 **Dry-nonacidified-fine**	Pen No. 25 **Dry-nonacidified-coarse**
Pen No. 2 **Dry-acidified-fine**	Pen No. 10 **Wet-acidified-coarse**	Pen No. 18 **Wet-nonacidified-coarse**	Pen No. 26 **Dry-acidified-fine**
Pen No. 3 **Wet-nonacidified-coarse**	Pen No. 11 **Dry-acidified-fine**	Pen No. 19 **Dry-acidified-coarse**	Pen No. 27 **Wet-nonacidified-coarse**
Pen No. 4 **Dry-nonacidified-fine**	Pen No. 12 **Wet-nonacidified-coarse**	Pen No. 20 **Wet-acidified-coarse**	Pen No. 28 **Dry-acidified-coarse**
Pen No. 5 **Wet-acidified-fine**	Pen No. 13 **Wet-acidified-fine**	Pen No. 21 **Dry-acidified-fine**	Pen No. 29 **Dry-nonacidified-fine**
Pen No. 6 **Dry-nonacidified-coarse**	Pen No. 14 **Dry-nonacidified-fine**	Pen No. 22 **Dry-nonacidified-coarse**	Pen No. 30 **Wet-nonacidified-fine**
Pen No. 7 **Wet-nonacidified-fine**	Pen No. 15 **Dry-acidified-coarse**	Pen No. 23 **Wet-acidified-fine**	Pen No. 31 **Wet-acidified-coarse**
Pen No. 8 **Dry-acidified-coarse**	Pen No. 16 **Wet-nonacidified-fine**	Pen No. 24 **Wet-nonacidified-fine**	Pen No. **32** **Wet-acidified-fine**

Appendix 2: Plan of the experimental design in grower phase

Pen 1 DRY	Pen 16 WET	Pen 17 DRY	Pen 32 WET	Pen 33 DRY	Pen 48 WET	Pen 49 DRY	Pen 64 WET
Pen 2 WET	Pen 15 DRY	Pen 18 WET	Pen 31 DRY	Pen 34 WET	Pen 47 DRY	Pen 50 WET	Pen 63 DRY
Pen 3 DRY	Pen 14 WET	Pen 19 DRY	Pen 30 WET	Pen 35 DRY	Pen 46 WET	Pen 51 DRY	Pen 62 WET
Pen 4 WET	Pen 13 DRY	Pen 20 WET	Pen 29 DRY	Pen 36 WET	Pen 45 DRY	Pen 52 WET	Pen 61 DRY
Pen 5 DRY	Pen 12 WET	Pen 21 DRY	Pen 28 WET	Pen 37 DRY	Pen 44 WET	Pen 53 DRY	Pen 60 WET
Pen 6 WET	Pen 11 DRY	Pen 22 WET	Pen 27 DRY	Pen 38 WET	Pen 43 DRY	Pen 54 WET	Pen 59 DRY
Pen 7 DRY	Pen 10 WET	Pen 23 DRY	Pen 26 WET	Pen 39 DRY	Pen 42 WET	Pen 55 DRY	Pen 58 WET
Pen 8 WET	Pen 9 DRY	Pen 24 WET	Pen 25 DRY	Pen 40 WET	Pen 41 DRY	Pen 56 WET	Pen 57 DRY

Appendix 3: Nutrient requirements of broilers as percent or units per kilogram of diet (90% dry matter)* (NRC, 1994)

Nutrient	Unit	Age		
		0-3 weeks	3-6 weeks	6-8 weeks
Protein and amino acids				
Crude protein[#],	%	23.00	20.00	18.00
Arginine	%	1.25	1.10	1.00
Glycine +serine	%	1.25	1.14	0.97
Histidine	%	0.35	0.32	0.27
Isoleucine	%	0.80	0.73	0.62
Lysine	%	1.10	1.00	0.85
Methionine	%	0.50	0.38	0.32
Methionine + cystine	%	0.90	0.72	0.60
Phenylalnine	%	0.72	0.65	0.56
Phenylalnine + tyrosine	%	1.34	1.22	1.04
Proline	%	0.60	0.55	0.46
Threonine	%	0.80	0.74	0.68
Tryptophan	%	0.20	0.18	0.16
Valine	%	0.90	0.82	0.70
Fat				
Linoleic acid	%	1.00	1.00	1.00
Macro-minerals				
Calcium[+]	%	1.00	0.90	0.80
Chlorine	%	0.20	0.15	0.12
Magnesium	mg	600.00	600.00	600.00
Non-phytate phosphorus	%	0.45	0.35	0.30
Potassium	%	0.30	0.30	0.30
Sodium	%	0.20	0.15	0.12
Trace minerals				
Copper	mg/kg	8.00	8.00	8.00
Iodine	mg/kg	0.35	0.35	0.35
Iron	mg/kg	80.00	80.00	80.00
Manganese	mg/kg	60.00	60.00	60.00
Selenium	mg/kg	0.15	0.15	0.15
Zinc	mg/kg	40.00	40.00	40.00
Fat soluble vitamins				
A	IU	1,500	1,500	1,500
D_3	ICU	200	200	200
E	IU	10	10	10
K	mg/kg	0.50	0.50	0.50
Water soluble vitamins				
B_{12}	mg/kg	0.01	0.01	0.007
Biotin	mg/kg	0.15	0.15	0.12
Choline	mg/kg	1,300.00	1,000.00	750.00

Folacin	mg/kg	0.55	0.55	0.50
Niacin	mg/kg	35.00	30.00	25.00
Pantothenic acid	mg/kg	10.00	10.00	10.00
Pyridoxine	mg/kg	3.50	3.50	3.00
Riboflavin	mg/kg	3.60	3.60	3.00
Thaimin	mg/kg	1.80	1.80	1.80

*The requirements are based on the dietary metabolizable energy concentration of approximately 3200 kcal/ kg. Different energy values may be appropriate depending on local ingredients prices and availability.

[#] Broiler chickens do not have a requirement for crude protein per se. There, however, should be sufficient crude protein to ensure an adequate nitrogen supply for synthesis of non-essential amino acids. Suggested requirements for crude protein are typical of those derived with corn-soybean meal diets and levels can be reduced when synthetic amino acids are used.

[+] The calcium requirement may be increased when diets contain high levels of phytate phosphorus (Nelson, 1984 as cited in the NRC, 1984)

Appendix 4: Nutrient contents (calculated) of the diets used in the experiment

Nutrient	g/ kg of feed
Dry matter	880.00
Metabolisable Energy (MJ/Kg)	12.00
Crude protein	208.21
Crude fat	60.35
Crude fiber	31.17
Starch	380.69
Ca	8.00
P	5.82
Available P	4.00
Ca/P	2.00
Na	1.30
K	8.56
Cl	2.39
Ileal digestible lysine	10.00
Ileal digestible methionine	4.61
Ileal digestible cystine	2.69
Ileal digestible methionine + cystine	7.30
Ileal digestible threonine	6.60
Ileal digestible tryptophan	2.07
Linoleic acid	27.67

Appendix 5: Feed intake of birds during starter phase, week wise

Treatment	Week 1		Week 2		Week 3		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAC	120	3	303	17	480	40	902	57
DAF	113	16	253	25	354	38	720	74
DNC	118	8	291	24	437	47	847	75
DNF	127	5	263	33	347	79	737	117
WAC	110	12	350	39	679	38	1139	89
WAF	138	2	392	13	711	33	1241	51
WNC	119	7	376	16	682	24	1174	45
WNF	126	9	375	25	692	30	1192	56

Appendix 6: Feed intake of birds during grower phase, week wise

Treatment	Week 4		Week 5		Week 6		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DACdry	631	214	815	19	972	54	2418	194
DACwet	878	83	1257	46	1518	85	3652	169
DAFdry	531	96	730	97	928	124	2189	287
DAFwet	718	79	1114	63	1370	54	3201	177
DNCdry	623	82	863	68	1085	114	2571	189
DNCwet	811	95	1179	82	1432	113	3422	263
DNFdry	479	109	671	147	851	176	2000	431
DNFwet	696	225	1161	149	1442	142	3299	497
WACdry	710	54	966	52	1132	56	2808	158
WACwet	990	40	1333	65	1534	101	3857	177
WAFdry	773	10	1015	42	1263	79	3050	101
WAFwet	1015	44	1314	27	1514	44	3842	74
WNCdry	711	48	1039	65	1241	75	2991	179
WNCwet	1013	89	1359	140	1567	158	3939	376
WNFdry	730	32	990	22	1185	43	2905	84
WNFwet	1008	24	1329	34	1522	47	3859	140

Appendix 7: Body weight of birds during starter phase, week wise

Treatment	Day 1		Week 1		Week 2		Week 3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAC	46	1	130	5	325	25	606	62
DAF	47	1	119	9	246	26	447	72
DNC	46	2	137	9	307	30	514	99
DNF	47	3	124	11	248	43	456	113
WAC	46	1	141	7	402	26	812	108
WAF	46	1	177	5	481	13	971	48
WNC	47	1	150	9	413	23	877	38
WNF	46	1	166	7	445	26	937	41

Appendix 8: Body weight of birds during grower phase, week wise

Treatment	Week 3		Week 4		Week 5		Week 6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DACdry	624	52	994	68	1473	60	1991	31
DACwet	610	56	947	95	2070	83	2882	84
DAFdry	448	81	809	114	1238	151	1755	193
DAFwet	459	80	736	101	1740	122	2520	131
DNCdry	534	137	981	101	1501	126	2136	151
DNCwet	526	130	861	124	1946	152	2746	167
DNFdry	453	105	721	195	1092	303	1544	418
DNFwet	481	140	745	251	1777	308	2601	354
WACdry	886	66	1273	72	1829	104	2409	141
WACwet	891	48	1209	47	2309	81	3079	111
WAFdry	1043	72	1385	17	1986	48	2637	87
WAFwet	941	125	1290	11	2385	13	3132	48
WNCdry	923	17	1283	32	1911	73	2584	93
WNCwet	863	91	1211	89	2370	157	3168	227
WNFdry	934	50	1340	71	1903	78	2537	61
WNFwet	934	50	1340	71	2315	92	3044	128

Appendix 9: Feed conversion ratio of birds during starter phase, week wise

Treatment	Week 1		Week 2		Week 3		Starter phase	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAC	1.4	0.06	1.56	0.09	1.7	0.17	1.6	0.09
DAF	1.6	0.06	2.00	0.10	1.8	0.28	1.8	0.16
DNC	1.3	0.15	1.77	0.31	2.5	1.42	1.9	0.35
DNF	1.7	0.10	2.18	0.34	1.7	0.19	1.9	0.21
WAC	1.2	0.05	1.34	0.09	1.7	0.31	1.5	0.16
WAF	1.1	0.03	1.29	0.02	1.5	0.07	1.3	0.02
WNC	1.2	0.04	1.43	0.03	1.5	0.02	1.4	0.02
WNF	1.1	0.03	1.35	0.05	1.4	0.02	1.5	0.32

Appendix 10: Feed conversion ratio of birds during grower phase, week wise

Treatments	Week 4		Week 5		Week 6		Grower phase	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DACdry	1.7	0.53	1.7	0.05	1.9	0.16	1.8	0.11
DACwet	1.3	0.12	1.6	0.06	1.9	0.06	1.6	0.02
DAFdry	1.5	0.20	1.7	0.08	1.8	0.14	1.7	0.10
DAFwet	1.3	0.14	1.5	0.06	1.8	0.07	1.6	0.06
DNCdry	1.4	0.35	1.7	0.04	1.7	0.06	1.6	0.13
DNCwet	1.2	0.20	1.6	0.09	1.8	0.06	1.5	0.10
DNFdry	1.8	0.23	1.9	0.19	1.9	0.12	1.9	0.16
DNFwet	1.3	0.17	1.5	0.09	1.8	0.09	1.6	0.10
WACdry	1.8	0.10	1.7	0.09	2.0	0.08	1.8	0.03
WACwet	1.6	0.07	1.7	0.01	2.0	0.06	1.8	0.04
WAFdry	2.4	0.73	1.7	0.08	1.9	0.09	1.9	0.14
WAFwet	1.5	0.20	1.8	0.08	2.0	0.13	1.8	0.12
WNCdry	2.0	0.15	1.7	0.02	1.8	0.06	1.8	0.03
WNCwet	1.5	0.12	1.7	0.04	2.0	0.04	1.7	0.06
WNFdry	1.8	0.11	1.8	0.04	1.9	0.16	1.8	0.06
WNFwet	1.5	0.15	1.8	0.08	2.1	0.07	1.8	0.10

Appendix 11: Water intake of birds (ml) from nipple during starter phase, week wise

Treatment	Week 1		Week 2		Week 3		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAC	215	72	492	118	791	95	1499	230
DAF	193	60	351	79	566	126	1110	261
DNC	241	78	445	47	734	67	1420	166
DNF	222	58	366	108	627	180	1215	337
WAC	134	22	356	87	525	77	1015	129
WAF	186	36	350	36	553	63	1114	127
WNC	136	39	293	32	471	39	900	63
WNF	147	12	305	52	515	102	967	158

Appendix 12: Water intake of birds (ml) from nipple during grower phase, week wise

Treatment	Week 4		Week 5		Week 6		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DACdry	NA	NA	1413	53	1725	71	3138	124
DACwet	NA	NA	863	18	1300	71	2163	88
DAFdry	NA	NA	1263	159	1538	159	2800	318
DAFwet	NA	NA	788	53	1075	71	1863	124
DNCdry	NA	NA	1525	71	1900	212	3425	283
DNCwet	NA	NA	875	NA	1100	NA	1975	NA
DNFdry	NA	NA	1263	477	1800	742	3063	1220
DNFwet	NA	NA	863	53	1125	141	1988	194
WACdry	NA	NA	1600	35	1838	88	3438	124
WACwet	NA	NA	925	71	1245	29	2170	99
WAFdry	NA	NA	1775	106	2275	71	4050	177
WAFwet	NA	NA	1025	NA	1475	NA	2500	NA
WNCdry	NA	NA	1775	177	2288	265	4063	442
WNCwet	NA	NA	875	35	1213	301	2088	336
WNFdry	NA	NA	1600	35	2075	71	3675	35
WNFwet	NA	NA	1225	106	1613	301	2838	407

*NA- not available

Appendix 13: Total water intake of birds (ml) during starter phase, week wise

Treatment	Week 1		Week 2		Week 3		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAC	215	72	492	118	791	95	1499	230
DAF	193	60	351	79	566	126	1110	261
DNC	241	78	445	47	734	67	1420	166
DNF	222	58	366	108	627	180	1215	337
WAC	256	22	706	109	1204	102	2166	195
WAF	339	39	742	46	1263	75	2370	143
WNC	268	44	668	46	1153	40	2080	101
WNF	287	18	680	73	1207	126	2173	213

Appendix 14: Total water intake of birds (ml) during grower phase, week wise

Treatment	Week 4		Week 5		Week 6		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DACdry	NA	NA	1413	53	1725	71	3138	124
DACwet	NA	NA	2080	22	2757	129	4837	151
DAFdry	NA	NA	1263	159	1538	159	2800	318
DAFwet	NA	NA	1947	110	2479	113	4425	223
DNCdry	NA	NA	1525	71	1900	212	3425	283
DNCwet	NA	NA	2039	NA	2530	NA	4569	NA
DNFdry	NA	NA	1263	477	1800	742	3063	1220
DNFwet	NA	NA	2030	189	2563	272	4593	462
WACdry	NA	NA	1600	35	1838	88	3438	124
WACwet	NA	NA	2204	101	2744	41	4948	142
WAFdry	NA	NA	1775	106	2275	71	4050	177
WAFwet	NA	NA	2358	NA	3027	NA	5385	NA
WNCdry	NA	NA	1775	177	2288	265	4063	442
WNCwet	NA	NA	2196	255	2757	570	4953	825
WNFdry	NA	NA	1600	35	2075	71	3675	35
WNFwet	NA	NA	2562	126	3151	345	5713	472

*NA- not available

Appendix 15: Feed water intake ratio (water from nipple only) of birds during starter phase, week wise

Treatment	Week 1		Week 2		Week 3		Starter phase	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAC	0.59	0.16	0.64	0.13	0.61	0.02	0.61	0.06
DAF	0.61	0.11	0.74	0.09	0.64	0.10	0.66	0.09
DNC	0.52	0.11	0.66	0.03	0.60	0.04	0.60	0.04
DNF	0.59	0.10	0.75	0.14	0.56	0.04	0.62	0.07
WAC	0.85	0.19	1.01	0.19	1.31	0.15	1.13	0.12
WAF	0.76	0.15	1.13	0.08	1.30	0.16	1.15	0.12
WNC	0.92	0.20	1.29	0.10	1.46	0.15	1.31	0.07
WNF	0.86	0.06	1.25	0.18	1.37	0.21	1.25	0.15

Appendix 16: Feed water intake ratio (water from nipple only) of birds during grower phase, week wise

Treatment	Week 4		Week 5		Week 6		Grower phase	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DACdry	NA	NA	0.57	0.04	0.54	0.03	0.56	0.04
DACwet	NA	NA	1.41	0.02	1.12	0.02	1.24	0.02
DAFdry	NA	NA	0.58	0.03	0.59	0.04	0.59	0.03
DAFwet	NA	NA	1.47	0.03	1.31	0.05	1.38	0.04
DNCdry	NA	NA	0.54	0.03	0.52	0.04	0.53	0.04
DNCwet	NA	NA	1.33	NA	1.30	NA	1.31	NA
DNFdry	NA	NA	0.54	0.08	0.49	0.07	0.51	0.08
DNFwet	NA	NA	1.35	0.08	1.28	0.04	1.31	0.01
WACdry	NA	NA	0.58	0.02	0.59	0.02	0.59	0.02
WACwet	NA	NA	1.39	0.07	1.20	0.02	1.28	0.04
WAFdry	NA	NA	0.57	0.05	0.56	0.04	0.57	0.04
WAFwet	NA	NA	1.30	NA	1.05	NA	1.15	NA
WNCdry	NA	NA	0.58	0.09	0.53	0.09	0.55	0.09
WNCwet	NA	NA	1.51	0.19	1.29	0.10	1.37	0.01
WNFdry	NA	NA	0.61	0.01	0.56	0.01	0.58	0.00
WNFwet	NA	NA	1.09	0.08	0.97	0.15	1.02	0.12

*NA- not available

Appendix 17: Feed water intake ratio (total water intake) of birds during starter phase, week wise

Treatment	Week 1		Week 2		Week 3		Starter phase	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAC	0.59	0.16	0.64	0.13	0.61	0.02	0.61	0.06
DAF	0.61	0.11	0.74	0.09	0.64	0.10	0.66	0.09
DNC	0.52	0.11	0.66	0.03	0.60	0.04	0.60	0.04
DNF	0.59	0.10	0.75	0.14	0.56	0.04	0.62	0.07
WAC	0.43	0.04	0.53	0.02	0.56	0.03	0.53	0.03
WAF	0.41	0.04	0.53	0.02	0.56	0.03	0.53	0.03
WNC	0.45	0.05	0.56	0.02	0.59	0.02	0.56	0.01
WNF	0.44	0.02	0.55	0.03	0.58	0.04	0.55	0.03

Appendix 18: Feed water intake ratio (total water intake) of birds during grower phase, week wise

Treatment	Week 4		Week 5		Week 6		Grower phase	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DACdry	NA	NA	0.57	0.04	0.54	0.03	0.56	0.04
DACwet	NA	NA	0.59	0.00	0.53	0.00	0.55	0.00
DAFdry	NA	NA	0.58	0.03	0.59	0.04	0.59	0.03
DAFwet	NA	NA	0.60	0.00	0.57	0.01	0.58	0.01
DNCdry	NA	NA	0.54	0.03	0.52	0.04	0.53	0.04
DNCwet	NA	NA	0.57	NA	0.57	NA	0.57	NA
DNFdry	NA	NA	0.54	0.08	0.49	0.07	0.51	0.08
DNFwet	NA	NA	0.57	0.01	0.56	0.01	0.57	0.00
WACdry	NA	NA	0.58	0.02	0.59	0.02	0.59	0.02
WACwet	NA	NA	0.58	0.01	0.55	0.00	0.56	0.01
WAFdry	NA	NA	0.57	0.05	0.56	0.04	0.57	0.04
WAFwet	NA	NA	0.57	NA	0.51	NA	0.54	NA
WNCdry	NA	NA	0.58	0.09	0.53	0.09	0.55	0.09
WNCwet	NA	NA	0.60	0.03	0.56	0.02	0.58	0.00
WNFdry	NA	NA	0.61	0.01	0.56	0.01	0.58	0.00
WNFwet	NA	NA	0.52	0.02	0.49	0.04	0.50	0.03

*NA- not available

Appendix 19: Body weight and absolute fresh weight (g) of different parts of GIT at the end of starter phase

Treatment	Body weight		Crop		Proventriculus		Gizzard		Duodenum*		Jejunum	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAC	570	147	1.8	0.66	3.1	0.65	16.5	2.96	8.7	1.94	10.1	3.62
DAF	448	81	1.4	0.50	2.6	0.51	13.5	3.01	6.8	0.92	8.0	1.18
DNC	425	107	1.6	0.36	2.5	0.48	14.4	2.16	6.9	1.07	7.7	1.43
DNF	403	115	1.4	0.50	2.3	0.46	12.0	1.62	6.2	1.08	7.3	1.75
WAC	860	131	2.4	0.50	4.3	0.70	21.1	3.20	11.8	2.70	15.2	2.50
WAF	938	108	2.5	0.35	4.3	0.55	16.6	1.74	12.0	1.66	14.0	2.43
WNC	848	81	2.4	0.36	4.9	0.76	21.7	2.29	13.1	1.05	15.2	1.98
WNF	901	65	2.8	0.60	4.4	0.51	19.7	1.75	11.7	1.64	13.6	2.00

*Including pancreas

Appendix 20: Body weight and absolute fresh weight (g) of different parts of GIT at the end of grower phase

Treatment	Body weight		Crop		Proventriculus		Gizzard		Duodenum*		Jejunum	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DACdry	1953	120	5.0	0.94	5.5	0.35	34.3	7.21	19.0	2.30	25.8	3.62
DACwet	2850	146	6.7	1.16	7.5	0.94	38.6	3.52	26.0	6.90	31.7	5.84
DAFdry	1923	237	4.8	0.32	6.1	1.93	41.1	8.03	20.7	4.04	27.2	3.23
DAFwet	2454	163	6.2	1.24	6.5	1.01	34.6	6.07	23.7	1.35	35.2	1.81
DNCdry	2141	189	4.9	0.76	7.1	0.71	41.4	5.38	19.8	1.31	28.1	4.61
DNCwet	2731	201	6.5	1.32	8.3	1.04	37.1	5.34	23.2	2.74	33.7	5.04
DNFdry	1624	578	4.4	1.15	5.5	0.79	32.9	3.46	17.4	5.69	23.3	4.83
DNFwet	2532	236	6.6	1.94	7.4	1.13	31.6	5.12	21.1	1.76	32.5	1.43
WACdry	2517	135	5.3	0.94	6.4	0.17	38.0	3.66	23.3	1.72	35.0	2.31
WACwet	2888	158	6.5	1.43	8.1	1.16	36.1	3.58	23.9	1.40	32.7	2.97
WAFdry	2739	150	6.7	1.03	7.2	0.79	37.3	3.29	21.4	1.77	30.1	1.91
WAFwet	3013	252	7.4	1.15	7.3	0.79	38.5	7.56	24.0	3.12	35.4	2.17
WNCdry	2503	154	6.4	0.92	6.6	0.51	40.1	2.40	24.9	2.08	32.3	1.66
WNCwet	3199	200	6.3	0.55	7.7	0.64	40.6	5.54	25.9	2.30	37.4	3.13
WNFdry	2421	101	5.4	0.60	6.3	0.50	35.5	1.58	19.0	1.40	27.4	3.06
WNFwet	2970	220	6.6	2.29	9.1	2.08	35.0	4.88	22.4	2.38	31.7	1.07

***** including pancreas