

**Wet and coarse diets in broiler nutrition:
Development of the GI tract and performance**

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**Wet and coarse diets in broiler nutrition:
Development of the GI tract and performance**

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ABSTRACT. Mai Anh Khoa. Wet and coarse diets in broiler nutrition: development of the GI tract and performance.

Diet structure and conformation during the starter phase play an important role in the functional development of the gastro-intestinal (GI) tract of broiler chicken, in particular the foregut segment. Feed structure has a significant effect on the development of the foregut segments in broiler chickens. The development of the gastro-intestinal tract in chickens is aimed to adapt and to develop in line with the body in order to meet animal's demands for production traits and it should avoid the GI tract malfunction and improper GI tract development. These can be done by changing (alternating) feed properties and in feeding strategies during the starter phase. Chicken's diet components may be treated in order to enable the crop, the proventriculus- gizzard system to function optimally. Thus they will optimize feed digestion and utilisation. Therefore, the focus of this thesis was: 1) To understand the impact of changes in feed properties like technological treatments on broiler's performance. The technological changes in feed were made to induce differences in structure (particle sizes) and appearance (solid or liquid diets) of the feed and how this feed during the starter phase (0 – 3 weeks of age) influences the development and functioning of the foregut (crop and proventriculus); 2) To develop a feeding regime which optimizes foregut development and function of the foregut segment during starter phase and to see if there is a lasting effect on the following period, in other words, whether there is a carry over effect on performance of broilers during the grower phase.

It has been shown that the starter period is critical for GI tract development. Feeding coarse diets during the starter phase improves the functional development of the proventriculus-gizzard system. Wet feeding improves feed intake and, as a consequence, broiler performance. Feeding a wet *and* coarsely ground diet provides a large improvement in feed intake, feed conversion and body weight gain, showing the most pronounced effects during the starter phase of broiler's life.

Key words: coarse, fine, liquid, solid, GI tract development, performance, broiler

Dedicated to those whom I care for

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CHAPTER 1

General Introduction

BROILER PRODUCTION

Modern poultry has undergone great changes in growth characteristics during the past few decades (Havenstein *et al.*, 1994). For example, average 42-day market weight of broiler chicks increased from about 1.7 kg in 1980 to almost 2.5 kg in 2004, while feed conversion improved markedly. These genetic changes in growth potential were motivated by the increased demand in developed countries for further processed poultry meat at the expense of pork and beef. Improvement in the growth potential of commercial broilers has not come without associated (health) problems. Moreover, as a consequence of a decreasing durational in growth period (going down from 42 to 35 days of age), broiler producers get hardly enough time to manage and adjust feeding practices during the flock's grower period. In recent years, therefore, researchers have tried to counterbalance these problems by adjusting feed characteristics and feeding management.

PHYSIOLOGICAL FUNCTION OF THE FOREGUT SEGMENT

In the wild state, the jungle fowl as the ancestor of modern poultry flocks, ingests a variety range of feeds. These feeds can be classified into mainly two categories: "high protein-low energy" materials, such as worms and snails (about 10% of daily dietary intake), and, on the other hand, high fibrous vegetable resources (low protein) like grass shoots and cereal seeds (about 90% of daily dietary intake). For millions of years, fowl has adapted to these types of feed by developing a gastro-intestinal system which perfectly fits to the properties necessary to digest in particular the second category of feeds.

Digestive anatomy and function in birds differs from that in mammals, in particular with respect to the gastric and caecal-colonic areas. The esophagus includes the crop for temporary feed storage. The crop produces mucus which aids in the swallowing process of feed particles. The gastric apparatus includes the glandular stomach or proventriculus and the muscular stomach or gizzard, respectively. The mucus, which is also secreted by the proventriculus, has a second, very important role in foregut function. It forms a protective coating on the mucosa to prevent damage from pepsin and HCl, the enzymatic fluids responsible for initial dietary protein break down. The principal function of the gizzard, however, is the mechanical grinding of coarse feed particles. The gizzard empties its contents when particle sizes have been reduced to about 15 - 40 µm (Duke, 1994; Hetland *et al.*, 2003).

FACTORS INFLUENCING THE FUNCTIONAL DEVELOPMENT OF THE FOREGUT

There are many animal and environmental factors that influence feed digestion and utilization in animals. The function of the alimentary tract and environmental factors such as feed properties are considered most important. In order to optimize feed digestibility and utilization, these factors should be optimal. Alimentary functions for digestion are the degradation of feed properties by enzymes excretion and the absorption of nutrients. In all avian species, part of the function of the gastro-intestinal tract is storage. It is thought that it is important that birds have a storage segment and a grinding segment in their digestive system for survival. However, the importance of the crop may be different to birds receiving more concentrated and those receiving less concentrated feed. Both organs are considered very important for the digestion process and these functions may differ compared to that of mammals. It is known that genetic and environmental influences such as feeding and nutrition determine the development of the various parts in the digestive tract. Especially, the digestive organs in broilers in the post hatch and early starter period are influenced by these factors. Diet structure and composition are causative factors to explain the variation in the functioning of the gastro-intestinal tract (Nir *et al.*, 1994, Gabriel *et al.*, 2003). A good "functioning" with regard to the diet can be defined as a diet, that is designed to match the intrinsic characteristics of the fowl's gastro-intestinal tract. For the newly hatched chicken, the first days after hatch (pre-starter period) are critical for its development and survival. The changes from yolk – the endogenous diet – to a solid – exogenous – diet will cause large metabolic and physiological transitions. Thus the chicken's gastro-intestinal tract undergoes rapid physical and functional developments after the chicken hatched (Uni, 2003).

The exogenous solid diets offered in practical poultry husbandry during early feeding can vary due to differences in both nutrients (carbohydrates, protein, fats and their functionality) and also in feed form due to technological modification (e.g. the effects of extrusion; Plavnik, 2003). This variation determines intake of feed in the first days and this will have a major impact on the development of the gastro-intestinal tract and of the other digestive organs.

The use of liquid diets during early life of broilers

For newly hatched chickens, the week after hatching (pre-starter period) is a critical period for their development and survival. It has been suggested that the moistening capacity of the crop (swallowing) during the first few weeks post-hatch for a standard solid diet could be a limiting factor for an optimal functioning of the foregut.

In these first 5 to 7 days, the weight of the proventriculus, gizzard and small intestine increases more rapidly in relation to body weight than other organs and tissues (Dror *et al.*, 1977; Sklan, 2001). Moreover, in the gastro-intestinal tract of post hatch birds, rapid morphological changes occur during the first phase of life in villus growth (Sklan, 2001). Also changes in the expression and activity of functional brush border enzymes take place. Pancreatic enzyme activities in conjunction with crypt formation and elevated number of enterocytes prepare the framework for the ingestion of exogenous feed during the first phase of life (Uni, 2003). Geyra *et al.* (2001) showed that during the pre-starter period early access of newly hatched chickens to feed will ensure a more rapid development of the intestine compared to later access. Dibner (1999) observed that chickens which were malnourished in this period had a delay in maturation of the enzymatic system. In addition they showed a retarded development of the immune system. These large morphological and functional changes are clearly related to the time at which chickens had first access to feed and are sensitive to a delay in nutrient supply.

The use of a liquid diet (wet feeding) for broilers has been reported to have promising effects on feed intake and feed utilization efficiency, due to the improvement of nutrient retention (Yasar and Forbes, 1995). Liquid diets (with 80% wheat) increased feed intake and weight gain up to 17 days of age by almost 20% (Scott, 2002). So, in the context of this study, wet feeding is a promising feeding strategy during the early age of broilers and we will therefore use it to study the gastro-intestinal development in animals.

The use of coarsely ground diets

According to Duke (1994), the properties of the fowl's foregut enable broiler diets (in the form of pellets) to be dissolved within a short time in the crop into very fine particles. It has been shown that finely ground diets may inhibit the contraction of the gastro-intestinal tract including the refluxing activity of the gut in commercially raised broiler chickens. Dissections of animals which have consumed finely ground diets show less developed gizzards as

compared to animals which had been fed coarsely ground diets. This shows that the gizzard will only function at this age as a transit organ rather than a grinding organ (Cumming, 1994). It can be hypothesized that for chickens, which consume diets with large particles which enter the gastric region and cause an increase in gut mobility, an increased feed intake is observed. As a consequence, their performance and gut health will be improved (Engberg *et al.*, 2002). A coarse diet enhances the development of the foregut. A good foregut will maintain pH barriers throughout the gut (Engberg *et al.*, 2003). This is beneficial for health and performance throughout the grower period.

The optimal dietary composition and feed structure can be defined as what is needed to sustain an adequate digestion of nutrients in the post-hatch period; the traits, however, are not precisely known yet. This should be known, however, to give the proper feed to the animal for stimulating a good development and/or to prevent an underdeveloped gastro-intestinal tract. Feed composition and structure are causative factors for maintaining a healthy gastro-intestinal tract during the starter/grower period of the chickens. Technological treatment of diets can modify both the physical and chemical characteristics of feed. Physical properties are those associated with *e.g.* viscosity, uniformity and particle sizes. Chemical properties are those concerning nutrient digestibility and utilization of *e.g.* amino acids. These changes occur as a result of combinations of both temperature and pressure during processing. This can occur during primary (diet ingredients) or secondary (complete diet) processing (Plavnik, 2003). Research has shown also the importance of particle size distribution of diets during entire growing period. A coarse diet structure increases gizzard size and function (Nir *et al.*, 1994) and also strengthens the gastro-intestinal tract defense system (Engberg *et al.*, 2003) compared to a fine diet structure.

As a result of environmental changes after hatching, young chickens have to cope with some stress factors. This can also affect the development of the gastro-intestinal tract during the first few days of the production cycle. The stress factors after hatching include the transport from hatchery to the production facilities and the time lag between hatching and first access to feed and to water. In addition, pathogens and non-hygienic environments can be stressors. Moreover, heat- or cold-stress conditions (van der Hel *et al.*, 1991; 1992) as well as nutritional mismanagement are considered important. Hatchling broilers are very susceptible to these stressors. Birds which are subjected to these stress factors tend to have a slow start in growth. Often they do not immediately begin with eating and drinking on their own. The consequences of a delay in feed and water intake after hatching result in slow gastrointestinal

and immune development. This delay increased mortality and can result in a poor performance (Vieira and Moran, 1999a; 1999b). After hatching, young broilers switch to exogenous solid diets and these diets are *e.g.* composed of carbohydrates. In order to adapt themselves to the new environment and the new diet, a dramatic physical development must take place, especially during the first two weeks post-hatch. The proper functional development of the foregut segment may play an important role in digestive process. This development influences the mechanical and chemical changes of the ingested feed before nutrients are absorbed in the small intestine. Mechanical changes include swallowing, maceration and grinding of feed in the gizzard. Chemical changes include the secretion of enzymes and mucus from the crop, proventriculus and pancreas, bile from the liver. In addition, bacterial activities in the crop have an effect on the ingested feed (Duke, 1994).

It is therefore that the foregut has been chosen as a focus in this study. We choose to study in detail how the foregut during the starter phase is affected by the feed factors such as structure (coarse vs. fine structure) and conformation (wet vs. dry diets). It will be also included how differences in gastro-intestinal tract occurring during early life from hatch till 17 and 21 days of age persist during the next period of 3 – 4 weeks.

SCOPE OF THE STUDY

The main objective of this study is to evaluate the impact of technological treatments on feed to induce differences in structure (particle sizes distribution) and conformation (appearance: solid or liquid diets) of the feed and to study the influences on the development and functioning of the foregut (crop and ventricula) during the starter phase 0 – 3 weeks of age. Also, the performance in the first growing period of broiler chickens and the possible impact on the subsequent grower period was studied.

The aim of the study is to develop ideas for a feeding regime to optimize the foregut development and function, and subsequently improves performance in broilers. It is aimed to understand and quantify dietary characteristics and feeding management with regard to the development of different gastro-intestinal sections by measuring the relevant physical, morphological and histological parameters after feeding with these traits. Performance parameters were also taken into account. The resulting insight may determine if adjustment of the broiler's diet is well with regard to these factors.

It was hypothesized that a technological modification of the diet may significantly influence the functional development of some parts of the digestive organs in poultry. We also hypothesized that a good development will contribute to a healthy chicken in the starter/grower period.

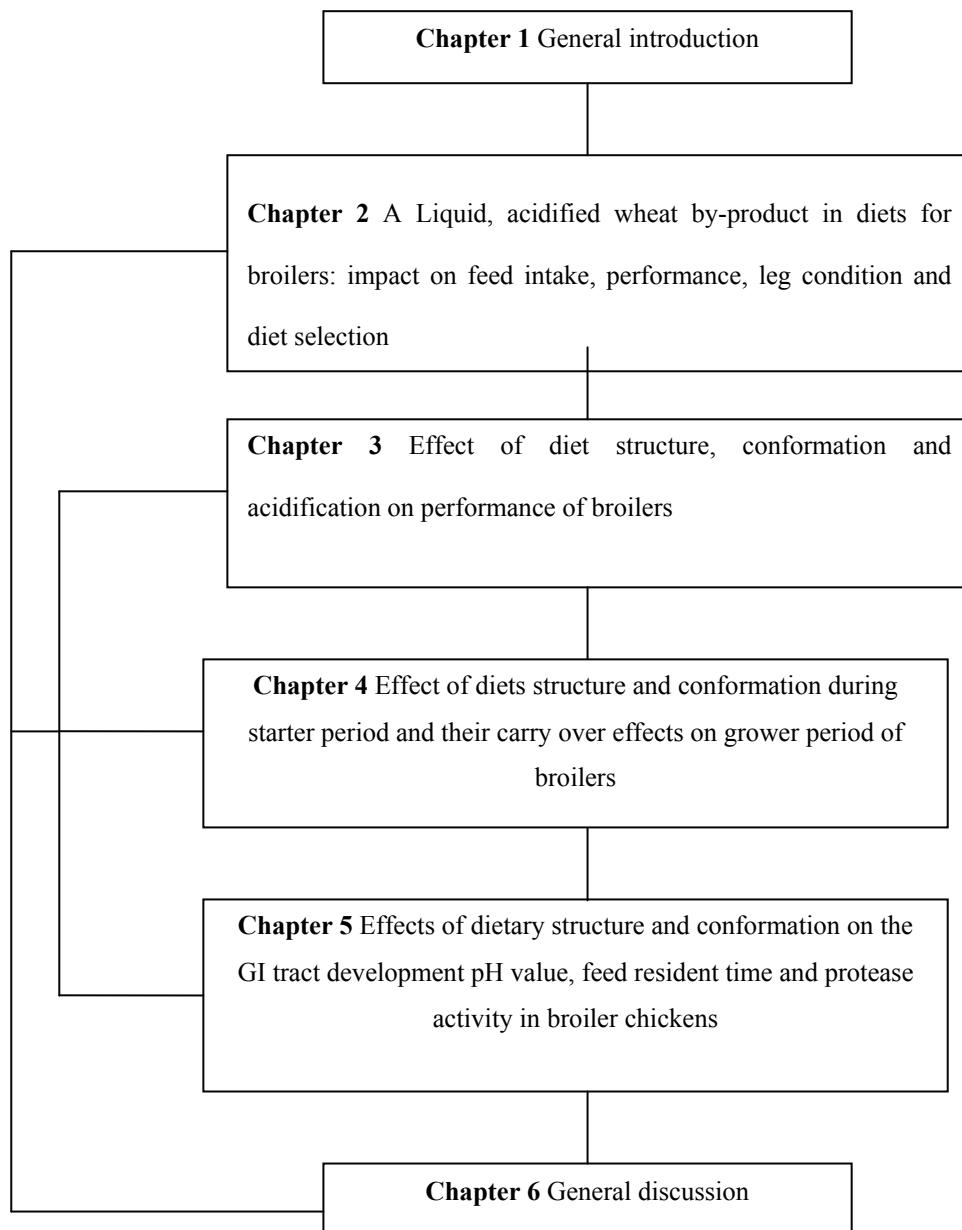
OUTLINE OF THIS THESIS

A series of experiments were carried out to quantify the effect of a liquid wheat by-product in a choice feeding system on diet selection, feed intake, foregut development and performance of broilers (Chapter 2). In addition, post-mortem analysis was carried out on number of birds per treatment: weights of crop, proventriculus, gizzard and duodenum were determined.

The results of a study on effect of diet structure, conformation, and acidification on feed intake, development of the foregut segments was described in Chapter 3.

In Chapters 4 and 5, an evaluating experiment was performed with regard to structure, conformation of feed on both production and development of chickens to explain the development during the starter (1 – 17 days) and the following grower period.

The results of different experiments are discussed in Chapter 6, a general discussion part of this thesis.



REFERENCES

- Cumming, R.B. (1994) Opportunities for whole grain feeding. *Proceeding of the 9th European Poultry Conference, 7-12 August 1994, Glassgow, UK*, 2: 219-222.
- Dibner, J.J. (1999) Feeding of hatchling poultry-avoid any delay. *Feed international*, 12: 30-34.
- Dror, Y., Nir, I. and Nitsan, Z. (1977) The relative growth of internal organs in light and heavy breeds. *British Poultry Science*, 18: 493-499.
- Duke, G.E. (1994) Anatomy and digestive function of the avian gut. *Proceeding of the 21st Annual Carolina Poultry Nutrition Conference, 7-8 December 1994, Charlotte, North Carolina, USA*: 46-51.
- Engberg, R.M., Hedemann, M.S. and Jensen, B.B. (2002) The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British Poultry Science*, 44: 569-579.
- Engberg, R.M., Bjerrum, L. and Pedersen, K. (2003) The influence of whole wheat feeding on the course of a *Salmonella Typhimurium* infection in broiler chickens. *Proceedings of the 14th European Symposium on Poultry Nutrition, Norway*: 167-168.
- Gabriel, I., Mallet, S. and Leconte, M. (2003) Differences in digestive tract characteristics of broiler chickens fed on complete pelleted diet or on whole wheat added to pelleted protein concentrate. *British Poultry Science*, 44 (2): 283-290.
- Geyra, A., Uni, Z. and Sklan, D. (2001) The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. *British Poultry Science*, 86: 53-61.
- Havenstein, G.B., Ferket P.R., Scheideler, S.E. and Larson, B.T. (1994) Growth, livability and feed conversion of 1957 vs 1991 broilers when fed “typical” 1957 and 1991 broiler diets. *Poultry Science*, 73: 1785-1794.
- Hetland, H., Svhuis, B. and Krogdahl, A. (2003) Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *British Poultry Science*, 44: 275-282.

- Nir, I., Hillel, R., Shefet, G., And Nitsan, Z. (1994) Effect of grain particle size on performance. 2. Grain texture interactions. *Poultry Science*, 74: 781-791.
- Plavnik, I. (2003) The use of feed technology to optimize feed structure and bird performance. *Proceedings of the 14th European Symposium on Poultry Nutrition*, Norway: 1-7.
- Scott, T.A. (2002) Impact of wet feeding wheat-based diets with or without enzyme on broiler chick performance. *Canadian Journal of Animal Science*, 82: 409-417.
- Sklan, D. (2001) Development of the digestive tract of poultry. *World's Poultry Science Journal*, 57: 415-427.
- Uni, Z. (2003) Methods for early nutrition and their potential. *Proceedings of the 14th European Symposium on Poultry Nutrition*, Norway: 254-260.
- Van der Hel, W., Verstegen, M.W.A., Henken, A. M. and Brandma, H.A. (1991) The upper critical temperature in neonatal chicks. *Poultry Science*, 70:1882-1887.
- Van der Hel, W, Verstegen, M.W.A., Pijls L. and van Kampen, M. (1992) Effect of two day temperature exposure of neonatal broiler chicks on growth performance and body composition during two weeks at normal conditions. *Poultry Science*, 71: 2014-2021.
- Vieira, S.L. and Moran, E.T. (1999a) Effects of egg of origin and chick post-hatch nutrition on broiler live performance and meat yields. *World's Poultry Science Journal*, 55: 125-142.
- Vieira, S.L. and Moran, E.T. (1999b) Effects of delayed placement and used litter on broiler yields. *Journal of Applied Poultry Research*, 8: 75-81.
- Yalda, A.Y. and Forbes, J.M. (1995) Food intake and growth in chickens given food in the wet form with and without access to the drinking water. *British Poultry Science*, 36: 357-369.

CHAPTER 2

**A liquid, acidified wheat by-product in diets for broilers: impact on
feed intake, performance, leg condition and diet selection.**

SUBMITTED TO BRITISH POULTRY SCIENCE

ABSTRACT

1. In this study, effects of the implementation of a liquid wheat by-product in a diet on broiler chicken performance parameters were evaluated.
2. 108 One-day-old male broiler chickens were randomly allocated to three pens. Within each pen the broiler chicken had access to two feeders filled with the control diet (CC treatment), experimental (EE treatment) or experimental and control diet (EC treatment). The control diet (C) had a 35% inclusion of wheat and was offered in a mash form. The experimental diet (E) was a combination of a liquid wheat by-product with 500 g starch/kg product and a supplement mash diet resulting in a diet dry matter content of 492 g/kg.

Both diets were isonitrogenous and isocaloric on a calculated dry matter basis. The birds could eat and drink *ad libitum*. Three days after arrival, the birds of each group were allocated in 6 pens of six birds per treatment.

3. Feed intake from each feeder was measured after each meal, whereas body weight, water intake and gait score were determined once a week. Chickens were euthanized at the end of the experiment and post-mortem examination was carried out.
4. Results showed an increased body weight and total water intake in the EE and EC treatments compared to the CC treatment. Feed conversion (g feed/g growth) was decreased for the birds from the EC group. Abdominal fat and carcass weight was higher in the EE and EC treatments relative to the CC treatment. Carcass weight relative to body weight was, however, higher for the CC treatment. Gait score was lower for birds of the EC treatment. The incidence of pericardial effusion was higher in birds of the EE and EC treatments. The birds of the EC treatment consumed in the first week more diet C and in the last three weeks more of diet E.
5. This study showed that inclusion of a liquid (starch-rich) wheat by-product showed a good performance of male broiler chickens.

Keywords: liquid wheat by-product, broilers, performance, diet selection, carcass traits

INTRODUCTION

The use of liquid by-products from the human food industry as an ingredient of pig diets has increased over the last decade (Scholten and Verdoes, 1997). Liquid by-products are relatively inexpensive and have been shown to reduce feeding costs of pigs by 10-17% (Scholten *et al.*, 1999). The role of liquid by-products or addition of water in poultry nutrition, however, has been small. As for the addition of water, Forbes and co-workers fed broilers dry feed with an addition of 1.3 to 2.0 kg of water per kg dry feed and observed an increase in feed intake and body weight gain (Yalda and Forbes, 1996; Yasar and Forbes, 2000). Yalda and Forbes (1995) reported that the effect of water addition starts somewhere above 0.75 to 1.5 kg water per kg air-dry feed. Depending on the poultry feed, the maximum amount of water added to the feed is the amount which results in a layer of water on the top of the feed, since this discourages feed intake, an effect that occurs somewhere between 2.25 and 3.0 kg water per kg air-dry feed, but could also be lower (Yalda and Forbes, 1995). Feeding liquid by-products to broilers can play an important nutritive and cost-reducing role in the future, if qualities related to feed intake, performance and health of broiler chickens are known.

In this study, a liquid wheat by-product was used as a diet ingredient for broiler chickens. This by-product arises from the wet processing of wheat. In addition to starch and protein it contains the soluble fraction of wheat flour and has a dry matter content of about 200 g/kg. The product is thickened and acidified for keeping quality during storage. Typical nutrient contents in the liquid by-product are (g/kg DM): crude protein 110; starch 500; sugar 200 and digestible phosphorus 2.6.

The objective of this study was to investigate the effect of the inclusion of a liquid wheat by-product in a diet for male broiler chickens on feed intake and performance. In addition, gait score and post-mortem analysis were performed that gives information about leg condition and health of the birds. Moreover, diet selection and performance of choice-fed broiler chickens were examined.

MATERIALS AND METHODS

Animals and housing

108 One-day-old male broiler chickens (Ross 308) were obtained from a commercial hatchery (Morren B.V., Lunteren, the Netherlands). Due to an error in gender determination, one female was included in the experiment. Since this was discovered while the experiment was running, the female was kept in the experiment and exposed to all measurements but was excluded from the analysis of data for feed intake and efficiency. Immediately after arrival the birds were randomly assigned to three treatment groups and placed in one pen per treatment. Up from the first day, broilers of the first and second treatment had access to two feeders filled with the experimental diet (EE treatment) or control diet (CC treatment), respectively. The third treatment (EC treatment) was a combination of the first and second treatment; one feeder was filled with the experimental diet and the other with the control diet. This gave birds the opportunity to select between the experimental or control diet. One day after arrival, each bird was weighed and got a small wing tag with an identification number. Three days after arrival, the birds of each treatment were weighed and randomly assigned to 6 floor pens with 6 birds in each of the 6 pens. All walls of each pen (1·5 m x 1·5 m per pen) were solid and 0·6 m high. The floor was covered with wood shavings. Each pen contained three drinking nipples with a cup underneath. All birds could eat and drink *ad libitum*. The temperature inside the room was initially maintained at 32⁰C from day 0 to 3 and gradually decreased by 0.5⁰C per day until it reached 21⁰C at day 21 and remain 21⁰C until day 43. Artificial light was provided initially at a schedule of 23-h light and 1-h dark. Three days after arrival, a schedule of 18 h continuous light and 6 h dark per day was maintained. The light period started at 7·30 h and no daylight entered the house.

Diets and feeding

The control diet (C) was least-cost formulated based on 10.0 g ileal digestible lysine, 27.7 g linoleic acid and about 380 g starch/kg diet. The control diet had a large inclusion of wheat and was offered in a mash form. The experimental diet (E) was a combination of a liquid wheat by-product and a dry supplement mash diet. The liquid wheat by-product had an average dry matter content of 186 g/kg product and a pH below 3.5. Both diets were isonitrogenous and isocaloric. The ingredient and nutrient compositions are given in Table 1.

Table 1. Ingredient (g/kg) and analysed nutrient composition (g/kg dry matter) of the control and experimental diet.

Composition	Diet	
	Control	Experimental
<i>Ingredients</i>		
Wheat by-product*	--	557.5
Water	--	2.3
Wheat	349.4	77.4
Maize	275.7	168.3
Toasted full fat soya beans	199.6	113.6
Soya bean meal 44/7	94.3	25.6
Fishmeal 66% CP	49.9	28.4
Ca CO ₃	11.8	6.5
L-Threonine	3.0	5.7
Broiler vitamin and mineral premix	5.0	5.0
Monocalciumphosphate	4.7	3.1
Phytase	3.0	1.7
Salt	1.7	--
Palm oil fatty acids	--	1.8
DL-Methionine	1.4	1.1
L-Lysine HCl	0.5	1.0
Soya bean oil	--	1.0
<i>Analysed nutrients</i>		
Crude protein	251.3	251.2
Crude fat	77.6	88.4
Total starch	407.0	383.1
Crude fibre	34.4	35.1
Ash	63.2	65.6

* Latico: 186 g DM/kg product; Duynie BV, Alphen a/d Rijn, the Netherlands.

The broiler chickens could eat and drink *ad libitum*. The experimental diet was freshly prepared each feeding time by mixing the liquid product with water and the supplement pellet in the ratio of 55.8 : 0.20 : 44.0 %, respectively. After homogeneous mixing it was offered in a clean feeder to the animals.

After a few minutes this feed was changed into a rather thick pasty consistency. The time interval between feed preparation and delivery of feed to the birds was approximately 30 minutes. Because of the high ambient temperatures ($\pm 30^{\circ}\text{C}$) in the first two weeks of the

experiment, the air could contain more water compared to the ambient temperatures during the remaining part of the experiment ($\pm 20^{\circ}\text{C}$). In this period water evaporation is higher from the feed to the air. Therefore, fresh feed was offered to all feeders of all treatments at 9·00, 13·00 and 19·00 h each day to prevent the formation of a solid layer on diet E, since this could make the experimental diet difficult to be consumed by young broiler chickens. Moreover, during these weeks small feeders were used in which birds had only access to the feed through small holes on top of the feeders. During the remaining part of the experiment, up from week 2, large open feeders were used and feeding times were at 9·00 and 19·00 h each day.

Feed and water intake

For measuring feed intake of each meal, the offered quantity of feed was corrected for the weight of the feed residues in the feeder. A single identical feeder was filled with feed E and placed in an empty pen to determine the evaporated amount of water. The weekly tabulated feed intake was expressed on a dry matter basis. Water consumption per pen was determined during the last four weeks of the experiment. It was not possible to determine the water intake accurately during the first two weeks because a few drinking nipples were leaking. Total water intake per week was calculated from weekly fresh water intake plus the water content of the feed consumed daily. Live weight of birds was recorded at 3 days of age and then every week starting at day 8.

Leg condition

The Wageningen University Committee on Animal Care and Use approved this experiment on the condition that when for a particular bird a gait score of three or higher was determined and when this finding was confirmed the next day, the individual bird had to be culled. The gait score has a six point scale: 0 = no detectable walking abnormalities, 5 = incapable of sustained walking on its feet (Kestin *et al.*, 1992). At the end of each day the gait of each bird was observed and once a week the gait score was recorded.

Post mortem measurements

On day 46 half of the birds were killed for post-mortem analysis, the other half on day 47. Three pens of each treatment were assigned to one of these days. Each bird was killed with

intravenous overdose of barbiturate (Euthanasate, Apharmo, Arnhem, The Netherlands) in the wing vein. Immediately thereafter, post-mortem examination was carried out to determine physical abnormalities. A prepared checklist (Bokkers and Koene, 2003) was followed during post-mortem examination. The entire digestive tract was removed and the abdominal fat was separated and weighed. The remaining carcass was weighed and frozen for future analyses to determine carcass quality. During post-mortem examination special attention was given to the presence of heart abnormalities (dilatation, heart fibrin or pericardial effusion), liver abnormalities (abnormal colour or shape), breast blisters, abnormalities of back (scoliosis or kinky back) and of tendons (degeneration) and condition of feet and hocks (swollen or burned).

Statistical analysis

Statistical analysis was performed using software from the SAS Institute Inc. (SAS, 1996). Data were analysed at pen level. The pen with the female broiler chicken was excluded from the statistical analysis for data measured at pen level. Data of feed and water intakes were analysed with an analysis of variance with the factor treatment included in the following model:

$$y_i = \mu + T_i + \varepsilon_i$$

where: y_i = variable; μ = overall mean; T_i = treatment effect ($i = 1, 2$ or 3); and ε_i = error term. Body weight data were analysed with an analysis of variance with the factor time as repeated measurement included in the model. A generalised linear mixed model with a logit link and binomial distribution was used to analyse the effect of treatment on the data of the gait score and post-mortem examination. The random effect of pen within treatment was included in this model. The effect of treatment was tested against the random effect of pen within treatment. When significant effects were found, pair-wise comparisons between treatments were made using post hoc analysis. Spearman rank correlations were calculated between gait, body weight and abnormalities.

RESULTS

Treatment EE vs CC

The effects of a liquid wheat by-product on broiler performance are presented in table 2.

Table 2. Feed and water intake (3 - 43 days), slaughter performance and physical abnormalities of male broiler chickens.

	Treatment*					
	CC		EE		EC	
	Mean	SEM	Mean	SEM	Mean	SEM
<i>Intake and efficiency</i>						
Feed intake (g DM/bird)	4214 ^a	85.9	4482 ^a	94.1	4788 ^b	85.9
Feed conversion (g/g)**	1.38 ^a	0.021	1.39 ^a	0.023	1.44 ^a	0.021
Water intake (l/bird)***	9.11 ^a	0.258	10.64 ^b	0.316	10.94 ^b	0.258
<i>Pooled SEM</i>						
<i>Slaughter performance</i>						
Slaughter weight (g)	3397 ^a		3668 ^b		3796 ^c	42.2
Carcass weight (g)	2139 ^a		2250 ^b		2333 ^b	30.9
Carcass weight (%)	63.0 ^a		61.3 ^b		61.5 ^b	0.38
Abdominal fat (g)	49.4 ^a		71.4 ^b		70.6 ^b	2.97
<i>Abnormality (%)</i>						
Pericardial effusion	0.09 ^a		0.33 ^b		0.41 ^b	0.065
Scoliosis	0.51 ^a		0.27 ^a		0.28 ^a	0.113
Liquid in tendon	0.11 ^a		0.06 ^a		0.03 ^a	0.036
Heart abnormality	0.46 ^a		0.47 ^a		0.38 ^a	0.070
Tendon degeneration	0.39 ^a		0.39 ^a		0.42 ^a	0.112
Gaitscore ≥ 1	0.60 ^a		0.81 ^b		0.94 ^b	0.049

Means within a row followed by different superscripts are significantly ($P<0.05$) different.

* Nr of pens involved in statistical analysis in intake & efficiency data: CC,6 ; EE, 6; EC, 5.

** Feed intake/growth (g DM/g)

*** Water consumption as measured in period 15-43 days

Body weight (BW) of birds of the EE treatment was higher compared to birds of the CC treatment at 36 and 43 days of age ($P<0.05$; Figure 1). It was observed that total body weight gain from day 3 to day 43 was higher ($P<0.05$) for birds of the EE treatment (3199 ± 43.7 g) compared to CC treatment (3059 ± 43.7 g).

Feed conversion (g feed intake/g growth) in the period 3 to 43 d (Table 2) was not significant different between the EE and CC treatment ($P>0.05$)

It was observed that total water intake was not affected by treatment in the first 29 d; thereafter, the CC treatment had a significant lower total water intake than the EE treatment in the last two weeks of the experiment ($P<0.01$). In the period 15 to 43 d total water intake was significant higher for the EE treatment compared to the CC treatment ($P<0.01$).

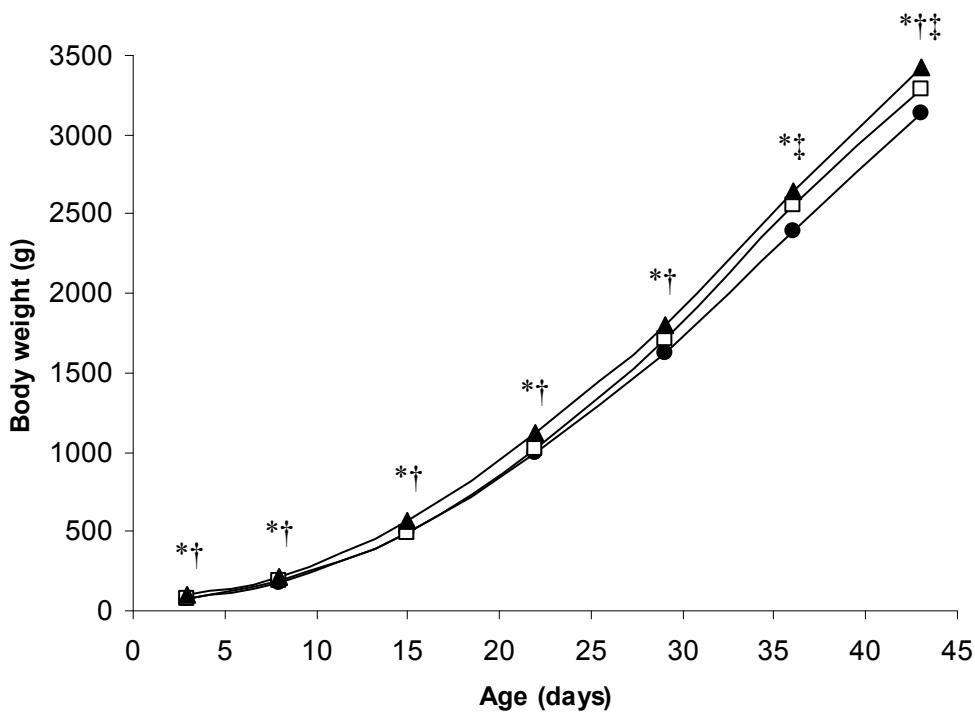


Figure 1. Body weight (g) of male broiler chickens over days of treatments CC (●), EE (□) and EC (▲). * Mean values of treatment EC were significant different from that of the CC treatment ($P<0.05$). † Mean values of treatment EC were significant different from that of the EE treatment ($P<0.05$). ‡ Mean values of treatment EE were significant different from that of the CC treatment ($P<0.05$).

There was no significant difference in body, carcass or abdominal fat weight between the two days (day 46 and 47) of post-mortem examination. Therefore, data of the post-mortem examination was pooled. Significant higher BW was found for the EE treatment compared to the CC treatment ($P<0.01$) (Figure 1). Carcass weight of the EE treatment was significant higher compared to the CC treatment ($P<0.05$). However the CC treatment had a significant higher carcass weight relative to body weight than the EE treatment ($P<0.01$). The fresh

weight of abdominal fat was significantly higher for the EE treatment than for the CC treatment ($P<0\cdot01$). Moreover, abdominal fat relative to carcass weight found to be significant higher for the EE treatment compared to the CC treatment ($P<0\cdot01$).

Abnormalities, efficiency and performance

One bird of 40 days of age from treatment EC was killed due to a gait score of 3 at 40 days of age. There were no significant differences found between treatment groups in number of birds with a gait score of 1 or higher. Also, no correlation was found between gait score and body weight. A significant effect of treatment ($P<0\cdot05$) was found for the occurrence of pericardial effusion. Post hoc analysis showed that treatment CC scored significantly lower than the EE or EC treatment. No effect of body weight at time of slaughtering was found on the presence of the physical abnormalities: no liver abnormalities, kidney abnormalities, hock irritation, kinky back or curved toes were observed during the post-mortem examination.

No tendon rupture or slipped tendons were found since these are disorders that would have leaded to gait scores > 2 and a broiler would therefore have been culled. No correlation was found between heart abnormalities with BW and tendon degeneration or scoliosis with gait score.

BW of birds of the EC treatment was significant higher than BW of birds of the CC and EE treatments ($P<0\cdot01$ and $P<0\cdot05$, respectively) (Table 2). Carcass weight of the EC treatment was significantly higher than the CC treatment as were the absolute weights of leg, back, breast and filet ($P<0\cdot05$) from birds of the EC group. Carcass weight relative to BW of the birds of the EC treatment was significantly lower than that of the CC treatment. It was observed that the fresh weight of abdominal fat and abdominal fat relative to carcass weight were significantly higher in the EC treatment than in the CC treatment.

Diet selection and performance of the birds of the EC treatment

From day 3 to 8 the birds of the EC treatment consumed more diet C than diet E (Figure 2). However, diet E was more consumed than diet C in the last three weeks of the experiment. In the total period (3 to 43 d), total feed intake of diet E was significant higher than diet C ($3301 \pm 125\cdot7$ g vs $1487 \pm 125\cdot7$ g). Birds of the EC treatment showed significant higher feed intake

than treatments CC and EE in the periods 3 to 8 d, 8 to 15 d and 15 to 22 d. In the last three weeks, feed intake of the CC treatment remained different from the EC treatment, but feed intake of the EC and EE treatments was not found to be statistically different.

Body weight gain from day 3 to day 43 was significantly ($P<0\cdot01$) higher for the EC treatment ($3330 \pm 43\cdot7$ g) compared to the CC treatment ($3059 \pm 43\cdot7$ g) but not significant higher than the EE treatment ($3199 \pm 43\cdot7$ g).

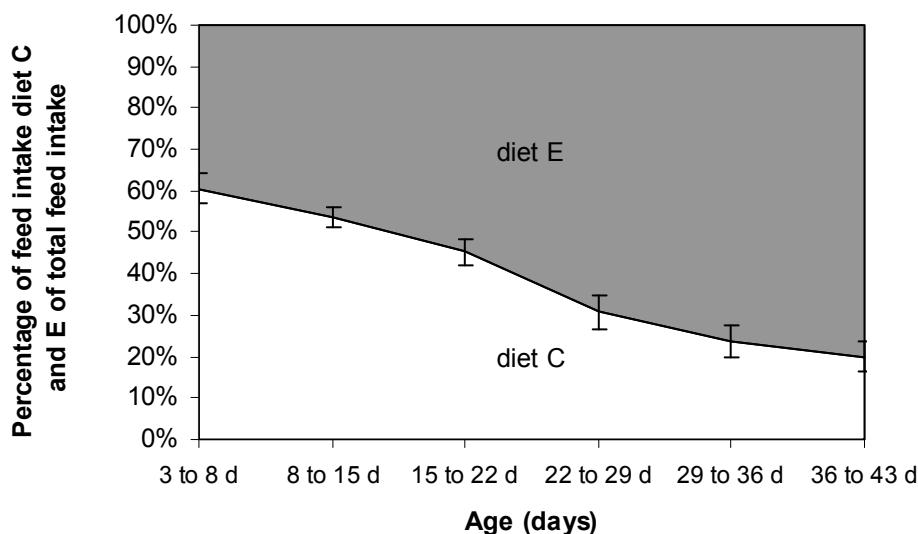


Figure 3 Diet selection of male broiler chickens of the EC treatment. I = standard deviation between replicates within treatments.

Total water intake was not affected by treatment in the period 15-22 d. Thereafter, birds of the EC and EE treatments had a significant higher total water intake than those of the CC in the last three weeks of the experiment. In the period 15-43 d total water intake for treatment EE and EC was significant higher compared to the CC treatment (10643 ± 316 g and 10940 ± 258 g vs 9114 ± 258 g, respectively). Ratio of water intake per feed intake was for the EC treatment did not differ significantly from EE ($2\cdot62 \pm 0\cdot03$ vs $2\cdot64 \pm 0\cdot04$).

DISCUSSION

This study was conducted to investigate the effect of the inclusion of a liquid wheat by-product in a diet for male broiler chickens on feed intake, performance, leg condition and diet

selection. The results of this investigation showed the experimental diet resulted in an increase in both water and feed intake.

Significant improvements in body weight gain resulting from high moisture diets have been observed previously (Yalda and Forbes, 1995, 1996; Yasar and Forbes, 1999, 2000). Feed conversion was not significantly affected by the experimental diet which is in accordance with the effect of wet feeding found by Yasar and Forbes (1999, 2000). This effect is a result of combined actions in the digestive tract: the efficiency of feed digestion in wet feeding is decreased since passage rate is increased due to a lower viscosity (Yasar and Forbes, 2000). However, faster penetration by the digestive enzymes and acids into the food particles increases nutrient digestibility and villi height in the digestive segments to result in an increased absorptive area in the digestive tract (Yasar and Forbes, 1999). It can be summarised that feed conversion in wet feeding is not affected by the combined effects of larger feed intake and potentially increased retention. In our experiment no effort was made to find evidence behind the performance of the broilers, but the results of feed intake and growth caused by wet feeding in our study suggests a similar mechanism as mentioned above.

An increase in total water intake as a result of wet feeding was also found by Yalda and Forbes (1995) and Yasar and Forbes (1998; 1999; 2000). However, the increase in this study of the ratio total water intake per g DM feed was not expected, because this effect was not observed by Yasar and Forbes (1999; 2000) and Scott (2002). It is possible that a liquid wheat by-product contained a higher amount of non-starch polysaccharides in the cell wall, which are able to bind more water and therefore stimulate water intake (Van der Klis *et al.*, 1993).

The results showed that the weight of the carcass in proportion to body weight was significantly higher for the CC than for the EE treatment. The results of the post-mortem examination showed a higher weight of the abdominal fat pad of the EE treatment compared to the CC treatment. Both results are probably caused by the combined effect of a different quantitative and qualitative feed consumption.

There were no high incidences observed of gait scores above 3 or a significant correlation found between gait score and live weight. This last result is in contrast with the correlations between gait score and body weight found by Sørensen *et al.* (1999). They found that lighter birds having a better gait score than heavy fast-growing broilers of the same age and strain. A possible explanation for this difference is that the activity level of the birds was increased due to meal feeding and low stocking density in our study. Birds were fed three times per day in

the first two weeks and thereafter two times per day. Both taking feeders out of the pens and placing the feeders back in the pen raised some activity of the birds in terms of attention (walking and/or standing). Stimulation of locomotor activity improves leg condition resulting in less leg weakness (Sørensen *et al.*, 1999; Bizeray *et al.*, 2002). Moreover, it is noted that the small number of birds per m² made it possible that the litter remained dry during the entire experiment. This has a positive effect on walking ability of the broiler chickens (Su *et al.*, 2000).

Pericardial effusion was found more often for the EE treatments compared to the CC treatment. This abnormality is a result of high growth rate (Van Voorst, 2004, personal communication). A higher BWG in the period 22 - 36 d for birds of the EE treatment compared to birds of the CC treatment is most likely the cause of the occurrence of this abnormality.

Results of diet selection of birds of the EC treatment showed a higher feed intake of the control diet from day 3 to 8, but thereafter, decreasing. In the last three weeks of the experiment a significant higher feed intake on dry matter basis of the experimental diet was observed. The shift in selection of the diet from C to E in the last three weeks of the experiment is possible caused by the fact that diet E was more attractive, but, because the pasty consistency, more difficult to be accessed and consumed by one or two week old broilers. It is observed that the taste buds of chickens are more stimulated by flavours when administered through water than through dry feed (Kare and Pick, 1960). Therefore, we think that the wet experimental diet had a more attractive flavour and had a higher potential to stimulate the taste buds compared to the dry control diet. The higher amount of diet E consumed by the EC treatment broilers suggests to be the cause of the similar results in total water intake, water feed ratio, carcass weight, abdominal fat weight and pericardial effusion observed in the EE treatment. However, feed intake and body weight of the choice fed broiler chickens was in the first week of the experiment already higher compared to the EE and CC treatments and this difference persisted throughout the experiment.

In conclusion, the inclusion of a liquid wheat by-product in a broiler diet showed an increased body weight of male broiler chickens but without an effect on feed conversion or walking difficulties. Moreover, choice fed broilers consumed more of the experimental diet and showed at least similar results as broilers fed with only the experimental diet.

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REFERENCES

- Bizeray, D., Leterrier, C., Constantin, P., Picard, M. and Faure, J.M. (2002) Sequential feeding can increase activity and improve gait score in meat-type chickens. *Poultry Science*, 81: 1798-1806.
- Bokkers, E.A.M. and Koene, P. (2003) Behaviour of fast- and slow growing broilers to 12 weeks of age and the physical consequences. *Applied Animal Behaviour Science*, 81: 59-72.
- Kare, M.R. and Pick, H.L. (1960) The influence of the sense of taste on feed and fluid consumption. *Poultry Science*, 39: 697-706.
- Kestin, S.C., Knowles, T.G., Tinch, A.E. and Gregory, N.G. (1992) Prevalence of leg weakness in broiler chickens and its relationship with genotype. *Veterinary Record*, 131: 190-194.
- SAS, 1996 Proprietary Software, Release 6.12. SAS Institute Inc., Cary, NC, USA.
- Scholten, R. and Verdoes, N. (1997) The Dutch benefit from a recycling role. *Pigs*, 13: 16-17.
- Scholten, R.H.J., Van der Peet-Schwering, C.M.C., Den Hartog, L.A., Vesseur, P.C. and Verstegen, M.W.A. (1999) Effect of liquid by-products on performance and health of pigs. *Pig News and Information*, 20: 81N-88N.
- Scott, T.A. (2002) Impact of wet feeding wheat-based diets with or without enzyme on broiler chick performance. *Canadian Journal of Animal Science*, 82: 409-417.
- Sørensen, P., Su, G. and Kestin, S.C. (1999) The effect of photoperiod:scotoperiod on leg weakness in broiler chickens. *Poultry Science*, 78: 336-342.
- Su, G., Sørensen, P. & Kestin, S.C. (2000) A note on the effects of perches and litter substrate on leg weakness in broiler chickens. *Poultry Science*, 79: 1259-1263.
- Van der Klis, J.D., Van Voorst, A. and Van Cruyningen, C. (1993) Effect of a soluble polysaccharide (carboxy methyl cellulose) on the physico-chemical conditions in the gastrointestinal tract of broilers. *British Poultry Science*, 34: 971-983.

- Yalda, A.Y. and Forbes, J.M. (1995) Food intake and growth in chickens given food in the wet form with and without access to drinking water. *British Poultry Science*, 36: 357-369.
- Yalda, A.Y. and Forbes, J.M. (1996) Effects of food intake, soaking time, enzyme and cornflour addition on the digestibility of the diet and performance of broilers given wet food. *British Poultry Science*, 37: 797-807.
- Yasar, S. and Forbes, J.M. (1998) Effects of wetting and enzyme supplementation of wheat-based foods on performance and gut responses of broiler chickens. *British Poultry Science*, 38: S43-S44.
- Yasar, S. and Forbes, J.M. (1999) Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. *British Poultry Science*, 40: 65-76.
- Yasar, S. and Forbes, J.M. (2000) Enzyme supplementation of dry and wet wheat-based feeds for broiler chickens: performance and gut responses. *British Journal of Nutrition*, 84: 297-307.

CHAPTER 3

**Effect of diet structure, conformation and acidification on
development of the foregut segment and performance of broilers**

SUBMITTED TO BRITISH SCIENCE

ABSTRACT

The effects of structure (coarse vs fine), conformation (wet vs dry) and acidification (acidified vs nonacidified) of a diet fed during the starter phase (0-21 days) and its carry over effect in the grower phase (22-42 days) while feeding a dry or wet grower diet were studied, using 308 male broilers. Animals were housed in floor pens. Birds were fed *ad libitum* in a meal feeding system for 3 times a day during the starter phase and 2 times a day during the grower phase, with photoperiods of 18 h light: 6 h dark and 16 h light: 8 h dark, respectively.

Wet feeding increased feed intake with about 48 % and body weight gain by 85 % compared to dry feeding during the starter phase. During the grower phase these traits increased 39% and 86 %, respectively in favour of the wet diets. A carry over effect of starter diet structure for body weight gain during grower phase was observed. There was a significant interaction between diet conformation and structure on feed intake and body weight gain during the starter phase. Water intake was reduced ($P < 0.05$) in the birds fed wet diets during the whole study period (0-42 days), but total water intake (free water + water added to feed) was significantly higher in the wet fed birds (approximately 67 %) than in the dry fed birds, especially during the starter phase. Water intake during the grower phase was similar among all treatments. Diet structure and acidification did not show any effect on water intake during either stage of this experiment. Fresh weights of the gastro-intestinal (GI) tract organs were influenced by wet feeding during the whole study period. Relative weight of the GI tract organs was significantly ($P < 0.05$) higher in the dry fed birds and differed approximately 21% during the starter phase and by 11 % during the grower phase compared to wet feeding. There was a carry over effect of diet conformation (larger organs) in the starter phase on the weight gain of the organs during the grower phase. Larger GI tract organs (approximately 10%) were found in birds that had been fed a coarse diet during the starter phase but it had no significant carry over effect during the grower phase.

Keywords: particle size, acidified, wet, dry, GI tract development, performance

INTRODUCTION

Several methods of feed manipulation and feeding strategy have been proposed to obtain better performances and health of broilers. Results from literature showed that the inclusion of whole wheat or the use of a coarse mash (larger particle sizes) were associated with large gizzards. Such enlarged gizzards were the result of feed structure (Nir *et al.*, 1995; Hetland and Svhuis, 2001). A large and muscular gizzard may further optimize the grinding and absorptive capacities of the gastrointestinal tract (Nir *et al.*, 1995) and this may have further effects on feed processing after intake. It may thus contribute to a better performance of broilers. Similarly, wet feeding for broilers in the early phase of life has promising effects on feed intake and feed utilization efficiency, due to the improvement of nutrient retention (Yalda and Forbes, 1995). Moreover, 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.*, 2007 unpublished). Thus, there may be reason for the development of a new feeding strategy for broilers based on these effects on gut functioning. The majority of the work done so far is limited to a specific time period in broilers' life. It was thought that there is still a lot of information missing with regard to different types of diets and their effects on broiler performance.

The objective of this 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 the carry over effects to dry and wet feeding in the grower phase (22-42 days).

MATERIALS AND METHODS

Bird, 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, with 10 chickens per pen. Chickens in each pen were weighed for initial weight and after weekly intervals.

All the birds had access to feed and water *ad libitum*. The feed was made available on a meal fed basis to change the wet diets after a certain interval. The feed trough with the wet feed would otherwise dry-out quickly and, as a consequence, prevent small chickens to properly ingest their feed. The experimental diets were offered 3 times a day at 8:00; 14:00 and 20:00 hours during the starter period and 2 times a day at 8.00 and 18.00 hours during the grower period. There were 3 nipple drinkers and 1 feeding trough placed in each pen to have easy access to feed and water for each chicken.

Full wood shavings covered the floor pens; each pen had dimensions of 1m x 1.5m and 0.6m height. The temperature inside the room was initially maintained at 32⁰C from day 0 to 7 and gradually decreased by 3⁰C per week until it reached 21⁰C at day 21. This temperature was set and maintained until day 42.

Artificial light was provided at a schedule of 18 hours light and 6 hours dark during the starter phase. Light was provided for 18 hours from 8:00 h (first meal) till 2:00 h, then followed by the dark period from 2:00 h till 8:00 h. In the first 3 days, light was provided 23 hours to enable the young chicken sufficient time to eat and drink. This was done to prevent birds from dehydration during the critical period of early life. After the starter phase at 21 days of age, the light schedule was rearranged to match 16 hours light and 8 hours dark during the grower phase. Light was provided from 8:00 h (first meal) till 24:00 h, followed by a dark period from 24:00 h to 8:00 h. Light intensity was maintained above 20 lux at the bird's level during the light periods.

Scheme 1, Experimental scheme during starter period (0 – 21 days of age)

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

Starting from day 22 (grower period), each of the 32 pens were split up to make 64 pens with 4 chickens in each pen. Two birds per pen were sacrificed at the end of the starter period. The grower diet was fed in either wet or dry form until 42 days of age to study carry over effects of the treatments during the starter phase on the grower phase. This means that all diets in the grower period were 'fine' and 'non-acidified'.

Experimental diets

The feed preparation and mixing was done outside the experimental shed to prevent any disturbance to the chickens. All feed troughs were taken out each time while offering feed in similar way to maintain uniformity. Wet diets were always offered completely new to have fresh feed at every meal. The same routine was used with the dry feeds.

All experimental diets (see Table 1) were formulated to meet the nutrient and energy requirements for broilers (NRC, 1994). The diet defined as ‘coarse’, was processed by a roller-mill with one roller pair and roller 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’ were added without hammer milling. Diets defined as ‘fine’ were processed by a hammer mill with an opening screen of 3.0 mm (modified after Hamilton and Proudfoot, 1995). Particle size distribution of the coarse and fine diets was assessed with wet sieve analysis (Goelema, 1999), and is shown in Figure 1.

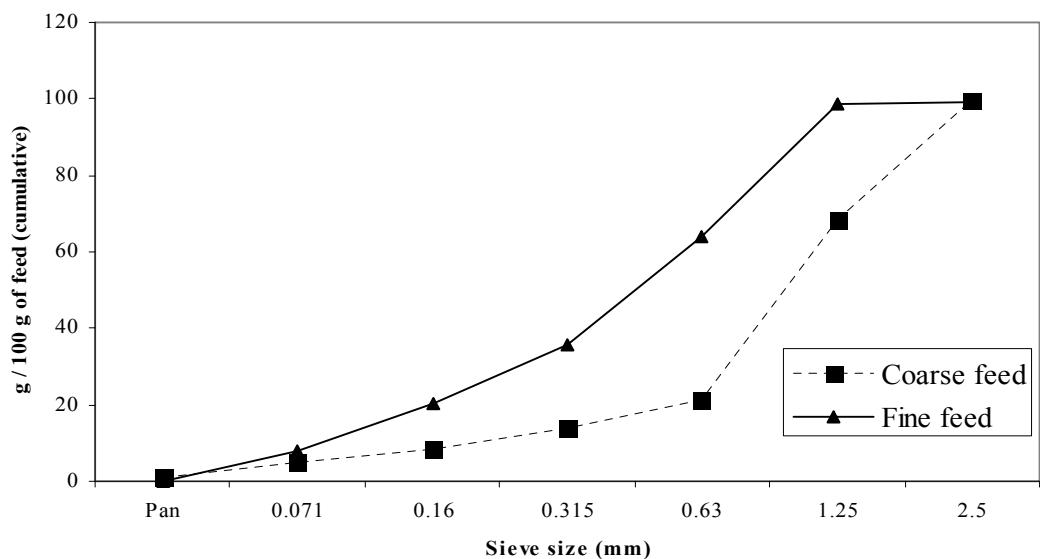


Figure 1. Particle size distribution of coarse and fine feeds (wet feeding method)

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

The wet diets were made by mixing 1 kg of dry feed with 1 kg of tap water 20 minutes prior to each feeding time (modified after Yasar and Forbes, 1999). In the first 3 days of age, feed to water ratio was 1:1.3. This somewhat higher moisture content of the wet diets was done to facilitate the young birds to pick and adapt easily to the wet diets while shifting from yolk nutrition to a ‘solid diet’.

Observations

Feed intake was determined by subtracting the weight of the refusals in the feeder from the given quantity of feed. Feed intake of the wet diet was calculated on the basis of the dry feed quantity offered to the chickens. The real ‘dry’ intake of the wet diet was corrected for water loss due to evaporation, by using a feed trough with a wet coarse diet and a feed trough with a wet fine diet in an empty pen and to measure weight losses of the full feed trough in coarse of time. Thus all of feed intake is based on a dry feed basis.

Body weight gain was determined by weighing the initial body weight of birds per pen and at weekly intervals.

The development of the GI tract was determined at the end of the starter and grower phase by weighing the empty crop, proventriculus, gizzard, and duodenum (with pancreas intact). The fresh weights of these organs were expressed as gram per 100 gram of body weight.

Table 1. Ingredient (g/kg) and analysed nutrient composition (g/kg dry matter) of the control and experimental diet.

Composition	Amount presented
<i>Ingredients</i>	
Wheat	349.4
Maize	275.7
Toasted full fat soya beans	199.6
Soya bean meal 44/7	94.3
Fishmeal 66% CP	49.9
Ca CO ₃	11.8
L-Threonine	3.0
Broiler vitamin and mineral premix	5.0
Monocalciumphosphate	4.7
Phytase	3.0
Salt	1.7
DL-Methionine	1.4
L-Lysine HCl	0.5
<i>Analysed nutrients</i>	
Crude protein	251.3
Crude fat	77.6
Total starch	407.0
Crude fibre	34.4
Ash	63.2

Statistical analysis

Analysis of data was performed using SPSS® software (SPSS Inc., 2003) with pen as experimental unit and treatment as factor. Similarly, carry over effects from 3 weeks onwards were analyzed as separate factors in addition to effect of wet and dry feed.

The data of the starter phase were analyzed using a full factorial model with 3 factors: diet conformation, structure and acidification. In total, there were 8 different treatments in the starter phase. In the grower phase the full factorial model was used. There were 4 factors: conformation, structure and acidification during starter phase (the carry over effect), and conformation (wet or dry) of grower phase diet. In total there were thus 16 different treatments. Significancies, if not stated otherwise, are based on a 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 = Carry over effect of structure (coarse / fine);
 γ_k = Carry over 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.

RESULTS

Starter phase (0 – 21 days of age)

Feed intake, body weight gain and feed conversion ratios

The effects of the different treatments on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers during the starter phase of the experiment are presented in Table 2. It shows that conformation of diet had a significant effect on feed intake (FI) of broilers as wet-fed birds consumed significantly more feed (about 48%) than dry-fed birds. Among the wet feeding groups, the highest FI was found in Wet-Fine-Acidified (WFA) diet group, while birds of Dry-Fine-Acidified (DFA) group consumed the lowest amount of feed during the whole starter phase. Diet structure and acidification did not affect FI of broilers. The interaction between conformation and structure of diet was significant for feed intake, indicating that a coarser diet increased FI under dry feed conditions while a finer diet increased FI under wet feed conditions.

Table 2. Effect of different treatments on feed intake (g), body weight gain (g) and feed conversion ratio of birds during 0- 21 days (Mean \pm SD)

Conformation	Acidification	Structure	FI	BWG	FCR	
Dry	Acidified	Coarse	902 \pm 57	560 \pm 61	1.62 \pm 0.09	
		Fine	720 \pm 74	401 \pm 71	1.82 \pm 0.16	
	Non acidified	Coarse	847 \pm 75	468 \pm 99	1.85 \pm 0.35	
		Fine	737 \pm 117	409 \pm 112	1.85 \pm 0.21	
Wet	Acidified	Coarse	1139 \pm 89	766 \pm 109	1.50 \pm 0.16	
		Fine	1241 \pm 51	925 \pm 48	1.34 \pm 0.02	
	Non acidified	Coarse	1174 \pm 45	830 \pm 37	1.41 \pm 0.02	
		Fine	1192 \pm 56	890 \pm 124	1.50 \pm 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 2. It can be observed from Figure 2 that there was an extra increase in FI rate during week 3 among the wet-fed birds. Increment of FI of dry-fed birds was almost the same throughout the whole period. Feed intake of birds on different structure and acidification followed a linear trend with age during whole starter period. It was showed that there was no separate effect of diet structure or acidification on FI of birds.

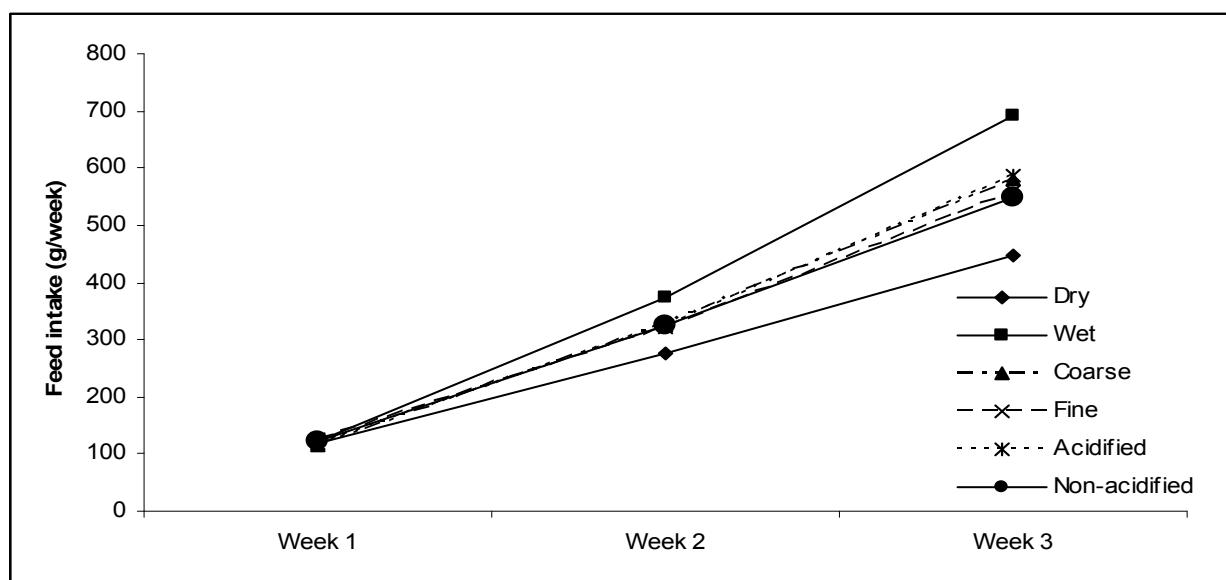


Figure 2. Effect of the main diet factors on feed intake during the starter phase, weekwise.

Water intake

During the whole starter phase, BWG tended to be highest in birds fed with the wet-fine-acidified diet, followed by the wet-fine-nonacidified group. Differences in treatments within the wet feeding groups were not significant. Compared to birds fed with dry diets, wet-fed birds showed a much higher BWG (85 %; $P<0.01$). Within the dry or wet feeding groups, BWG was similar, so diet structure and acidification did not affect BWG. These findings gave a strong evidence of the effect of conformation of the diets on BWG. Similar to FI, there was a highly significant interaction between structure and conformation on BWG.

FCR showed a similar trend compared to FI and BWG: conformation of the diet had a significant effect on FCR, but no effect of diet structure or diet acidification could be detected. Non-significancies were partly due to the large variation among individual pens (some birds could better cope with smaller quantities of diet and still gain weight than other birds). Wet-fine-acidified-fed birds had the lowest FCR of all treatment, followed by the wet-coarse-non acidified fed birds.

Feed to water intake ratio

There was a large difference in feed intake among the different treatments, so therefore, water intake was expressed as feed to water intake ratio (FWIR).

FWIR was calculated in two different ways, as it is presented in Table 3. Firstly, FWIR from the nipple only (free water) was considered. Secondly, water mixed in the feed in addition to water from the nipple (= total water intake) was also calculated as birds of the wet feeding groups consumed water via this route as well.

The feed to ‘free’ water intake ratio was between 0.60 – 0.66 for all dry-fed groups, and between 1.13 – 1.31 for all wet-fed groups: the latter groups reduced their free water intake by almost 50% due to the ‘wetness’ of their diets. Considering ‘total’ water intake, the wet-fed groups consumed approximately 21% more water than the dry-fed groups. FWIR was not affected by structure or acidification. Only a small interaction in FWIR could be observed between conformation and acidification for free water intake.

Table 3. Feed water intake ratio of birds in different treatments during starter phase

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
Structure		0.773
Acidification		0.110
Conformation x Structure		0.406
Structure x Acidification		0.434
Acidification x Conformation		0.021

Weights of parts of the foregut

Fresh empty weights of different parts of the foregut at 21 days are presented as relative weights (as g per 100 g body weight of birds). The results are shown in Table 4.

Table 4. Relative fresh organ weights of different parts of gastro-intestinal tract (g/100g BW of birds) during starter phase (Mean \pm SD)

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

*Including pancreas

It can be seen from Table 4 that the relative weights of all parts of the foregut were significantly higher in the dry-fed birds as compared to the wet-fed birds (18, 13, 37, 13, and 9 % higher for crop, proventriculus, gizzard, duodenum and jejunum, respectively). Relative weights of the gizzard, duodenum and jejunum were higher for the birds that were fed a coarse diet compared to those that were fed a fine diet (14, 8, and 8 % higher for gizzard, duodenum and jejunum, respectively). On the other hand, relative weights of the proventriculus and gizzard were lower for the birds that were fed an acidified diet compared to those that were fed a non-acidified diet (5 and 9 % lower for proventriculus and gizzard, respectively). It is interesting to note that there was an effect of all the three diet factors (conformation, structure and acidification) on relative gizzard weight.

Two interaction terms were significant: for both proventriculus and jejunum, relative weights on a coarse diet were somewhat lower under dry-fed conditions but quite higher under wet-fed conditions.

Grower phase (22 – 42 days of age)

Feed intake, body weight gain and feed conversion ratio

Conformation of the diets in both the starter and grower phase had a tremendous effect on FI, BWG and FCR in the grower phase, as illustrated in Table 5.

Table 5. Effect of different treatments on feed intake (g), bodyweight gain (g) and feed conversion ratio of birds during grower phase (Mean \pm SD).

Starter diet			Grower diet	FI	BWG	FCR	
Dry	Acidified	Coarse	Dry	2418 \pm 194	1367 \pm 65	1.77 \pm 0.11	
			Wet	3652 \pm 169	2272 \pm 130	1.61 \pm 0.02	
		Fine	Dry	2189 \pm 287	1307 \pm 152	1.67 \pm 0.10	
	Nonacidified	Coarse	Wet	3201 \pm 177	2061 \pm 66	1.55 \pm 0.06	
			Dry	2571 \pm 189	1602 \pm 122	1.61 \pm 0.13	
		Fine	Wet	3422 \pm 263	2220 \pm 123	1.54 \pm 0.10	
Wet	Acidified	Coarse	Dry	2000 \pm 431	1091 \pm 320	1.87 \pm 0.16	
			Wet	3299 \pm 497	2120 \pm 223	1.55 \pm 0.10	
		Fine	Dry	2808 \pm 158	1523 \pm 104	1.84 \pm 0.03	
	Nonacidified	Coarse	Wet	3857 \pm 177	2187 \pm 91	1.76 \pm 0.04	
			Dry	3050 \pm 101	1598 \pm 153	1.92 \pm 0.04	
		Fine	Wet	3842 \pm 74	2191 \pm 149	1.76 \pm 0.14	
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	

FI and BWG during the grower phase of the wet-fed birds in the grower phase, irrespectively their nutritional history in conformation type of diet, were 39 and 49 % higher, respectively, as compared to their dry-fed counterparts. FCR followed this pattern. Irrespective of the type of diet fed during the grower phase, FI and BWG in the grower phase were 20 and 10 % higher, respectively, for birds that had been fed wet diets in contrast to dry diets in the starter phase. Moreover, irrespective of the type of diet fed during the grower phase, FI and BWG in the grower phase were 5 and 7 % higher, respectively, for birds that had been fed coarse diets in contrast to fine diets in the starter phase.

The interaction term for conformation during the starter phase and conformation during the grower phase was significant: feeding a wet diet instead of a dry diet during the grower phase resulted in a more than 60 % higher BWG under dry-fed conditions in the starter phase and about 35 % higher BWG under wet-fed conditions in the starter phase, as a result of a carry-over effect. Dry-fed birds during early life compensated FI during later life if they receive a wet-based diet that is easily to be ingested. This is illustrated by the weekly BWG data in Figure 3.

Although FI and BWG were both higher for the wet-fed birds during the grower phase, FCR's became somewhat worse in these groups, regardless whether these diets had been fed in the starter or grower phase.

In contrast to the significant effects on performance due to conformation and structure, no effects of diet acidification could be detected on performance during the grower phase.

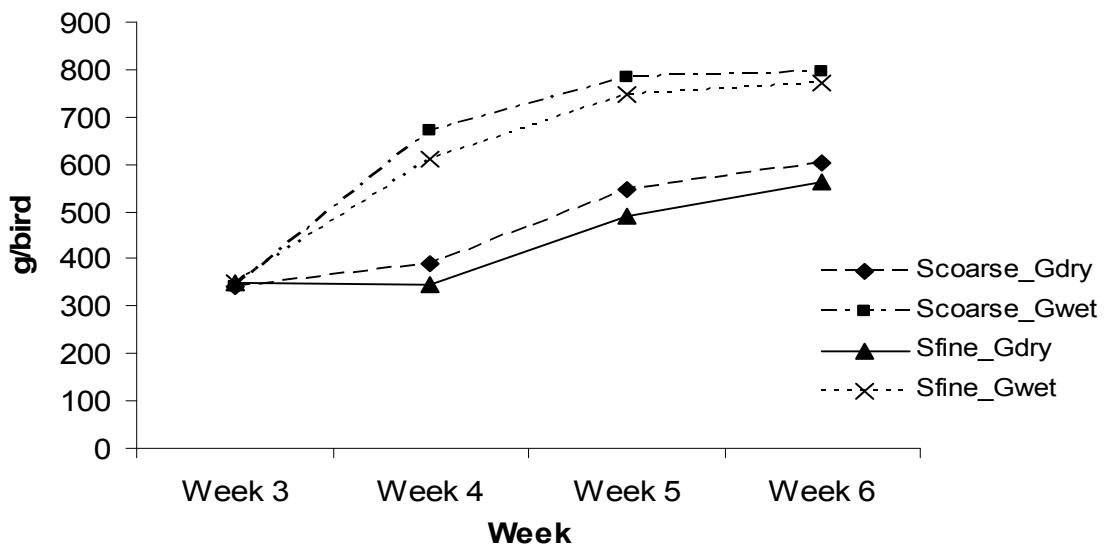


Figure 3. Effect of diet structure in the starter phase on body weight gain in the grower phase, week wise (S = starter; G = grower)

Weights of parts of the foregut

Empty weights of the different parts of the foregut differed among the treatment groups, mainly as a result of effects of conformation during the starter and grower phase (Table 6).

Empty relative weights of gizzard, duodenum and jejunum at the end of the grower phase of the wet-fed birds during the grower phase were, irrespective their nutritional history in conformation type of diet in the starter phase, 24 % (gizzard), 9 % (duodenum) and 7 % (jejunum) lower as compared to their dry-fed counterparts. Irrespective of the type of diet fed during the grower phase, empty relative weights of all foregut segments at the end of the grower phase were 8 % (crop), 11 % (proventriculus), 15 % (gizzard), 11 % (duodenum) and 10 % (jejunum) higher for birds that had been fed wet diets in contrast to dry diets in the starter phase. Moreover, irrespective of the type of diet fed during the grower phase, relative weight of the proventriculus at the end of the grower phase was 7 % higher for birds that had

been fed a non-acidified diet in contrast to those that had been fed an acidified diet in the starter phase.

The interaction term for conformation during the starter phase and conformation during the grower phase was significant for the relative empty weight of the gizzard: feeding a wet diet instead of a dry diet during the grower phase resulted in a 27 % lower relative gizzard weight under dry-fed conditions in the starter phase and in a 21 % lower relative gizzard weight under wet-fed conditions in the starter phase. This carry-over effect may be due to a tremendous increase in BWG of wet-fed birds in the grower phase after a dry-fed starter phase, than as a result of large differences in gizzard development as such in the grower phase.

There were no significant effects of diet structure during the starter phase on the empty weights of all foregut segments at the end of the grower phase.

Table 6. Relative fresh weights of different organs of gastro-intestinal tract (g/100g BW) during grower phase (mean \pm SD)

		Starter phase		Grower diet		Crop		Proventriculus		Gizzard		Duodenum*		Jejunum		
Dry	Acidified	Coarse	Fine	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	
Nonacidified	Coarse	Dry	0.25 ± 0.04	0.28 ± 0.04	0.26 ± 0.05	1.76 ± 0.35	0.97 ± 0.15	1.32 ± 0.22	Fine	0.24 ± 0.04	0.26 ± 0.05	1.35 ± 0.09	0.91 ± 0.21	1.11 ± 0.18	1.42 ± 0.10	
		Dry	0.25 ± 0.03	0.32 ± 0.07	2.14 ± 0.66	1.07 ± 0.10	1.43 ± 0.12	1.42 ± 0.10		0.25 ± 0.05	0.41 ± 0.23	0.97 ± 0.06	1.43 ± 0.12	1.31 ± 0.16	1.43 ± 0.12	
		Wet	0.23 ± 0.05	0.33 ± 0.01	1.93 ± 0.26	0.92 ± 0.02	1.36 ± 0.13	0.85 ± 0.08		0.30 ± 0.02	0.30 ± 0.02	1.36 ± 0.13	0.85 ± 0.08	1.23 ± 0.17	1.23 ± 0.17	
		Wet	0.24 ± 0.05	0.34 ± 0.07	2.02 ± 0.63	1.01 ± 0.17	1.43 ± 0.29	1.43 ± 0.29		0.27 ± 0.04	0.29 ± 0.05	1.25 ± 0.22	0.83 ± 0.04	1.28 ± 0.17	1.28 ± 0.17	
	Fine	Dry	0.26 ± 0.06	0.29 ± 0.05	1.25 ± 0.22	0.83 ± 0.04	1.25 ± 0.19	0.93 ± 0.05		0.21 ± 0.03	0.25 ± 0.02	1.51 ± 0.19	0.93 ± 0.05	1.39 ± 0.04	1.39 ± 0.04	
		Wet	0.26 ± 0.06	0.29 ± 0.05	1.25 ± 0.22	0.83 ± 0.04	1.25 ± 0.19	0.93 ± 0.05		0.21 ± 0.03	0.25 ± 0.02	1.51 ± 0.19	0.93 ± 0.05	1.39 ± 0.04	1.39 ± 0.04	
		Dry	0.24 ± 0.04	0.26 ± 0.02	1.36 ± 0.10	0.78 ± 0.10	1.10 ± 0.06	1.10 ± 0.06		0.23 ± 0.04	0.28 ± 0.03	1.25 ± 0.08	0.83 ± 0.07	1.13 ± 0.11	1.13 ± 0.11	
		Wet	0.25 ± 0.03	0.24 ± 0.03	1.28 ± 0.19	0.80 ± 0.07	1.17 ± 0.13	1.17 ± 0.13		0.24 ± 0.04	0.26 ± 0.02	1.25 ± 0.08	0.83 ± 0.07	1.13 ± 0.11	1.13 ± 0.11	
Wet	Coarse	Dry	0.23 ± 0.04	0.28 ± 0.03	1.25 ± 0.08	0.83 ± 0.07	1.25 ± 0.08	1.25 ± 0.08		0.23 ± 0.04	0.26 ± 0.02	1.25 ± 0.08	0.83 ± 0.07	1.25 ± 0.08	1.25 ± 0.08	
		Wet	0.24 ± 0.04	0.26 ± 0.02	1.36 ± 0.10	0.78 ± 0.10	1.10 ± 0.06	1.10 ± 0.06		0.24 ± 0.03	0.28 ± 0.03	1.28 ± 0.19	0.80 ± 0.07	1.17 ± 0.13	1.17 ± 0.13	
		Dry	0.25 ± 0.03	0.24 ± 0.03	1.28 ± 0.19	0.80 ± 0.07	1.17 ± 0.13	1.17 ± 0.13		0.26 ± 0.04	0.26 ± 0.02	1.60 ± 0.19	0.99 ± 0.08	1.29 ± 0.07	1.29 ± 0.07	
		Wet	0.25 ± 0.03	0.24 ± 0.03	1.27 ± 0.22	0.81 ± 0.11	1.17 ± 0.06	1.17 ± 0.06		0.20 ± 0.01	0.24 ± 0.03	1.27 ± 0.22	0.81 ± 0.11	1.17 ± 0.06	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	1.13 ± 0.09		0.22 ± 0.07	0.31 ± 0.06	1.18 ± 0.14	0.75 ± 0.06	1.07 ± 0.08	1.07 ± 0.08	
		Wet	0.22 ± 0.07	0.31 ± 0.06	1.18 ± 0.14	0.75 ± 0.06	1.07 ± 0.08	1.07 ± 0.08		0.20	0.029	<0.001	<0.001	<0.001	<0.001	
		Dry	0.951	0.784	0.313	0.722	0.459	0.539		0.200	0.390	0.204	<0.001	0.005	0.684	
		Wet	0.390	0.656	0.459	0.722	0.459	0.539		0.853	0.051	0.005	0.337	0.156	0.762	
Probability level of contrast		Conformation starter	0.020	0.001	<0.001	<0.001	0.001	<0.001	Acidification starter	0.951	0.029	0.313	0.722	0.005	0.684	
		Structure starter	0.722	0.784	0.313	0.722	0.459	0.539	Conformation grower	0.200	0.390	0.204	<0.001	0.005	0.684	
		Conformation starter x Conformation grower	0.390	0.656	0.459	0.722	0.459	0.539	Acidification starter x Conformation grower	0.853	0.051	0.005	0.337	0.156	0.762	
		Structure starter x Conformation grower	0.459	0.784	0.313	0.722	0.459	0.539	Conformation grower	0.425	0.882	0.298	0.156	0.495	0.884	

Probability level of contrast

DISCUSSION AND CONCLUSIONS

There are a number of published studies (Reece *et al.*, 1985; Rogel *et al.*, 1987; Cumming, 1994; Munt *et al.*, 1995; Kwakkel *et al.*, 1997; Hetland and Svhuis, 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; Yalda and Forbes, 1995; Yalda and Forbes, 1996; Slade and Forbes, 1997; Yalda and Forbes, 1999; Versteegh and Jongbloed, 1999; Preston *et al.*, 2000) with regard to optimization of broiler performance as a result of different type of diets. Several reports concluded that there are benefits of using coarse diets, acidified diets and or wet diets in the first weeks of life with regard to the enhancement of feed intake and body weight gain. These studies have shown some variation in results, mainly due to differences in experimental conditions. There are, however, no other studies found that combine all three above mentioned factors (coarse vs fine feed, acidified vs nonacidified feed and wet vs dry feed), as was done in the present trial.

Considering the need for future broiler feeding strategy, we set lighting hours and other management factors as per the interest and objectives of this study.

Generally, the influence of feed conformation (wet versus dry feeding) on different parameters in the present study was much greater than that of diet structure (coarse vs fine) and acidification (acidified vs non acidified). The effect of particle size differed with regard to different parameters, it had a large effect on gizzard development. The effect of acidification of the diet was not visible in almost all parameters of this study, except on the development of the gizzard and proventriculus. This effect was not maintained during grower period.

Wet feeding improved broiler growth rate during both starter and grower phase, which was associated with an increased in feed intake (FI). This is in accordance with the other authors (Pittard, 1996; Yalda and Forbes, 1995; Yalda and Forbes, 1996; Slade and Forbes, 1997; Yasar and Forbes, 1999). This effect may be explained by the facilitation of access of enzymes resulting in a better nutrient retention (Yalda and Forbes, 1995).

A second explanation of the beneficial effects of wet feeding may be attributed to a decreased viscosity of gut contents. So, there may be a better development of the layer of the villi in the digestive segments and a reduced crypt cell proliferation rate in the crypts (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 retention time remained longer in the GI tract (Yasar and Forbes, 2000).

Similarly, Preston *et al.* (2000) working with dry and wet mash diet found that birds on wet mash showed higher FI and FCR, with almost 50% higher BWG compared to birds fed with dry mash. Outperforming growth (about 85%) was also found in the present study.

Scott (2002) stated that broilers cannot eat enough of a 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 difference between dry and wet fed birds in the present study, compared to other reports may be associated to the lighting schedule of our experiment. Feeds offered in our study were based on *ad libitum* meal feeding with 18 hr light and 6 hours dark during the starter phase. So there were lesser hours available for the birds to have access to the feeders compared to most literature studies.

An explanation may be that swallowing of a wet diet into the crop may be faster than with dry feed. Thus a higher FI can be expected when birds have a limited time (light) for eating. This reasoning was supported by Savory (1976), who found that broilers kept on a lighting schedule that includes periods of dawn and darkness will increase their feeding activity during the dawn 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 lower FCR. In contrast, dry-fed birds require more time and energy for feed intake and digestion leading to poor growth and FCR. It also seems 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 in the dry-fed birds.

There was no significant effect of diet structure on FI and growth of the birds; this contradicts the finding of several workers (Nir *et al.*, 1990; Nir *et al.*, 1994). These workers found that FI and BWG improved positively with increased particle size. It may be due to the dominant effect of conformation of the diet involved in our experiment. On the other hand, some other studies were done with diets in pelleted form. In pellets, however, the ratio of fine particles is larger compared to coarse mash diets. The diets used in this study were in the mash form.

We were not able to confirm a positive result from acidification of the diet, which was against our expectation. Literature on 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 a trial conducted by Research Institute for Animal Husbandry, the Netherlands. They reported better growth and feed conversion ratio with less mortality both in starter and grower period by using dry feed acidified with this product. In another field trial in Brazil, it was found that this acidifier has by far outperformed a probiotic compound in growth and feed conversion ratio.

Studies in literatures have shown that acidification of diets may have favorable effect on poultry production by creating a healthy gut environment (Heres *et al.*, 2003; Heres *et al.*, 2004; Iba & Berchieri, 1995; Berchieri and Barrow, 1996; Thompson and Hinton, 1997), rather than directly increasing FI. Thus, this study remained inconclusive on the effect of acidification of feed, because GIT microbiology and pathology was not studied here.

Although fresh WI (water intake from the nipple only) was lower in birds given wet feed, total WI (from the nipple plus that from the wet feed) was significantly higher in wet-fed birds than in dry-fed birds. Similarly, DM intake was higher in wet-fed birds. These findings are in accordance with those of Yasar and Forbes (1999). Water consumption is influenced by several factors, including size and age of birds and type and amount of feed consumed (Duke, 1986). Water is essential for the digestion and for metabolism and the voluntary intake of water is usually closely related to dry matter intake in most species, including poultry (Patrick and Ferrise, 1962). This is supported by the data of present study.

Empty fresh weight of individual gut segments tended to increase with wet feeding. No significant differences between wet and dry feeding on GI tract development were found in the study of Yasar and Forbes (2000). We found a significant effect of diet conformation on GI tract development. On the other hand, there was a clear effect of diet structure and acidification on the development of the GI tract organs. Heavier gizzards were found in birds fed coarse diets compared to birds fed 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 Svhuis, 2001; Engberg *et al.*, 2002; Gabriel *et al.*, 2003; Dahike *et al.*, 2003) were also in the same line, their results showed positive correlation of feed particle size and development of the gizzard in broilers.

Sizes of duodenum and jejunum in birds fed with a fine diet were bigger compared to those in birds fed with a coarse diet. This is similar with the findings of other workers (Gabriel *et al.*, 2003; Dahike *et al.*, 2003). In our study the influence of diet structure was not prominent for the development of proventriculus, unlike results of studies reported by Gabriel *et al.* (2003). Similarly, acidification did not show a clear effect on the growth of the duodenum while it had an effect on the reduction in relative weight of the proventriculus, which is in contrast with the finding of Bosch *et al.* (2007 unpublished). This phenomenon may be due to the acidification of the diet preventing the proventriculus to develop its acid secretion. The weight of these organs was much more affected by the conformation of diet than by acidification. Birds with a dry diet had relative bigger proventriculus, duodenum and jejunum during both starter and grower phase.

The differences observed in the GI tract development imply that feed 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 by Nir and Ptichi (2001) that when fed a fine mash, feed flows quicker through the stomachs to the duodenum and small intestine. This phenomenon was accompanied by a marked atrophy of the gizzard and mild hypertrophy of the small intestine. Our study does not support this argument. It seems that the rapid development of GI tract in the some fine-diet-fed birds has a visible effect on the growth of the GI tract. From these results it seems that definition of coarse and fine need to be much more precise.

It can be concluded that overall performance of broilers was much better in all wet diet groups as compared to the dry diet groups. There was a better growth in the wet fed birds associated with a higher FI and lower FCR during the entire broiler production cycle, even when a limited time of feeding was offered. Structure and acidification of diet had mixed effects on performance and GI tract development of broilers, which need further study.

There was a carry over effect of the starter phase diet conformation on the overall performance and GI tract development of broilers during the 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, feeding a coarse diet in wet form during the starter phase and wet fine diets during grower phase may be used as a better strategy.

Wet feeding of diet has shown a promising effect on the performance and GI tract 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 GI tract. Similarly, the interaction effect between diet structure and conformation need to be studied.

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REFERENCES

- Berchieri, Jr.A. and Barrow, P.A. (1996) Reduction in incidence of experimental fowl typhoid by incorporation of a commercial formic acid preparation (Bio-Add TM) into poultry feed. *Poultry Science*, 75:339-341.
- Bosch, G., Khoa, M.A., van der Poel, A.F.B., Koene P., and Bokkers, E.A.M. (2007). A liquid, acidified wheat by-product in diets for broilers: impact on feed intake, performance, leg condition and diet selection. *British Poultry Science*, submitted.
- Cumming, R.B. (1994) Opportunities for whole grain feeding. *Proceeding of 9th European Poultry Conference, Glasgow*, Volume II, p. 219. World Poultry Science Association.
- Dahike, F., Ribeiro, A.M.L., Kessler, A.M., Lima, A.R. and Maiorka, A. (2003) Effects of corn particle size and physical form of the diet on the gastrointestinal structure of broiler chickens. *Brazilian Journal of Poultry Science*, 5: 61-67.
- Duke G.E. (1986) Alimentary canal: secretion and digestion, special digestive functions and absorption in avian physiology. *4th edition. (P. D. Sturkie, Ed.) New York β Springer-Verlag*: 289-302.
- Engberg, M.S., Hedemann, M.S. and Jensen, B.B. (2002) The influence of grinding and peletting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British Poultry Science*, 44: 569-579.
- Gabriel, I., Mallet, S. and Leconte, M. (2003) Differences in the digestive tract characteristics of broiler chickens fed on complete pelleted diet or whole added to pelleted protein concentrate. *British Poultry Science*, 44: 283-290.
- Goelema, J.O., (1999). Processing of legume seeds: effects on digestive behaviour in dairy cows. PhD Thesis, Animal Nutrition group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands, 222 pp.
- Hamilton, R.M.G. and Proudfoot, F.G. (1995) Ingredient particle-size and feed texture- effects on the performance of broiler-chickens. *Animal Feed Science and Technology*, 51: 203-210.

- Heres, L., Engel, B., van Knapen, F., Wagenaar, A. and Urlings, B.A.P. (2003) Effect of fermented feed on the susceptibility for *Campylobacter jejuni* colonisation in broiler chickens with and without concurrent inoculation of *Salmonella enteritidis*. *International Journal of Food Microbiology*, 87(1-2): 75-86.
- Heres, L., Engel, B., Urlings, H.A.P., Wagenaar, J.A. and van Knapen, F. (2004) Effect of acidified feed on susceptibility of broiler chickens to intestinal infection by *Campylobacter* and *Salmonella*. *Veterinary Microbiology*, 99 (3-4): 259-267.
- Hetland, H. and Svhuis, B. (2001) Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British Poultry Science*, 42: 354-361.
- Iba, A.M. and Berchieri, Jr.A. (1995) Studies on the use of formic acid - propionic acid mixture (Bio-AddTM) to control experimental *Salmonella* infection in broiler chickens. *Avian pathology*, 24:303-311.
- Kwakkel R, Williams, B.A. and van der Poel, A.F.B. (1997) Effects of fine and coarse particle diets on gizzard growth and fermentation characteristics of the caecal contents in broiler chickens. *Proceedings of the 11th European Symposium on Poultry Nutrition (WPSA), Faaborg, Denmark*, August 24-28, 1997: 249-251.
- Munt, R.H.C., Dingle, J.G. and Sumpa, M.G. (1995) Growth, carcass composition and profitability of meat chickens given pellets, mash or free-choice diet. *British Poultry Science*, 36: 277-284.
- Nir, I., Melcion, J.P. and Picard, M. (1990) Effect of particle size of sorghum grains on feed intake and performance of young broilers. *Poultry Science*, 69: 2177-2184.
- Nir, I., Hillel, R. Shefet, G. and Nitsan, Z. (1994) Effect of grain particle size on performance. 2. Grain texture interactions. *Poultry Science*, 73: 781-791.
- Nir, I., Hillel, R. Ptichi, I. and Shefet, G. (1995) Effect of particle size on performance. 3. Grinding pelleting interactions. *Poultry Science*, 74: 771-783.
- Nir, I. and Ptichi, I. (2001) Feed particle size and hardness: Influence on performance, nutritional, behavioural and metabolic aspects. Advances in Nutritional Technology 2001. *Proceedings of the 1st World Feed Conference, 7th to 8th November, 2001*: 157–186.
- NRC (1994) Nutrient requirements of chickens. *National Research Council*, 1994.

- Patrick, H., Ferrise, A. (1962) Water requirements of broilers. *Poultry Science*, 41: 1363-1367.
- Pittard, E.W. (1996) Automatic poultry feeding devices with water sprayed feed mix. *USA*, 3 (443): 205.
- Preston, C.M., McKracken, K.J. and McAllister, A. (2000) Effect of diet form and enzyme supplementation on growth, efficiency and energy utilization of wheat-based diet for broilers. *British Poultry Science*, 41: 324-331
- Reece, F.N., Lott, B.D. and Deaton, J. W. (1985): The effect of feed form, grinding method, energy level, and gender on broiler performance in moderate (21°C environment. *Poultry Science*, 64: 1834-1839.
- Rogel, A.M., Balnave, D. Bryden, W.L. and Annison, E.F. (1987) Improvement of raw potato starch digestion in chickens by feeding oat hulls and other fibrous feedstuffs. *Australian Journal of Agricultural Research*, 38: 629-637.
- Savory, C.J. (1976) Broiler growth and feeding behaviour in three different lighting regimes. *British Poultry Science*, 17: 557-560.
- Scott, T. (2002) Impact of wet feeding wheat-based diets with or without enzyme on broiler chick performance. *Canadian Journal of Animal Science*, 82: 409-417.
- Slade, R. and Forbes, J.M. (1997) The effect (and duration effect) of wet feeding broiler chicks from day 1 to day 10 of life. Unpublished report to Dalgety Agriculture Ltd.
- SPSS® (2003) SPSS 12.0.1 for Window by SPSS Inc. 2003.
- Thompson, J.L. and Hinton, M. (1997) Antibacterial activity of formic acid and propionic acids in the diet of hens on salmonellas in the crop. *British Poultry Science*, 38: 59-65.
- Versteegh, H.A.J. and Jongbloed, A.W. (1999) Lactic acid has a positive effect on broiler performance. *World poultry*, 15: 16-17.
- Yalda, A.Y. and Forbes, J.M. (1995) Food intake and growth in chickens given food in the wet form with and without access to drinking water. *British Poultry Science*, 36: 357-369.

- Yalda, A.Y. and Forbes, J.M. (1996) Effect of food intake, soaking time, enzyme and corn flour addition on the digestibility of the diet and performance of broilers given wet food. *British Poultry Science*, 37: 797-807.
- Yasar, S. and Forbes, J.M. (1999) Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. *British Poultry Science*, 40: 65-76.
- Yasar, S. and Forbes, J.M. (2000) Enzyme supplementation of dry and wet wheat-based foods for broilers chickens: performance and gut responses. *British Journal of Nutrition*, 84: 297-307.
- Yasar, S. and Forbes, J.M. (2001) In vitro estimation of the solubility of dry matter and crude protein of wet feed and dry. *Turkish Journal of Vet. and Animal Science*, 25: 149 – 154.

CHAPTER 4

Effect of dietary structure and conformation during starter and grower phases on growth performance and GIT development in broiler chickens

ABSTRACT

A total of 288 one-day-old male broiler chickens were used to investigate the effect of diet conformation (wet or dry diets) and diet structure (fine or coarse diets) during both starter and grower phase on foregut segments development and growth performance of broilers.

Wet feeding significantly improved feed intake by 12 % on average, daily weight gain by 11 %, but not feed conversion ratio. A coarse diet had a significant effect on the development of the gizzard in both the starter phase and grower phase as compared to birds being fed on a fine diet (wet coarse vs. wet fine). For the starter phase, it was 2.90 g/100 g body weight for coarse and 2.70 g/100 body weight fine for fed birds, respectively. For the grower phase, it was 1.50 g/100 body weight for wet coarse diet and 1.36 g/100 g body weight, respectively.

There were carry over effects (an extra effect) of wet coarse diet during starter phase on the daily weight gain during grower phase. Wet fine fed birds during starter phase significantly increased feed and water intake compared to dry fine fed birds during starter phase, but not showing the improvement in daily body weight gain. Birds which received a wet coarse diet during the starter phase did not increase feed and water consumption during the grower phase but showed a significantly increased daily bodyweight gain compared to the control group. Diet structure during starter phase did not affect the development of the foregut segment during the grower phase.

Best growth performances during the entire cycle of broiler production were found when wet coarse diets were fed in both the starter as well as the grower phase.

Keywords: coarse, fine, diet, wet, dry, GI tract development, growth

INTRODUCTION

In many parts of both the developed and developing world, growth rate is still the most important trait in establishing an optimal production programme for raising broilers (North and Bell, 1990). Rapid growth not only saves labor and feed, but also allows the annual production cycle of broilers to be more efficient; thereby, minimizing many of the fix costs of production (Austic and Nesheim, 1990).

Early feed intake of post-hatch chickens not only boost growth, but it also increases the relative growth of internal organs such as the small intestine, liver and pancreas in poultry. A proper early development of the foregut segments, that plays an important role in the digestion process, may contribute to an optimal feed digestion and utilization at later ages. Optimal development reached with a proper feed structure resulted in a large and muscular gizzard (Nir *et al.*, 1995). Thus feed structure may increase the grinding and absorptive capacities of the gastrointestinal tract, and, as a consequence, enhances growth. Similarly to that of diet structure, diet conformation (wet feeding) for broilers, especially during young ages, may have promising effects on feed intake and feed utilization. This may be related to an improvement of a better nutrient retention (Yalda and Forbes, 1995). The effects of feed structure and conformation during early ages on functional development of the foregut segments and its carry over effects on performance is not known in detail.

The objective of this experiment was to gain insight in the effects of diet conformation (wet versus dry diets) and diet structure (coarse versus fine diets) on developmental characteristics of the foregut segments and growth performance during the starter and grower phase in broilers.

MATERIALS AND METHODS

Birds, housing and care

A total of 288 day-old male broiler chickens (Ross 308) was 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 24 pens, with 12 chickens per pen. Chickens in the pen were weighed for initial BW.

All birds had free access to feed and water. 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 chickens.

Full wood shavings covered the floor pens. The temperature inside the room was initially maintained at 32°C from day 0 to 3 and gradually decreased by 0.5°C per day until it reached 24°C at day 21 which was (tried to be) maintained until day 35 (summer time).

Broilers were housed in a dark house. Artificial light was provided at a schedule of 23 hours light and 1 hour dark throughout both periods. Light intensity maintained above 20 lux at the bird's level during all lighting periods throughout the experiment.

All birds were fed 3 times daily during the starter phase (day 0-17) and 2 times daily during the grower phase (day 18-35). Feeding times during the starter phase were at 8.00 h, 14.00 h and 20.00 h. Feeding times during the grower phase were at 8.00 h and 18.00 h.

Experimental design

The experimental design is shown in Table 1. A dry diet in combination with a fine structure was in both starter and grower phases regarded as the 'control' situation. Several combinations were tested during starter and grower phases in order to see which factor: 'conformation' or 'structure' was more affecting growth in a certain phase.

Table 1: Experimental design.

Treatment no.	Age in days		Number of replicate pens
	0 - 17 (starter phase)	18 – 35 (grower phase)	
1 ('control')	Dry + Fine	Dry + Fine	3
2	Dry + Fine	Wet + Fine	4
3	Dry + Fine	Wet + Coarse	3
4	Wet + Fine	Dry + Fine	4
5	Wet + Fine	Wet + Fine	3
6	Wet + Coarse	Dry + Fine	3
7	Wet + Coarse	Wet + Coarse	4

Experimental diets

All diets were formulated to meet the nutrient and energy requirements for broilers (NRC, 1994). Ingredient and nutrient composition are shown in Table 2.

Table 2. Ingredient (g/kg) and analysed nutrient composition (g/kg dry matter) of the experimental diet.

Composition	Amount presented
<i>Ingredients</i>	
Wheat	349.4
Maize	275.7
Toasted full fat soya beans	199.6
Soya bean meal 44/7	94.3
Fishmeal 66% CP	49.9
Ca CO ₃	11.8
L-Threonine	3.0
Broiler vitamin and mineral premix	5.0
Monocalciumphosphate	4.7
Phytase	3.0
Salt	1.7
DL-Methionine	1.4
L-Lysine HCl	0.5
<i>Analysed nutrients</i>	
Crude protein	251.3
Crude fat	77.6
Total starch	407.0
Crude fibre	34.4
Ash	63.2

The diet defined as ‘coarse’, was processed by a roller-mill with one roller pair and a roller 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 hammer mill with opening screen of 3.0 mm (modified after Hamilton and Proudfoot, 1995). Particle size distribution of the coarse and fine diets were assessed in a similar diet as it was shown in Chapter 3 with wet sieve analysis (Goelema, 1999).

The wet diets were made by mixing 1 kg of dry feed with 1 kg of tap water 20 minutes prior to the every feeding time (Mai *et al.*, 2007 unpublished). The preparation and mixing of the feed was done outside the experimental shed to prevent any disturbance to the chickens.

Observations

Feed intake and BW gain were determined at weekly intervals. Final data are presented per phase of production. Feed intake of the wet diets was calculated on the basis of the dry feed intake. The real ‘dry’ intake of the wet diet was corrected for water loss due to evaporation, calculated in the same way as reported in the previous paper (Mai *et al.*, 2007 submitted; Chapter 3).

Development of the GI tract was determined at the end of the starter phase (17 days of age; 12 birds per treatment) and grower phase (35 days of age; 4 birds per treatment) by weighing the empty crop, proventriculus + gizzard, duodenum (with pancreas intact), jejunum and ileum. The fresh weights of these organs were expressed as grams per 100 gram of BW.

Statistical analysis

Analysis of data was performed by using SPSS software (SPSS Inc., 2003) with pen as experimental unit and treatment as factors. Carry-over effects were analyzed by comparing certain treatment groups.

The model used for analysis was $Y_{j(j=1,3)} = \mu + F_j + e_j$

Where,

Y_j = Individual performance of bird with

F_j = Effect of treatment

e_j = Error

The experimental design and treatment of animals were approved by the Animal welfare and Ethical Committee of Wageningen University and Research Centre.

RESULTS

Starter phase

The results of feed intake, total water intake, BW gain and feed conversion ratio (FCR) of broilers during the starter phase are presented in Table 3.

Table 3. Effect of dietary treatment on feed intake, water intake, BW gain and feed conversion ratio (FCR) of broilers during the starter phase.

Treatment	Trait			
	Feed intake (g/bird/day)	Total water intake (ml/bird/day)	BW gain (g/bird/day)	FCR
Dry + Fine	43.2 ± 0.5 a	71.6 ± 1.1 a	34.6 ± 0.5 a	1.25 ± 0.02
Wet + Fine	48.6 ± 0.6 b	84.4 ± 1.4 b	38.9 ± 0.7 b	1.25 ± 0.02
Wet + Coarse	47.5 ± 0.6 b	82.6 ± 1.4 b	38.3 ± 0.7 b	1.24 ± 0.02

a,b Means within columns that do not have identical letters differ significantly (P<0.05)

It is shown in Table 3 that during the starter phase feed intake, total water intake and BW gain of wet-fed birds were significantly higher compared to dry-fed birds (P<0.01), but no difference could be detected for FCR. Within the wet-fed group, there was no difference found between coarse and fine-fed birds in all of these traits.

The empty weights of the GI tract organs were examined at the end of the starter phase (17 days of age) and are presented in Table 4. From this table it can be seen that the wet+fine fed birds had the smallest proventriculus+gizzard as compared to the other two groups. Within the wet fed group, birds that received the coarse diet showed a higher proventriculus+gizzard weight than birds fed the fine diet, the difference being 7.4% (p<0.05).

Table 4. Effect of dietary treatment on empty weight of digestive organs

Treatment	Organ (empty weight in g per 100 g BW)		
	Crop	Proventriculus + Gizzard	Small intestine
Dry + Fine	0.52 ± 0.03	2.92 ± 0.08 a	3.98 ± 0.14 a
Wet + Fine	0.49 ± 0.04	2.70 ± 0.09 b	4.15 ± 0.17 b
Wet + Coarse	0.54 ± 0.03	2.90 ± 0.08 a	4.24 ± 0.14 b

a,b Means within columns that do not have identical letters differ significantly (P<0.05)

Birds that received a wet diet showed the heaviest small intestine compared to those that received a dry diet. The differences are 4.2 and 6.8 % (p<0.05) in the fine and coarse fed groups, respectively. Within the wet fed group, feed structure did not show any effect on the development of the small intestine. None of the treatments gave any effect on the development of the crop.

Table 5. The correlation of body weight and the length of different small intestine segments

Treatment/ Segments	Dry fine	Wet fine	Wet coarse
Duodenum	0.425	0.305	-0.119
Jejunum	-0.062	-0.038	-0.017
Ileum	0.210	0.077	0.368

Correlation coefficients between BW and lengths of segments of the small intestine (Table 5) show a relatively strong positive relationship between BW and length of duodenum for both fine diets and between BW and length of the ileum for the wet+coarse diet.

Grower phase

The performance of broiler chickens during the grower phase is presented in two ways:

1. carry over effects of dietary treatments from the starter on the grower phase (Table 6);
2. effects of changes in dietary treatments in the grower phase, following a starter phase with identical treatments (Tables 7 and 8).

Table 6. Carry over effects of different starter diets to identical grower diets on feed and water intake, BW gain and feed conversion ratio (FCR) of broilers during the grower phase (g/day).

Starter	Grower	Feed intake	Water intake	BW gain (g/d)	FCR
Dry + Fine	Dry + Fine	132.1 ± 1.8 a	202.1 ± 7.2 a	87.7 ± 1.4 a	1.51 ± 0.08
Wet + Fine	Dry + Fine	140.6 ± 1.6 b	235.5 ± 6.2 b	89.5 ± 3.4 a	1.59 ± 0.07
Wet + Coarse	Dry + Fine	136.7 ± 1.8 b	226.5 ± 7.2 b	94.9 ± 3.9 b	1.44 ± 0.08

a.b Means within columns that do not have identical letters differ significantly (P<0.05)

From Table 6 it can be seen that feed intake, water intake and, to some extent, BW gain were increased during the grower phase for birds on a dry+fine diet, if they had been fed a wet diet during the previous starter phase (P<0.05). Although non-significantly different, it is remarkable to see that among the wet diets that had been fed in the starter phase, these birds had a lower feed and water intake, a higher BW gain, and, as a consequence, a better FCR in the grower phase, if they had been fed a coarse diet in the starter phase. No statistical differences were observed for FCR.

The effects on performance for birds receiving different dietary treatments in the grower phase, after identical treatments in the starter phase, is presented in Table 7.

Table 7. Comparisons between different dietary treatments during the grower phase, after similar starter diets, on feed and water intake, BW gain and feed conversion ratios (FCR) of broilers during the grower phase.

Starter	Grower	Feed intake (g/d)	Water intake (g/d)	BW gain (g/d)	FCR
Dry + Fine	Dry + fine	132.1 ± 1.8 a	202.1 ± 7.2 a	87.7 ± 1.4 a	1.51 ± 0.08
Dry + Fine	Wet + fine	146.0 ± 1.9 b	261.3 ± 4.7 b	97.2 ± 1.2 b	1.50 ± 0.03
Dry + Fine	Wet + coarse	145.7 ± 2.2 b	261.7 ± 5.5 b	95.7 ± 1.4 b	1.52 ± 0.03
Wet + Fine	Dry + Fine	140.6 ± 0.9 b	235.5 ± 3.5 c	89.5 ± 5.0 ab	1.58 ± 0.10
Wet + Fine	Wet + Fine	146.7 ± 3.2 b	268.4 ± 1.8 b	97.6 ± 1.5 b	1.50 ± 0.02
Wet + Coarse	Dry + Fine	136.7 ± 1.7 a	226.5 ± 19.6 c	94.9 ± 1.9 b	1.44 ± 0.03
Wet + Coarse	Wet + Coarse	147.2 ± 1.1 b	269.8 ± 10.1 b	101.9 ± 2.0 b	1.44 ± 0.04

a.b Means within columns that do not have identical letters differ significantly (P<0.05)

From the results in Table 7, it is obvious that, in general terms, birds fed on a wet diet during the grower phase, irrespectively what diet they had received during the starter phase (dry or wet), perform better in this phase than their dry fed counterparts. Diet structure (coarse/fine) did not show any effect on performance of broiler chickens. Not all of the contrasts could be identified as being statistically significant, probably due to the small number of replicates.

The effects of the dietary treatments on the empty weights of foregut segments at the end of the grower phase is presented in Table 8. It can be seen from this table that the dietary treatments did not show any significant effect on the weight of the crop. For the proventriculus-gizzard, it seems that feeding a coarse diet, regardless in which phase the coarse diet had been fed, results in heavier weights for this segment complex. A fine diet, particularly in wet form and then fed in both phases, gives the smallest proventriculus-gizzard weights.

Table 8. Comparisons between different dietary treatments during the grower phase, after similar starter diets, on foregut development of broilers at the end of the grower phase (relative empty weights per 100 g BW).

Starter	Grower	Crop	Proventriculus-gizzard
Dry + Fine	Dry + Fine	0.32 ± 0.03	1.36 ± 0.09 a
Dry + Fine	Wet + Fine	0.34 ± 0.03	1.36 ± 0.07 a
Dry + Fine	Wet + Coarse	0.35 ± 0.03	1.50 ± 0.06 b
Wet + Fine	Dry + Fine	0.34 ± 0.02	1.41 ± 0.09 ab
Wet + Fine	Wet + Fine	0.28 ± 0.02	1.26 ± 0.06 a
Wet + Coarse	Dry + Fine	0.30 ± 0.01	1.43 ± 0.05 ab
Wet + Coarse	Wet + Coarse	0.30 ± 0.03	1.43 ± 0.08 ab

a.b Means within columns that do not have identical letters differ significantly (P<0.05)

Entire growth period

In order to investigate the effects of all combinations of dietary treatments in both phases on overall performance of broiler chickens during the entire production period (0 – 35 days of age), data were calculated and presented in Table 9.

Table 9. Overall comparisons of all treatments over the entire experiment (0 - 35 days of age).

Starter	Grower	Feed intake (kg)	Water intake (kg)	BW gain (kg)	FCR	Water/feed ratio
Dry + Fine	Dry + Fine	2.86 ± 0.05 c	4.86 ± 0.12 d	2.20 ± 0.02 a	1.34 ± 0.01 a	1.54 ± 0.01 ac
Dry + Fine	Wet + Fine	3.10 ± 0.04 a	5.40 ± 0.09 b	2.35 ± 0.02 a	1.32 ± 0.02 a	1.74 ± 0.02 b
Dry + Fine	Wet + Coarse	3.17 ± 0.05 ab	5.59 ± 0.11 b	2.32 ± 0.03 a	1.37 ± 0.02 a	1.76 ± 0.02 b
Wet + Fine	Dry + Fine	3.03 ± 0.03 a	5.12 ± 0.13 a	2.26 ± 0.05 a	1.35 ± 0.03 a	1.69 ± 0.04 a
	Wet + Fine	3.21 ± 0.05 b	5.70 ± 0.13 c	2.44 ± 0.04 b	1.31 ± 0.03 ab	1.78 ± 0.03 b
Wet + Coarse	Dry + Fine	3.01 ± 0.04 a	5.15 ± 0.15 a	2.33 ± 0.02 a	1.29 ± 0.01 bc	1.71 ± 0.05 ab
	Wet + Coarse	3.11 ± 0.04 ab	5.60 ± 0.11 bc	2.50 ± 0.04 b	1.24 ± 0.03 c	1.80 ± 0.02 b

a,b Means within columns that do not have identical letters differ significantly ($P<0.05$)

It can be seen from Table 9 that, on average, broilers fed on a wet diet during the grower phase had a better performance at the end of the growing period than their dry fed counterparts within each comparison. If these broilers also had received a wet and coarse diet during the starter phase, than their growth characteristics were again much better than the growth of the birds that had received a dry and fine diet during the starter phase. A wet and coarse diet in both phases seems to be the best overall treatment (in terms of BW gain and FCR).

DISCUSSION AND CONCLUSIONS

Early nutrition may have clear influences on overall performance of broiler chickens up to 35 days. In the present study, chickens showed to have a great nutritional benefit from wet and coarse feeding at early ages of life. This strategy resulted in a larger feed intake, increased BW gain and the development of a larger proventriculus-gizzard system. A better development of the proventriculus-gizzard system may result in a better grinding and mixing capacity of this GI tract system. So birds can handle feed to optimize digestion process which takes place in the small intestine (Nir *et al.*, 1995, Hetland and Svhuis, 2001).

In this study, we found that the development of the proventriculus-gizzard system in wet-coarse fed birds during the starter phase was 7.4 % larger than that of the wet-fine fed birds. These results are in line with the finding of several authors (Yalda and Forbes, 1995; Nir *et al.*, 1995; Hetland *et al.*, 2002; Yalda and Forbes, 1996). Wet feeding during starter phase also gave a better development of the small intestine. This early development in association with viscosity reduction of wet feeding is the major factor contributing to enzyme response thus will contribute to a better digestion and absorption of nutrients in the small intestine (Bedford and Classen, 1992) and will improve nutrient retention (Yalda and Forbes, 1995). The intestine is the nutrient primary supply organ, the sooner it achieves its functional capacity, the sooner the young bird can utilize dietary nutrients and efficiently grow at its genetic potential and resist infectious and metabolic disease.

The carry over effect of wet+coarse feeding has been found in birds in term of BW gain. This improvement could be explained by coarser diets during the starter phase that might initiate maturation and functional development of the GI tract quicker than in birds of the treatments

wet+fine and dry+fine groups. A proper development of the GI tract, especially the development of the gizzard, can be obtained by feeding the chickens with larger particle sizes during the starter phase. These larger particles sizes are associated with larger gizzards (Nir *et al.*, 1995; Hetland and Svhuis, 2001). A large and muscular gizzard may further optimizes the grinding and thus facilitate absorption (Nir *et al.*, 1995). It may thus contribute to a better performance of broilers during later stages of growth.

Wet feeding improved broiler growth rate during both starter and grower phase, which is clear from increased feed intake and better feed conversion ratio (FCR). This result is in accordance with other studies (Pittard, 1996; Yalda and Forbes, 1995; Yalda and Forbes, 1996). The effect may be explained by the facilitation of the access of enzymes and then a better nutrient retention (Yalda and Forbes, 1995). Birds fed wet coarse diet showed the best performance in terms of feed intake, body weight gain and FCR. These results agree with literature (Nir *et al.*, 1995) in which broiler chickens performed best with the medium or coarse particle sizes of wheat diets. Similarly, Proudfoot and Hulan (1989) reported that birds fed with very coarse mash diets were heavier than those received fine mash diets. The results suggest that grinding cereal grains into very fine particles before feeding should not be a preferred procedure for feeding broiler chickens. The better FCR's found in wet coarse diets compared to fine diets caused an increase in total transit time associated with increased feed intake (Hetland and Svhuis, 2001). The results of the present study are in accordance and showed that in wet fine fed birds consumed the high amount of feed compared to the coarse fed birds (with a higher feed conversion).

The results of the correlation between BW and the length of different parts of the small intestine suggested that the longer gut length, specially the ileum length maybe beneficiated for nutrients absorption resulting in heavier body weight. There is a trend to have a longer ileum length in wet coarse treated birds during the starter phase. The results of this study also suggested that the combination of wet and coarse diet is not only positive for the development of the gizzard but also for the length of the small intestine. Some reports have supported the idea that the use of wet and large particle size diets can lengthen the small intestine (Yalda and Forbes, 1995; Bedford and Classen, 1992).

Total water intake (from the nipple plus that from the wet feed) was significantly higher in wet-fed birds than in dry-fed birds. Similarly, dry matter intake was higher in wet-fed birds. These findings are in accordance with those of Yasar and Forbes (1999). Water consumption

is influenced by several factors, including size and age of the birds and type and amount of feed consumed (Duke, 1986). The voluntary intake of total water was usually closely related to dry matter intake in this study as well indicated by Patrick and Ferrise (1962).

From the results of this study, it can be concluded that a wet and coarse diet during the starter phase improves both feed intake and the development of the GI tract, and thus greatly contributes to the performance of broiler chickens during the production cycle, and especially result in a better final BW and improved FCR.

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REFERENCES

- Austic, R. E. and Nesheim, M. C. (1990) Poultry Production. 13th ed. Lea & Febiger 200 Chesterfield Parkway, Malvern, PA.
- Bedford, M.R., Classen, H.L., (1992) Reduction of intestinal viscosity through manipulation of dietary and pentosane concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. *Journal of Nutrition*, 122: 560–569.
- Duke, G.E (1986) Alimentary canal: secretion and digestion, special digestive functions and absorption. *Avian physiology*, 4th edition, New Yorkß Springer-Verlag: 289-302.
- Goelema, J.O., (1999) Processing of legume seeds: effects on digestive behaviour in dairy cows. PhD Thesis, Animal Nutrition group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands, 222 pp.
- Hamilton, R.M.G. and Proudfoot, F.G. (1995) Ingredient particle-size and feed texture- effects on the performance of broiler-chickens. *Animal Feed Science and Technology*, 51: 203-210.
- Hetland H., Svhuis, B. (2001) Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British Poultry Science*, 42: 354-361.
- Hetland, H., Svhuis, B. and Olaisen, V. (2002) Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *British poultry Science*, 2: 416-423.
- Mai, A.K., Jha R., Kwakkel R.P., Van der Poel, T.F.B. and Verstegen, M.W.A. (2007) Effect of diet structure, confirmation and acidification on performance and gastrointestinal tract development of broilers. (Unpublished). *Wageningen University, Department of Animal Nutrition, the Netherlands*.

- Nir I., Hillel, R., Ptichi, I. and Shefet, G. (1995) Effect of particle size on performance. 3. Grinding pelleting interactions. *Poultry Science*, 74: 771-783.
- North, M. O. and Bell, D. D. (1990) Commercial Chicken Production Manual. 4th edition Chapman & Hall, One Penn Plaza, New York, NY.
- NRC (1994) Nutrient requirements of chickens. National Research Council, 1994.
- Patrick, H., Ferrise, A. (1962) Water requirements of broilers. *Poultry Science*, 41: 1363-1367.
- Pittard, E.W. (1996) Automatic poultry feeding devices with water sprayed feed mix. USA, 3 (443): 205.
- Proudfoot, F.G. and Hulan, H.W. (1989) Feed texture effects on the performance of roaster chickens. *Canadian Journal of Animal Science*, 69: 801.
- SPSS® (2003) SPSS 12.0.1 for Window by SPSS Inc. 2003.
- Yalda A.Y. and Forbes, J.M. (1995) Food intake and growth in chickens given food in the wet form with and without access to drinking water. *British Poultry Science*, 36: 357-369.
- Yalda, A.Y. and Forbes, J.M. (1996) Effects of food intake, soaking time, enzyme and cornflour addition on the digestibility of the diet and performance of broilers given wet food. *British Poultry Science*, 37: 797-807.
- Yasar S. and Forbes J.M. (1999) Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. *British Poultry Science*, 40: 65-76.

CHAPTER 5

**Effects of dietary structure and conformation on pH in the GI tract, feed
resident time and protease activity in broiler chickens**

ABSTRACT

A study with 288 male broiler chickens was undertaken to investigate the effect of diet conformation (wet or dry diets during the starter phase) and diet structure (impact of coarse diets thereafter) affected feed resident time, protease activity in the proventriculus and the pH in chyme of the gastro intestinal (GI) tract.

Treatments consisted of 3 different groups during the starter phase and 7 different groups during the grower phase, in both phases diets differed in structure and conformation.

Diet structure (coarse feeding) significantly improved protease activity, lowered the pH value of the proventriculus-gizzard during the starter phase. There was no effect of diet structure on protease activity during the grower phase. Diet conformation (wet feeding) had no effect on protease activity in both the starter and the grower phases.

Feeding a wet coarse diet during starter phase caused a carry over effect (an extra effect) on the protein content of the proventriculus during the grower phase.

Key words: wet, coarse, fine, residence time, pH, protease activity, diet.

INTRODUCTION

The development of the gastro-intestinal (GI) tract of the chickens already starts during the incubation period (Romanoff 1960). Immediately after hatch the weight of the proventriculus, the gizzard and small intestine increases more rapidly in relation to body weight than other organs and tissues. In chickens, the maximal relative size of these digestive organs occurred at 3 to 7 days of age (Cook and Bird, 1973; Dror *et al.*, 1977). In fast growing chickens, there is a different gastro-intestinal tract development and this difference depends on feed processing technologies. A proper development of the GI tract can be obtained by feeding strategies such as choice feeding (Erener *et al.*, 2003), sequential feeding (Rose *et al.*, 1995), feeding coarse diets (Nir *et al.*, 1995) and/or the inclusion of whole grain (Hetland *et al.*, 2002). However, these feeding schemes still present some problems to young chickens. They may induce health problems in growing chickens such as ascitis and tibial dyschondroplasia (Havenstein *et al.*, 1994). The effective utilization of feed is influenced by a number of factors. This includes the duration of time that digesta is exposed to digestive enzymes. Moreover, the relative activity of digestive enzymes and the efficiency of nutrient absorption (Cherry and Siegel, 1978) can be affected by this factor. Gastrointestinal function influences growth of broiler chickens (Nitsan *et al.*, 1991). Therefore, the development and secretion of digestive enzymes may be a limiting factor for digestion and subsequent growth of chickens.

Different feeding techniques not only affect the development of the GI tract but also affects the passage of feed through the GI tract. When the gizzard is well developed it contributes to a greater reflux of chyme in the GI tract (Bird, 1971). This can improve digestion by both animal and the natural enzymes activities present in the feed as the feed will stay in the upper digestive tract for a longer duration of time (Taylor, 1998). Many studies have been made to achieve an optimal development of the GI tract in broiler chickens. It is, however, still unclear how during early feeding diet structure and conformation will affect the feed residence time. Also it is not clear how pH of chyme and proventricular protease activities in broiler chickens are affected by these factors. The objective of this study is to investigate the effect of early feeding diet structure and conformation on feed resident time in the foregut segment, its pH value and protease activities in the proventriculus of broiler chickens.

MATERIAL AND METHOD

Animals, housing and care

A total of 288 day-old male broiler chickens (Ross-308) were obtained from a commercial hatchery (Morren Breeders B.V., Lunteren, The Netherlands). After arrival, the chickens were wing tagged for identification. After that, chickens were assigned randomly to 24 pens of 12 chickens each and to dietary treatment of dry fine, wet fine and wet coarse. All birds had access to feed and water *ad libitum*. The experimental diets were offered 3 times a day during the starter period of 17 days. After that, the animals were assigned to either continue with the starter dietary treatments or switched to dry fine or wet fine diets to make the combination of the grower phase with the starter phase in a 2 x 3 factorial design; ‘coarse vs. fine’ and ‘entire period wet feed vs. starter period wet feed vs. grower period wet feed’ and an added control group. 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.

Wood shavings were used as bedding. The temperature inside the room was initially maintained at 32⁰C from day 0 to 3 and gradually decreased by 0.5⁰C per day until it reached 24⁰C at day 21 which was (tried to be) maintained until day 35 (summer time).

Artificial light was provided at schedule of 23 hours light and 1 hour dark throughout the test period. Light intensity was maintained above 20 lux at the bird’s level during all lighting period throughout the experiment.

Experimental design

The experimental design is shown in Table 1. A dry diet in combination with a fine structure was in both starter and grower phases regarded as the ‘control’ situation. Several combinations were tested during starter and grower phases in order to see which factor: ‘conformation’ or ‘structure’ was more affecting growth in a certain phase.

Table 1: Experimental design.

Treatment no.	Age in days		Number of replicate pens
	0 - 17 (starter phase)	18 – 35 (grower phase)	
1 ('control')	Dry + Fine	Dry + Fine	3
2	Dry + Fine	Wet + Fine	4
3	Dry + Fine	Wet + Coarse	3
4	Wet + Fine	Dry + Fine	4
5	Wet + Fine	Wet + Fine	3
6	Wet + Coarse	Dry + Fine	3
7	Wet + Coarse	Wet + Coarse	4

Experimental diets

All diets were formulated to meet the nutrients and energy requirements for broilers (NRC, 1994). The diets were formulated with the composition as is shown in Table 2.

Table 2. Ingredient (g/kg) and analysed nutrient composition (g/kg dry matter) of the diet.

Composition	Amount presented
<i>Ingredients</i>	
Wheat	349.4
Maize	275.7
Toasted full fat soya beans	199.6
Soya bean meal 44/7	94.3
Fishmeal 66% CP	49.9
Ca CO ₃	11.8
L-Threonine	3.0
Broiler vitamin and mineral premix	5.0
Monocalciumphosphate	4.7
Phytase	3.0
Salt	1.7
DL-Methionine	1.4
L-Lysine HCl	0.5
<i>Analysed nutrients</i>	
Crude protein	251.3
Crude fat	77.6
Total starch	407.0
Crude fibre	34.4
Ash	63.2

The diet defined as ‘coarse’, was processed by 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’ will be added without hammer milling. Diets defined as ‘fine’ was processed by hammer mill with opening screen of 3.0 mm (modified after Hamilton and Proudfoot, 1995). Particle size distribution of the coarse and fine diets were assessed in a similar diet with wet sieve analysis (Goelema, 1999), as it was shown in Chapters 3 and 4.

The wet diets were made by mixing 1 kg of dry feed with 1 kg of tap water 20 minutes prior to the every feeding time (Mai *et al.*, 2007 unpublished). The preparation and mixing of the feed was done outside the experimental shed to prevent any disturbance to the chickens.

Collecting of the samples

GIT contents

At the end of the starter period (at day 17), 12 chickens from each treatment were randomly sampled to measure the feed residence time by using Titanium dioxide as the marker. Twelve birds from each treatment were administrated with 2 pills of Ti0₂. Each pill contains 150 mg Ti0₂ or 90mg pure Titanium. After Ti0₂ pills had been administrated, all chickens had access to feed and water as usual and were assigned to 4 time periods of 3 birds each. Then after 30 minutes, 60 minutes, 90 minutes and 120 minutes 1 group of 4 birds was euthanized and dissected to obtain the contents of the crop, the proventriculus-gizzard and the small intestine. The contents were collected and stored at -20° C and after that the samples were freeze dried for Titanium recovery determination. For pH measurements, another 4 birds were used per treatment

Sample of the proventriculus

From each treatment, 16 birds during the starter phase and 4 birds during the grower phase were euthanized and dissected to obtain the proventriculus samples for protease activity determination. After the GI tract was removed from the body, the proventriculus was immediately separated from the GI tract. Then its content was removed and placed on an ice-cold glass tray. Alimental fat was removed and ice-cold saline was used to wash out the

contents of the proventriculus and small intestine. Each proventriculus was bottled dry, weighed and stored at -20⁰C.

Recovery of titanium

Titanium content in the GI tract content in different parts was determined after drying and digestion. This method has been employed in digestibility studies in chickens (Short *et al.*, 1996). This method has been used in the Animal Science Group of Wageningen University, Netherlands and also by Myers *et al.* (2004). The titanium recovery is almost 100%, rapid and reproducible.

A standard or calibration curve was prepared by pipetting 0.0, 10.0, 20.0, 30.0, 40.0 and 50.0 ml of standard solution (The equation of the standard solution was (NH₄)₂TiF₆ in H₂O. 1000mg/l TiO₂: Merck, Germany) into a plastic test tube and diluted with water to achieve 0.0 (5.0 ml of water), 20.0 (0.1 ml of TiO₂ + 4.9 ml of water), 40.0 (0.2 ml of TiO₂ + 4.8 ml of water), 60.0 (0.3 ml of TiO₂ + 4.7 ml of water), 80.0 (0.4 ml of TiO₂ + 4.6 ml of water) and 100.0 mg/l (0.5 ml of TiO₂ + 4.5 ml of water) respectively. Thereafter, 0.2 ml (200 µl) of 30 % hydrogen peroxide (Merck, Germany) was added to each plastic test tube containing different concentrations of titanium. This was then mixed thoroughly with fortex mixer (Heidolph, REAX top, Germany). These solutions were analysed using a UV-visible spectrophotometer (Varian, CARY 50 probe) and absorbance was measured at 408 nm. The standard containing 0.0 mg of titanium was used to zero calibrate the instrument. A linear standard curve was produced with a regression equation in which absorbance is plotted against titanium content:

$$Y = mX + c$$

Where, Y is the absorbance or extension which is determined by X, m is the slope, the X is the variable amount of titanium in the standard solution samples while c is the intercept.

Protease activity measurement

Protease activity was measured at the end of the starter and grower phase. The procedure to measure protease activity was adapted from Susbilla *et al.* (2003). The proventriculus was thawed on ice, weighed and cut into small pieces. Then it was homogenised with polytron/ultra turrax (13500 rpm) for 2 times 8 – 10 seconds in 10 mM sodium phosphate buffer pH 7 on ice and then added to one gram tissue 3 ml buffer. These samples were then centrifuged at 12.400 x g for 60 min at 4⁰C. The supernatant was stored at -20⁰ C before being analysed for enzyme activity. After thawing, the supernatant was diluted 20 times in 10mM

sodium phosphate buffer (pH 7). 25 µl of the diluted supernatant was added to a glass test tube containing 1ml of a bovine haemoglobin (Stock solution 2.5 g haemoglobin in 100ml H₂O; 4 ml solution is acidified with 1ml 0.2 M HCl to have a pH of 2.1 – 2.2). Samples were incubated in a shaking water bath at 41⁰C for 10 minutes. The incubation was stopped by adding 1.7 ml 5% (w/v) TCA. Samples were then centrifuged for 10 minutes at 10.000 x g. Exactly 100 µl supernatant was transferred to a plastic 1 ml cuvette and then neutralized with 100µl saturated Na₂CO₃ (350g Na₂CO₃ in 1 litre H₂O of 70-80⁰C). Tyrosine released through hydrolysis of the haemoglobin was measured at 750nm with Folin and Ciocalteau's phenol reagent. Activity was expressed as µmole tyrosine released/min/g tissue.

Statistical analysis

Analysis of data was performed by using SPSS software (SPSS Inc., 2003) with pen as experimental unit and treatments as factors. The carry over effect was analyzed as separate factors in addition to effect of wet and dry feed.

The model used for analysis was $Y_{ij} = \mu + F_j + e_{ij}$

Where,

Y_{ij} = Individual performance of bird with

F_j = Effect of treatments ($j = 1,3$)

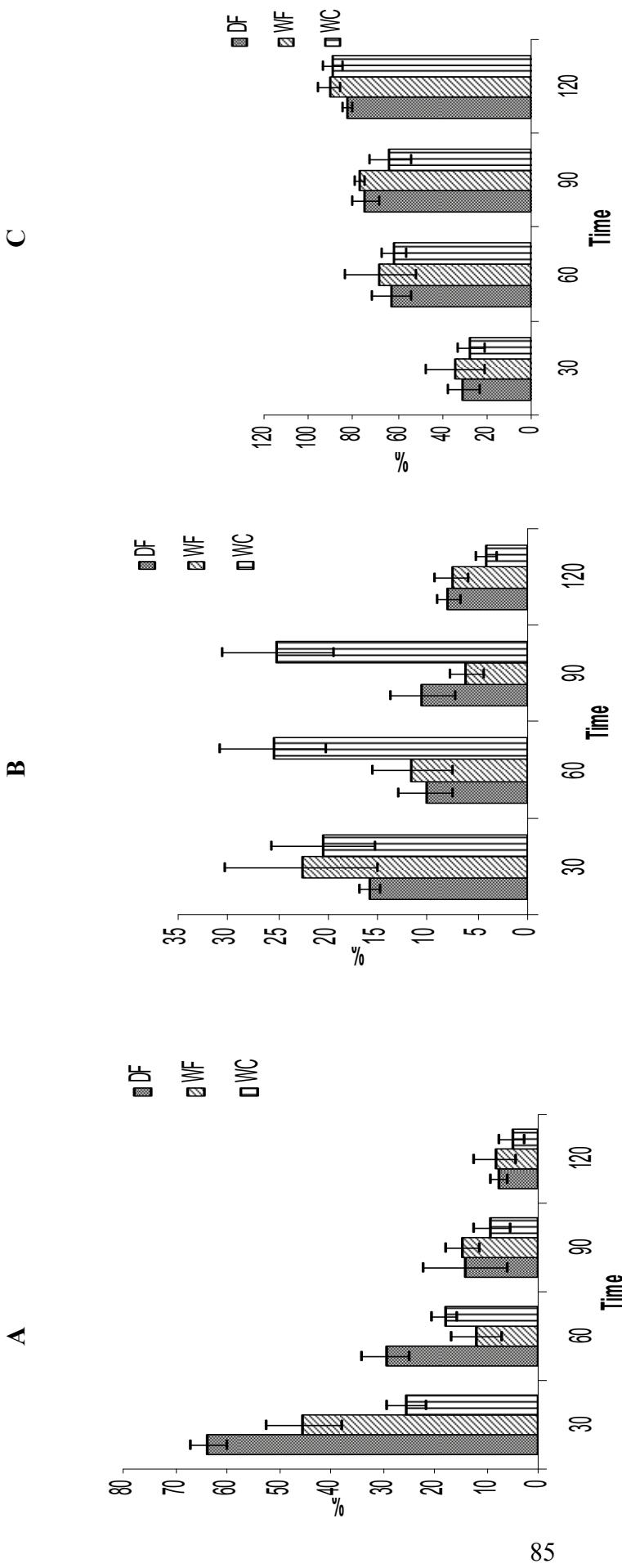
e_{ij} = Error

The experimental design, protocol and treatments of animal were approved by the Animal Welfare and Ethical Committee of Wageningen University.

RESULTS

Resident time of digesta in crop, proventriculus - gizzard and small intestine

The recovery of titanium in digesta is expressed as the percentage of titanium recovered after 30, 60, 90 and 120 minutes of titanium pill administration, and is shown in Figure 2. The recovery of titanium in the crop of birds fed a dry+fine diet after 30 minutes was significant higher than that in birds fed a wet+coarse or wet+fine diet ($p<0.05$). Within the wet fed group titanium dioxide found was higher in the fine fed birds ($p<0.05$). Samples taken after 60 minutes had similar titanium contents within wet groups. After 90 and 120 minutes the content was similar in all groups.



(DF = Dry fine; WF = Wet fine; WC = Wet coarse)

Figure 1. The % of titanium recovered in the crop (A), the proventriculus-gizzard (B) and the small intestine (C) over times

The recovery of titanium dioxide after 60 and 90 minutes in the proventriculus – gizzard contents was highest in the wet+coarse fed birds ($p<0.05$). After 120 minutes the recovery of titanium was lowest in the wet+coarse fed birds ($p<0.05$). There were no differences in titanium recovery in the small intestine after 30 and 60 minutes (Figure 2). But after 90 minutes and 120 minutes, the recovery of titanium was lower in the wet+coarse fed animals compared to wet+fine and dry+fine fed animals ($p<0.05$). And after 120 minutes the recovery was lowest in the dry fine group ($p<0.05$).

Protease activities

Starter phase

The protease activity is expressed as the amount (μmol) of tyrosine released from haemoglobin during 10 minutes of incubation at 41°C . The effect of diet treatments on protease activity during starter phase was expressed per gram of the fresh proventriculus tissue and results are shown in Table 3.

Table 3. Effect of diet treatment during the starter phase on protease activity.

Treatment	Protein content (mg/g tissue)	Activity ($\mu\text{mol tyrosine/g tissue}$)	Activity ($\mu\text{mol tyrosine/mg protein}$)	Relative activity ($\mu\text{mol tyrosine/100g BW}$)
Dry + Fine	155.4 ± 6.4	1445 ± 107 ab	9.3 ± 0.6 a	212.4 ± 15.3 a
Wet + Fine	139.0 ± 6.8	1332 ± 114 a	9.6 ± 0.6 a	178.3 ± 16.3 b
Wet + Coarse	138.9 ± 6.6	1661 ± 110 b	12.2 ± 0.8 b	225.6 ± 15.8 a

a.b Means within columns that do not have identical letters differ significantly ($P<0.05$)

It can be seen in Table 2 that the protein contents of the proventriculus (expressed in mg per g tissue) were similar between all different treatment groups, although the average protein content of the proventriculus in the dry fed group was higher than the protein content of the proventriculus in the wet fed groups. There was no difference in protease activity of the proventriculus between wet fine and dry fine fed birds. Within the wet fed groups, protease activity per gram of organ tissue of the proventriculus and relative activity in coarse fed birds was 24.7% higher than that in fine fed birds ($p<0.05$). The protease activity expressed as μmol of tyrosine/g tissue was not different between dry fine and wet coarse diets, but they were different when the activity was expressed as μmol of tyrosine/mg protein ($p<0.05$).

Grower phase

The effect of dietary treatments during the grower phase and the carry over effect of dietary treatments during the starter phase on protease activity during the grower phase are presented in Table 4.

Table 4. Protein contents and protease activity in the proventriculus in the grower phase and carry over effect of different treatments during starter phase on the protease activity during the grower phase.

Starter	Grower	Protein content (mg/g tissue)	Activity (μmol tyrosine/g tissue)	Activity (μmol tyrosine/mg protein)	Relative activity (μmol tyrosine/100g BW)
Dry + Fine	Dry + Fine	56.7 ± 4.0 a	1930 ± 181	34.4 ± 4.2 b	87.9 ± 10.0 a
Dry + Fine	Wet + Fine	58.9 ± 2.8 a	2082 ± 346	30.6 b ± 2.9 b	72.8 ± 12.0 b
Dry + Fine	Wet + Coarse	67.1 ± 4.0 b	1796 ± 244	29.9 b ± 7.2 b	88.5 ± 17.0 a
Wet + Fine	Dry + Fine	59.4 ± 4.3 a	1820 ± 196	28.4 ± 3.0 b	69.9 ± 11.8 b
Wet + Fine	Wet + Fine	70.4 ± 2.7 b	2159 ± 239	30.4 ± 2.4 b	83.7 ± 10.7 a
Wet + Coarse	Dry + Fine	73.7 ± 4.7 b	2029 ± 215	29.8 ± 1.0 b	88.2 ± 10.8 a
Wet + Coarse	Wet + Coarse	61.2 ± 1.6 b	2245 ± 96	36.7 ± 1.3 a	88.3 ± 5.1 a

a.b Means within columns that do not have identical letters differ significantly (P<0.05)

There were no carry over effect of dietary treatments during the starter phase on protease activity in the grower phase. There was only a carry over effect of coarse diet during the starter phase found to have an effect on protein content of the proventriculus in the growing period (p<0.05).

There were no significant differences in protease activity between all treatment groups during grower phase. Protein content of the proventriculus after the grower period was higher in wet coarse group compared to wet fine and dry fine groups (p<0.01 and p<0.05) but there was no differences in protease activity expressed as μmol tyrosine/g tissue. But when expressed this activity in μmol tyrosine/mg protein, the protease activity was higher in dry fine group compared to wet fine and wet coarse groups (p<0.05). When expressed the protease activity as the relative activity/100 g BW, the protease activity was significantly higher in wet coarse diet than in wet fine diet groups (p<0.05). Wet coarse fed group during the entire experimental period had an increased protease activity compared to wet fine group (p<0.05).

Effect of dietary treatments on pH value of the different GI tract segments after starter period

Starter phase

Results on effect of different dietary treatments on the pH value of chyme in different GI tract segments are presented in Table 5.

Table 5. Effect of dietary treatments on pH value of the ingesta.

Treatment	Crop	Prov-gizz	Duodenum	Jejunum	Ileum
Dry + Fine	4.53 ± 0.07 a	2.50 ± 0.07 a	5.78 ± 0.06	5.62 ± 0.08	5.09 ± 0.13 a
Wet + Fine	3.91 ± 0.09 b	2.71 ± 0.09 b	5.81 ± 0.08	5.58 ± 0.11	5.61 ± 0.17 b
Wet + Coarse	4.48 ± 0.08 a	2.30 ± 0.08 c	5.89 ± 0.07	5.80 ± 0.09	5.70 ± 0.15 b

a.b Means within columns that do not have identical letters differ significantly (P<0.05)

From this Table it can be seen that the pH value of the crop in wet fine diet treatment group was lower than that of wet coarse group (p<0.01) whereas the pH value of the proventriculus-gizzard system was lower in wet coarse fed group (p<0.01). The treatment wet and dry feeding only showed differences in pH value of the ileum content (p<0.05), whereas pH of chyme in the other GI tract segments was similar between treatments.

Grower phase

The effect of dietary treatments during grower phase on pH of the content and the carry over effect of dietary treatments during starter phase on grower phase are presented in Table 6.

Table 6. Effect of dietary treatments in grower phase on pH value of different GI tract segment's contents during the grower phase.

Starter	Grower	Crop	Pro-gizz	Duodenum	Jejunum	Ileum
Dry + Fine	Dry + Fine	3.85 ± 0.20 b	3.76 ± 0.20 a	5.49 ± 0.12	4.98 ± 0.22	5.45 ± 0.31 a
Dry + Fine	Wet + Fine	4.02 ± 0.15 a	3.65 ± 0.12 a	5.31 ± 0.10	4.82 ± 0.22	4.70 ± 0.29 b
Dry + Fine	Wet + Coarse	3.77 ± 0.17 b	3.34 ± 0.15 a	5.34 ± 0.12	5.30 ± 0.26	5.72 ± 0.35 a
Wet + Fine	Dry + Fine	4.28 ± 0.20 a	3.80 ± 0.20 a	5.34 ± 0.12	5.22 ± 0.22	5.57 ± 0.31 a
Wet + fine	Wet + Fine	4.05 ± 0.13 a	4.06 ± 0.11 b	5.35 ± 0.12	5.15 ± 0.17	4.84 ± 0.35 b
Wet + Coarse	Wet + Coarse	4.26 ± 0.13 a	3.38 ± 0.11 a	5.60 ± 0.12	5.20 ± 0.17	5.05 ± 0.35 a
Wet + Coarse	Dry + Fine	4.21 ± 0.20 a	3.62 ± 0.20 a	5.46 ± 0.12	5.00 ± 0.22	5.16 ± 0.31 a

a.b Means within columns that do not have identical letters differ significantly (P<0.05)

There were no carry over effects of the dietary treatments during the starter phase on the pH value in different GI tract segments during the grower phase.

The effect of dietary treatment (coarse and fine) during grower phase on the pH value of the ileum chyme was significant ($p<0.05$). But there was no effect of treatment on pH in the other segments of the GI tract. With regard to treatments applied during the entire experimental period, the pH value of the crop in birds which received wet diet was higher compared to dry diet fed birds ($p<0.05$). These treatments, however, have no effect on pH value of other GI tract segments. The pH value of the proventriculus-gizzard chyme was lower in birds fed with wet coarse diet ($p <0.01$). There were no effects of coarse and fine diet treatments on the pH value of other GI tract segments.

DISCUSSION AND CONCLUSIONS

In this study we estimated the residence time of digesta in the gastro-intestinal tract by estimate the percentage of Titanium dioxide recovered from digesta in different segments of the foregut. The resident time of ingested feed will influence the hydrolysis and absorption of nutrients in chickens (van der Klis and van Voorst, 1993; Uni *et al.*, 1995). The digestive and absorptive capacity of the chickens is thus dependent on the duration of time in which ingesta stays in the gut. The longer it stays in the gut, the opportunities for the digesta to contact with digestive enzymes and bile salts. The time available for contacting between digested particles and absorptive surfaces are likely to influence the hydrolysis and thus the absorption of nutrients and consequently also the energy uptake by the chicken. Growth performance and nutrient absorption are influenced by rate of passage of digesta in chickens (van der Klis and van Voorst, 1993; Uni *et al.*, 1995; Hetland and Svhuis, 2001).

For a proper digestion and absorption of nutrients, the digesta first undergoes several mechanical and chemical treatments. Mechanical changes include swallowing, maceration and subsequently particle size reduction of feed in the muscular stomach. Chemical digestion includes the secretion of enzymes from the mouth, stomach, intestines and pancreas, bile acid from the liver, of hydrochloric acid from stomach and hydrolysis. Also bacterial action will occur (Duke, 1986). After intake by the mouth, the oesophagus and the crop are responsible for residing and transport of feed. Both organs produce mucus for lubrication. Initial stages of carbohydrate digestion may occur in the crop of due to mode of action of salivary amylase

(Klasing, 1998). In our study, we hypothesized that the resident time of digesta in the foregut segment depends upon the structure and conformation of the feed. As dry feed needs to be moisturized in the crop before it could be transferred to the proventriculus-gizzard system for particle size reduction and enzyme hydrolysis (Duke, 1986), it may stay for longer period of time in the crop compared to wet feed (Figure 1). Before leaving the gizzard the ingested feed particles are reduced to a certain particles size (Nir *et al.*, 1994, 1995, Hetland *et al.*, 2003). It is logic that in our study the feed residence time in the crop of wet coarse feed was shorter and was longer in the proventriculus-gizzard system. Thus there may be sufficient opportunities for the ingesta particle to be reduced to a proper size and there will be sufficient contact with digestive enzymes excreted from the proventriculus-gizzard system (Turk, 1982).

The amount and activity of digestive enzymes in poultry change with diet as well as with age of animals (Pubols, 1991). Each enzyme probably has a different optimum condition (Rothman, 1977). The effective utilization of feed is influenced by a number of factors including the relative activity of the digestive enzymes and the efficiency of nutrient absorption (Cherry and Siegel, 1978; Iji *et al.*, 2001). In our study, the activity of protease in the proventriculus was taken into account. It has been shown that the protease activity was more influenced by the dietary treatments (especially effect of diet structure) during the starter phase rather than during the grower phase (see Tables 3 and 4).

Although there was no difference between the protein contents of the proventriculus in between birds fed coarse or fine diet groups in the starter phase. Protease activity was highest in animals fed a coarse diet. There was no difference in protease activity between wet and dry treatment groups. Although the average protein content of this organ was higher in dry treatment group. That may be due to the large variation in protease activity was found within the treatments. In the grower phase there was only a carry over effect of coarse diet treatment during the starter phase on the protein content of the proventriculus during the grower phase. Dietary treatments during entire experimental period effected on the protein content of the organ but it had no effect on protease activity. This is in accordance with results from Susilla *et al.* (2003).

pH is one of the most important factors influences the enzymes activity (McDermid *et al.*, 1988). In the current study, the pH value of chyme the crop and proventriculus-gizzard in wet coarse fed birds was lowest (pH 2.30 at the starter phase and 3.30 at the grower phase). This is quite favorable for the optimal enzyme activity in the proventriculus (Murakami *et al.*, 1979). The status of the GI tract lumen has a strong effect on the bacterial proliferation in the gut

(Vahjen *et al.*, 1998). In poultry, pathogen bacteria e.g. salmonella enter the GI tract via the crop. The environment of the crop with respect to microbial composition and pH seems to be very important in relation to the resistance to pathogens. High amounts of lactobacilli and a low pH in the crop show maybe negative for salmonella survival in the crop (Hinton *et al.*, 2000).

A lower pH of the crop contents and in the proventriculus-gizzard in wet coarse fed groups maybe related to the longer resident time of the ingesta in the upper part of the GI tract. Thus the digesta have a chance to receive and absorbed large amounts of HCl excreted from the proventriculus. This was supported by some gastric gland secretion activity studies done by Turk (1982).

From the results of this study, it can be concluded that wet coarse feeding during the starter period increased protease activity. Moreover it has a carry over effect on the protein content of the proventriculus during the grower phase. The residence time of the digesta in the foregut segment was dependent on the structure of the ingested feed.

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REFERENCES

- Bird, F.H. (1971) Distribution of trypsin and amylase activities in the duodenum of the domestic poult. *British Poultry Science*, 12: 373-378.
- Cherry, J.A., Siegel, P.B. (1978) Selection for body weight at eight weeks of age. 15. Feed passage and intestinal size of normal and dwarf chicks. *Poultry Science*, 57: 336–340.
- Cook R.H. and Bird, F.H. (1973) Duodenal villus area and epithelial cellular migration in conventional and germ-free chicks. *Poultry Science*, 52: 2276-80.
- Duke G.E. (1986) Alimentary canal: secretion and digestion, special digestive functions and absorption in avian physiology. *4th edition. (P. D. Sturkie, Ed.) New York β Springer-Verlag*: 289-302.
- Dror, Y., Nir, I. and Nitsan, Z. (1977) The relative growth of internal organs in light and heavy breeds. *British Poultry Science*, 18: 493-496.
- Erener, G., Ocak, N., Ozturk, E. and Ozdas, A. (2003) Effect of different choice feeding methods based on whole wheat on performance of male broiler chickens. *Animal Feed Science and Technology*, 106: 131–138.
- Hamilton, R.M.G. and Proudfoot, F.G. (1995) Ingredient particle-size and feed texture- effects on the performance of broiler-chickens. *Animal Feed Science and Technology*, 51: 203-210.
- Goelema, J.O., (1999) Processing of legume seeds: effects on digestive behaviour in dairy cows. PhD Thesis, Animal Nutrition group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands, 222 pp.
- Havenstein, G.B., Perket, P.R., Scheideler, S.E. and Larson, B.T. (1994) Growth, liveability, and feed conversion of 1957 vs 1991 broilers when fed “typical” 1957 and 1991 broiler diets. *Poultry Science*, 73: 1785 – 1794.
- Hetland, H. and Svihus, B. (2001) Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British Poultry Science*, 42: 354-361.

- Hetland, H., Svhuis, B. and Olaisen, V. (2002) Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *British Poultry Science*, 2: 416-423.
- Iji P.A., Saki, A. and Tivey D.R. (2001) Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. *British Poultry Science*, 42: 514-522.
- Klasing, K.C. (1998) Anatomy and Physiology of the Digestive system. *Comparative Avian Nutrition*, CAB International: 9-35.
- McDermid, A.S, McKee, A.S. and Marsh, P. D. (1988) Effect of environmental pH on enzyme activity and growth of *Bacteroides gingivalis* W50. *Infection and Immunology*, 56(5): 1096-1100.
- Murakami, T., Suzuki, Y. and Murachi, T. (1979) An acid protease in human erythrocytes and its localization in the inner membrane. *European Journal of Biochemistry*, 96, 221-227.
- Myers, W.D. Ludden, P.A. Nayigihugu V. and Hess B.W. (2004) A procedure for the preparation and quantitative analysis of samples for titanium dioxide. *Journal of Animal Science*, 82: 179-183.
- Nir, I., Hillel, R. Shefet, G. and Nitsan, Z. (1994) Effect of grain particle size on performance. 2. Grain texture interactions. *Poultry Science*, 73: 781-791.
- Nir, I., Hillel, R., Ptichi, I. and Shefet, G. (1995) Effect of particle size on performance. 3. Grinding pelleting interactions. *Poultry Science*, 74: 771-783.
- Nitsan, Z., Ben-Avraham, G., Zoref, Z. and Nir, J. (1991) Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. *British Poultry Science*, 32: 515-523.
- NRC (1994) Nutrient requirements of chickens. *National Research Council*, 1994.
- Pubols, M.H. (1991) Ratio of digestive enzymes in the chick pancreas. *Poultry Science*, 70: 337-342.
- Romanoff, A. C. (1960) The Avian Embryo. *MacMillan Co., New York, NY*.
- Rose, S.P., Fielden, M., Foote, W.R., and Gardin, P. (1995) Sequential feeding of whole wheat to growing broiler chickens. *British Poultry Science*, 36: 97-111.

- Rothman, S.S. (1977) The digestive enzymes of the pancreas: a mixture of inconstant proportions. *Annual Review of Physiology*, 39: 373-389.
- Short, F.J., Gorton, P., Wiseman, J. and Boorman, K.N. (1996) Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science Technology*, 59: 215-221.
- Susbilla, J.P., Tarvid, I., Gow, C.B. and Frankel, T.L. (2003) Quantitative feed restriction or meal-feeding in broiler chicks alter functional development of enzymes from protein digestion. *British Poultry Science*, 44: 689-709.
- SPSS® (2003) SPSS 12.0.1 for Window by SPSS Inc. 2003.
- Taylor, R.D. (1998) Production, physiological and metabolic response to alternative methods of calcium presentation to laying hens. *PhD thesis, University of New England, Armidale*,
- Turk, D.E. (1982) The anatomy of the avian digestive tract as related to feed utilization. *Poultry Science*, 61: 1225-1244,
- Uni, Z., Noy, Y. & Sklan, D. (1995) Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. *Poultry Science*, 74: 1622-1629.
- Vahjen, W., Glaser, K., Schafer, K. and Simon, O. (1998) Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. *Journal of Agricultural Science, Cambridge*, 130: 489-500.
- Van der Klis, J.D. & van Voorst, A. (1993) The effect of carboxy methyl cellulose (a soluble polysaccharide) on the rate of marker excretion from the gastrointestinal tract of broilers. *Poultry Science*, 72: 503-512.
- Yasar S. and Forbes J.M. (1999) Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. *British Poultry Science*, 40: 65-76.

CHAPTER 6

General discussion

INTRODUCTION

In literature there are a number of reports on investigations on the development tract during early life of broiler chickens by nutritional means. The general aim was to derive feeding strategies which favor good development. It is clear that early access to feed and water after hatching enhances the functional development of the alimentary tract and the foregut segments in particular. It is assumed that in this way, diet digestibility and feed utilization are also optimized. The development of the gastro intestinal tract starts during the embryonic (incubation) period, when the egg white and egg yolk supply all the nutrients. The digestive tract will develop further after hatching, and dramatic changes occur in both the physical size of the digestive tract and in the mucosal morphology for the first 6 – 8 days (Cook and Bird, 1973; Uni *et al.*, 1995, 1996 and 1999). After hatching, the birds must make the transition from using energy supplied by the endogenous nutrients provided by the yolk, to the utilization of nutrients of an exogenous carbohydrate-rich feed. In order to be able to obtain nutrients from exogenous feed, the birds must have a proper functional developed GI tract. It is thought that in feed which has undergone some physical and eventually chemical treatments like grinding, pelleting etc. before ingestion by the animal, starch and protein may be hydrolysed at a higher rate compared to untreated feed (Hetland *et al.*, 2002). This can ensure that nutrients are released from feed particles at a rate and level which are higher. These can then be absorbed by the GI tract and used in the body. Growth of the GI tract is often expressed as relative growth. This is the increase in weight of a digestive segment in relation to size of the body. In young growing birds, the intestinal growth rate is higher than the growth rate of the body. The process of rapid relative growth achieves its maximum at about 6 – 8 days in broilers (Murakami *et al.*, 1992; Pinchasov and Noy, 1994). After this period, the relative weight of the gizzard and intestines in relation to total body weight will decline. The relative length of the small intestine, however, continues to increase with age (Iji *et al.*, 2001). Therefore the limiting factors to the functional development of the GI tract may be determined at a very early age of life. From literature it is clear that coarse diets at a young age can increase the relative weight of the gizzard and intestines (Kwakkel *et al.*, 1997). However these investigations all had more factors which varied than coarseness alone. Therefore we aimed our studies at different ages and at different factors like coarseness wetness and acid or not. We hypothesized that at least until a few weeks of age these limiting factors such as the relative weight of the gizzard and intestines may be influenced by diet structure (coarse) and diet conformation (wet). Researchers have made efforts to get the birds

as quick as possible eating feed and water after hatch. For instance, Noy and Sklan (1999) held newly hatched birds for 48 hours in a so-called “oasis”, this is a box in which feed is provided. They found that this box (which is used during transportation of the birds) improved body weight in chickens at 4 days of age compared to animals in transport boxes without feed. This early feeding strategy for broiler chickens at young age may also help the functional development of the foregut segments. Consequently is thought to affect feed utilization and performance after feeding of day-old animals. Therefore we hypothesize that feed factors in addition to nutrient composition like feed structure and its conformation can be used for influencing development of the foregut segments.

A feeding strategy to influence the foregut segments should include

1. Compose a diet which can supply the nutrients required by the bird
2. Supply the nutrients in a feed form which is ingested at a high level such as wet feeding. Wet feeding may increase or stimulate feed intake and help maintain gut health at a very young age in broiler chickens.
3. Technological treatment of the diet: This should result in physical diet properties such as good particle size distribution. It is well known that sufficient large particles can stimulate the development of the proventriculus-gizzard system.

Different feeding strategy approaches have been applied in this thesis. It is aimed to have sufficient nutrients in the diet so that is not a variable between diets. Variables which were varied were; particle size; wet or dry and acid or not acid. These later are studied with regard to the morphological development of the foregut segments and broiler and also with regard to performance. Effects of diet treatments on the retention time of the ingesta in the foregut segment as well as some gut health properties were investigated. In the following paragraphs the results of the studies will be compared with other studies in literature which have aspects similar to our study. At the end we will give conclusions and suggestions for further research.

Development of the foregut segment

About 18 hours after the beginning of the incubation period the alimentary tract starts to appear (Romanoff, 1960) and it develops further throughout the incubation period. During the immediate post hatching period, the proventriculus, gizzard, and small intestinal weight increase much more rapidly in weight in relation to body weight than other organs and tissues

do. In chicken, the maximal relative size of these digestive organs occurs at 3 to 7 days of age (Cook and Bird, 1973; Dror *et al.*, 1977). The small intestines increase in weight more rapidly than the body mass during the first week post hatch (Katanbaf *et al.*, 1988; Sell *et al.*, 1991; Akiba and Murakami, 1995; Sklan, 2001). Microscopic examination of the small intestine revealed that rapid morphological changes occur after hatch. Villus volume increases three to five fold during the first week post-hatch and there are different ontogenetic timetables for villus growth in the duodenum, jejunum and ileum respectively (Uni *et al.*, 1998; Sklan, 2001). This development can be seen from Figure 1.

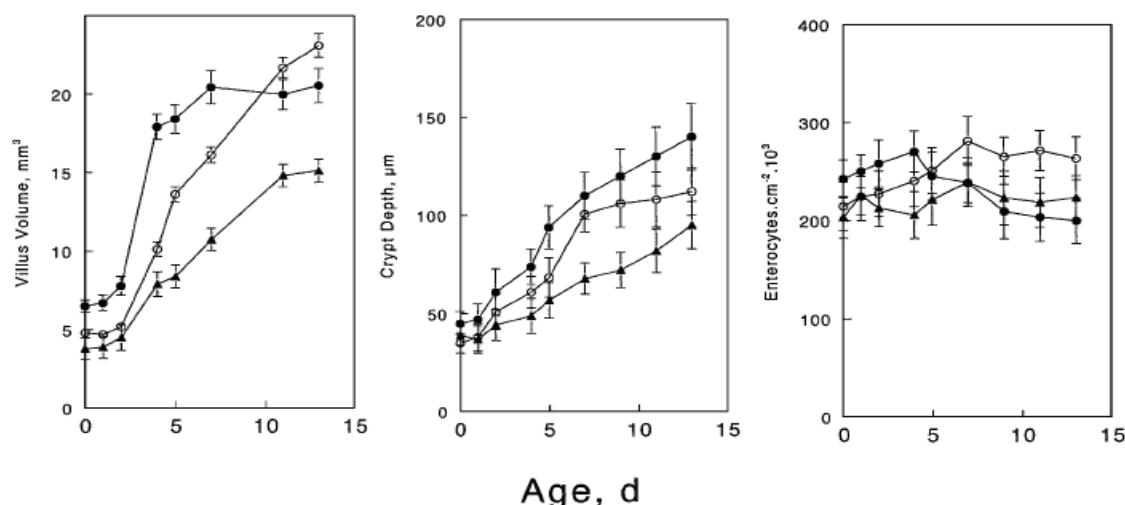


Figure 1. Values for villus volume ,crypt depth and no of enterocytes in duodenum (●), jejunum (○), and ileum (▲) from hatch until 14 days. (Uni *et al.*, 1998)

It can be seen that villus volume was greater in the duodenum than the jejunum until about 9-10 d, villus volume was greater in the jejunum than the ileum from 4 days onwards. Crypt depth was not different in the duodenum and jejunum, but was lower in the ileum than in the duodenum and jejunum between 7 and 11 days.

The profile of enzymatic activity in the small intestine adapts to accommodate the exogenous feed substrates (Marchaim and Kulka, 1967). Their studies showed that concentration of all pancreatic enzymes in the ileal chyme increase after hatch, although the rate of increase was different for each enzyme. The pancreatic lipase concentration in the chyme increased linearly until 16 days after hatching and then reached a plateau (Noy and Sklan, 1998). Brush-border enzyme activities, which are present in the intestines of neonatal chicks, increase rapidly (Black, 1978; Black and Moog, 1978; Uni *et al.*, 2003).

The role of the foregut segment in digestion process

The process of digestion involves all of the mechanical and chemical changes that ingested feed undergoes before the nutrients are released from it and are absorbed. Changes due to mechanical action occur during and after swallowing, by maceration and during particle size reduction of the feed in the muscular stomach. Chemical digestion includes the secretion of enzymes from the mouth, stomach, intestines and pancreas, bile acid from the liver, of hydrochloric acid from stomach, and bacterial action (Duke, 1986). After ingestion by the beak, the oesophagus and the crop are responsible for storage and for transport of feed to other parts of they GI tract. Initial phase of carbohydrate digestion can occur in the crop of some species due to salivary amylase (Klasing, 1998).

Compared to layer chicken, broiler chicken grows much faster and has a different rate of body development. Thus they will also have a different gastro-intestinal development. Feed for broiler has undergone a different range of processing variables compared to feed for layer chickens. In general feed for broilers is processed by hammer mill and feed for layer chickens is roller mill.

Development can be influenced by feed intake and thus by special feeding schemes such as choice feeding (Erener *et al.*, 2003), sequential feeding (Rose *et al.*, 1995), feeding pelleted diets (Nir *et al.*, 1995) and the whole grain feeding (Hetland *et al.*, 2002).

The crop

The crop is a very important storage organ in chickens. Some digestion occurs in the crop as a result of both enzymatic and bacterial activity (Duke, 1986). Amylase has been found in the crop of chicken, but it may not have originated from there. Amylase originates from salivary glands, crop mucosa, bacteria in the crop; feed in the crop or from intestine and pancreas via reflux. The pH in the crop is most favorable for amylase activity and, probably, both bacterial and nonbacterial amylase are involved (Duke, 1986). About 25% of ingested starch is already hydrolysed to sugar in the crop (Pritchard, 1972). Sugars may also be hydrolysed by bacteria. Parts of these sugars disappear from the crop (about 10%). They may either have been absorbed or used by crop bacteria (Pritchard, 1972). The crop wall secretes mucus and moisturizes ingesta by this mucus fluid and prepares it for the gastric and intestinal digestion. These contributions to the digestive process in the crop may be more important than any “direct” digestion occurring in the crop (Duke, 1986). Removal of the crop (“Cropectomy”),

however, only has a small negative effect on digestion in highly concentrated diets. These diets contain compounds which are digested at a high rate; easy digested. This means that at least in these ``normal`` diets, the crop is not a limiting factor for digestion. In a bulky ration, however, the crop may be more important because the feed takes a longer time to digest and the animal uses more storage. In addition the animal increases its feeding frequency with bulky feeds (Smith and Pilz, 1971).

The proventriculus-gizzard system

The proventriculus-gizzard system plays a very important role in the digestion process. The proventriculus is the glandular stomach that secretes both acid and pepsinogen from the chief cells and mucus from single mucosal cells (Duke, 1986). The low pH ($\text{pH} < 2$) in the proventriculus-gizzard system is ideal for pepsinogen which is secreted into the proventriculus and then changes into pepsin. The pH of pure gastric secretion is about 2, but the pH of the gastric content is usually higher because the secretions are diluted and buffered by ingesta. HCl and pepsin have been found in the gastric secretion of all the bird species. Other enzymes (e.g. lipase) have been detected in gastric contents, probably resulting from the duodenal reflux (Klasing, 1988).

Beside the proventriculus, the gizzard as the organ for particle size reduction also contributes to the digestion process to a large extends. Particle size reduction is considered very important for grain-eating birds: before leaving the gizzard, all feed particles need to be ground to a critical size this is needed in order to be reached by the enzymes. Then feed components will be hydrolysed and nutrients are subsequently released and can be absorbed by the small intestine (Nir *et al.*, 1994, 1995; Hetland *et al.*, 2003).

Digestive capacity of an individual chicken

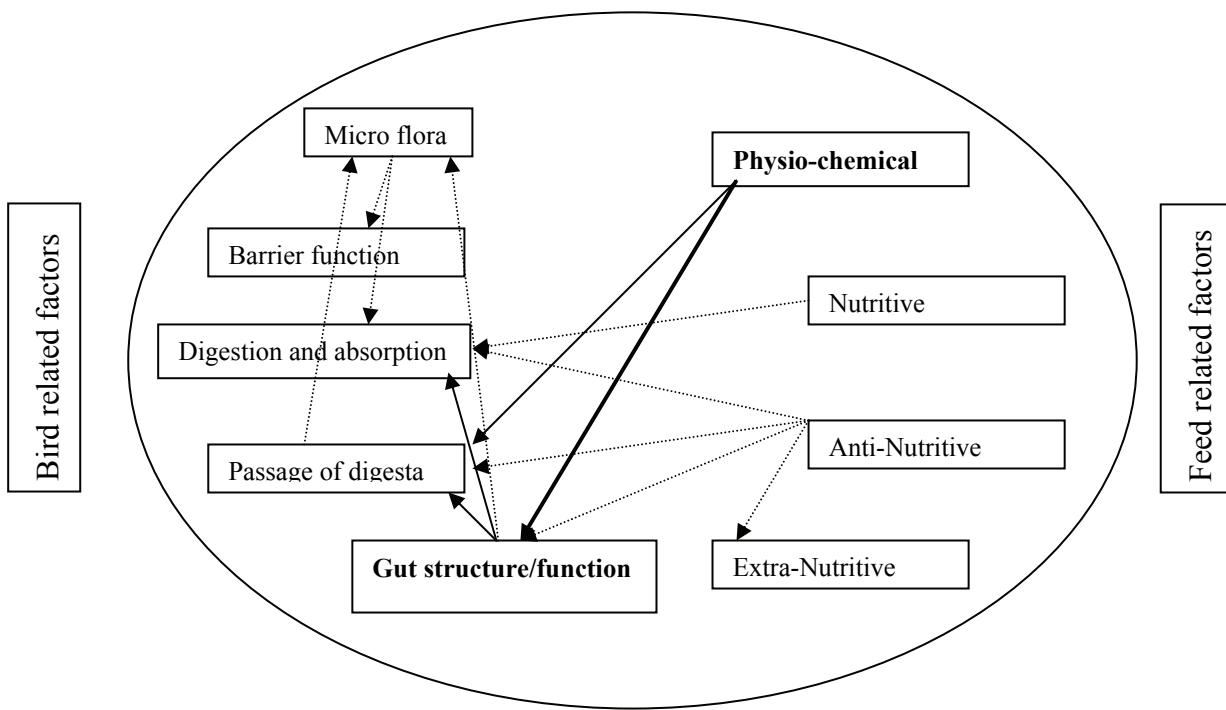


Figure 2. A schematic digestive capacity integration of bird-related and feed-related factors.

The direction of arrows indicates the possible impact (Modified from Hughes, 2001).

In Figure 2, a survey is given of the relation between animal and dietary factors which are related to each other and which influence each other during digestion according to Hughes (2001). It can be seen from Figure 2 that the digestive functioning of an individual chicken is dependent on several factors. The animal factors involve a number of influences like age which influence gut structures and gut functions like passage of ingesta, digestion and absorption of feed, and probably also micro flora abundances. Of the feed factors, it may well be that, beside nutrient active factors in the feed, and feed physical and chemical properties are limiting the digestive capacity of the chickens. In our studies we have chosen the age period in which the development of the GI tract undergoes most changes in morphology. And we speculate that this will also hold for its function.

We found that diet structure during the starter phase stimulated the development of the proventriculus-gizzard system especially when a coarse diet was offered to the birds up to 21 days of age (Chapter 3) we concluded that we may shorten the stimulation period from 21 to 17 days of age (Chapter 4). Wet feeding clearly increased the weight of the gastro-intestinal tract probably due to the higher intake of dry matter compared to dry feeding. Wet feeding

applied only during the starter phase had a carry over effect on the weight of these organs during grower phase. It is however not known yet whether there will be long lasting effects afterwards. Wet feeding stimulated the development of the GI tract. The bigger GI tract (approximately 10%) at the end of the starter period were also found in birds fed a coarse diet during the starter phase. But these effects on the GI tract development disappeared during grower phase (Chapter 3). This can mean two things

- Animals need continuous stimulation of the GI tract to maintain its development
- The GI tract is developed sufficiently to enable hydrolysis of various compounds to nutrients in various kinds of feeds

In table 1 some data on GI tract development in the starter period are given.

Table 1. Effect of early feeding strategies on development of the GI tract at the end of the starter phase (g/100gBW)

	This thesis		Other studies in literature	
	Structure (Coarse)	Conformation (wet)	Structure (Coarse)	Conformation (Wet)
Crop	0.32	0.49	0.39*	NA
Proventriculus-gizzard	3.44	2.70	3.12*/3.15**	NA
Small intestine	4.24	4.15	3.92*	NA

* Nir et al., 1995

** Kwakkel, 1998

It can be seen from table 1 that diet structure (coarse) clearly effected the development of the GI tract during the starter phase, thus it contributed to digestion and utilization of feed during grower phase (Chapters 3 and 4).

Feeding level after hatching

Within the first 2-3 d after hatching, chickens undergo a transition from yolk-derived lipid to dietary starch as the primary source of energy (Vieira and Moran, 1999a). The intake of high carbohydrate diets soon after hatching increases blood glucose levels and decreases the chick's dependence upon gluconeogenesis (Donaldson and Christensen, 1991). Under commercial rearing conditions, chickens have a delay in the access to feed and water. This situation increases the risk of dehydration (Vieira and Moran, 1999b). Chickens of broiler

strains have been selected for rapid weight gain and efficient utilization of feed. Broilers are usually fed on an *ad libitum* basis to ensure a rapid development to market size. Therefore, after hatching, it is very important for the bird to have a rapid adaptation of the digestive system in order to be able to digest the ingested feed and to replace nutrients from the yolk sac within a few days after hatching.

Early dietary access of post-hatch chickens does not only boost early intake and growth, but it also increases the relative (in relation to bodyweight) growth of internal organs such as the small intestine, liver and pancreas in poultry (Knight and Dibner, 1998). In Figure 3 the ratio of dry matter intake to body weight as calculated from data of our experiment in Chapter 4 is used to estimate the feeding level post hatching. It can be seen from this Figure 3, that the intake of exogenous nutrients increases rapidly during a period of 8 days and that the level of dry matter intake and bodyweight was highest at day 8.

In comparison with the energy and protein requirement for maintenance (estimated according to Rosy and Harper, 1996) of the chickens accounted for body weight (included activity and composition of the bird) and loss of body heat, the energy intake in MJ per day of our experimental birds was calculated based on the equation: $E = 0.35 W^{0.75} + (a - bT)W^{0.75}$ where: W is bodyweight in kg and T is effective ambient temperature (in degree Celsius). The result of actual energy intake and requirement per day in our experiment 4 is presented in Figure 4. It can be seen from Figure 3 that the intake of energy for growth rapidly increased after hatching. This high feeding level coincides with the yolk sac removal period (Turro-Vincent et al., 1994). Immediately few days after hatch the yolk sac, which comprises approximately 20% of the body weight, has disappeared completely (Noy and Sklan, 1998). Thus the chickens had to increase feed intake to compensate for the shortage of nutrients which are now no longer provided by the yolk.

Figure 4 suggests that when the yolks are gone the chicks' GI tract is able to accommodate and digest exogenous feed very efficiently (Turro-Vincent et al., 1994). Our study in Chapter 4 shows that in this period, the energy intake per chicken was 0.432 MJ for dry diet fed birds and 0.468 MJ for wet diets birds or 2.79 MJ/kg body weight and 2.84 MJ/kg body weight, respectively. This was about 400% of the maintenance energy level required for the birds.

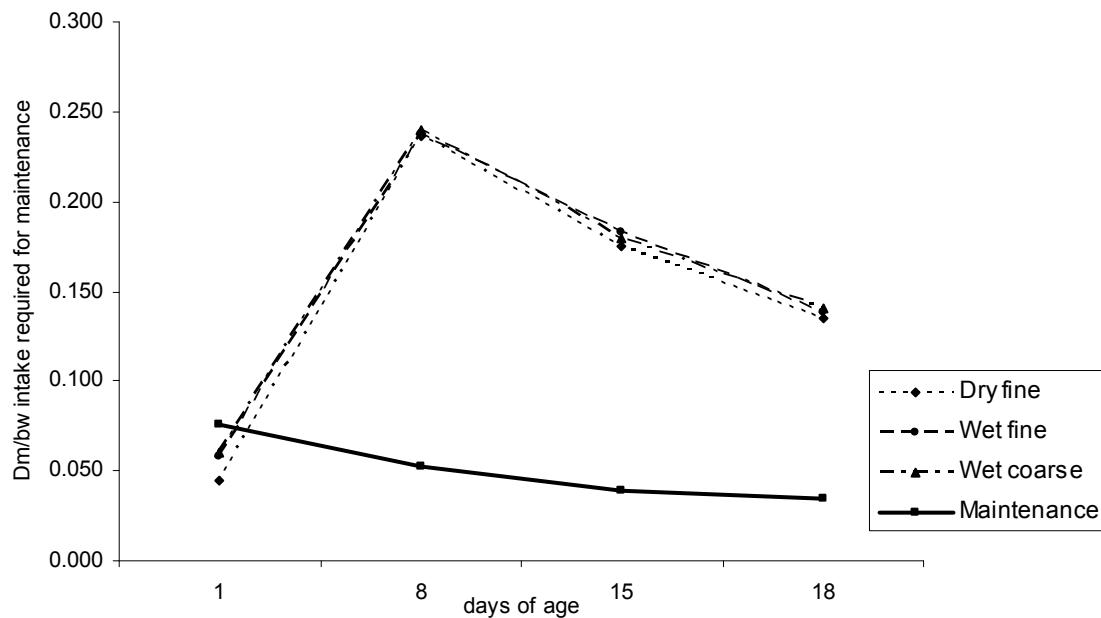


Figure 4. Ratio of dry matter intake/bodyweight and energy requirement for maintenance of broiler at day 1, 8, 15 and 18 (g dry matter/g body weight). Assumed is 12.8 MJ ME/kg DM of feed

Factors influence the development and function of the foregut segment

1. Diet composition

Factors associated with diet composition can influence the morphology and function of the gastro intestinal tract. For example: type of cereal (Jamroz *et al.*, 1992; Sharma *et al.*, 1997), fibre content (Langhout *et al.*, 1999), vitamins (Uni *et al.*, 2000), antinutritional factors (Rubio *et al.*, 1990), enzyme supplementation (Sharma *et al.*, 1997; Yasar and Forbes, 1997).

In our study, which has been reported in Chapter 2 a Liquid wheat by-product was used as replacement of normal wheat (starch). This liquid by-product is inexpensive; therefore it has been excessively used in pig nutrition and it has been shown to reduce feed costs (Van Brakel *et al.*, 1996; Scholten *et al.*, 1999). In the present study, an investigation was made by replacement of the dry wheat starch in commercial broiler diet with a liquid wheat by-product. The inclusion of liquid wheat by-product in a broiler diet increased body weight of male broiler chickens but showed no effect on feed conversion. This means that mostly the intake is affected and less the utilization. In order to check whether the rapid growth influenced the activity of birds we also measured walking ease but found no effect of the liquid wheat by-product on the ease of walking.

We did find an effect of the liquid wheat by-product on morphological development of the duodenum. This may reflect the activity of the pancreas. The duodenum was 10% bigger consuming the liquid by-product compared to consuming the solid feed. It should be noted that with fermented wheat starch two factors are changed: both wet feeding and acidification showed an increased feed intake of birds which was in accordance with the other authors (Yalda and Forbes, 1995; Yalda and Forbes, 1996).

In order to check whether this was an impact of feed acidification or also of liquid feeding we repeated the acidification part for this purpose by adding 1% of organic acid into the feed (Chapter3). But we found that acid did not show any effect in the very young birds. The acid used Calprona AL® (Anonymus, 2004) has been tested before and the results of the unpublished tests suggested that lactic acid stimulates digestibility and feed intake by stimulating the secretion of pancreatic enzymes. This claim was supported by a trial conducted by Research Institute for Animal Husbandry, the Netherlands (Anonymus, 2005), where a better growth and feed conversion ratio in both starter and grower period was found in broilers using a dry feed acidified with Calprona AL®. In our study, we also did not find any clear effect of the acidification on the feed intake of birds, our results are in line with the findings of Gentle (1971). The difference between our studies and those in literature is that in our control animals intake was already very high. So acidification may work in less favorable conditions

Other studies showed that acidification of diets may have favorable effect on poultry production by creating a healthy gut environment (Heres *et al.*, 2003; Heres *et al.*, 2004; Iba & Berchieri, 1995; Berchieri and Barrow, 1996; Thompson and Hinton, 1997).

2. Diet structure and conformation

Literature has shown often that feed texture properties have a clear effect on the development of the gastrointestinal tract of the chickens. Birds fed a coarsely ground diet had a gizzard twice as heavy as found in birds fed a fine ground diet (Kwakkel *et al.*, 1997). When the chicken is fed with the coarse feed, feed intake increases and body weight gain improves in comparison to birds fed a fine feed (Nir *et al.*, 1994).

In our experiments (Chapters 3, 4, and 5) feed processing was carried out according to Hamilton and Proudfoot (1995). We found that birds fed coarse diets had a heavier gizzard, had an increased feed intake and body weight gain compared to the birds fed with fine diets.

It is expected that a muscular gizzard will provide a better grinding of feed particles thus a better handling of ingesta (Rogel *et al.*, 1987; Hetland *et al.*, 2002; Hetland *et al.*, 2003). The muscular gizzard thus may optimize the reflux of chyme in the gastro intestinal tract (Bird, 1971), thereby optimizing digestion. This occurs both via the birds “and the grains” natural enzyme activities as the feed stays in the upper digestive tract for a long time (Taylor, 1998). Optimum particle sizes also enhance digesta motility and back flow within the gastrointestinal tract (Williams *et al.*, 1997; Hetland and Svhuis 2001). In our experiment (Chapter 5) titanium dioxide was used as marker to investigate the resident time of feed in the different foregut segments. We found that the percentage of titanium recovery in coarse feed was the highest in the gizzard through a course of time after 60 and go and 90 minutes. This means that the coarse feed need to be finely ground to a certain particle size before it passed through to the small intestine (Hetland *et al.*, 2002). Also it may increase the duration of time of enzymes to act on feed compounds. Also proper particle size may accommodate the reflux within the gizzard duodenum system.

Yasar and Forbes (1999) suggested that compared to normal feed, wet feeding leads to a more rapid solubilisation of dry matter and crude protein in the crop. In that case the outcome is similar to that when large dietary particles are retained in the crop for some time. This potential influence of the crop for enzymatic activities so far has not been investigated well. Pettersson and Aman (1989) working with rye and wheat, indicated that both gizzard function, through feed grinding, and bacterial and/or endogenous enzymes are mainly responsible for fibre degradation. Moreover the effect of exogenous enzymes may be influenced as well. This supports the argument of aiming towards an increased digestive function with enhanced gastro-intestinal development. In Chapter 3 and 4, the diets conformation and structure were taken into account to examine on the functional development of the foregut segment.

In this study, wetting of diets significantly increased body weight gain and lowered feed conversion feed intake was not much effected. This was in accordance with the findings of Yasar and Forbes (1999). The advantages of increased retention of dry matter and protein with wet feeding can either be exploited by feeding conventional diets and expecting to see an improved feed conversion, or by reducing the cost of the diet by reducing its content of digestible nutrients (Forbes, 2003).

The overall performance of broiler chickens was presented in Table 2.

Table 2. Diet structure and conformation and overall performance of broiler chickens.

Starter phase	Dry/fine		Wet + coarse	Wet/coarse
Grower phase	Dry/fine	Wet/coarse	Dry/fine	Wet/coarse
Body weight gain	2.20 ^a	2.32 ^b	2.33 ^b	2.50 ^c
Feed intake	3.16 ^a	3.17 ^a	3.01 ^b	3.11 ^a
Water intake	4.86 ^a	5.59 ^b	5.15 ^{ab}	5.60 ^b
Feed conversion	1.43 ^a	1.37 ^a	1.29 ^b	1.24 ^b

Different superscript letters in a row are significant different ($p<0.05$)

It can be seen from Table 2 that the highest in bodyweight gain of chickens which received wet coarse diet during the whole experimental period, followed by those which received a wet coarse diet during the starter phase and a dry fine diet during the grower phase.

Carry-over effect of early GI tract development on broiler performance

Broiler performance during the grower phase is influenced by post hatch feeding (Saki, 2005). This is not due to the age of the parents stock and the day old chicken weight (Pezeshkian, 2002). In our studies, there was a carry-over effect of feeding strategies during starter phase on performance during grower phase when wet coarse diets were used to stimulate the functional development of the gastro intestinal tract (Chapters 3 and 4).

This means a development of the gastro intestinal tract which results in increased enzymes secretions from the GI tract and or better excess of enzymes to feed. The effects of these enzymes on feed digestion and absorption are dependent on the duration of ingesta stay in the GI tract (Griffiths *et al.*, 1977). In our study, the protease activity from the proventriculus (μmol of tyrosine/gram of tissue) in wet coarse fed chicken was the highest compared to other treatments. There was only a carry-over effect of wet coarse diet during starter phase on the protein content of the organ during grower phase.

Main conclusions

- Feeding coarse diets during the starter phase improves the functional development of the proventriculus-gizzard system.
- Wet feeding improves feed intake and, as a consequence, broiler performance.

- Feeding a wet *and* coarsely ground diet provides a large improvement in feed intake, feed conversion and body weight gain, showing the most pronounced effects during the starter phase of broiler's life.

Practical implication

Wet and coarse feeding of broilers during the starter phase shows a promising effect on their performance and GI tract development. Our studies shows that wet feeding may help birds in their first phases of life for development in addition to structure of the diet. Before application, however, other aspects of wet feeding – on farm – should be taken into account. In such a liquid feeding system, both the coarse feed and the water have to be transported to automatic mixing/stirring devices. These devices should not only regularly provide the wet diet to the animals but also – preferably – produce this diet in a relative silent way. This is to avoid stress to the animals, triggered by the freshly mixed diet becoming available to the animals and the potential “fighting” which may occur.

REFERENCES

- Akiba, Y. and Murakami, H. (1995) Partitioning of energy and protein during early growth of broiler chicks and contribution of vitelline residue. In: *10th European symposium on poultry nutrition*, (ed. Analia, Kurkey).
- Anonymus (2004) Calprona AL. Product information sheet, Verdugt B.V., the Netherlands (Unpublished).
- Anonymus (2005) Evaluation of antibiotic alternatives in broilers. A trial report by Research Institute for Animal Husbandry, the Netherlands (Unpublished).
- Benett, C. D., Classen, H.L. and Riddell, C. (2002) Feeding broiler chickens wheat and barley diets containing whole, ground and pelleted grain. *Poultry Science*, 81: 995-1003.
- Berchieri, A.Jr. and Barrow, P.A. (1996) Reduction in incidence of experimental fowl typhoid by incorporation of a commercial formic acid preparation (Bio-Add TM) into poultry feed. *Poultry Science*, 75:339-341.
- Black, B.L. (1978) Morphological development of the epithelium of the embryonic chick intestine in culture: Influence of thyroxine and hydrocortisone. *American Journal of anatomy*, 153: 573-599.
- Black, B.L. and Moog, F. (1978) Alkaline phosphatase and maltase activity in embryonic chick intestine in culture. Influence of thyroxine and hydrocortisone. *Developmental Biology*, 66: 232-249.
- Cook, R.H. and Bird, F.H. (1973) Duodenal villus area and epithelial cellular migration in conventional and germ-free chicks. *Poultry Science*, 52: 2276-80.
- Donaldson, W.E. and Christensen, V.L. (1991) Dietary carbohydrate level and glucose metabolism in turkey poult. *Comparative Biochemistry Physiology*, 98A: 347- 350.
- Dror, Y., Nir, I. and Nitsan, Z. (1977) The relative growth of internal organs in light and heavy breeds. *British Poultry Science*, 18: 493-496.

- Duke G.E. (1986) Alimentary canal: secretion and digestion, special digestive functions and absorption. *Avian physiology 4th edition*, New Yorkß Springer-Verlag: 289-302.
- Erenler, G., Ocak, N., Ozturk, E. and Ozdas, A. (2003) Effect of different choice feeding methods based on whole wheat on performance of male broiler chickens. *Animal Feed Science and Technology*, 106: 131–138.
- Forbes J.M. (2003) Wet foods for Poultry. *Avian and Poultry Biology Reviews*, 14 (4): 175-193.
- Gentle, M.J. (1971) Taste and its importance to the domestic chicken. *British Poultry Science*, 12: 77-86.
- Griffiths, L., Leeson, S. and Summers, J.D. (1977) Fat deposition in broilers: Effect of dietary energy to protein balance and early life caloric restriction of productive performance and abdominal fat pad size. *Poultry Science*, 72: 243-250.
- Hamilton, R.M.G. and Proudfoot, F. G. (1995) Ingredient particle size and feed texture: effects on the performance of broiler chickens. *Animal Feed Science and Technology*, 51 (3-4): 203-210.
- Heres, L., Engel, B., van Knapen, F. Wagenaar, A. and Urlings, B.A.P. (2003) Effect of fermented feed on the susceptibility for *Campylobacter jejuni* colonisation in broiler chickens with and without concurrent inoculation of *Salmonella enteritidis*. *International Journal of Food Microbiology*, 87(1-2): 75-86.
- Heres, L., Engel, B., Urlings, H.A.P., Wagenaar, J.A. and van Knapen, F. (2004) Effect of acidified feed on susceptibility of broiler chickens to intestinal infection by *Campylobacter* and *Salmonella*. *Veterinary Microbiology*, 99 (3-4): 259-267.
- Hetland, H. and Svihus, B. (2001) Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British Poultry Science*, 42: 354-361.
- Hetland, H., Svihus, B. and Olaisen, V. (2002) Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *British Poultry Science*, 43: 416-423.

- Hetland, H., Svhuis, B. and Krogdahl, A. (2003) Effect of hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *British Poultry Science*, 44: 275-282.
- Hughes, R.J. (2001) Variation in the digestive capacity of the broiler chicken. *Recent Advances in Animal Nutrition in Australia*, 13: 153-161.
- Iba, A.M. and Berchieri, Jr.A. (1995) Studies on the use of formic acid - propionic acid mixture (Bio-AddTM) to control experimental *Salmonella* infection in broiler chickens. *Avian pathology*, 24:303-311.
- Iji, P.A., Hughes, R.J., Choct, M. and Tivey, D.R. (2001) Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. *Asian-Australasian Journal of Animal Sciences*, 14: 54-60.
- Jamroz, D., Skorupinska, J., Wilickzkiewicz, A. and Schleicher, A. (1992) Effect of feed composition and antibiotic growth promoters on blood chemistry, blood cells and histology of the small intestinal wall of broilers. *Viewer Tierarztliche Monatsschrift*, 1: 13-19.
- Katanbaf, M.N., Dunnington, E.A. and Siegel, P.B. (1988) Allomorphic relationships from hatching to 56 days in parental lines and F1 crosses of chickens selected 27 generations for high or low body weight. *Growth, development and aging*, 52: 11- 21.
- Klasing, K.C. (1998) Anatomy and Physiology of the Digestive system. *Comparative Avian Nutrition*, CAB International: 9-35.
- Knight, C.D. and Dibner, J.J. (1998) Nutritional programming in hatchling poultry: Why a good start is important. *Poultry Digestion*, Aug/Sept: 20-26.
- Kwakkel, R.P. (1998) 25th Carolina Poultry Nutrition Conference, December 15 & 16, 1998, Raleigh NC, USA.
- Kwakkel, R.P., Williams, B.A. and van der Poel, A.F.B. (1997) Effect of fine- and coarse particle diets on gizzard growth and fermentation characteristics of the caecal contents in broiler chickens. *Proceeding of the 11th European Symposium on Poultry Nutrition (WPSA), Faaborg, Denmark*, August 1997: 249-251.

- Langhout, D.J., Schutte, J.B., Van Leeuwen, P., Wiebenga, J. and Tamminga, S. (1999) Effect of dietary high- and low- methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. *British Poultry Science*, 40: 340-347.
- Marchaim, U. and Kulka, R.G. (1967) The none-parallel increase of amylase, chymotrypsinogen and procarbonypeptidase in the developing chick pancreas. *Biochimica et Biophysica Acta*, 146: 553-562.
- Murakami, H., Akiba, Y. and Horiguchi, M. (1992) Growth and utilization of nutrients in newly-hatched chicks with or without removal of residual yolk. *Growth, Development and Aging*, 56: 75–84.
- Nir, I., Hillel, R., Shefet, G., and Nitsan, Z. (1994) Effect of grain particle size on performance. 2. Grain texture interactions. *Poultry Science*, 74: 781-791.
- Nir, I., Hillel, R., Ptichi, I. and Shefet, G. (1995) Effect of particle size on performance. 3. Grinding and pelleting interactions. *Poultry Science*, 74: 711-783.
- Nir, I., Hillel, R., Ptichi, I. and Shefet, G. (1995) Effect of particle size on performance. 3. Grinding pelleting interactions. *Poultry Science*, 74: 771-783.
- Noy, Y. and Sklan, D. (1998) Yolk utilization in the newly hatched poult. *British Poultry Science*, 39: 446-451.
- Noy, Y. and Sklan, D. (1999) Energy utilization in newly hatched chicks. *Poultry Science*, 78: 1750–1756.
- Pettersson, D. and Aman, P. (1989) Enzyme supplementation of poultry diet containing rye and wheat. *British Journal of Nutrition*, 62: 139-149.
- Pezeshkian, S. (2002) Utilization of standard – prestarter feed formulation flour corn, calcium propionate in primary nutrition of chicken. *Jahan Poultry*, 9: 13-15.
- Pinchasov, Y. and Noy, Y. (1994) Early postnatal amylolysis in the gastrointestinal tract of turkey poult (Meleagris gallopavo) *Comparison Biochemistry and Physiology*, 106: 221-225.

- Pritchard, P.G. (1972) Digestion of sugar in the crop. *Comparative Biochemistry and Physiology*, 43: 195-201.
- Rogel, A.M., Balnave, D. Bryden, W.L. and Annison, E.F. (1987) Improvement of raw potato starch digestion in chickens by feeding oat hulls and other fibrous feedstuffs. *Australian Journal of Agricultural Research*, 38: 629-637.
- Rose, S. P. and Harper, A. (1996) Principles of Poultry Science. *Agricultural College, Shropshire, UK*. (Chapter 8: Nutrition and feeding: 99 - 115).
- Rose, S.P., Fielden, M., Foote, W.R., and Gardin, P. (1995) Sequential feeding of whole wheat to growing broiler chickens. *British Poultry Science*, 36: 97-111.
- Rubio, L.A., Brenes, A. and Castano, M. (1990) The utilization of raw and autoclaved faba beans (*Vicia faba L. minor*) and faba bean fractions in diets for growing broiler chickens. *British Journal of Nutrition*, 63: 419-430.
- Saki A.A. (2005) Effect of post hatched feeding on broiler performance. *International Journal of Poultry Science*, 4: 4-6.
- Scholten, R.H.J., Van der Peet-Schwering, C.M.C., Den Hartog, L.A., Vesseur, P.C. and Verstegen, M.W.A. (1999) Effect of liquid by-products on performance and health of pigs. *Pig News and Information*, 20: 81N-88N.
- Sell, J.L., Angel, C.R., Piquer, F.J., Mallarino, E.G. and Al-Batshan, H.A. (1991) Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. *Poultry Science*, 70: 1200-1205.
- Sharma, R., Fernandez, F., Hinton, M. and Schumacher, M. (1997) The influence of diet on the mucin carbohydrate in chick intestinal tract. *Cellular and Molecular Life Sciences*, 53: 935-942.
- Sklan, D. (2001) Development of the digestive tract of poultry. *World's poultry Science journal*, 57: 415-427.
- Smith, C.J.V., and Pliz, D.R. (1971) Feeding behavior of chickens; effect of cropectomy. *Poultry Science*, 50: 226-232.

- Thompson, J.L. and M. Hinton (1997) Antibacterial activity of formic acid and propionic acids in the diet of hens on salmonellas in the crop. *British Poultry Science*, 38: 59-65.
- Turro-Vincent, I., Nitsan, Z., Picard, M., Dunnington, E. A. and Siegel, P. B. (1994) Removal of residual yolk at hatch influences food choice and feeding activity in lines of chickens selected for high or low juvenile body weight. *Reproduction Nutrition and Development*, 34: 449-460.
- Uni, Z., Ganot, S. and Sklan, D. (1998) Post-hatch development of mucosal function in the broiler small intestine. *Poultry Science*, 77: 75–82.
- Uni, Z., Noy, Y. and Sklan, D. (1995) Post hatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. *Poultry Science*, 74: 1622-1629.
- Uni, Z., Noy, Y. and Sklan, D. (1996). Development parameters of the small intestines in heavy and light strain chicks pre- and post-hatch. *British Poultry Science*, 36: 63-71.
- Uni, Z., Ganot, S., and Sklan, D. (1998) Post hatch development of mucosa function in the broiler small intestine. *Poultry Science*, 77:75–82
- Uni, Z., Noy, Y. and Sklan, D. (1999) Post hatch development of small intestinal function in the poult. *Poultry Science*, 78: 215-222.
- Uni, Z., Zaiger, G., Gal-Garber, O., Pines, M., Rozenboim, I. and Reifen, R. (2000) Vitamin A deficiency interferes with proliferation and maturation of cells in chicken small intestine. *British Poultry Science*, 41: 410-415.
- Uni, Z., Tako, E., Gal-Garber, O. and Sklan, D. (2003) Morphological, molecular and functional changes in the chicken small intestine in the late term of embryo. *Poultry Science*, 82:1747-1754.
- Van Brakel, C.E.P., Scholten, R.H.J. & Backus, G.B.C. (1996) Economische evaluatie van het voeren van natte bijproducten aan vleesvarkens. [Economic evaluation of

feeding wet byproducts to fattening pigs]. Report P1.147, Research Institute for Pig Husbandry, Rosmalen, the Netherlands.

- Vieira, S.L. and Moran, E.T. (1999a) Effects of egg of origin and chick post-hatch nutrition on broiler live performance and meat yields. *World's Poultry Science Journal*, 55: 125-142.
- Viera, S.L., and Moran, E.T. (1999b) Effects of delayed placement and used litter on broiler yields. *Journal of Applied Poultry Research*, 8: 75-81.
- Williams, B.A., van Osch, L.J.M. and Kwakkel, R.P. (1997) Fermentation characteristics of the caecal contents of broiler chickens fed fine and coarse particle diets. *British Poultry Science*, 38: 41-S42.
- Yalda, A.Y. and Forbes, J.M. (1995) Food intake and growth in chickens given food in the wet form with and without access to drinking water. *British Poultry Science*, 36: 357-369.
- Yalda, A.Y. & Forbes, J.M. (1996) Effects of food intake, soaking time, enzyme and cornflour addition on the digestibility of the diet and performance of broilers given wet food. *British Poultry Science*, 37: 797-807.
- Yasar, S. and Forbes, J.M. (1997) Effects of wetting and enzyme supplementation of wheat-based foods on performance and gut responses of broiler chickens. *British Poultry Science*, 38 (suppl): S43-S44.
- Yasar, S. and Forbes, J.M. (1999) Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. *British Poultry Science*, 40: 65-76.

SUMMARY

SUMMARY

Modern poultry has undergone great changes in growth characteristics during the past few decades (Havenstein *et al.*, 1994). For example, average 42-day market weight of broiler chicks increased from about 1.7 kg in 1980 to almost 2.5 kg in 2004, while feed conversion improved markedly. These genetic changes in growth potential were motivated by the increased demand in developed countries for poultry meat at the expense of pork and beef.

This improvement in growth potential of commercial broilers has not come without associated (health) problems in the gastro-intestinal (GI) tract (malabsorption syndrome, necrotic enteritis) and with postabsorptive metabolism (ascitis). Moreover, as a consequence of a decreased duration in growing period (going down from 42 to 35 days of age), broiler producers get hardly enough time to manage and adjust feeding practices during the flock's grower period. In recent years, researchers have tried to counterbalance these problems by adjusting feed characteristics and feeding management.

The main aim of the studies described in this thesis was to evaluate the impact of changes in feed properties like technological treatments on broiler performances. The technological changes in feed were made to induce differences in structure (particle sizes) and conformation (appearance: solid or liquid diets) of the feed and how this feed during the starter phase (0 – 3 weeks of age) influences the development and functioning of the foregut (crop and ventriculi). We also studied possible carry-over effects of treatment during the starter period on performance in the subsequent grower period.

This study mainly focused on the impact of a number of selected feed characteristics (solid vs. liquid diets; finely vs. coarsely ground diets) in the diet of broiler chickens on gut characteristics and performance. The aim of the study was to develop a feeding regime which optimizes foregut development and function of the foregut segment during the starter phase and to see if there is a carry over effect on performance of broilers during the grower phase. Our investigations were meant to study the following hypothesis: Particle size distribution of feed, acidification of feed and wet feeding in the starter phase enhance functional development and health of the gastrointestinal tract and subsequently affect broiler performance during the grower phase.

In the first study, the effects of a solid diet (C) versus a liquid/acidified diet (E) on the development of the GI tract (specially the foregut segment) and on broiler performance were described and evaluated (Chapter 2).

In this experiment, all replicate pens of the three different treatment groups had access to two feed troughs. The control group had diet C in both troughs (group CC), whereas the two other groups had either only diet E (group EE) in both troughs or both diets C and E in the separate troughs (group EC), in a choice feeding design. Feed intake and performance were recorded. In addition, gait score and post-mortem analysis were performed that gives information about leg condition and health of the birds. Moreover, diet selections of the choice-fed broiler chickens were examined. Results showed an increased body weight (BW) and total water intake in the EE and EC treatments compared to the CC treatment. Feed conversion ratio (FCR: g feed/g growth) was decreased for the birds from the EC group. Abdominal fat and carcass weights were higher in the EE and EC treatments relative to the CC treatment. Various GI tract segments were weighed and related to total BW. Carcass weight relative to BW was higher for the CC treatment. Gait score was better for birds of the EC treatment. The incidence of pericardial effusion was higher in birds of the EE and EC treatments. The birds of the EC treatment consumed in the first week more of diet C and in the last three weeks more of diet E. This study showed that the inclusion of a liquid (starch-rich) wheat by-product resulted in better performances of male broiler chickens than if a standard solid diet had been fed. Conclusively, liquid or wet feeding improved feed intake and broiler performance, but it did not affect the development of the foregut segments. Because the wet feeding was accompanied with the acidified starch, it may be that the acid helped digestion and thus there was no need for the gut system to work harder (increase in size) with more feed ingested.

Liquid acidified feeding showed to have a favorable effect on feed intake and BW gain. Thus, there may be reason for the development of a new feeding strategy for broilers based on these effects on gut functioning. The majority of the work done so far is limited to a specific time period in broiler's life. It was thought that there is still a lot of information missing with regard to different types of diets and their effects on development in term of physiological function of the foregut segments.

In the second study, we split the effect of liquid (wet) feeding and acidification. The effects of diet structure (coarse vs. fine), conformation (wet vs. dry) and acidification (acidified vs. non-acidified) fed during the starter phase (0-21 days) and their carry over effect on dry and wet fed growers (22-42 days) were studied and described in Chapter 3. The diet defined as

'coarse', was processed by 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 a hammer mill with an opening screen of 3.0 mm. 'Wet' diets were the result of adding water to a solid feed in a 1:1 ratio. The results showed that during the starter phase wet feeding increased feed intake by about 48 % and BW gain by 85 %, as compared to dry feeding. During the grower phase, these traits increased by 39 and 86 %, respectively as a result of wet feeding.

There was a carry over effect of diet conformation during the starter phase on overall performance and GI tract development of broilers during the grower phase, while that of diet structure was limited to FI and BW gain, with highest BW gain in wet+coarse diet fed birds. There was for some traits a significant interaction between diet conformation and diet structure during the starter phase, in a way that in dry diets coarseness improved feed intake and BW gain, whereas in wet diets coarseness decreased feed intake and BW gain. As expected, water intake from the nipples was reduced in wet fed birds during the whole study period; however, total water intake (from wet feed plus nipples) was approximately 67 % higher in wet fed birds than in dry fed birds (especially during the starter phase). Water intake during the grower phase was similar among all treatments. Diet structure and acidification did not show any effect on water intake during either stage of this experiment. The major differences in feed intake and consequently BW gain between wet and dry fed birds may be partly due to the restricted light period (18L:6D).

Relative weights of the GI tract organs were significantly higher in dry fed birds by approximately 21% during the starter phase and by 11 % during the grower phase compared to those of the wet fed birds. Heavier GI tract organs (approximately 10%) were found in birds fed coarse diets during the starter phase, but it had no significant carry over effect during the grower phase.

It can be concluded that wet feeding helps processing of feed in the GI tract of the birds and coarse diets will let organs work harder thereby enlarging the proventriculus and gizzard.

In the follow up experiment (described in the Chapters 4 and 5), no treatments with an acidification of the diet were included anymore, due to limited responses in the previous experiments (separate from wet feeding). The starter phase lasted for 17 days and the grower

phase ended at day 35. The focus was now to test both diet structure (coarse or fine) and conformation (wet or dry) in both phases of growth (starter and grower).

The results showed that feeding a wet diet in a non-restricted light scheme (23L:1D) also significantly improved feed intake, daily weight gain, but it had no effect on feed conversion ratio. A coarse diet structure clearly increased relative weight of the gizzard (g per 100 g BW) during the starter phase. There was a carry over effect of feeding a wet coarse diet during the starter phase on daily weight gain during the grower phase. This means that the birds still benefited from a well developed gizzard after the wet coarse feeding had stopped. The wet diet during the starter phase significantly affected feed intake and daily BW gain. Best performances during the entire production cycle of broiler chickens were found when a wet+coarse diet was fed in both the starter as well as the grower phase.

Functional development of the GI tract was described in Chapter 5. In this Chapter, the investigation on the effects of diet conformation (wet or dry diets during early ages) and diet structure (impact of coarse diets thereafter) on feed resident time, protease activity of the proventriculus and pH value of the GI tract contents was carried out to evaluate the functional development of the GI tract. The results showed that a coarse diet structure significantly improved protease activity and lowered the pH value of the content of the ventriculi during the starter phase; no effect, however, on protease activity during the grower phase could be observed. The feeding of a wet diet (in contrast to a dry diet) had no effect on protease activity in both starter and grower phases. There was also no carry over effect of feeding a coarse diet during the starter phase on protease activity during the grower phase. There was only a carry over effect of a wet+coarse diet during the starter phase on the protein content of the proventriculus during the grower phase. Apparently this is beneficial for growth performance.

The General Discussion (Chapter 6) focused on four main issues: a) Factors influencing GI tract development in the starter phase of broiler chickens; b) The roles of the foregut segment in the digestion process; c) Effects of feeding strategies on development of the GI tract during the starter phase; d) Comparative analysis of results obtained from the development of the GI tract and performance of broilers (Results presented in Chapters 2 to 5)

Conclusions from these studies were as follows

- Feeding coarse diets during the starter phase improves the functional development of the proventriculus-gizzard system.

- Wet feeding improves feed intake and, as a consequence, broiler performance.
- Feeding a wet *and* coarsely ground diet provides a large improvement in feed intake, feed conversion and body weight gain, showing the most pronounced effects during the starter phase of broiler's life.

SAMENVATTING

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Moderne vleeskuikens hebben een veel grotere groeipotentie dan de rassen of merken die enkele tientallen jaren geleden werden gebruikt (Havenstein *ea*, 1994). Om een voorbeeld te geven: een gemiddeld slachtgewicht van een kuiken op 42 dagen leeftijd was in 1980 zo'n 1.7 kg, terwijl dat gewicht tegenwoordig al rond de 2.5 kg ligt (een stijging van zo'n 50%), voornamelijk als gevolg van een sterk verbeterde voederconversie. Deze verbeteringen in groeipotentie werden ingegeven door de sterk toegenomen vraag naar kuikenvlees, met name in de westerse wereld.

Deze verbetering in groeipotentie in commercieel gehouden vleeskuikens bleek echter ook nadelige effecten met zich mee te brengen: de gezondheid van het kuiken en met name het maagdarmkanaal ging achteruit; kuikens vertoonden frequenter het malabsorptie syndroom, necrotische enteritis en/of ascites. Bovendien, als gevolg van een verkort mesttraject (van 42 dagen terug naar 35 dagen), hebben kuikenmesters tegenwoordig nauwelijks meer tijd om de koppel bij te sturen indien er iets mis dreigt te gaan. De laatste jaren probeert men via een verbeterd voermanagement dit soort problemen te ondervangen.

De in dit proefschrift beschreven experimenten hadden als belangrijk doel: het beschrijven en analyseren van de effecten van het variëren van voerkarakteristieken als structuur ('grofheid' van het voer) en verschijningsvorm (als droge korrel of als brijvoer) op productiekenmerken (groei, voeropname en voederconversie) en op de ontwikkeling van de (voor)magen (krop, klier- en spiermaag). Tegelijkertijd werd er bestudeerd of er carry-over effecten waren van behandelingen tijdens de startfase op productiekenmerken tijdens de groefase. Uiteindelijk doel van de studie is om te komen tot een voerstrategie/voermethode die een optimale ontwikkeling van het magensysteem (als belangrijkste component in het verteringsproces) bevordert, alsook een goede prestatie van het kuiken laat zien.

De hypothese luidde dan ook aldus: Een grove structuur van het startvoer, verstrekt in brijvorm, verbetert de ontwikkeling van het maagdarmkanaal en daarmee de uiteindelijke prestaties van het kuiken gedurende de gehele mestperiode.

In het eerste experiment werden de effecten bestudeerd van twee type voeders op de ontwikkeling van het (voor)maagsysteem en op productiekenmerken (Hoofdstuk 2). Een normale droge kruimel (voer C) werd vergeleken met een kruimel waaraan een vloeibaar aangezuurd tarwe bijproduct was toegevoegd (voer E). De kuikens in elk grondhok hadden de

beschikking over 2 voerbakken. De controle groep kreeg voer C in beide voerbakken verstrekt (proefgroep CC); beide andere proefgroepen kregen ofwel het experimentele voer in beide voerbakken verstrekt (proefgroep EE) ofwel in de ene voerbak voer C en in de andere voerbak voer E (proefgroep EC). Laatsgenoemde proefgroep had dus de keuze uit twee voeders. Voeropname en groei, alsook gait score (een maat voor de mogelijkheid om zich als kuiken voort te bewegen (= pootconditie)) werden bestudeerd. Daarnaast werd gekeken of de EC-kuikens een specifieke voorkeur hadden voor een bepaald voer in afhankelijkheid van de leeftijd.

EE- en EC-dieren vertoonden een hogere groei, meer buikvet, een hogere incidentie van pericardiale effusie en een hogere totale wateropname dan de CC-dieren. De voederconversie was duidelijk slechter in de EC-groep. Ofschoon de CC-dieren een lager absoluut karkasgewicht lieten zien, was het karkasgewicht in relatieve zin (tov lichaamsgewicht) juist wel weer zwaarder dan bij de andere twee groepen dieren. De gait score was duidelijk beter in de EC-groep. De groep die de mogelijkheid tot keuzevoedering had (groep EC) bleek in de eerste weken vooral een voorkeur te hebben voor voer C en in de laatste 3 weken voor voer E. Het uitdrogen van het E-voer kan hierbij een rol hebben gespeeld.

Uit de resultaten van dit eerste experiment blijkt dat het voeren van een vloeibaar (zetmeelrijk) aangezuurd tarwe bijproduct (gemengd met een korrel) de groeiprestatie van het vleeskuiken verbetert ten opzichte van een normale kruimel. Echter, de ontwikkeling van het voormagensysteem werd niet positief beïnvloed door het E-voer, wellicht als gevolg van het feit dat de aanzuring reeds voldoende ‘hielp’ bij de afbraak van voedingstoffen en een vergroting van het maagsysteem dus niet nodig was.

In het tweede experiment (Hoofdstuk 3) werden de verstrengelde effecten van ‘aanzuring’ en verschijningsvorm (brijvoer versus droog voer) uit het eerste experiment afzonderlijk bestudeerd. In dit experiment werden de effecten van structuur (groot versus fijn), verschijningsvorm (brij versus droog), en het wel of niet aangezuurd zijn van het voer getoetst in een $2 \times 2 \times 2$ factorieel experiment, waarbij de proeffactoren werden opgelegd tijdens de startfase van de groei (0 – 21 dagen). Observaties werden verricht tijdens zowel de start- als groefase (22 – 42 dagen). Tijdens de groefase werd elke groep weer opgesplitst in een droog voer en brijvoer-groep (met fijne structuur en niet aangezuurd). Het ‘grote’ voer werd verkregen door tarwe en mais (als belangrijkste grondstoffen) met een walsenstoel te malen, met gebruikmaking van respectievelijk één en twee rolpalen voor beide grondstoffen. Het ‘fijne’ voer werd gemalen met behulp van een hamermolen. Het ‘brijvoer’ was het resultaat

van het toevoegen van water aan een droog meelvoer in de verhouding 1:1. Het aangezuurde voer werd verkregen door 1 deel zuurmengsel (melkzuur, fosforzuur en azijnzuur) te mengen met 99 delen voer.

Brijvoedering verhoogde de voeropname met respectievelijk 48 en 39 % tijdens de start-en groefase van de kuikens ten opzichte van droogvoerverstrekking. Dientengevolge waren de brijvoer-dieren aan het eind van beide fasen zo'n 85% zwaarder dan de droogvoer dieren. Het effect van brijvoedering, en in iets mindere mate grof voer, tijdens de startfase bleek voor veel kenmerken nog steeds een na-ijlend effect te vertonen in de groefase. Dieren die in de startfase droogvoer verstrekten hadden gekregen, lieten in de groefase op brijvoer zeer lage voederconversies zien. Enkele interacties bleken interessant tijdens de startfase: bij droogvoer dieren werd de voeropname en de groei verhoogd, indien er grof gemalen werd, en in de brijvoer dieren werd de voeropname en de groei juist verlaagd indien er grof gemalen werd. Voerderconversies tijdens de startfase waren duidelijk lager voor de brijvoerkuikens. Totale wateropname tijdens de startfase was duidelijk hoger voor de brijvoerkuikens; terwijl er in de groefase weinig verschillen te zien waren. Structuur van het voer en aanzuring hadden in beide fasen geen effect op de totale wateropname. De grote verschillen in voeropname en groei tussen dieren op brijvoer en op droogvoer kan voor een deel verklaard worden uit het gemak waarmee de jonge kuikens brijvoer opnemen in het beperkte daglichtschema (18L:6D). De droogvoerkuikens bleven dan ook aanmerkelijk achter in vergelijking tot praktijkkoppels van hetzelfde merk.

Het maagdarmpakket (uitgedrukt in g per 100 g lichaamsgewicht) van droogvoer kuikens bleek respectievelijk 21 en 11 % zwaarder op 21 en 42 dagen leeftijd in vergelijking tot brijvoer kuikens. Ook grofheid van voer tijdens de startfase gaf een zwaarder maagdarmkanaal; tijdens de groefase was dit effect afwezig.

In het laatste experiment (Hoofdstukken 4 en 5) werden geen proefvoeders meer aangezuurd, vanwege het geringe effect dat deze voerders hadden laten zien in het voorgaande experiment. In dit experiment werd voerstructuur (grof versus fijn) en verschijningsvorm (brij versus droog) getest in zowel de start- (0 - 17 dagen) als de groefase (18 - 35 dagen). Ook nu bleek weer dat brijvoedering in de startfase de voeropname verhoogt en daarmee de groei verbetert, zelfs bij een praktisch volledige daglengte (23L:1D). Voederconversie werd evenwel niet verbeterd. Een betere structuur (grof) van het startvoer deed het gewicht van de spiermaag (in g per 100 g lichaamsgewicht) toenemen. Het voeren van een grof brijvoer tijdens de startfase had positieve naderingen op de groei tijdens de groefase (vanaf 18 dagen): kuikens op een

droog en fijn voer tijdens de groefase vernoonden de beste prestaties in termen van groei en voederconversie, indien deze dieren in de startfase een grof brijvoer verstrekt hadden gekregen, hetgeen kan betekenen dat de dieren nog steeds een voordeel ondervinden van een goed ontwikkelde spiermaag. De uiteindelijk beste prestaties aan het eind van de mestronde werden verkregen door kuikens zowel tijdens de start- alsook tijdens de groefase een grof brijvoer te voeren.

Functionele ontwikkeling van het maagdarmkanaal is beschreven in Hoofdstuk 5 van dit proefschrift. In dit hoofdstuk werden de effecten van verschijningsvorm en structuur van het voer onderzocht op kenmerken als retentietijd van het voer, protease activiteit van de spiermaag, en pH van de chymus op diverse plaatsen in het maagdarmkanaal. De resultaten laten zien dat een grovere voerstructuur in de startfase de protease activiteit verhoogt en de pH van de chymus in de ventriculi verlaagt. De protease activiteit werd niet beïnvloed door grover voer tijdens de groefase; evenmin had het voeren van het brijvoer tot het droogvoer een effect op de protease activiteit (in beide fasen). Er kon slechts een carry over effect worden aangetoond van het voeren van grof brijvoer tijdens de startfase op de eiwithoeveelheid in de kliermaag aan het eind van de groefase. Welke fysiologische effecten dit met zich meebrengt, dient nog nader onderzocht te worden.

De Afsluitende Discussie (Hoofdstuk 6) behandelt vier aspecten: a) Factoren die de ontwikkeling van het maagdarmkanaal beïnvloeden tijden de vroege jeugd van kuikens; b) De rol van het magensysteem in het verteringsproces; c) Effecten van voerstrategieën op de ontwikkeling van het maagdarmkanaal tijdens de startfase; d) Een vergelijkende analyse van de resultaten zoals beschreven in de verschillende hoofdstukken in dit proefschrift.

Samengevat hebben de resultaten, zoals beschreven in dit proefschrift, het volgende aangetoond:

- Het voeren van grof gemalen voeders tijdens de startfase van de mestperiode verbetert de functionele ontwikkeling van het magensysteem (proventriculus – gizzard).
- Brijvoeding verhoogt duidelijk de voeropname, en als een resultante daarvan, verbetert het ook de groei van vleeskuikens.
- Het voeren van grove voerdeeltjes in brijvoervorm resulteert in een sterke verbetering van de voeropname, de voerefficiëntie en de groei van kuikens, met name indien deze voerstrategie wordt toegepast tijden de eerste 3 levensweken van het kuiken.

TÓM TẮT

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Trong vài thập kỷ gần đây ngành chăn nuôi gia cầm đã gặt hái được những thành tựu to lớn trong năng suất chăn nuôi (Havenstein *et al.*, 1994). Ví dụ: vào thập kỷ 80, khối lượng trung bình của gà thịt thương phẩm đạt 1,7 kg ở 42 ngày tuổi. Trong khi đó, đến năm 2004, cũng vào thời điểm 42 ngày tuổi, gà thương phẩm đạt đến mức 2,5 kg và hiệu suất sử dụng thức ăn được cải tiến đáng kể. Những những nghiên cứu với mục đích nâng cao các tính trạng về khả năng tăng trưởng ở gà thịt được kích thích bởi sự gia tăng nhu cầu về thịt gia cầm ở các nước phát triển, song song với nhu cầu về thịt của các loài gia súc khác như thịt bò và thịt lợn.

Sự cải tiến về khả năng tăng trưởng của gà công nghiệp luôn đi kèm với nhiều vấn đề nảy sinh khác liên quan đến hệ tiêu hóa ở gia cầm (Hội chứng giảm tiêu hóa, hấp thu do hoại tử đường ruột) và các rối loạn trao đổi chất khác (Ascitis). Hơn nữa, kết quả của việc rút ngắn thời gian nuôi thịt (từ 42 ngày xuống còn 35 ngày), người chăn nuôi không có đủ thời gian để điều chỉnh thức ăn cho gia cầm trong giai đoạn nuôi tăng trưởng. Những năm gần đây, nhiều nhà nghiên cứu đã tìm cách cân đối những vấn đề này bằng việc thay đổi đặc tính của thức ăn và phương pháp quản lý cho ăn.

Mục đích chính của đề tài nghiên cứu trong cuốn luận văn này là đánh giá sự ảnh hưởng của những thay đổi về các đặc tính của thức ăn cho gà thịt, ví dụ như phương pháp chế biến làm thay đổi về đặc tính cấu trúc của thức ăn (hạt thô hoặc hạt mịn), tính chất (ướt hoặc khô) dùng cho gà thịt trong giai đoạn khởi động (từ 0 – 3 tuần) ảnh hưởng đến sự phát triển và vai trò của hệ bộ máy hóa trước (gồm có diều và dạ dày tuyến, dạ dày cơ). Chúng tôi cũng nghiên cứu khả năng ảnh hưởng của thức ăn trong giai đoạn khởi động ảnh hưởng đến khả năng tăng trưởng của gà trong giai đoạn tăng trưởng.

Nghiên cứu này chủ yếu tập trung đến đánh giá sự ảnh hưởng của một số đặc tính chọn lọc của thức ăn cho gà thịt (TA khô, TA ướt; TA nghiên mịn, TA nghiên thô) đến đặc tính của hệ tiêu hóa và khả năng tăng trưởng của gà. Mục đích của đề tài là để tìm và phát triển một dạng thức ăn làm tối ưu hóa sự phát triển và vai trò của bộ máy tiêu hóa trước ở gà thịt trong giai đoạn khởi động để thấy được liệu có sự kéo dài ảnh hưởng của thức ăn trong giai đoạn khởi động đến khả năng sinh trưởng phát triển trong giai đoạn tăng trưởng của gà thịt. Các nghiên cứu của chúng tôi với mục đích để kiểm định giả thuyết sau: Độ thô của Thức ăn, độ lên men hóa và thức ăn dạng ướt cho gà thịt trong giai đoạn khởi động làm tăng khả năng phát triển,

nâng cao vai trò của hệ tiêu hóa và sau đó làm tăng khả năng tăng trưởng của gà thịt trong giai đoạn tăng trưởng.

Thí nghiệm đầu tiên chúng tôi nghiên cứu về sự ảnh hưởng của thức ăn khô và thức ăn ướt được men hóa đến sự phát triển của bộ máy tiêu hóa (đặc biệt là bộ máy tiêu hóa trước) và khả năng tăng trưởng của gà thịt. Thực nghiệm này đã được đánh giá và mô tả ở chương 2. Trong thí nghiệm được mô tả ở Chương 2, chúng tôi sử dụng sản phẩm phụ của lúa mì đã được acid hóa bằng cách cho lên men nhằm nghiên cứu ảnh hưởng của thức ăn ướt lên men đến khả năng tiêu thụ thức ăn và khả năng tăng trưởng của gà. Ở thí nghiệm này chúng tôi chia gà thí nghiệm làm 3 nhóm. Nhóm thứ nhất được bố trí thức ăn khô và là nhóm đối chứng (CC). Nhóm thứ hai được bố trí thức ăn ướt (trộn với sản phẩm phụ lúa mì lên men) làm nhóm thí nghiệm 1 (EE). Nhóm thứ 3 được bố trí cả hai loại thức ăn như ở nhóm thí nghiệm 1 và nhóm đối chứng (EC) làm nhóm thí nghiệm 3. Ngoài các chỉ tiêu về khả năng tiêu thụ thức ăn và tăng trưởng, chúng tôi cũng đánh giá các chỉ tiêu về sự đi lại, dáng đi để đánh giá sức khỏe của gà.Thêm nữa, sự lựa chọn thức ăn của gà ở nhóm thí nghiệm 3 cũng được đánh giá kiểm tra. Kết quả của thí nghiệm cho thấy, nhóm thí nghiệm 1 (EE) và 2 (EC) cho khả năng tăng trưởng và mức độ tiêu thụ nước tăng rõ rệt so với nhóm đối chứng (CC). Sự tiêu tốn thức ăn (g thức ăn/ g tăng trưởng) giảm rõ rệt ở nhóm thí nghiệm 2 (EC). Tỷ lệ mỡ bụng và khối lượng giết mổ ở nhóm TN 1 và 2 cao hơn ở nhóm đối chứng CC. Hiện tượng tích dịch màng tim cao hơn ở nhóm TN1 và 2 so với nhóm đối chứng. Trong tuần thứ nhất của TN, gà ở nhóm EC tiêu thụ nhiều thức ăn đối chứng (C) hơn so với thức ăn thực nghiệm (E). Trong hai tuần tiếp theo gà tiêu thụ thức ăn E hơn so với thức ăn C. Thí nghiệm này cho thấy sản phẩm phụ lúa mì lên men dạng ướt đã làm tăng khả năng tăng trưởng của gà thịt. Nhìn chung, thức ăn dạng ướt làm tăng khả năng tiêu thụ thức ăn, tăng khả năng tăng trưởng ở gà thịt nhưng nó không có ảnh hưởng nhiều đến sự phát triển của bộ máy tiêu hóa trước. Bởi vì thức ăn dạng ướt đã được lên men trong đó có các thành phần men hữu cơ đã giúp cho quá trình tiêu hóa dẫn đến hệ tiêu hóa không phải hoạt động nhiều để giúp cho quá trình tiêu hóa.

Thức ăn dạng ướt men hóa đã có những ảnh hưởng tích cực đến sự tăng trưởng và phát triển ở gà thịt, do vậy nó có thể là lý do để phát triển một phương pháp nuôi dưỡng mới cho gà thịt dựa trên những ảnh hưởng của nó đến những tính trạng này. Phần lớn các nghiên cứu trước đó đều có sự hạn chế nhất định liên quan đến các giai đoạn quan trọng của quá trình phát triển trong vòng đời của gà thịt. Hiện tại còn thiếu rất nhiều thông tin quan trọng khác liên quan đến sự ảnh hưởng của các loại thức ăn khác nhau ảnh hưởng đến sự phát triển và vai trò của bộ máy tiêu hóa trước. Do vậy ở thí nghiệm thứ 2 chúng tôi đã phân chia thức ăn dạng ướt

thành hai loại. Loại thức ăn ướt acid hóa và loại thường. Chúng tôi đã tiến hành nghiên cứu sự ảnh hưởng của cấu trúc thức ăn (Nghiền thô so với nghiền mịn), dạng thức ăn (ướt so với khô) và acid hóa (acid hóa so với không acid hóa) trong giai đoạn khởi động (0-21 ngày tuổi) và sự kéo dài ảnh hưởng của nó đến sự tăng trưởng của gà trong giai đoạn tăng trưởng (22-42 ngày tuổi). Kết quả của nghiên cứu này được trình bày trong Chương 3. Trong Chương 3, thức ăn thí nghiệm loại thô được chế biến bằng máy nghiền trực lăn với một cặp trực lăn đối với lúa mỳ. Khoảng cách con lăn khi nghiền là 1,6 mm (vận tốc con lăn 1 là 480 vòng/phút, con lăn 2 là 1022 vòng/phút) . Đối với ngô, chúng tôi dung máy nghiền 2 cặp trực lăn (với vận tốc cặp 1 là: trực lăn 1: 480 vòng/phút, trực lăn 2: 1214 vòng/phút; cặp con lăn thứ 2 là: trực lăn 1: 480 vòng/phút, trực lăn 2: 1244 vòng/phút). Các nguyên liệu chế biến khác như "khô đậu tương" và "khô dầu đậu tương 44/7" được trộn vào trực tiếp không thông qua máy nghiền búa. Thức ăn loại nghiền mịn được chế biến bằng máy nghiền búa với kích cỡ sàng là 3.0 mm (Áp dụng phương pháp của Hamilton và Proutfoot, 1995). Kết quả thí nghiệm cho thấy trong giai đoạn khởi động, thức ăn dạng ướt làm tăng 48 % khả năng tiêu thụ thức ăn và tăng trưởng của gà tăng 85% so với gà được cho ăn thức ăn dạng khô. Trong giai đoạn tăng trưởng những tính trạng này tiếp tục tăng lần lượt là 39 và 86 % trên gà được cho ăn thức ăn dạng ướt. Thức ăn dạng nghiền thô trong giai đoạn khởi động có sự kéo dài ảnh hưởng đến giai đoạn tăng trưởng đối với các tính trạng về khả năng tăng trưởng của gà. Sự tương tác giữa thức ăn nghiền thô và thức ăn dạng ướt được quan sát thấy trên các tính trạng về khả năng tiêu thụ thức ăn và khả năng tăng trưởng trong giai đoạn khởi động của gà. Khả năng tiêu thụ nước uống của gà được cho ăn thức ăn dạng ướt giảm trong thời gian thí nghiệm (từ 0 – 42 ngày tuổi). Nhưng khi tính toán tổng lượng nước tiêu thụ (bao gồm nước từ bình chύ và nước từ thức ăn) thì gà được cho ăn thức ăn dạng ướt tiêu thụ nhiều hơn rõ rệt so với gà được cho ăn thức ăn dạng khô (xấp xỉ 67%), sự khác biệt này đặc biệt rõ rệt trong giai đoạn khởi động. Lượng nước tiêu thụ của gà trong giai đoạn tăng trưởng không có sự khác biệt giữa các lô thí nghiệm và lô đối chứng. Thức ăn nghiền thô và thức ăn men hóa không có ảnh hưởng đến lượng nước tiêu thụ của gà trong cả hai giai đoạn khởi động và tăng trưởng. Thức ăn dạng ướt có ảnh hưởng đến khối lượng các cơ quan thuộc bộ máy tiêu hóa của gà trong suốt thời gian thí nghiệm.

Khối lượng tương đối của các cơ quan thuộc bộ máy tiêu hóa ở gà được cho ăn thức ăn dạng khô tăng rõ rệt (27% trong giai đoạn khởi động, 11% trong giai đoạn tăng trưởng) so với gà được cho ăn thức ăn dạng ướt. Có sự ảnh hưởng kéo dài của thức ăn dạng ướt trong giai đoạn khởi động đến khối lượng các cơ quan thuộc bộ máy tiêu hóa ở gà. Khối lượng bộ máy tiêu

hóa (tăng xấp xỉ 10%) được thấy trên gà được cho ăn thức ăn hạt thô trong giai đoạn khởi động, nhưng không có sự kéo dài ảnh hưởng của thức ăn dạng hạt thô đến khói lượng của bộ máy tiêu hóa trong giai đoạn tăng trưởng.

Từ những kết quả trên, chúng tôi có kết luận là, có sự trợ giúp của thức ăn dạng ướt đến quá trình tiêu hóa của thức ăn và bộ máy tiêu hóa của gà phải hoạt động tích cực hơn để tiêu hóa thức ăn dạng hạt thô do vậy dạ dày tuyến và dạ dày cơ phát triển mạnh hơn.

Trong thí nghiệm tiếp theo (Trình bày ở Chương 4), chúng tôi đã loại bỏ yếu tố thí nghiệm acid hóa thức ăn. Giai đoạn khởi động của gà được rút ngắn xuống còn 17 ngày và giai đoạn tăng trưởng chỉ kéo dài đến ngày thứ 35. Ở thí nghiệm này chúng tôi quan tâm nhiều hơn đến cấu trúc thức ăn (hạt thô) và dạng thức ăn (ướt hoặc khô) với mục đích để nghiên cứu sự ảnh hưởng của dạng thức ăn (thức ăn ướt hoặc khô trong giai đoạn khởi động) và cấu trúc thức ăn (ảnh hưởng của thức ăn hạt thô), cũng như thời điểm thích hợp để chuyển đổi từ giai đoạn khởi động sang giai đoạn tăng trưởng, ở thời điểm mà cơ quan tiêu hóa của gà đã được phát triển một cách ổn định về mặt kích thước cũng như chức năng, do đó nó sẽ đóng góp một cách tích cực cho khả năng sinh trưởng phát triển của gà trong giai đoạn tăng trưởng. Kết quả của nghiên cứu cho thấy dạng thức ăn (thức ăn dạng ướt) giúp gà nâng cao khả năng tiêu thụ thức ăn, khả năng tăng trưởng một cách rõ rệt, nhưng nó không có ảnh hưởng đến hiệu quả sử dụng thức ăn. Cấu trúc thức ăn (hạt thô) làm tăng khói lượng của dạ dày cơ một cách rõ rệt do vậy nó có ảnh hưởng đến sự phát triển của dạ dày cơ trong giai đoạn khởi động. Kết quả tính toán từ khói lượng tương đối của dạ dày cơ/100 g khói lượng gà cho thấy gà được cho ăn thức ăn hạt thô có khói lượng tương đối của dạ dày cơ lớn hơn so với khói lượng dạ dày cơ của gà được cho ăn thức ăn dạng hạt mịn. Có sự kéo dài ảnh hưởng (ảnh hưởng phụ) của thức ăn hạt thô dạng ướt trong giai đoạn khởi động đến khả năng tăng trưởng hàng ngày của gà trong giai đoạn tăng trưởng. Điều đó có nghĩa gà đã được hưởng lợi từ sự phát triển của dạ dày cơ sau khi ngừng cho ăn thức ăn dạng hạt thô trong giai đoạn tăng trưởng. Thức ăn dạng ướt trong giai đoạn khởi động có ảnh hưởng rõ rệt đến khả năng tiêu thụ thức ăn, khả năng tăng trưởng hàng ngày. Thức ăn hạt thô trong giai đoạn khởi động không có sự ảnh hưởng đến sự phát triển của cơ quan tiêu hóa trước trong giai đoạn tăng trưởng. Khả năng tăng trưởng tốt nhất trong toàn bộ thời gian thí nghiệm được thấy trên gà được cho ăn thức ăn hạt thô dạng ướt trong cả hai giai đoạn khởi động và tăng trưởng.

Sự phát triển của chức năng cơ quan tiêu hóa được trình bày ở Chương 5. Trong chương này, chúng tôi nghiên cứu sự ảnh hưởng của dạng thức ăn (ướt hoặc khô trong giai đoạn khởi động) và cấu trúc hạt của thức ăn (ảnh hưởng của dạng hạt thô) đến thời gian lưu giữ thức ăn,

hoạt lực của men tiêu hóa protein và pH của chất chúa dạ dày ruột để đánh giá sự phát triển chức năng tiêu hóa của bộ máy tiêu hóa. Kết quả nghiên cứu cho thấy thức ăn dạng hạt thô nâng cao hoạt lực của men tiêu hóa protein một cách rõ rệt trong giai đoạn khởi động. Loại thức ăn này không có sự ảnh hưởng đến hoạt lực của men tiêu hóa protein trong giai đoạn tăng trưởng. Dạng thức ăn (dạng ướt) không có sự ảnh hưởng đến hoạt lực của men tiêu hóa protein trong cả hai giai đoạn khởi động và tăng trưởng. Không có sự kéo dài ảnh hưởng của thức ăn hạt thô trong giai đoạn khởi động đến hoạt lực của men tiêu hóa protein trong giai đoạn tăng trưởng. Chỉ có sự kéo dài ảnh hưởng của thức ăn hạt thô dạng ướt trong giai đoạn khởi động đến hàm lượng protein của dạ dày tuyến trong giai đoạn tăng trưởng. Rõ ràng sự kéo dài ảnh hưởng này đã có lợi cho sự sinh trưởng phát triển của gà.

Phần thảo luận chung được tập trung thảo luận các điểm chính sau: a) Các yếu tố ảnh hưởng đến sự phát triển của cơ quan tiêu hóa ở gà trong giai đoạn khởi động; b) Vai trò, ảnh hưởng của bộ máy tiêu hóa trước đến quá trình tiêu hóa; c) Ảnh hưởng của các phương pháp chế biến thức ăn cho gà trong giai đoạn khởi động và sự phát triển của bộ máy tiêu hóa; d) So sánh các kết quả nghiên cứu về sự phát triển của bộ máy tiêu hóa và các kết quả về khả năng tăng trưởng phát triển của gà (Các kết quả trình bày từ chương 2 đến chương 5).

Kết luận chung của các nghiên cứu trong đề tài này được rút ra như sau:

1. Việc cho ăn thức ăn hạt thô trong giai đoạn khởi động làm tăng khả năng phát triển của bộ máy tiêu hóa trước, đặc biệt là sự phát triển của hệ thống dạ dày tuyến-cơ.
2. Thức ăn dạng ướt làm tăng khả năng tiêu thụ thức ăn, tăng khả năng sinh trưởng phát triển.
3. Sự kết hợp làm ướt thức ăn dạng hạt thô làm tăng khả năng tiêu thụ thức ăn, tăng hiệu suất sử dụng thức ăn và khả năng tăng trưởng.

PUBLICATIONS AND ORAL PRESENTATIONS

1. Mai, A.K., R.P. Kwakkel, A.F.B. van der Poel. Liquid and solid wheat starch in diets for broilers: effect on feed intake, foregut development and performance. Proceeding on the 15th European Symposium on Poultry Nutrition, Balatonfured-Hungary, 25 – 29 Sept. 2005: 389-391.
2. Bosch, G., A.K. Mai, A. F.B. van der Poel, Paul Koene and Eddie A.M. Bokkers. A liquid, acidified wheat by-product in diets for broilers: impact on feed intake, performance, leg condition and diet selection. *British Poultry Science In press* (2007).
3. Mai, A.K., R. Jha, R. P. Kwakkel, A.F.B. van der Poel, and M.W.A. Verstegen. Effect of diet structure, conformation and acidification on development of the foregut segment and performance of broilers. *British Poultry Science Submitted* (2007).
4. Mai, A.K., L. Baarslag, E. den Hertog, R.P. Kwakkel, A.F.B. van der Poel, and M.W.A. Verstegen. Effect of dietary structure and conformation during starter and grower phases on growth performance and GIT development in broiler chickens. *Poultry Science Submitted* (2007).
5. Mai, A.K., E. den Hertog, L. Baarslag, R.P. Kwakkel, A.F.B. van der Poel, and M.W.A. Verstegen. Effects of dietary structure and conformation on pH in the GI tract, feed resident time and protease activity in broiler chickens. *Poultry Science Submitted* (2007).
6. Mai, A.K., R. P. Kwakkel, A.F.B. van der Poel. Liquid and solid wheat starch in diets for broilers: effect on feed intake, performance, welfare and diet. **Oral presentation**. Annual Meeting of Dutch Speaking Nutritionists 2005. Gent University, Belgium: page 23-24.
7. Mai, A.K., R. Jha, R.P. Kwakkel, A.F.B. van der Poel, and M.W.A. Verstegen. Effect of diet structure, conformation and acidification on performance of broilers. **Oral presentation**. Annual Meeting of Dutch Speaking Nutritionists 2006. Rotterdam, the Netherlands: page 56-57.
8. Mai, A.K., L. Baarslag, E. den Hertog, R.P. Kwakkel, A.F.B. van der Poel, and M.W.A. Verstegen. Effect of dietary structure and conformation on the GI tract

development, feed resident time, pH of the contents in broiler chickens at 18 days of age. **Oral presentation.** Annual Meeting of Dutch Speaking Nutritionists 2007. Gent University, Belgium: page 56-57.

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Mai Anh Khoa

June, 2007

About the author

Mai Anh Khoa was born on the 5th of April, 1975 in Thainguyen, Vietnam. He started his higher education studies in 1991 at Thainguyen University of Agriculture and Forestry (Thainguyen, Vietnam) and received a BSc degree in the field of Animal Sciences in 1996. In the same year, he was appointed as a Research assistant at the Experimental and Practical Station of Thainguyen University of Agriculture and Forestry. Meanwhile, he also continued his studies and obtained his MSc degree in the same field in 2000. After completing the Master studies, he was appointed as a lecturer at the Department of Animal Sciences in Thainguyen University of Agriculture and Forestry and worked there for 3 years. In 2003, he was awarded a scholarship by the Ministry of Education and Training, Vietnam, allowing him to pursue his Ph.D study abroad. May 2003, he started his Ph.D project at the Animal Nutrition Group, Wageningen University and Research Centre, the Netherlands. He is now returning to his assigned duty as an academic staff.

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Training and Supervision Plan		Graduate School WIAS	
Name PhD student	Mai Anh Khoa		
Project title	<i>Wet and coarse diets in broilers nutrition: development of the GI tract and performance</i>		
Group	Animal Nutrition		
Period	May 2003 - June 2007		
Supervisor	Prof.Dr.Ir. M.W.A. Verstegen		
Daily supervisors	Dr.Ir. A.F.B. Van der Poel, Dr.Ir. R.P. Kwakkel		
The Basic Package		year	ECTS*
WIAS Introduction Course		2003	1.5
Course on philosophy of science and/or ethics		2004	1.5
SUBTOTAL			3.0
Scientific Exposure		year	ECTS
<i>International conferences</i>			
14th European Symposium on Poultry Nutrition - Norway (4 days)		2003	1.2
15th European Symposium on poultry Nutrition - Hungary (4 days)		2005	1.2
29th Dutch speaking Nutritionist - Belgium (1 day)		2005	0.3
30th Dutch speaking Nutritionist - Netherlands (1 day)		2006	0.3
31st Dutch speaking Nutritionist-Belgium (1 day)		2007	0.3
<i>Seminars and workshops</i>			
WIAS Science day 2004, 2005, 2006, 2007		2004-07	1.2
PhD retreat 2004 (2 days)		2004	0.6
Seminar on protein turnover - Wageningen - NL (1 day)		2005	0.3
Seminar on use of Isotopes in nutrition research - Wageningen - NL (1 day)		2005	0.3
<i>Presentations</i>			
WIAS Science day (Oral)		2004	1.0
15th European Symposium on poultry Nutrition - Hungary (Poster)		2005	1.0
29th Dutch speaking Nutritionist (Oral)		2005	1.0
30th Dutch speaking Nutritionist (Oral)		2006	1.0
31st Dutch speaking Nutritionist (Oral)		2007	1.0
SUBTOTAL			10.7
In-Depth Studies		year	ECTS
<i>Disciplinary and interdisciplinary courses</i>			
WIAS debating course		2003	1.5
Advanced statistics courses (M. Grossman and W. Koops 2005)		2005	1.0
Eco-physiology of the gastro-intestinal tract (VLAG course)		2006	1.5
<i>Undergraduate courses</i>			
Case study Animal Nutrition		2003	6.0
Feed technology		2004	6.0
SUBTOTAL			16.0
Professional Skills Support Courses		year	ECTS
WIAS Course Techniques for Scientific Writing		2003	1.2
Use of Laboratory Animals/Utrecht		2004	3.0
Project and time management		2006	1.0
SUBTOTAL			5.2
Research Skills Training		year	ECTS
Preparing own PhD research proposal		2003	4.0
SUBTOTAL			4.0
Didactic Skills Training		year	ECTS
<i>Supervising MSc thesis</i>			
Rajesh Jha (MSc major thesis)		2005	2.0
Ellen den Hertog (BSc thesis)		2006	1.0
Linda Baarslag (BSc thesis)		2006	1.0
SUBTOTAL			4.0
Education and Training Total			42.9



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