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The Effects of Different Thermal Treatments and Organic Acids Levels in Feed on Nutrient Digestibility and Gut Microbiota in Broilers

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To My Love NARGES

You Are The Best Thing That Ever Happened To ME

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LIST OF ABBREVIATIONS

AA	Amino Acid
AMEN	Apparent Metabolizable Energy adjusted for Nitrogen
ANOVA	Analysis Of Variance
BCSFA	Branched Short Chain Fatty Acids
BWG	Body Weight Gain
CF	Crude Fiber
CFU	Colony Forming Unit
CP	Crude Protein
E110	Expanding at 110°C
E130	Expanding at 130°C
FCR	Feed Conversion Ratio
FI	Feed Intake
GLM	Generalized Linear Model
L	Long Term Conditioning
LSD	Least Square Differences
P	Pelleting
qPCR	quantitative Polymerase Chain Reaction
SCFA	Short Chain Fatty Acid
SEM	pooled Standard Error of Mean
TSCFA	Total Short Chain Fatty Acids

1 CHAPTER 1: GENERAL INTRODUCTION

There are considerable evidences that feed ingredients and animal feed are frequently contaminated with foodborne bacterial pathogens like *Campylobacter* species and non-Typhi serotypes of *Salmonella enterica*, Shiga toxin–producing strains of *Escherichia coli*, and *Yersinia enterocolitica* (Mead et al., 1999; Crump et al., 2002). The microbial contamination of feed ingredients and animal feed may occur during the harvesting, handling, transportation, processing and/or storage (Mahrous et al., 2001; Maciorowski et al., 2006) and can stay for months in stored feed (Davies and Wray, 1995), and would thereby be taken up by livestock. In 1994, the FDA reported that 25% of the investigated feed samples, collected from feed mills and farms were contaminated by *Salmonella enterica* during the harvesting, handling, transportation and processing (Crump et al., 2002) Therefore, animal feed could be a potential vector in the transmission of pathogens and toxins to the poultry and livestock. The slaughtering and/or processing facilities can be contaminated by carrier or infected carcasses which lead to transmit of pathogens through the food chain to human (Cox et al., 1986). The high risk ingredients in the industrial compound feed are oil seed meals, vegetable proteins and animal derived proteins (EFSA, 2008). For instance fish meal, meat and bone meal, and soy products have been shown to have a relatively high prevalence of *Salmonella* (Jones and Richardson, 2004). The role of contaminated feed for food safety is discussed, however, a positive correlation between the presence of *Salmonella* in the poultry feed and their isolation on carcasses has been observed (Bains and MacKenzie, 1974). This is important as over 50% of all traceable human cases of salmonellosis have been associated with the consumption of eggs and other poultry products (Ebel et al., 1992).

The need for biological safety of the poultry products initiated a worldwide search to find an economically efficacious feed management strategy, which decontaminates feed, and meanwhile, would be putatively beneficial for the gut microbiota and have no negative impact on the animal welfare, performance and consumer health.

Several studies have shown the potential of thermal processes in the reduction of microbial contaminations of feedstuffs (Mossel et al., 1967; Furuta et al., 1980a; Jones et al., 1991; Adams et al., 1996), however, feed treated with heat is at the risk for recontamination during the cooling process. Organic acids are often used in feed production to reduce bacterial contaminations and to prevent recontamination (Ricke, 2003; Martin et al., 2005; Ricke, 2005) and both strategies may act synergistically (Tabib et al., 1984). Thermal processing of feed and the inclusion of organic acids can improve the stability and hygiene of feed, might alter the chemical and physical characteristics of its constitutive ingredients, can improve the nutritional value of animal feeds, might have beneficial effects on the gastrointestinal function and the microbial status of gastrointestinal tract (Furuta et al., 1980a; Duffus and Duffus, 1991; Jongbloed et al., 2000; Dibner and Buttin, 2002; Engberg et al., 2002; Rehman et al., 2007; Jones, 2011; Abdollahi et al., 2013).

Despite a promising nutritional value, there is not enough knowledge regarding the effect of organic acid supplementation on performance and particularly feed digestibility in broilers. The interaction of thermal treatment strategies and the supplementation of organic acids have not been widely investigated. Therefore, the first part of the present PhD thesis was performed to study the effect of different types of thermal processes including pelleting, long-term conditioning and expansion at two different temperatures (110°C and 130°C) and organic acid inclusion levels (0, 0.75 and 1.5%) and their interactions on hygienic status of broiler feed, on performance and feed digestibility in broilers.

The effects of different types of thermal processes on the gut microbiota and possible interactions with supplementation of organic acids as feed additives have not been widely studied. The second part of the present PhD thesis was performed to investigate the effect of different thermal treatments including pelleting, long-term conditioning and expansion at two different temperatures (110°C and 130°C) and also different organic acid inclusion levels (0, 0.75 and 1.5%) and their interactions on bacterial composition and activity in gastrointestinal tract of broiler chicks.

2 CHAPTER 2: LITERATURE REVIEW

2.1 *Feed Production*

The feed cost constitutes up to 70 percent of the poultry production costs (Abdollahi et al., 2013). The most significant part of this cost is for the feed ingredients. About 95% are required for meeting energy and protein requirements, about 3-4% for mineral and vitamin needs and 1-2% for various feed additives (Ravindran, 2013), and the minor but still significant part is for feed processing. The feed processing consists any treatment that poultry feed is gone through prior to ingestion (Maier and Bakker-Arkema, 1992) and refers to the basal feed processing including the practices of receiving, grinding, batching, mixing, loading, and delivering of constitutive feed ingredients of the diet and a supplementary feed processing called "thermal processing". Regarding the fact that after the basal feed processing (receiving to mixing) a complete feed is already available and further feed processing raises up cost of the final product, the thermal processing has to be economically justified. However the available thermal treatments for poultry feed can improve the stability and hygiene of the feed and also may positively affect the chemical and physical characteristics of the constitutive ingredients, and positively affect animal performance (Behnke, 1996; Thomas et al., 1999; Behnke and Beyer, 2002; Abdollahi et al., 2013). In recent three decades, several feed processing techniques in poultry feed production have been developed, however, the cost and benefits of each technique has to be carefully weighed and justified (Behnke, 1996).

2.1.1 *Mash Feed*

For mash feed production, the particle size of the majority of feed ingredients, particularly coarse cereals grains is commonly reduced via grinding and then the ground seeds are mixed with the protein meals and micro feed ingredients like amino acids (AA), vitamins, etc. The first step of particle size reduction is the disruption of outer seed coats and the second is the exposure of endosperm (Koch, 1996; Amerah et al., 2007a). In general, due to the increasing in the number of particles and the surface area per unit volume, grinding or particle size reduction cause greater access of the digestive enzymes to the nutritional components (Goodband et al., 2002; Koch, 1996), as well as better homogeneity, blending ability and less segregation during and after mixing (Koch, 1996). After the thermal processing, grinding is the second greatest energy cost in the feed processing (Reece et al., 1985).

2.1.2 *Mash Quality*

Commonly, the mash quality is assessed by the homogeneity, uniformity and size of its particles. One of the main challenges in the poultry feed manufacturing is the uniformity. The feed industry has a desire to produce a consistent homogenized and uniform feed mixture. The non-uniform mash feed, with different particle sizes, give the more aggressive birds the chance to consume only the bigger feed particles and leave the smaller fine particles for less aggressive birds (Behnke and Beyer, 2002). To achieve the ideal mash form, a proper grinding and mixing is required. The particle size of the ground seeds can change based on the industry demands by feed mill setting, however, as the protein meals are supplied pre-ground, and the micro-ingredient are generally supplied in the fine form (mostly as a powder), there is a little control over their particle size (Amerah et al., 2007a). Therefore, producing a mash with an absolute uniform particle size for all of the diet constitutive ingredients seems to be very critical. It has been reported that different factors like particle size, particle number, particle shape, density, electrostatic charge, dustiness, hygroscopicity and flowability can significantly affect the quality of the feed mixture (Axe, 1995). However, by far, particle characteristics, particularly particle size, can be the most important and limited factor for uniformity. Sorting and selecting of large particle by the aggressive birds can make homogenized and balanced mash diets failing in the meeting of the nutritional requirements for the entire herd (Abdollahi et al., 2013). Furthermore, a mixture of ingredients with different particle sizes is subjected to segregation. In a segregated mixture, the random mixing cannot be achieved and one component may have a greater probability of being in the one part of the mixture than other components (Rhodes, 1990; Axe, 1995). Thus, the uniformity of particle sizes in a complete balance diet is necessary to optimize nutrients reception and utilization. The failure in providing a homogenous and uniform diet through feed production could lead to a poor animal performance (Behnke, 1996); however, practically it seems being impossible to achieve an ideal feed mixture (Axe, 1995).

2.2 Thermal Processes

The agglomeration of the individual ground ingredients into the larger particles could be one of the practical solutions for this problem. When a pelleted feed is presented to birds, practically they are prevented from choosing between different particle sizes. It has been observed that, broiler performance was more dramatically affected by the particle size when birds fed mash diets than fed pelleted diets (Amerah et al., 2008). It could be due to the better uniformity, less ingredients segregation and finally a better assimilation for receiving the micro and macro ingredients. Concerning the present status of the poultry feed manufacturing and regarding the difficulties for the appropriate homogenized mash production, recently many of the farms use the different forms of the thermal processed feed, like crumbled, pelleted or expanded feed. During thermal processing of the feed, the application of heat, moisture, pressure and shear alter the physico-chemical properties of feedstuffs, thereby affecting their digesting behavior (Nielsen, 1994; Thomas and van der Poel, 1996; Goelema et al., 1999). In fact, the interaction between the constitutive feed ingredients of the diet and the thermal conditioning variables such as machinery, temperature, pressure, time and steam determines the chemical reactions between nutrients, the adhesive properties on the surfaces of feed particles and the final physico-chemical structure of the feed. In terms of returning the invested energy (electrical, steam energy and etc.), hydrothermally processed feed could be more profitable compared to mash feed, when balancing against the gain in the available feed energy (Peisker, 2006). The thermal processing can decrease feed wastage, reduce the time and energy spending for prehension, reduce ingredient segregation, improve hygienic status of the feed, prevent selecting feed component by the chickens, and might improve protein and starch digestibility (Behnke, 1994; Jones et al., 1995; Behnke, 1996; Vilarino et al., 1996; Beyer et al., 2000; Behnke and Beyer, 2002), however, specific consideration regarding the heat sensitive ingredients like AA, vitamins and feed additives like probiotics and enzymes, must be given, when applying thermal processes (Peisker, 2006).

The technology of poultry feed thermal processing consist a broad range of methods like pelleting, long term thermal treatment, extrusion and expansion.

2.2.1 Long Term Conditioning

In order to eliminate, inactivate or reduce the anti-nutrients and pathogens in the animal feed ingredients and feed, a simple heat processing method have been used for decades and still is being used in many animal feed manufactories (Rehman and Shah, 2005). This simple heat processing method could be dry or moist, and based on the purposes of the usage and its technical characteristics (machinery, temperature, retention time and etc.) can be classified as cooking, roasting, toasting, autoclaving and long term conditioning. The basic principles of this heat processing are comparatively high temperature and long retention time (Rehman and Shah, 2005). In studies which terms of "roasting", "toasting" and "autoclaving" have been used, the applied temperatures have been higher than 90°C and the applied retention times have been longer than 10 min (Almas and Bender, 1980; Kadam et al., 1987; Sosulski et al., 1988; Rowe et al., 1999; Newkirk and Classen, 2002; Gracia et al., 2003; Newkirk et al., 2003). These types of thermal treatments could sometimes cause some unfavorable reactions, chemical and physical alterations in feed (like Maillard reaction and degradation of heat-labile AA, enzymes and vitamins), which could impair the nutritional value of the animal feed (Almas and Bender, 1980; Newkirk and Classen, 2002; Newkirk et al., 2003). The observations were not consistent because, the beneficial and impairing effects of mentioned thermal treatments are related to several variables including the type of feed ingredients, the heating temperature, processing time at the given temperature, the initial moisture and the volume of water during the treatments (Almas and Bender, 1980; Rehman and Shah, 2005).

In the typical long term conditioning, which is commonly used in order to decontaminate feed, the feed is processed at lower temperature and shorter time compared to roasting, toasting and autoclaving. Although this type of long term conditioning has been the basis of the modern thermal processes, it has not been widely studied and there is a dearth of information on performance and nutrients digestibility of long-term conditioned feed in poultry and other monogastric animals.

2.2.2 Pelleting Process

Pelleting is the most prevalent hydrothermal processing in the poultry feed production. It agglomerates small particles into larger particles by the means of a mechanical process in combination with moisture, heat and pressure (Falk, 1985; Behnke and Beyer, 2002). Almost at the end of the 1920's, the so-called "flat die" pelleting machine and "ring die" pellet mill were developed.

The early pelleting process involved mixing the feed ingredients and pelleting them with no further treatment (Calet, 1965; Thomas and van der Poel, 1996). In modern pellet processes, the mash is pelleted in a roller-die pellet press, both vertically and horizontally (Thomas and van der Poel, 1996), with preliminary treatments such as mixing with pellet binders (Waldroup et al., 2002), conditioning with steam (Skoch et al., 1981), expanding (Veenendaal, 1990; Behnke, 1996) and post treatments like spraying of enzymes, vitamins and oils on the final product (McCracken, 2002). In addition to the roller-die pellet mill itself, auxiliary equipments including conditioners, cooler, dryers, and related process equipment are the indispensable part of the pellet production processing in the animal feed manufacturing.

In the modern pelleting process the conditioning of mash prior to pelleting is the major step (Skoch et al., 1981), which means injecting steam to the mash feed. After this step, the conditioned mash flows from conditioner into the pelleting chamber and is formed by pressing through a metal die. The last step of pelleting process is the cooling and drying. The temperature of the final pelleted feed before cooling and drying is varying from 80 - 90°C at about 15 – 17% moisture. The cooling and drying reduce the temperature to about 8°C above the ambient temperature and decrease the moisture to about 10 – 12% (Zimonja et al., 2007; Abdollahi et al., 2013).

Improving feed intake (**FI**) and consequent body weight gain (**BWG**) are the major advantages and motivation for pelleted feed compared to mash feed (Engberg et al., 2002; Svihus et al., 2004; Abdollahi et al., 2013). Furthermore, as explained above, pelleting process prevents selection of larger particles by aggressive birds and also causes less particle falling from the beak into the water or onto the floor and finally reduces the feed wastage (Calet, 1965; Axe, 1995; Jensen et al., 2000; Abdollahi et al., 2013).

It has been reported that broiler chicks fed mash spent approximately 15% of the time for feed ingestion and those fed pelleted feed spent approximately 4% of the time for the ingestion (Nir et al., 1994). Productive energy is an estimation of energy per kg of the feed used for protein and lipid synthetize (Reddy et al., 1962; Abdollahi et al., 2013). It has been shown that mash feed contained less productive energy than the pelleted feed (Reddy et al., 1962). This is explained by increasing the diet density through pelleting process (Jensen et al., 2000). The heat increment during the ingestion and utilization was lower for the chickens fed pelleted diet compared to those fed mash diet, therefore more energy was available for productive purposes when pelleted feed was fed to broiler chicks (Latshaw and Moritz, 2009; Abdollahi et al., 2013). Due to the beneficial effect of pelleting process on the feed wastage, heat increment and available energy of the feed for productive purposes, there is a general agreement on higher potential of the pelleted feed compared to the mash, for improving FI, BWG and feed efficiency. However the observed results regarding the effects of the thermal processes, particularly pelleting, on broiler performance, nutrient digestibility, energy retention and the apparent metabolizable energy of the feed have been inconsistent (Jensen et al., 1965; Peisker, 1994; Behnke and Beyer, 2002; Svihus et al., 2004; Peisker, 2006; Amerah et al., 2007b; Cutlip et al., 2008; Abdollahi et al., 2013). This inconsistency might be due to the differences in the proportion of specific feed ingredients in the experimental diets (oil content, cereal source like wheat, corn or barley based diets etc.) or could be because of differences in the applied machinery, processing temperatures and times (Peisker, 2006; Abdollahi et al., 2013; Liu et al., 2013). For instance in terms of the proportion of specific feed ingredients in the experimental diets and temperature in pelleting, it was shown that pelleting of wheat-based diets at 80–85°C improved broiler performance, but increasing conditioning temperatures above 85°C impaired performance (Silversides and Bedford, 1999). With a barley–maize–soy diet, increasing temperature from 60 to 75°C improved FI and BWG, whereas conditioning temperatures of 90°C impaired broiler performance (Samarasinghe et al., 2000). A pelleted maize–soy diet treated at 65°C improved BWG compared to diets pelleted at 75 and 85°C (Kirkpinar and Basmacioglu, 2006). In contrast, a decreased FI, similar BWG and better feed conversion ratio (**FCR**) were shown for broilers fed maize–soy diets pelleted at 93.3°C compared to those fed diets pelleted at 82.2°C (Cutlip et al., 2008). Conditioning wheat-based diets with a temperature above 60°C reduced FI and BWG, but birds fed maize-based diets conditioned at 60 and 90°C had higher FI and BWG than those fed the diet conditioned at 75°C (Abdollahi et al., 2010a). With maize- and sorghum-based diets, increasing temperature did not affect FCR, while increasing the conditioning temperature from 60 to 75°C reduced the BWG, but the BWG was restored at 90°C (Abdollahi et al., 2010b). Improved pellet quality, in terms of durability and hardness at a higher conditioning temperature, might cause the restoration of FI and BWG at 90°C (Abdollahi et al., 2013). With a wheat-based mash starter diet, increasing temperatures per se above 60°C reduced nutrient utilization and performance of broiler chicks. However, with pelleting at temperatures above 60°C, better pellet quality was achieved and restored the performance (Abdollahi et al., 2011). It was concluded that the high temperatures negatively affect nutrient availability and positively affect pellet quality; therefore, performance responses of broilers fed diets conditioned at different temperatures reflect a balance between the nutrient availability and the pellet quality (Abdollahi et al., 2013).

The pelleting process is one the effective strategies for the feed decontamination and reduction of the bacterial load in feed (Cover et al., 1984). However, heat resistance between microbial species varies greatly. While the majority of Salmonellae and coliforms can be eliminated by pelleting at temperatures above 80°C, spore formers are resistant to pelleting process even at 90°C (Obi, 1978; Furuta et al., 1980b; Cox et al., 1986; Veldman et al., 1995; Jones and Richardson, 2004). The decontamination effect of pelleting on *Enterobacteriaceae* in a standard feed mixture, at two different temperatures rang (80-83^{o}C and 51-67^{o}C) was investigated. Pelleting process at temperature under 80°C reduced the bacterial count by a factor of $l0^3$ and pelleting at temperature over 80°C eliminated the bacteria by a factor of $l0^5$ (Mossel et al., 1967). Pelleting of a commercial cattle concentrate feed at 70°C for 20 and 120 seconds resulted in 1.3 and 2.2 log reductions in *Escherichia coli* O157, respectively (Hutchison et al., 2007). A study comparing the prevalence of Salmonellae in broiler flocks fed either pelleted feed at 60-80° C and pelleted feed at 80-82° C indicated a significant reduction in the occurrence of Salmonellae in the flocks fed the more intensively heated pellets (Voeten and Leest, 1989). However, the decontaminating efficacy of thermal treatments appears to be influenced by the moisture content of the feed. The pelleting process of poultry feed with 15% moisture at 82.2° C for 2.2 seconds eliminated *Salmonella enteritidis* by 4.5 log units, however in a similar feed containing 5% moisture only a 1.5 log reduction was observed (Himathongkham et al., 1996). In the modern thermal processing, the generated steam in the boiler is a medium through which heat energy can be transferred to the mash feed. The heat transference is accompanied by condensation of the steam as well as an increase in feed moisture. The conditioning of mash feed prior to pelleting influences the potential of the applied processes in feed decontamination.

2.2.3 Extrusion Process

Extrusion in food and feed production has been used for almost one century. Extrusion is a thermal treatment according to the high temperature, short time (HTST) principles and applies a combination of moisture, pressure, temperature, shaping kneading and shearing forces. In feed processing equipments which work with the HTST principle, the steam conditioning and the friction in the screw generate a high temperature and after passing the resistor in the outlet gate, the high pressure drops to atmosphere pressure (Armstrong, 1994). This release of pressure and the spontaneous evaporation of the moisture makes the end product porous with a low density (Heidenreich, 1994), allowing oil and feed additives to be sprayed on the feed after the processing.

The HTST equipments like extruders and expanders demand comparatively high energy expenditures and high investment cost, therefore a substantial capital investment is required. Extruders could be classified as moist or dry, single or twin screw extruder. The moist extrusion usually implies process moisture of 30% or more, while dry extrusion generally processes feed at less than 18% moisture level. Extrusion has the ability to minimize the nutrient degradation while it eliminates microorganisms, inactivates enzyme inhibitors and destroys anti-nutritional factors of the feed (Riaz, 2000). The transfer of mechanical energy to thermal energy results in temperatures of up to 100°C, leading to improved starch gelatinization, protein denaturation and feed texture (Fancher et al., 1996; Thomas et al., 1997). Extruders have been mainly implemented for the production of pet and fish feed (Rokey, 1994). Extruded animal feed must subsequently be more intensively cooled and dried compared to other types of thermal processing (Heidenreich, 2001).

Broiler chicks fed pelleted and extruded feed had higher BW compared to those fed mash diets (Kidd et al., 2005). Birds fed a crumbled extruded starter diet had a higher BWG compared to chicks fed a crumbled pelleted starter diet. It was claimed that extrusion can cause protein and AA destruction; therefore, extruded broiler feed might need to be formulated with slightly higher nutrient density to compensate for the nutrient losses during extrusion (Jones et al., 1995).

Regarding the effects of extrusion process on the hygienic status of a standardized feed formulation, it has been shown that *Salmonella typhimurium* was eliminated from mash feed processed in an extruder at 83°C with 28.5% moisture and a retention time of 7 seconds (Okelo et al., 2006). The same standard feed was inoculated with *Salmonella typhimurium* and *Bacillus stearothermophilus* and subjected to an extrusion process at 77-110°C, 24.5-34.5% moisture and 3-11 seconds retention time. Only *Bacillus stearothermophilus* could be recovered from the processed feed over the entire range of extrusion conditions (Okelo et al., 2008).

2.2.4 Expansion Process

Expanders have been introduced more recently in the animal feed industry. Expander and extruder are similar regarding the design and function. The expander consists of a conveying screw with mixing bolts which exerts a mixing, transport and shearing action into the mash feed. The expander is considered as a single screw extruder with a moving die installed at the outlet (Fancher et al., 1996; Thomas et al., 1997). There are small functional differences between a single screw extruder and an expander. The temperature of expanders can rise up to more than 150°C, however the common range is between 100 - 130°C, whereas temperatures range in extruders varies from 80 to 200°C (Fancher et al., 1996; Thomas et al., 1999; Lundblad et al., 2011; Sørensen, 2012; Prestløkken and Fôrutvikling, 2013). The moisture content, shear action and production cost in the extrusion process is higher than the expansion. Extrusion processing lines needs a dryer and a cooler while, for expansion processing a cooler seems to be sufficient

(Fancher et al., 1996; Thomas et al., 1999; Prestløkken and Fôrutvikling, 2013). With an expander, it is possible to produce a pellet shaped feed without using a pelleting mill (Riaz, 2000; Riaz, 2007). Cooking during the expansion process siginificantly gelatinizes starch into an elastic adhesive that glues the feed particles and results in a puffed and low-density formation for feed (Camire et al., 1990). Additional benefits of the expansion process can include improvement of the pellet quality, lower feed moisture, higher diet formulation flexibility, better feed hygiene, higher mill throughput, manipulation of the feed bulk density and particle size, the ability to crumble without pelleting, and longer life for die (Fancher et al., 1996; Thomas et al., 1997).

The observations regarding the effects of expanded broiler diets on poultry performance are inconsistent, which might be due to the temperatures and feed ingredients used in different studies (Vest and Calhoun, 1997; Peisker, 2006; Liu et al., 2013). It has been reported that the expansion process does not act on all ingredients in the same manner (Peisker, 2006). Expanding is well known as a technology which increases the fiber solubility, fat stability, starch gelatinization and metabolisable energy due to more available starch and fat, and in comparison to pelleting, can be more flexible for diet formulation (Armstrong, 1994; Peisker, 1994; 2006). This technology allows using a high level of oils, molasses and milling by-products, which usually reduce pellet quality (Armstrong, 1994; Fancher et al., 1996). Thus, the differences in the proportion of specific feed ingredients in the experimental diets like oil and cereals, could be one of the main factors for the final results of the experiments and the observed inconsistency (Peisker, 2006; Liu et al., 2013).

In a former study, the effects of pelleting (P) and expanding plus pelleting (E+P) on the performance variables of broiler chicks were investigated. The E+P group had a lower BW and better FCR compared to the P group (Smith et al., 1995). Average daily gain and average daily FI were higher for broiler chickens fed pelleted diets compared to those fed expanded diets (Lundblad et al., 2011). Birds fed pelleted feed tended to have a better BWG and FCR compared to those received expanded-pelleted feed (Zimonja and Svihus, 2005). Feeding an expanded diet improved performance variables of broiler chicken by 5-10%, compared to those fed pelleted diet (Beyer et al., 2000). An improvement in growth and FCR was observed for male turkeys fed expanded compared to those fed pelleted diet (Fancher et al., 1996). In other studies, no significant differences in the final BW and FCR were reported between broilers fed pelleted and expanded feed (Nissinen et al., 1993; Peisker, 2006). Regarding the results of the mentioned studies, the destruction of the certain heat sensitive nutrients during the expansion process should not be a restrictive factor for using this kind of processes in poultry feed production (Coelho, 1994; Broz et al., 1997).

Expansion process could be considered as an effective tool in order to improve the hygienic quality of the feed (Fancher et al., 1996; Broz et al., 1997; Beyer et al., 2000; Behnke and Beyer, 2002). It has been reported that processing temperatures higher than 110°C reduced the bacterial count of naturally occurring *Enterobacteriaceae* by a factor of 10^5, and ensured the elimination of *Salmonella* spp. (Koenig, 1995). Similarly Heidenreich (1994) reported bacterial decontamination rates of 10^5-10^6 cfu/g.

2.3 Water and Pressure in Thermal Processing

In thermal processes, heat, water and pressure are the main variables. The quality and quantity of these factors directly influence the final product of hydrothermal processing and have the main role determining the product quality.

2.3.1 Water

Water in thermal processing is known as a binding agent between different particles, especially with capillary forces. Increased moisture can improve physical quality of the processed feed (Moritz et al., 2001; Moritz et al., 2002; Moritz et al., 2003). During steam conditioning, the feed particles are covered with a thin film of water. This thin film of water with its effect on the surface structure of the materials may exhibit bonds via capillary sorption between the particles (Rumpf, 1958; Thomas and van der Poel, 1996). The intensity of the binding bridges between the particles is depending to the amount and surface tension (Knacke and Pohl, 1959). More moisture bridges took place between particles when the amount of water in the steam conditioning step increased and it caused more binding forces (Knacke and Pohl, 1959). Overload of the water saturation during the feed processing fills all the pores between the particles and prevents the capillary forces and structural binding establishment (Knacke and Pohl, 1959; Thomas and van der Poel, 1996). Due to the different machinery design and the type of processing, the optimum amount of required steam during processing can be highly variable; however, the overload of the injected steam may block the dies and considerably decreases the efficiency of the processing (Knacke and Pohl, 1959; McBain, 1966).

The feed components and ingredients exhibit different interactions when water and heat are combined (Thomas et al., 1997). During thermal processing, degradation, denaturation, solubilization and gelatinization of AA, vitamins, proteins and starch can occur and different binding bridges can be formed between the feed particles (Wood, 1987; Mommer and Ballantyne, 1991; Thomas and van der Poel, 1996).

In modern feed thermal processing, steam instead of liquid state of the water is commonly used (Thomas and van der Poel, 1996; Abdollahi et al., 2013). The application of steam is the routine way to insert heat, pressure and water in the same time (Smallman, 1996; Thomas et al., 1997; Abdollahi et al., 2013). In the steam conditioning, the live steam is injected into the feed mash as it is conveyed through the conditioner which generally consists of a cylindrical tube with a rotating shaft. The condensing steam increases the temperature and moisture content of the mash (Smallman, 1996). Steam is homogeneously and fast scattered and distributed through the feed particles (Thomas et al., 1999). The steam conditioning increases the pellet production rate, improves the pellet durability, lengthens the roller- and die-life, reduces the energy costs, decreases fine particles, reduces the fine particles recycling, and decreases the dust during handling, transportation and feeding (Skoch et al., 1981; Moritz et al., 2001; Moritz et al., 2002; Cutlip et al., 2006; Vukmirović et al., 2010; McBain, 1966).

Dry-pelleting caused higher temperature in the final product compared to steam-conditioning-pelleting (Skoch et al., 1981). It is obvious that less mechanical friction can increase the life of the die and roller assembly (Skoch et al., 1981; Moritz et al., 2001; McBain, 1966). Furthermore, steam conditioning before pelleting can reduce separation of fine particles during scalping (Bartikoski, 1962; Skoch et al., 1981; Moritz et al., 2001; McBain, 1966).

Steam conditioning has some disadvantages. Pelleted feed with higher moisture content had higher durability and lower hardness (Vukmirović et al., 2010). Furthermore, the moisture addition during the feed processing might increase the weight per volume of the feed and causes a higher transportation cost (Dozier, 2001). However, the moisture content of the final product is changed by cooling and drying. The ambient atmospheric condition and the quality of cooling process as well as the nutrient composition of the diet greatly affect the final moisture content in the processed feed (Cutlip et al., 2006; Cutlip et al., 2008). Steam conditioning decreased the availability of several AA, specifically lysine digestibility, due to the Maillard reaction (Smith and Circle, 1978).

2.3.2 Pressure

One of the important developments in the feed processing technology was the processing system in which the conditioner and die cavities were pressurized. The pressure inside of the chamber (barrel conditioner) is directly related to the volume and temperature of the injected steam during the conditioning (Skoch et al., 1981; Stevens, 1987). The pressure during the feed processing is originated from the motor power and steam (Stevens, 1987; Thomas and van der Poel, 1996; Thomas et al., 1999). The motor power and steam pressure affect durability and hardness of the processed feed (Thomas et al., 1999), most probably because of the densification effect on the mash feed (Thomas et al., 1998). It has been reported that the steam pressure did not affect pellet quality as dramatic as conditioning temperature, however it was still remarkable (Cutlip et al., 2008).

The applied pressure during the thermal processes can de-aerate the feed mash and also can decrease its porosity, which finally causes improvement in the energy consumption of the subsequent compaction by pellet press and also causes stronger binding forces between the neighboring particles due to the shorter radiuses (Rumpf, 1958; Ouchiyama and Tanaka, 1985; Thomas et al., 1997). In feed with high viscous ingredients, the solid-solid interactions between the different particles are the main adhering forces that stick particles together (Thomas and van der Poel, 1996). The high pressure decreases the distance between the particles and these interactions could more easily come into effect. Therefore, high pressure for feed mixtures which contain highly viscous ingredients can increase hardness, but on the other hand, due to the reduction in flexibility and redistribution of the binding agents around particles, it can also impair durability (Thomas and van der Poel, 1996).

Low steam pressure during conditioning (injection of more water relative to heat) should only be used when diets contain high level of the starch or heat sensitive material like dry milk powder, sugar and whey (Thomas et al., 1997). However, in feed processing systems with low steam pressure, the system may not be able to sufficiently remove sediments, thus, gradually; a wet choke can block the die holes (Briggs et al., 1999).

On the other hand, when relatively low water volumes and high temperatures are needed, high steam pressure conditioning could be the first choice (Thomas et al., 1997). High steam pressure processing should be used for diets which contain high concentrations of fiber, protein and anti-nutrient factors, as well as when high hygienic status of the feed is required (Maier and Gardecki, 1993; Thomas et al., 1997; Cutlip et al., 2008). With applying high pressure steam conditioning, a temperature higher than 80°C can easily be reached. However it might economically be inefficient (Thomas et al., 1997). The difference between the enthalpies of the saturated vapor steam at 138 and 552 kPa is just 2.2% (Briggs et al., 1999). Steam pressures between 241 to 276 kPa for conditioning have been recommended (Briggs et al., 1999). Furthermore, Stevens (1987) and Briggs et al. (1999) reported that a high steam pressure did not significantly affect pellet durability, production rate, moisture gain and temperature rise across the die. The achieved pellet quality with various steam condition pressures has not been as drastic as the pellet quality associated with different temperatures (Cutlip et al., 2008).

2.4 Organic Acids

In terms of feed decontamination, chemical treatments are often used. As advantage, organic acids provide some residual protection against recontamination, depending on the product implemented, the application rate, the thoroughness of the application, as well as the persistence of the substance in the feed (Hinton and Linton, 1988; Rouse et al., 1988) (Hinton and Linton, 1988; Rouse et al., 1988). Formic, propionic and acetic acid are the main agents used in animal feed production to reduce bacterial contaminations and to prevent recontamination (Ricke, 2003; Martin et al., 2005; Ricke, 2005).

The organic acids have a long history of use as preservatives and functional feed additives (Vogt et al., 1982; Jongbloed et al., 2000; Dibner and Buttin, 2002; Ricke, 2003; Ricke, 2005). The utilization of organic acids in the animal feed industry was originally started to serve as fungistats (Paster, 1979; Dixon and Hamilton, 1981), however, in past 40 years these organic acids individually or in various combinations have also been used for their antimicrobial activity and growth promoting effects (Hinton and Linton, 1988; Izat et al., 1990b; Berchieri and Barrow, 1996; Thompson and Hinton, 1997; Dibner and Buttin, 2002).

2.4.1 Antimicrobial Activity of Organic Acids

The mechanism of bacteriostatic and bactericidal activities of organic acids is not fully elucidated and it is related to the physiological status of the targeted bacteria and the physicochemical characteristics of the external environment (Ricke, 2003; Ricke, 2005). Regarding the fact that the common antimicrobial organic acids are weak acids and have a pKa (the pH at which the acid is half dissociated) between 3 and 5 (Dibner and Buttin, 2002), the pH of the external environment, by its effect on the dissociation of the organic acids, is the main primary determinant factor. At higher pH of the external environment, organic acid will be increasingly dissociated (Davidson, 2001; Dibner and Buttin, 2002; Ricke, 2003). The undissociated from of organic acids is lipophilic and can penetrate across the cell membrane of the bacterial and mold cells, and when they are into the neutral environment of the cytoplasm, dissociate into protons and anions (Eklund, 1985; Cherrington et al., 1990; Cherrington et al., 1991; Davidson, 2001; Ricke, 2003). Bacterial cells needs a near neutral pH in the cytoplasm, therefore a reduced cytoplasmic pH can disrupt enzymatic reactions and nutrient transportations (Cherrington et al., 1991; Dibner and Buttin, 2002; Ricke, 2003; van Immerseel et al., 2006). Furthermore, unloading the cells of the excess free protons requires energy and consumption of ATP, which may cause discharge of cellular energy and depletion of energy availability for cell proliferation (Davidson, 2001; Dibner and Buttin, 2002; Ricke, 2003). The other proposed antimicrobial mechanisms of organic acids are relating to the interference of organic acid with the cell membrane protein, cell membrane structure and eventually subsequent disruption of pH and electrical gradients across the cell membrane (Sheu and Freese, 1972; Sheu et al., 1972; Salmond et al., 1984; Russell, 1992; Davidson, 2001).

2.4.2 Bacterial Resistance to Antimicrobial Activities of Organic Acids

The sensitivity of bacteria to the certain organic acids can differ greatly among the forms (endospore versus vegetative cell) and strains (Heinzel, 1998; Dibner and Buttin, 2002; Ricke, 2003). For instance, vegetative cells are very susceptible and spores are the most resistant forms (Heinzel, 1998). It might be due to the fact that some bacteria under effect of organic acids are capable of allowing their cytoplasmic pH to decline (Russell, 1992) or could be because of less antimicrobial activities of some organic acids (Dibner and Buttin, 2002; Ricke, 2003). The antibacterial effect and the minimal inhibitory concentration (MIC) of one acid versus another can be highly different (Hsiao and Siebert, 1999; van Immerseel et al., 2006). For instance *E. coli* is approximately 10 times more resistant to malic, tartaric and citric acid compared to acetic, butyric, lactic and caprylic acid and on the other hand the MIC of acetic acid for *Bacillus subtilis* is 250 times lower than for lactobacilli (Hsiao and Siebert, 1999). The different antimicrobial abilities of organic acids might be explained by the different abilities of organic acids in disruption of cell membrane permeability, interference with nutrient transportation, interruption in macromolecular synthesis, creating damage in cell membrane and subsequent membrane leakage (Cherrington et al., 1991; Alakomi et al., 2000; Davidson, 2001; Ricke, 2003).

Each individual organic acid is better known for certain spectrum of antimicrobial activity (Dibner and Buttin, 2002). For instance, lactic acid is more known for antibacterial activity and sorbic acid is more effective against molds; however butyric, propionic and formic acid are known to be effective against fungi, bacteria and yeasts and have a wider antimicrobial spectrum (Doerr et al., 1995; Partanen and Mroz, 1999; Dibner and Buttin, 2002). It has been reported that mixtures of organic acids have synergistic antimicrobial activity *in vitro* (Dibner and Buttin, 2002; Huyghebaert, 2005). Thus, there is a tendency in feed industry to use a blend of organic acid (Mroz, 2000).

2.4.3 Organic Acids as Feed Additives

The use of organic acids, especially formic and propionic acid as well as their mixture, as feed additives in poultry nutrition is practically important for feed preservation, however, nutritional or health benefits are debated controversially (Canibe et al., 2001; Ricke, 2003; do Vale et al., 2004). Acids exert their effects in the feed prior to the consumption and/or upon ingestion by chicken, as feed is moistened by the bird's alimentary secretions, and comes into contact with the crop, proventriculus and intestinal endogenous secretions and acids (Cherrington et al., 1991). The organic acids in the crop and gizzard may reduce the bacterial activity and total bacterial load and, are specifically effective against *Campylobacter jejuni*, *Escherichia coli* and *Salmonella* spp. (Izat et al., 1990b; Hadorn et al., 2001; Dibner and Buttin, 2002). In several studies it has been reported that both formic and propionic acids were able to reduce *Salmonella* spp., *E. coli* and coliforms in the small intestinal, caecum and fecal contents of chickens (Hinton and Linton, 1988; Rouse et al., 1988; Izat et al., 1990a; McHan and Shotts, 1992; Ricke, 2003). Hinton and Linton (1988) and Berchieri and Barrow (1996) reported, that formic acid alone or in combination with propionic acid at a concentration of 0.6% in poultry diet were able to prevent infection with *Salmonella kedougou* and *Salmonella gallinarum*.

Less microbial proliferation in the small intestine causes less competition for the endogenous nitrogen between the host and microflora (Dibner and Buttin, 2002) and can decrease the microbial bile acid deconjugation (Klaver and van der Meer, 1993; Engberg et al., 2002). Microbial bile acid deconjugation can impair lipid digestion resulting in a poor broiler performance (Partanen et al., 2001). Therefore, the antimicrobial role of organic acids in the crop, ileum and caecum could be partly different. However, the major reason for using organic acids in poultry diet could be as aid in controlling horizontal bacterial transmission from the crop (Ewing and Cole, 1994).

2.4.4 Organic Acids in the Gastrointestinal Tract

The transportation and diffusion of organic acids mainly occur in undissociated form. The relatively low pH of the proximal part of the gastrointestinal tract in poultry tends to be supportive for the antimicrobial activity of organic acids and also their diffusion into the gut epithelium (Mroz, 2000; Dibner and Buttin, 2002). If organic acids reach to the distal part of the gut, the pH in the distal part of the gastrointestinal tract could be in favor of the dissociated form of organic acid, thus, the diffusion of organic acids reduces, however, the epithelial surface of gastrointestinal tract has an acidic microenvironments which permit the transportation of undissociated acid into the enterocytes and bacteria (Von Engelhardt et al., 1989; Dibner and Buttin, 2002). It has been reported that butyric, formic and propionic acid are readily metabolized and absorbed in the proximal part of the digestive tract and do not reach the distal part in sufficient quantities to be effective (Bolton and Dewar, 1965; Hume et al., 1993; Thompson and Hinton, 1997; Leeson et al., 2005; van Immerseel et al., 2006). Therefore, direct effects of organic acids on bacterial composition and metabolism in poultry may be assumed in the crop and proximal part of small intestine.

2.4.5 The Benefits of Organic Acids in Poultry Nutrition

The benefits of using organic acids in animal nutrition, specifically poultry nutrition, could be beyond their antimicrobial activities and modification of gut microbiota. It has been reported that individual organic acid and blends of organic acids, apart from their antimicrobial activity, reduce the digesta pH, improve in digestive enzymes and microbial phytase activity, increase the pancreatic secretion, have beneficial effects on the gastrointestinal microbiota and morphology, stimulate gastrointestinal cell proliferation and have trophic effects on the gastrointestinal mucosa (Jongbloed et al., 2000; Dibner and Buttin, 2002). Variables such as buffering capacity of dietary ingredients, the pancreatic secretion, presence of other antimicrobial compounds, the hygienic status of production environment, and finally the heterogeneity of the gut microbiota make the benefits of using organic acids in poultry diets less predictable (Dibner and Buttin, 2002).

Several studies have investigated the effects of organic acids supplementation in diets on poultry performance. Some findings indicated a negative influence on palatability, appetite and FI (Cave, 1984). Moreover, it has been shown that formic and propionic acid, their salts, lactic and fumaric acid could have a positive effect on feed efficiency (Runho et al., 1997; Paul et al.,

2007) and propionic, tartaric and sorbic acid could improve growth performance (Vogt et al., 1982).

Supplementation of 0.5 and 1% fumaric acids in a broiler diet improved BWG and had no effect on FI (Patten and Waldroup, 1988). The supplementation of male broiler chicks with 0.125, 0.25 and 0.50% fumaric acid improved FCR and BWG (Skinner et al., 1991). Broiler chicks fed a diet containing 0.5 to 1% fumaric acid had significantly lower FI and better feed efficiency compared to the control group (Runho et al., 1997). Adding 0.2% of butyric acid to a broiler diet had no significant effect on FI, however, in vaccinated chicken challenged with coccidia, was helpful in order to maintain performance of broiler chicks comparable to the control group (Leeson et al., 2005). The supplementation of the propionic and formic acid mixture below 1% in the broiler diets had no significant effect on the final performance variables (Kaniawati et al., 1992; do Vale et al., 2004; Isabel and Santos, 2009). A blend of 2.1 g/kg propionic acid and 5 g/kg formic had no significant effect on performance variables after the second week of the broiler chick's fattening period (Isabel and Santos, 2009). An identical observation was reported in the study in which 5 or 10 g formic acid/kg was investigated (Hernandez et al., 2006). Under hygienic conditions, dietary formic acid did not have a positive effect on the performance of broiler chickens, even if there was a very slight positive effect on the apparent ileal digestibility of dry matter (Hernandez et al., 2006). Buffered propionic acid at 0, 0.2, 0.4, and 0.8% had no positive effect on BWG and feed utilization (Izat et al., 1990b). In a study, in which broiler chickens received 0, 0.25, 0.50, 1.0 and 2.0% of a mixture with formic and propionic acid, it was shown that the effective doses of this blend for the control of *Salmonella* spp. did not affect broiler performance. The inclusion of 1% organic acids in the diet resulted in a performance similar to that of untreated birds (do Vale et al., 2004). Similarly to this study, the supplementation of broiler diets with 1% of a blend of formic and propionic acid (Kaniawati, 1993) and 1% formic acid or 1.45% calcium formate (Izat et al., 1990a) did not influence growth and feed efficiency in broilers at 42 d of age. Ammonium formate or calcium propionate (3g/kg diet) inclusions improved the BW at d 21 and the feed efficiency of broiler chicks at d 42 of age, whereas no significant effect on BW was observed at d 42 of age. It was reported that the use of the salt form of these organic acids in broiler diets lowered feed consumption, but the growth was comparable with control birds and FCR was improved (Paul et al., 2007).

3 CHAPTER 3: *AIMS AND OBJECTIVES*

Thermal treatment of feed has an impact on the feed hygiene, while simultaneously might affect feed digestibility, subsequent nutrient availability for the gut microbiota and the performance of broilers. Organic acids supplementation might conserve feed from subsequent contaminations and may simultaneously affect gut function, gut microbiota and the feed digestion process. However, the interactions of different thermal processes and organic acid levels have not been investigated to date. Despite a promising nutritional effect, the interaction of thermal processing methods and the addition of organic acids have not been widely studied and there is a little information assessing the effect of organic acid supplementation, solely or/and in combination with thermal processing, on gut microbiota, performance and particularly feed digestibility in broilers. Therefore, the aim of the present dissertation was to evaluate the effect of different thermal treatments including pelleting, long-term conditioning at 85°C for 3 minutes, or expanding at 110°C and 130°C for 3-5 seconds without or with 0.75 and 1.5% organic acids supplementation (63.75% formic acid, 25.00% propionic acid and 11.25% water) and their interactions on:

1. Hygienic status of broiler feed
2. Bacterial composition in crop, ileum and caecum of broilers
3. Bacterial metabolism in crop, gizzard, ileum and caecum of broilers
4. Relative weights of the proventriculus, gizzard, duodenum, ileum, caecum and pancreas
5. Nutrient digestibility of broiler feed
6. Performance variables of broiler chicks

The results of the present thesis have been reported and described in the published manuscripts of the following chapters **(Chapter 4 and Chapter 5)**.

4 CHAPTER 4: The Effects of Different Thermal Treatments and Organic Acids Levels on Nutrient Digestibility in Broilers

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5 CHAPTER 5: The Effects of Different Thermal Treatments and Organic Acids Levels in Feed on Microbial Composition and Activity in Gastrointestinal Tract of Broilers

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6 CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

The results on hygienic status of feed, performance and nutrients digestibility, bacterial composition and activity in gastrointestinal tract of broiler chicks have been described and discussed in detail in chapters 4 and 5.

In accordance with the results of other studies, the findings of the decontamination trial in the current study demonstrated that the applied thermal treatments and organic acids inclusion were effective strategies in order to decontaminate broiler feed (Mossel et al., 1967; Cover et al., 1984; Israelsen et al., 1996; Ricke, 2003; Martin et al., 2005; Ricke, 2005; Okelo et al., 2006; van Immerseel et al., 2006).

The inclusion of organic acids mixture in the broiler diet linearly improved FCR at 7 d of age ($P \leq 0.05$), but did not affect performance variables after the first week. The organic acids inclusion had no significant effect on the pH of the gastrointestinal tract, relative organ weights and ileal digestibilities of AA and crude protein. The effects of organic acids supplementation on the metabolites and bacterial cell numbers were remarkable in the crop, but appeared to be reduced along the length of the gut. The organic acids inclusion drastically reduced the overall bacterial activity in the crop. The dominant bacterial group in the crop was lactobacilli, and although lactic acid was reduced due to organic acids inclusion, no differences were observed for lactobacilli cell counts. However the organic acids supplementation slightly reduced the ileal lactobacilli and enterobacteria cell numbers as well as moderately increased the ileal acetate concentration. The same study was also conducted to investigate the effects of thermal treatments (P, L and E130) and organic acids inclusion levels (0 and 1.5%) on the apparent ileal absorption of calcium, phosphorus, sodium, potassium, magnesium, copper, iron, zinc and, manganese as well as various tibial quality parameters and retention of mentioned minerals in tibia and liver of broiler chicks (Hafeez et al., 2014). The inclusion of organic acids had no marked effect on the apparent ileal absorption of the investigated trace elements and minerals as well as the tibial mineral concentrations, tibial quality parameters and retention of investigated mineral in hepatic tissue ($P > 0.05$). The reason for these findings might be provided by taking into account that the ingested formic and propionic acid are readily metabolized and absorbed in the proximal part of the gastrointestinal tract and do not reach the distal part in sufficient quantities to be significantly effective (Hume et al., 1993; Thompson and Hinton, 1997; van Immerseel et al., 2006). Therefore, the beneficial effects of organic acids in poultry nutrition can only be assumed for the parameters and variables related to the upper part of gastrointestinal tract, specifically, in the crop, gizzard and proximal small intestine. The observed effects in the distal part of digestive tract in the present study could probably be due to the changes in the crop microbiota that reached the small intestine.

The different thermal treatments in the present study had no remarkable effect on broiler performance variables. However, the long-term thermal conditioning significantly impaired the apparent ileal digestibilities of crude protein and AA, except cysteine ($P \leq 0.05$). A high temperature in combination with moisture and shear would provide favorable conditions for the Maillard reaction to occur (Mauron, 1981; Cheftel, 1986). Moreover, a high temperature may cause marked degradation of the most heat-labile AA, lysine, followed by threonine, arginine and serine (Papadopoulos, 1989; Svihus and Zimonja, 2011). The apparent ileal absorption of calcium, sodium and phosphorus was significantly higher in E130 group compared to L group (Hafeez et al., 2014). In contrast to the ileal apparent absorption and digestibility results, the performance variables were not negatively affected by the thermal processing treatments, indicating that the safety margins for the minerals and AA recommended allowances for broiler are obviously high enough to cover the observed differences in the mineral absorption and AA digestibilities. In fact the total amounts of absorbed minerals and digested AA in the ileum were sufficient to cover the needs of broiler chicks at high growth rates.

The cell number of *Lactobacillus* spp. in the crop and ileum were higher in E130 group compared to groups P and L, while clostridia and enterobacteria in the crop seemed unaffected by thermal processes. The cell number of *Bifidobacterium* spp. in the crop was lower for groups P and E130 in comparison to group E110 ($P \leq 0.05$), but this effect was reversed in the ileum. Furthermore, in the crop, a high numeric increase for lactate as well as a significant increase for acetate was found for the E130 group compared to all other thermal processes. The ileal SCFA concentrations remained identical but the ileal lactate concentrations increased in expansion groups. Therefore, the impact of the different thermal treatments in the crop and the small intestine was mostly confined to lactobacilli and their metabolism. On the other hand, the relative weights of jejunum and small intestine were significantly higher in the E130 group compared to P and L ($P \leq 0.05$).

Therefore, the fermentation activity in the proximal part of gastrointestinal tract and the relative weights of jejunum and small intestine were higher in the birds fed expanded diets compared to those fed pelleted and long term conidtioned diets. It might be explained by the fact that expansion changes the size of microstructural particles, reduces the density of feed particles and increases the fiber solubility and starch availability of the diet (Armstrong, 1994; Peisker, 1994). Soluble fibers are considered as anti-nutritional factor contributing to the high viscosity of digesta, inhibiting digestion, impairing absorption and thereby reducing nutrient digestibility (Smits and Annison, 1996). The soluble fibers compared to insoluble ones are generally more rapidly and easily fermented in the gut and this makes their influence on the bacterial composition and activity significant (Choct et al., 1996; Langhout, 1998; Bach Knudsen, 2001; Montagne et al., 2003; Hetland et al., 2004). Furthermore it has been reported that the soluble fibers could cause some morphological changes in the small intestine and increase its weight (Smits et al., 1997; Iji, 1999; Iji et al., 2001). It seemed that the expansion process in the present study increased the portion of soluble fiber in the broiler diets which might cause marked improvement in the accessibility of the fermentable carbohydrates for bacteria, in general, and for lactobacilli, in particular. Thus, the production of lactic acid by lactobacilli could be increased and subsequently might change the morphology of the small intestine.

In conclusion, the results of the present dissertation indicated that the applied thermal and organic acid treatments were efficient strategies in order to hygienize the poultry feed. The long-term thermal conditioning might decrease the ileal nutrient digestibility, while pelleting and expansion, without or/and with organic acids supplementation, seem to be less critical. While expansion increased lactobacilli and lactate in the crop and ileum, organic acids addition remarkably influenced bacterial composition and activity in the crop. Regarding the observed effects of applied thermal and organic acids treatments on the microbiology of gastrointestinal tract, they might have modification effects on the bacterial composition of the upper gastrointestinal tract in broiler chicks that may offer interesting perspectives to achieve a better control of intestinal bacterial colonization in broilers. These indicate the potential of expansion process and organic acids addition for hygienization of broiler feed.

7 CHAPTER 7: SUMMARY

Title of the PhD thesis: **The Effects of Different Thermal Treatments and Organic Acids Levels in Feed on Nutrient Digestibility and Gut Microbiota in Broilers.**

Poultry feed could be a potential vector in the transmission of pathogens to the poultry. The processing facilities of poultry products can be contaminated by carrier or infected carcasses which lead to transmit of pathogens through the food chain to human. There is a correlation between the presence of *Salmonella* in poultry feed and their isolation on poultry meet. On the other hand, over 50% of all traceable human cases of salmonellosis have been associated with the consumption of eggs and other poultry products. The need for biological safety of the poultry products initiated a worldwide search to find an economically efficacious feed management strategy, which decontaminates feed, and meanwhile, would be putatively beneficial for the gut microbiota and have no negative impact on the animal welfare, physiology, performance, nutrient digestibility and consumer health (**Chapter 1**).

In **Chapter 2**, a summary of the literature on different types of feed management strategies is given. The main objective of the present thesis was to investigate the effects of different thermal treatments including pelleting, long-term conditioning at 85°C for 3 minutes, or expanding at 110°C and 130°C for 3-5 seconds without or with 0.75 and 1.5% organic acid supplementation (63.75% formic acid, 25.00% propionic acid and 11.25% water) and their interactions on hygienic status of broiler feed, nutrient digestibility and bacterial composition and activity in gastrointestinal tract of broiler chicks. The detailed information on the main aims and hypotheses of the present study can be found in **Chapter 3**.

In **Chapter 4 and 5**, the effects of different thermal treatments including pelleting (P), long-term conditioning at 85°C for 3 minutes (L), or expanding at 110°C (E110) and 130°C for 3-5 seconds (E130) without or with 0.75 and 1.5% organic acids supplementation on hygienic status of feed, performance, nutrient digestibility, gastrointestinal microbiota and organ weights of broilers are investigated an disccused. In total, 960 one-day-old broiler chicks were randomly assigned to 8 replicates using a 3 × 4 factorial arrangement. Performance variables were determined, and the relative organ weights, ileal and total amino acid (AA) digestibilities were measured at d 35. Also, at d 35, bacterial cell numbers in the crop, ileum and caecum, and bacterial metabolites in the crop, gizzard, ileum and caecum were determined. The organic acids inclusion linearly improved feed efficiency in the first week ($P \leq 0.05$). The acid inclusion levels and thermal treatments had no significant effect on the performance variables at later intervals of the growing period. The inclusion of organic acids had a quadratic effect on total and ileal digestibility of isoleucine ($P \leq 0.05$); while it had no significant effect on the ileal and total digestibility of other AA and nutrients. The inclusion of 1.5% organic acids increased cell numbers of all clostridial clusters in the crop. The organic acids supplementation increased the propionic acid concentration in the crop and gizzard whilst there was a decrease in lactic acid concentration. In the ileum, the 0% organic acids group had the highest numbers of *Lactobacillus* spp. and enterobacteria. Inclusion of 1.5% organic acids increased ileal acetate concentration. The L group showed the lowest ileal AA and crude protein digestibility. The relative weights of jejunum and small intestine were significantly higher in the E130 group compared to P and L ($P \leq 0.05$). Increasing the feed processing temperature led to an increase of lactobacilli in the crop

and ileum, while clostridia and enterobacteria seemed unaffected. Similarly, lactate concentrations increased in the ileum, but short chain fatty acids remained identical. In the crop, an increase for acetate was found for the E130 group compared to all other thermal treatments.

In conclusion, the results of the present thesis demonstrated that the applied thermal and organic acids treatments were efficient strategies in order to hygienize broiler feed. The long-term thermal conditioning might decrease the ileal nutrient digestibility, while pelleting and expansion, without or/and with organic acids supplementation, seemed to be less critical. While expansion increased lactobacilli and lactate in the crop and ileum, organic acid addition remarkably influenced bacterial composition and activity in the crop. Taking into account all the observed effects of applied thermal and organic acids treatments on the microbiota of gastrointestinal tract, the evaluated treatments in the present study might have positive effects on the bacterial composition and activity of the upper gastrointestinal tract in broiler chicks that might offer interesting perspectives to achieve a better control of intestinal bacterial colonization in poultry. These indicate the potential of expansion process and organic acids addition for the hygienization of broiler feed (**Chapter 6**).

8 KAPITEL 8: ZUSAMMENFASSUNG

Titel der Dissertation: **Der Einfluss verschiedener Wärmebehandlungen und Zulagen organischer Säuren im Futter auf die Nährstoffverdaulichkeit und die Mikrobiota des Darms von Broilern.**

Geflügelfutter stellt eine potenzielle Übertragungsmöglichkeit von pathogenen Erregern auf das Geflügel dar. Zur Verbesserung der Sicherheit bei Geflügelprodukten sind ökonomische Fütterungsstrategien erforderlich, die das Futter dekontaminieren, vorteilhaft für die Mikrobiota des Darms sind und die keine negativen Auswirkungen auf die Tiergesundheit, die Physiologie und Leistung der Tiere, die Nährstoffverdaulichkeit und die Sicherheit der Lebensmittel haben (**Kapitel 1**).

In **Kapitel 2** sind verschiedene Fütterungsstrategien aus der Literatur zusammengefasst. Ziel dieser Arbeit war es den Einfluss verschiedener Wärmebehandlungen, darunter das Pelletieren, die Langzeit-Aufbereitung bei 85 °C für 3 Minuten, oder die Expansion bei 110 °C und 130 °C für 3-5 Sekunden mit oder ohne den Zusatz von 0,75 und 1,5 % organischer Säure (63,75 % Ameisensäure, 25,00 % Propansäure und 11,25 % Wasser) und deren Wechselwirkungen auf den Hygienestatus von Broilerfutter, die Nährstoffverdaulichkeit und bakterielle Zusammensetzung und die Aktivität des Magen-Darm-Trakts von Broilerküken zu untersuchen. Detaillierte Informationen zu den Hauptzielen und Hypothesen dieser Arbeit sind in **Kapitel 3** aufgeführt.

In **Kapitel 4 und 5** wurden die Einflüsse verschiedener Wärmebehandlungen, darunter das Pelletieren (P), Langzeit-Aufbereitung bei 85 °C für 3 Minuten (L), oder Expansion bei 110 °C (E110) und 130 °C für 3-5 Sekunden (E130) mit oder ohne den Zusatz von 0,75 und 1,5 % organischer Säure auf den Hygienestatus des Futters, die Leistung, Nährstoffverdaulichkeit, die Mikrobiota des Magen-Darm-Trakts und die Organgewichte von Broilern untersucht und diskutiert. Insgesamt wurden 960 Eintagsküken randomisiert in einem 3 × 4 faktoriellen Versuchsplan acht Wiederholungen zugeordnet. Die Leistungsvariablen wurden ermittelt und die relativen Organgewichte sowie die ileale und die gesamte Verdaulichkeit der Aminosäuren an Tag 35 gemessen. An Tag 35 wurden zudem die bakteriellen Zellzahlen in Kropf, Ileum und Caecum sowie die bakteriellen Metabolite in Kropf, Muskelmagen, Ileum und Caecum bestimmt. Der Zusatz von organischen Säuren führte zu einer Verbesserung der Futtereffizienz in der ersten Woche ($P \leq 0.05$). Die verschiedenen Mengen an zugeführten organischen Säuren und die Wärmebehandlungen zeigten zu späteren Zeitpunkten der Wachstumsphase keine signifikanten Auswirkungen auf die Leistungsvariablen. Der Säurenzusatz zeigte einen quadratischen Effekt auf die ileale und die Gesamtverdaulichkeit von Isoleucin ($P \leq 0.05$), hatte jedoch keinen signifikanten Einfluss auf die anderen Aminosäuren und Nährstoffe. Der Zusatz von 1,5% eines Produkts mit organischen Säuren erhöhte die Zellzahl aller Clostridiengruppen im Kropf. Durch die Zugabe erhöhte sich die Propionsäurekonzentration in Kropf und Muskelmagen bei einem gleichzeitigen Absinken der Milchsäurekonzentration. Im Ileum zeigte die Gruppe ohne Säurenzusatz die höchsten Zellzahlen an *Lactobacillus* spp. und Enterobakterien. Der Zusatz organischer Säuren erhöhte die ileale Acetatkonzentration. Die L-Gruppe zeigte die geringste ileale Aminosäuren- und Rohproteinverdaulichkeit. Die relativen Gewichte des Jejunums und des gesamten Dünndarms waren in der E130 Gruppe signifikant höher als in den Gruppen P und L ($P \leq 0.05$). Eine Erhöhung der Verarbeitungstemperatur des Futters führte zu einer erhöhten Anzahl an Milchsäurebakterien in Kropf und Ileum, wobei Clostridien und Enterobakterien unbeeinflusst schienen. Zudem erhöhten sich die Laktatkonzentrationen im Ileum, die kurzkettigen Fettsäuren blieben jedoch unverändert. Im Kropf konnte ein erhöhter Acetatgehalt in der E130 Gruppe im Vergleich zu allen anderen Wärmebehandlungen gezeigt werden.

Schlussfolgernd konnten die Ergebnisse dieser Arbeit zeigen, dass die angewandten Wärmebehandlungen und der Einsatz organischer Säuren effiziente Strategien einer Hygienisierung von

Broilerfutter darstellen. Die Langzeiterhitzung verringert möglicherweise die ileale Nährstoffverdaulichkeit, während Pelletierung und Expansion mit oder ohne Zugabe organischer Säure weniger bedenklich erscheinen. Während die Expansion Milchsäurebakterien und Laktat in Kropf und Ileum erhöhte, beeinflusste die Zugabe organischer Säuren die bakterielle Zusammensetzung und Aktivität im Kropf.

Aufgrund der beobachteten Effekte von Wärmebehandlung und organischen Säuren auf die Mikrobiota des Magen-Darm-Trakts führen diese beiden Ansätze möglicherweise zur Veränderung der bakteriellen Zusammensetzung des oberen Magen-Darm-Trakts bei Broilerküken, wodurch sich interessante Aspekte für eine Kontrolle der bakteriellen Besiedlung beim Geflügel ergeben.

9 REFRENCES

Abdollahi, M. R., V. Ravindran, T., Wester, G., Ravindran, and D. Thomas. 2010a. Influence of conditioning temperature on the performance, nutrient utilisation and digestive tract development of broilers fed on maize-and wheat-based diets. Br. Poult. Sci. 51:648-657.

Abdollahi, M. R., V. Ravindran, T., Wester, G., Ravindran, and D. Thomas. 2011. Influence of feed form and conditioning temperature on performance, apparent metabolisable energy and ileal digestibility of starch and nitrogen in broiler starters fed wheat-based diet. Anim. Feed Sci. Technol. 168:88-99.

Abdollahi, M. R., V. Ravindran, and B. Svihus. 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. Anim. Feed Sci. Technol. 179:1-23.

Abdollahi, M. R., V. Ravindran, T. J. Wester, G. Ravindran, and D. Thomas. 2010b. Influence of conditioning temperature on performance, apparent metabolisable energy, ileal digestibility of starch and nitrogen and the quality of pellets, in broiler starters fed maize- and sorghum-based diets. Anim. Feed Sci. Technol. 162:106-115.

Adams, C., H. A. Vahl, and A. Veldman. 1996. Interaction between nutrition and Eimeria acervulina infection in broiler chickens: diet compositions that improve fat digestion during Eimeria acervulina infection. Br. J. Nutr. 75:875-880.

Alakomi, H.-L., E. Skyttä, M. Saarela, T. Mattila-Sandholm, K. Latva-Kala, and I. Helander. 2000. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Appl. Environ. Microbiol. 66:2001-2005.

Almas, K., and A. E. Bender. 1980. Effect of heat treatment of legumes on available lysine. J. Sci. Food Agric. 31:448-452.

Amerah, A., V. Ravindran, R., Lentle, and D. Thomas. 2007a. Feed particle size: Implications on the digestion and performance of poultry. World's Poult. Sci. J. 63:439-455.

Amerah, A., V. Ravindran, R., Lentle, and D. Thomas. 2007b. Influence of feed particle size and feed form on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters. Poult. Sci. 86:2615-2623.

Amerah, A., V. Ravindran, R., Lentle, and D. Thomas. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat-and corn-based diets. Poult. Sci. 87:2320-2328.

Armstrong, H. 1994. Nutritional implications of expanded feed. Feed Mix 45:385-385.

Axe, D.E. 1995. Factors affecting uniformity of a mix. Anim. Feed Sci. Technol. 53:211-220.

Bach Knudsen, K.E. 2001. The nutritional significance of "dietary fibre" analysis. Anim. Feed Sci. Technol. 90:3-20.

Bains, B., and M. A. MacKenzie. 1974. Transmission of *Salmonella* through an integrated poultry organisation. Poult. Sci. 53:1114-1118.

Bartikoski, R. 1962. The effect of steam on pellet durability. Cost Reductions Through In-Plant Production Controls. Feed Production School, Kansas City, MO, 42-47.

Behnke, K. 1994. Factors affecting pellet quality, Proceedings-Maryland Nutrition Conference. Dept. of Poultry Science and Animal Science, College of Agriculture, Univ. of Maryland, College Park.

Behnke, K.C. 1996. Feed manufacturing technology: Current issues and challenges. Anim. Feed Sci. Technol. 62:49-57.

Behnke, K. C., and R. S. Beyer. 2002. Effect of feed processing on broiler performance. In: VIII. International Seminar on Poultry Production and Pathology , Santiago, Chile. Accessed Mar. 17. 2014. http://nmfeed.com/Files/Posts/Portal1/processing_1.pdf

Berchieri, A., and P. Barrow. 1996. Reduction in incidence of experimental fowl typhoid by incorporation of a commercial formic acid preparation (Bio-Add™) into poultry feed. Poult. Sci. 75:339-341.

Beyer, R., J. Moritz, K., Wilson, and K. Cramer. 2000. The effect of feed processing and feed form on animal performance, Proceedings of 61st Minnesota Nutrition Conference, Bloomington, MN, USA, Citeseer. Pages 225-235.

Bolton, W., and W. Dewar. 1965. The digestibility of acetic, propionic and butyric acids by the fowl. Br. Poult. Sci. 6:103-105.

Briggs, J. L., D. E. Maier, B. A., Watkins, and K. C. Behnke. 1999. Effect of ingredients and processing parameters on pellet quality. Poult. Sci. 78:1464-1471.

Broz, J., E. Schai, and M. Gadient. 1997. Micronutrient stability in feed processing. Report No. FT42. American Soybean Association, Singapore

Calet, C. 1965. The relative value of pellets versus mash and grain in poultry nutrition. World's Poult. Sci. J. 21:23-52.

Camire, M. E., A. Camire, and K. Krumhar. 1990. Chemical and nutritional changes in foods during extrusion. Crit. Rev. Food Sci. Nutr. 29:35-57.

Canibe, N., R. M. Engberg, and B. B. Jensen. 2001. An overview of the effect of organic acids on gut flora and gut health. Accessed 2013 May: Accessed 2013 May http://pure.au.dk/portal/en/publications/an-overview-of-the-effect-of-organic-acids-on-gut-flora-and-gut-health(46cc4cb0-05c2-11db-aa3e-000ea68e967b).html

Cave, N.A. 1984. Effect of dietary propionic and lactic acids on feed intake by chicks. Poult. Sci. 63:131-134.

Cheftel, J. C. 1986. Nutritional effects of extrusion-cooking. Food Chem. 20:263-283.

Cherrington, C., I. Chopra, and M. Hinton. 1991. Acid treatment of feed for the control of *Salmonella* infections in poultry. Veterinary Annual 31.

Cherrington, C. A., M. Hinton, and I. Chopra. 1990. Effect of short-chain organic acids on macromolecular synthesis in Escherichia coli. J. Appl. Microbiol. 68:69-74.

Choct, M., R. Hughes, J. Wang, M. Bedford, A. Morgan, and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci. 37:609-621.

Coelho, M. 1994. Comparison with pelleting and extrusion: vitamin stability in expanders. Feed management. 45:10-15.

Cover, M. S., J. T. Gary, and S. F. Binder. 1984. Reduction of standard plate counts, total coliform counts and *Salmonella* by pelletizing animal feed. Proceedings of the International

Symposium on *Salmonella*. New Orleans, LA, July 19–20, 1984. G. H. Snoeyenbos, ed. American Association of Avian Pathologists, Kennett Square, PA. Pages 221–231.

Cox, N. A., D. Burdick, J. S. Bailey, and J. E. Thomson. 1986. Effect of the steam conditioning and pelleting process on the microbiology and quality of commercial-type poultry feeds. Poult. Sci. 65:704-709.

Crump, J. A., P. M. Griffin, and F. J. Angulo. 2002. Bacterial contamination of animal feed and its relationship to human foodborne illness. Clin. Infect. Dis. 35:859-865.

Cutlip, S., J. Hott, N. Buchanan, and J. Moritz. 2006. Effect of steam conditioning practices on pellet quality and growing broiler nutritional value. Poult. Sci. 85:51-52.

Cutlip, S. E., J. M. Hott, N. P. Buchanan, A. L. Rack, J. D. Latshaw, and J. S. Moritz. 2008. The Effect of Steam-Conditioning Practices on Pellet Quality and Growing Broiler Nutritional Value. J. Appl. Poult. Res. 17:249-261.

Davidson, P.M. 2001. Chap. 29. Chemical preservatives and natural antimicrobial compounds. Food Microbiology—Fundamentals and Frontiers. 2nd ed. M. P. Doyle, L. R. Beuchat, and T. J. Montville. Pages 593–627.

Davies, R. H., and C. Wray. 1995. Observations on disinfection regimens used on *Salmonella* enteritidis infected poultry units. Poult. Sci. 74:638-647.

Dibner, J., and P. Buttin. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res. 11:453-463.

Dixon, R. C., and P. B. Hamilton. 1981. Effect of feed ingredients on the antifungal activity of propionic acid. Poult. Sci. 60:2407-2411.

do Vale, M. M., J. F. M. Mentende, S. C. D. Morais, and M. M. de Almeida Brainer. 2004. Mixture of formic and propionic acid as additives in broiler feeds. Scientia Agricola 61, 371-375.

Doerr, J., F. Attard, E., Doerr, and W. Robey. 1995. Possible anti-fungal effects of hydroxy-methylthio-butanoic acid (HMB). Poult. Sci 74: 23.

Dozier, W., 2001. Cost-effective pellet quality for meat birds. Feed Management 52:21-24.

Duffus, C. M., and H. J. Duffus. 1991. Toxic substances in crop plants. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge CB4 4WF, Cambridge (1991). Pages 1–21.

Ebel, E. D., M. J. David, and J. Mason. 1992. Occurrence of *Salmonella enteritidis* in the US commercial egg industry: report on a national spent hen survey. Avian Dis. 646-654.

Eklund, T. 1985. Inhibition of microbial growth at different pH levels by benzoic and propionic acids and esters of *p*-hydroxybenzoic acid. Int. J. Food Microbiol. 2:159-167.

Engberg, R. M., M. S. Hedemann, and B. B. Jensen. 2002. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. Br. Poult. Sci. 43:569-579.

Ewing, W., and D. Cole. 1994. Micro-flora of the gastro-intestinaltract. The Living Gut—An Introduction to Micro-organisms in Nutrition. Context Publication, Tyrone, UK. Pages 45–65.

Falk, D., 1985. Pelleting cost center. Feed manufacturing technology III. 3:167-190.

Fancher, B. I., D. Rollins, and B. Trimbee. 1996. Feed processing using the annular gap expander and its impact on poultry performance. J. Appl. Poult. Res. 5:386-394.

Furuta, K., S. Morimoto, and S. Sato. 1980a. Bacterial contamination in feed ingredients, formulated chicken feed and reduction of viable bacteria by pelleting. Lab Anim. 14:221-224.

Furuta, K., I. Oku, and S. Morimoto. 1980b. Effect of steam temperature in the pelleting process of chicken food on the viability of contaminating bacteria. Lab Anim. 14:293-296.

Goelema, J., A. Smits, L., Vaessen, and A. Wemmers. 1999. Effects of pressure toasting, expander treatment and pelleting on *in vitro* and *in situ* parameters of protein and starch in a mixture of broken peas, lupins and faba beans. Anim. Feed Sci. Technol. 78:109-126.

Goodband, R. D., M. D. Tokach, and J. L. Nelssen. 2002. The effects of diet particle size on animal performance. MF-2050 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University. 6 pages.

Gracia, M., M. Latorre, M. Garcia, R. Lazaro, and G. Mateos. 2003. Heat processing of barley and enzyme supplementation of diets for broilers. Poult. Sci. 82:1281-1291.

Hadorn, R., H. Wiedmer, and D. Feuerstein. 2001. Effect of different dosages of an organic-acid mixture in broiler diets. Archiv fur Geflugelkunde 65:22-27.

Hafeez, A., A. Mader, F. Goodarzi Boroojeni, I. Ruhnke, I. Röhe, K. Männer, and J. Zentek, J. 2014. Impact of thermal and organic acid treatment of feed on apparent absorption and metabolism of minerals and tibia quality in broilers. Poul. Sci. 93:1754–1763.

Heidenreich, E. 1994. Operation strategies for expansion cooking. Feed Mix 2, 32-34.

Heidenreich, E. 2001. Futtermittelhygiene. In: Strauch D, Böhm R (Eds). Reinigung und Desinfektion in der Nutztierhaltung und Veredelungswirtschaft. Stuttgart: Enke Verlag.

Heinzel, M. 1998. Phenomena of biocide resistance in microorganisms. Int. Biodeterior. Biodegrad. 41:225-234.

Hernandez, F., V. Garcia, J. Madrid, J. Orengo, P. Catala, and M. D. Megias. 2006. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. Br. Poult. Sci. 47:50-56.

Hetland, H., M. Choct, and B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. World's Poult. Sci. J. 60:415-422.

Himathongkham, S., M. das Graças Pereira, and H. Riemann. 1996. Heat destruction of *Salmonella* in poultry feed: effect of time, temperature, and moisture. Avian Dis. 72-77.

Hinton, M., and A. H. Linton. 1988. Control of *Salmonella* infections in broiler chickens by the acid treatment of their feed. Vet. Rec. 123:416-421..

Hsiao, C. P. and K. J. Siebert. 1999. Modeling the inhibitory effects of organic acids on bacteria. Int. J. Food Microbiol. 47:189-201.

Hume, M. E., D. E. Corrier, G. W. Ivie, and J. R. Deloach, J.R., 1993. Metabolism of [C-14] propionic-acid in broiler chicks. Poult. Sci. 72:786-793.

Hutchison, M., D. Thomas, and S. Avery. 2007. Thermal death of *Escherichia coli* O157: H7 in cattle feeds. Lett. Appl. Microbiol. 44:357-363.

Huyghebaert, G. 2005. Alternatives for antibiotics in poultry. In: N. Zimmermann (Ed). Proceedings of the 3rd Mid-Atlantic Nutrition Conference. 36-57.

Iji, P., 1999. The impact of cereal non-starch polysaccharides on intestinal development and function in broiler chickens. World's Poult. Sci. J. 55:375-387.

Iji, P.A., A. Saki, and D. R. Tivey. 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br. Poult. Sci. 42:505-513.

Isabel, B., and Y. Santos. 2009. Effects of dietary organic acids and essential oils on growth performance and carcass characteristics of broiler chickens. J. Appl. Poult. Res. 18:472-476.

Israelsen, M., J. Busk, M. Virsoe, and I. Hansen. 1996. Reduction of *Salmonella* in compound feed by expanding and pelleting. Kraftfutter.

Izat, A. L., R. E. Hierholzer, J. M. Kopek, M. H. Adams, J. P. Mcginnis, and M. A. Reiber. 1990a. The Use of Propylene-Glycol and or Lactic-Acid in Chill Water for Reducing Salmonellae on Broilers. J. Food. Process. Press. 14:369-374.

Izat, A. L., N. M. Tidwell, R. A. Thomas, M. A. Reiber, M. H. Adams, M. Colberg, P. W. Waldroup. 1990b. Effects of a buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. Poult. Sci. 69:818-826.

Jensen, L., A. Aumaitre, B. Lee, and J. Ha. 2000. Influence of pelleting on the nutritional needs of poultry, Proceedings of 2000 International Symposium Recent Advances in Animal Nutrition, Seoul, Korea, 20-22 April 2000., Pages 35-46.

Jensen, L., G. Ranit, R. Wagstaff, and J. McGinnis. 1965. Protein and lysine requirements of developing turkeys as influenced by pelleting. Poult. Sci. 44:1435-1441.

Jones, F., K. Anderson, and P. Ferket. 1995. Effect of extrusion on feed characteristics and broiler chicken performance. J. Appl. Poult. Res. 4: 00-309.

Jones, F., R. Axtell, D. Rives, S. Scheideler, Jr. F. Tarver, R. Walker, and M. Wineland. 1991. A survey of *Salmonella* contamination in modern broiler production. J. Food Prot. 54.

Jones, F., Richardson, K., 2004. *Salmonella* in commercially manufactured feeds. Poult. Sci. 83:384-391.

Jones, F. T. 2011. A review of practical *Salmonella* control measures in animal feed. J. Appl. Poult. Res. 20:102-113.

Jongbloed, A., Z. Mroz, R. van der Weij-Jongbloed, and P. Kemme. 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livest. Prod. Sci. 67:113-122.

Kadam, S. S., R. R. Smithard, M. D. Eyre, and D. G. Armstrong. 1987. Effects of heat treatments of antinutritional factors and quality of proteins in winged bean. J. Sci. Food Agric. 39:267-275.

Kaniawati, S. 1993. Effects of feeding organic acids to broilers on performance and *Salmonella* colonization of the ceca and/or contamination of the carcass. University of Arkansas.

Kidd, M. T., A. Corzo, S. M. Hill, C. D. Zumwalt, E. H. Robinson, and W. A. Dozier. 2005. Growth and meat yield responses of broilers provided feed subjected to extrusion cooking. J. of Appl. Poult. Res. 14:536-541.

Kirkpinar, F., and H. Basmacioglu. 2006. Effects of pelleting temperature of phytase supplemented broiler feed on tibia mineralization, calcium and phosphorus content of serum and performance. Czech J. Anim. Sci. 51:78.

Klaver, F., and R. van der Meer. 1993. The assumed assimilation of cholesterol by Lactobacilli and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. Appl. Environ. Microbiol. 59:1120-1124.

Knacke, O., and H. Pohl. 1959. Experimenteller Beitrag zu den Grundlagen des Pelletisierens. Chem. Ing. Tech. 31:50-54.

Koch, K. 1996. Hammermills and roller mills. MF-2048 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University. 8 pages.

Koenig, H. 1995. *Salmonella* decontamination with an expander. Muehle+ Mischfuttertechnik 132.

Langhout, D. J. 1998. The role of the intestinal flora as affected by non-starch polysaccharides in broiler chicks. PhD Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

Latshaw, J. D., and J. S. Moritz. 2009. The partitioning of metabolizable energy by broiler chickens. Poult. Sci. 88:98-105.

Leeson, S., H. Namkung, M. Antongiovanni, and E. Lee. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci. 84:1418-1422.

Liu, S. Y., P. H. Selle, and A. J. Cowieson. 2013. Strategies to enhance the performance of pigs and poultry on sorghum-based diets. Anim. Feed Sci. Technol. 181:1-14

Lundblad, K., S. Issa, J. Hancock, K. Behnke, L. McKinney, S. Alavi, E. Prestløkken, J. Fledderus, and M. Sørensen. 2011. Effects of steam conditioning at low and high temperature, expander conditioning and extruder processing prior to pelleting on growth performance and nutrient digestibility in nursery pigs and broiler chickens. Anim. Feed Sci. Technol. 169:208-217.

Maciorowski, K., P. Herrera, F. Jones, S. Pillai, and S. Ricke. 2006. Cultural and immunological detection methods for *Salmonella* spp. in animal feeds–a review. Vet. Res. Commun. 30:127-137.

Mahrous, S., A. Shahin, and B. Youssef. 2001. Production of aflatoxin B1 in wheat grains under different environmental storage conditions. Egypt. J. Rad. Sci. Applic 14:111-129.

Maier, D., and F. Bakker-Arkema. 1992. The counterflow cooling of feed pellets. J. agri. Eng. Res. 53:305-319.

Maier, D., and J. Gardecki. 1993. Evaluation of pellet conditioning: understanding steam. Feed Management 44:15.

Martin, H., P. Maris, G. Jehannin, A. Rault, and R. Fresnel. 2005. An assessment of the bactericidal and fungicidal efficacy of seventeen mineral and organic acids on bacterial and fungal food industry contaminants. Sci. Aliments 25:105-127.

Mauron, J. 1981. Maillard reaciton in food; a critical review from the nutritional standpoint. Prog. Food Nutr. Sci. 5(1-6):5-35.

McBain, R. 1966. Pelleting animal feed. American Feed Manufacturers Association. Arlington, VA. 1-28.

McCracken, K. 2002. Effects of physical processing on the nutritive value of poultry diets, in: Mcnab, J.M. & Boorman, K.N. (Eds) Poultry Feeds, Supply, Composition and Nutritive Value, New York, CAB International. Pages 301-316

McHan, F., and E. B. Shotts. 1992. Effect of feeding selected short-chain fatty acids on the *in vivo* attachment of *Salmonella typhimurium* in chick ceca. Avian Dis. 139-142.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607–625.

Mommer, R., and D. Ballantyne. 1991. Reasons for pelleting. A Guide To Feed Pelleting Technology. Hess and Clark, Inc., Ashland, OH. Pages 3–6.

Montagne, L., J. Pluske, and D. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108:95-117.

Moritz, J., R. Beyer, K. Wilson, K., Cramer, L. McKinney, and F. Fairchild. 2001. Effect of moisture addition at the mixer to a corn-soybean-based diet on broiler performance. J. Appl. Poult. Res. 10:347-353.

Moritz, J., K. Wilson, K. Cramer, R., Beyer, L. McKinney, W. Cavalcanti, and X. Mo. 2002. Effect of formulation density, moisture, and surfactant on feed manufacturing, pellet quality, and broiler performance. J. Appl. Poult. Res. 11:155-163.

Moritz, J. S., K. R. Cramer, K. J. Wilson, and R. S. Beyer. 2003. Feed manufacture and feeding of rations with graded levels of added moisture formulated to different energy densities. J. Appl. Poult. Res. 12:371-381.

Mossel, D., M. van Schothorst, and E. Kampelmacher. 1967. Comparative study on decontamination of mixed feeds by radicidation and by pelletisation. J. Sci. Food Agric. 18:362-367.

Mroz, Z. 2000. Supplementary organic acids and their interactive effects with microbial phytase in diets for pigs and poultry, Proc. Annu. Conf. Phytase in Animal Nutrition. Lublin, Poland.

Newkirk, R. W., and H. L. Classen. 2002. The effects of toasting canola meal on body weight, feed conversion efficiency, and mortality in broiler chickens. Poult. Sci. 81:815-825.

Newkirk, R. W., H. L. Classen, T. A. Scott, and M. J. Edney. 2003. The digestibility and content of amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci. 83:131-139.

Nielsen, I. 1994. Effect of expander treatment on protein quality in different raw materials and mixtures, Intercoop Feedstuffs Congress. Pages 23-26.

Nir, I., Y. Twina, E. Grossman, and Z. Nitsan. 1994. Quantitative effects of pelleting on performance, gastrointestinal tract and behaviour of meat-type chickens. Br. Poult. Sci. 35:589-602.

Nissinen, V., M. Peisker, and F. Liebert. 1993. Feed treatment and enzyme addition in poultry feeds. Kraftfutter 9:364-367.

Obi, S. K. 1978. The effects of pelleting and gamma irradiation on the microbial population of animal diets. Zentralblatt für Veterinärmedizin Reihe B 25:178-185.

Okelo, P. O., S. W. Joseph, D. D. Wagner, F. W. Wheaton, L. W. Douglass, and L. E. Carr. 2008. Improvements in Reduction of Feed Contamination: An Alternative Monitor of Bacterial Killing During Feed Extrusion. J. Appl. Poult. Res. 17:219-228.

Okelo, P. O., D. D.Wagner, L. E. Carr, F. W. Wheaton, L.W. Douglass, S. W., Joseph. 2006. Optimization of extrusion conditions for elimination of mesophilic bacteria during thermal processing of animal feed mash. Anim. Feed Sci. Technol. 129:116-137.

Ouchiyama, N., and T. Tanaka., 1985. Kinetic analysis of the compaction process of a pellet in granulation, 4th Int. Symposium on Agglomeration, Toronto, Canada. Pages 635-641.

Papadopoulos, M. C. 1989. Effect of processing on high-protein feedstuffs: a review. Biol. Waste. 29:123-138.

Partanen, K., T. Jalava, J. Valaja, S. Perttilä, H. Siljander-Rasi, and H. Lindeberg. 2001. Effect of dietary carbadox or formic acid and fibre level on ileal and faecal nutrient digestibility and microbial metabolite concentrations in ileal digesta of the pig. Anim. Feed Sci. Technol. 93:137-155.

Partanen, K. H., and Z. Mroz. 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12: 117.

Paster, N. 1979. A commercial scale study of the efficiency of propionic acid and calcium propionate as fungistats in poultry feed. Poult. Sci. 58:572-576.

Patten, J.D., Waldroup, P.W., 1988. Use of organic acids in broiler diets. Poult. Sci. 67:1178-1182.

Paul, S. K., G. Halder, M. K. Mondal, and G. Samanta. 2007. Effect of organic acid salt on the performance and gut health of broiler chicken. J. Poult. Sci. 44:389-395.

Peisker, M., 1994. Influence of expansion on feed components. Feed Mix 2:26-31.

Peisker, M. 2006. Feed processing—Impacts on nutritive value and hygienic status in broiler feeds. Proceedings of the 18th Australian Poultry Science Symposium. Sydney, Australia. Pages 7–16.

Prestløkken, E., and F. Fôrutvikling. 2013. Expander treatment. HFE 305 Feed Manufacturing Technology. http://www.umb.no/statisk/iha/kurs/nova/feed_technology/4.pdf

Ravindran, V. 2013. Poultry feed availability and nutrition in developing countries. Main ingredients used in poultry feed formulations. Poultry Development Review Food and Agriculture Organization of the United Nations 67-69.

Reddy, C., L. S. Jensen, L. H. Merrill, and J. McGinnis. 1962. Influence of mechanical alteration of dietary density on energy available for chick growth. J. Nutr. 77:428-432.

Reece, F. N., B. D. Lott, J. W. Deaton. 1985. The Effects of Feed Form, Grinding Method, Energy-Level, and Gender on Broiler Performance in a Moderate (21-C) Environment. Poult. Sci. 64:1834-1839.

Rehman, H. U., W. Vahjen, W. A. Awad, and J. Zentek. 2007. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. Arch. Anim. Nutr. 61:319-335.

Rehman, Z.-u., and W. Shah. 2005. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. Food Chem. 91:327-331.

Rhodes, M. J. 1990. Principles of powder technology. John Wiley & Sons, New York. Pages 193–225.

Riaz, M. N. 2000. Extruders in food applications. CRC Press. Technomic Publishing, Lancaster, PA.

Riaz, M. N., 2007. Extruders and expanders in Pet Food, Aquatic and Livestock Feeds. Agrimedia GmbH, Clenze, Germany. Page 387.

Ricke, S. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82:632-639.

Ricke, S. 2005. Ensuring the safety of poultry feed. Food Safety Control in the Poultry Industry. G. C. Mead, ed. Woodhead Publishing, Cambridge, UK. Pages 174–194

Rokey, G. 1994. Petfood and fishfood extrusion, The technology of extrusion cooking, Springer. Pages 144-189.

Rouse, J., A. Rolow, and C. Nelson, C., 1988. Research note: Effect of chemical treatment of poultry feed on survival of *Salmonella*. Poult. Sci. 67:1225-1228.

Rowe, J., M. Choct, and D. Pethick. 1999. Processing cereal grains for animal feeding. Crop. Pasture. Sci. 50:721-736.

Rumpf, H. 1958. Grundlagen und methoden des granulierens. Chem. Ing. Tech. 30:144-158.

Runho, R., N. Sakomura, S. Kuana, D. Banzatto, O. Junqueira, and J. Stringhini. 1997. Use of an organic acid (fumaric acid) in broiler rations. Rev. Bras. Zootec. 26.

Russell, J. 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. J. Appl. Microbiol. 73:363-370.

Salmond, C. V. R. G. Kroll, and I. R. Booth. 1984. The effect of food preservatives on pH homeostasis in Escherichia coli. J. Gen. Microbiol. 130:2845-2850.

Samarasinghe, K., R. Messikommer, and C. Wenk. 2000. Activity of supplemental enzymes and their effect on nutrient utilization and growth performance of growing chickens as affected by pelleting temperature. Arch. Anim. Nutr. 53:45-58.

Sheu, C. W., and E. Freese. 1972. Effects of fatty acids on growth and envelope proteins of *Bacillus subtilis*. J. Bacteriol. 111: 16-524.

Sheu, C. W., W. N. Konings, and E. Freese. 1972. Effects of acetate and other short-chain fatty acids on sugar and amino acid uptake of *Bacillus subtilis*. J. Bacteriol. 111:525-530.

Silversides, F., and M. Bedford. 1999. Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. Poult. Sci. 78:1184-1190.

Skinner, J. T., A. L. Izat, and P. W. Waldroup. 1991. Research note: Fumaric acid enhances performance of broiler chickens. Poult. Sci. 70:1444-1447.

Skoch, E. R., K. C. Behnke, C. W. Deyoe, and S. F. Binder. 1981. The Effect of Steam-Conditioning Rate on the Pelleting Process. Anim. Feed Sci. Technol. 6:83-90.

Smallman, C. 1996. Maximising conditioning potential. Feed milling international. 190:30-31.

Smith, A. K., and S. J. Circle. 1978. Soybeans: chemistry and technology. Volume 1. Proteins. Avi Publishing Company Inc. Westport, CT.

Smith, P., J. Firman, and N. Dale. 1995. Effects of feed processed in an annular gap expander on subsequent broiler performance. Poul. Sci. 74:145.

Smits, C. H., and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition-towards a physiologically valid approach to their determination. World's Poult. Sci. J. 52:203-222.

Smits, C. H., A. Veldman, M. W. Verstegen, and A. C. Beynen. 1997. Dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. J. Nutr. 127:483-487.

Sørensen, M. 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquac. Nutr. 18:233-248.

Sosulski, F.W., L. A. Minja, and D. A. Christensen. 1988. Trypsin inhibitors and nutritive value in cereals. Plant Foods Hum. Nutr. 38:23-34.

Stevens, C.A. 1987. Starch gelatinization and the influence of particle size, steam pressure and die speed on the pelleting process. Ph.D.Dissertation. Kansas State University, Manhattan, KS.

Svihus, B., K. H. Klovstad, V. Perez, O. Zimonja, S. Sahlstrom, R. B. Schuller, W. K. Jeksrud, and E. Prestlokken. 2004. Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. Anim. Feed Sci. Technol. 117:281-293.

Svihus, B., and O. Zimonja. 2011. Chemical alterations with nutritional consequences due to pelleting animal feeds: a review. Anim. Prod. Sci. 51:590-596.

Tabib, Z., F. T. Jones, and P. B. Hamilton. 1984. Effect of pelleting of poultry feed on the activity of molds and mold inhibitors. Poult. Sci. 63:70-75.

Thomas, M., P. T. H. J. Huijnen, T. van Vliet, D. J. van Zuilichem, and A. F. B. van der Poel. 1999. Effects of process conditions during expander processing and pelleting on starch modification and pellet quality of tapioca. J. Sci. Food Agric. 79:1481-1494.

Thomas, M., and A. F. B van der Poel. 1996. Physical quality of pelleted animal feed 1. Criteria for pellet quality. Anim. Feed Sci. Technol. 61:89-112.

Thomas, M., T. van Vliet, and A. F. B. van der Poel. 1998. Physical quality of pelleted animal feed 3. Contribution of feedstuff components. Anim. Feed Sci. Technol. 70:59-78.

Thomas, M., D. J. van Zuilichem, and A. F. B. van der Poel.1997. Physical quality of pelleted animal feed. 2. contribution of processes and its conditions. Anim. Feed Sci. Technol. 64:173-192.

Thompson, J. L., and M. Hinton. 1997. Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonellas* in the crop. Br. Poult. Sci. 38:59-65.

van Immerseel, F., J. Russell, M. Flythe, I. Gantois, L. Timbermont, F. Pasmans, F. Haesebrouck, F., and R. Ducatelle. 2006. The use of organic acids to combat *Salmonella* in poultry: a mechanistic explanation of the efficacy. Avian Pathol. 35:182-188.

Veenendaal, J. 1990. Extrusion in the compound feed industry. Adv. Feed Technol 3:60-72.

Veldman, A., H. Vahl, G. Borggreve, and D. Fuller. 1995. A survey of the incidence of *Salmonella* species and Enterobacteriaceae in poultry feeds and feed components. Vet. Rec. 136:169-172.

Vest, L., and G. Calhoun. 1997. Expanders and their influence on broiler performance. American Soybean Association. Brussels Belgium.

Vilarino, M., M. L. Picard, J. P. Melcion, and J. M. Faure. 1996. Behavioural adaptation of laying hens to dilution of diets under mash and pellet form. Br. Poult. Sci. 37:895-907.

Voeten, A., and L. Leest. 1989. Influence of the pelleting temperature used for feed on *Salmonella* infection in broilers. Archiv für Geflügelkunde 53.

Vogt, H., S. Mathes, and S. Harnisch. 1982. The effect of organic acids on productivity of broilers. Archives fur Geflugelkunde 46:223-227.

Von Engelhardt, W., K. Rönnau, G. Rechkemmer, and T. Sakata. 1989. Absorption of short-chain fatty acids and their role in the hindgut of monogastric animals. Anim. Feed Sci. Technol. 23:43-53.

Vukmirović, Đ., D. Ivanov, R. Čolović, B. Kokić, J. Lević, O. Đuragić, and S. Sredanović. 2010. Effect of steam conditioning on physical properties of pellets and energy consumption in pelleting process. J. Proc. Energy Agr. 14:106-108.

Waldroup, P., S. Watkins, J. Skinner, and A. Desai. 2002. Stability of Bacitracin Methylene Disalicylate in the Presence of Pellet Binding Agents in Poultry Feed. Int. J. Poult. Sci. 1:13-15.

Wood, J.F., 1987. The functional properties of feed raw materials and their effect on the production and quality of feed pellets. Anim. Feed Sci. Technol. 18:1-17.

Zimonja, O., A. Stevnebø, and B. Svihus. 2007. Nutritional value of diets for broiler chickens as affected by fat source, amylose level and diet processing. Can. J. Anim. Sci. 87:553-562.

Zimonja, O., and B. Svihus. 2005. A comparison of pelleting and expander treatment on nutritional value of a broiler diet containing two different fat sources, Proceedings of the 15th European Symposium on poultry nutrition, BalatonfÃ¼red, Hungary, 25-29 September, 2005. World's Poultry Science Association (WPSA). Pages 473-475.

PUBLICATION LIST

Published and accepted publications (peer reviewed)

1- **Goodarzi Boroojeni, F.,** A. H. Samie, M. A. Edriss, M. Khorvash, G. Sadeghi, A. van Kessel and J. Zentek. 2011. Replacement of corn in the diet of broiler chickens using foxtail millet produced by 2 different cultivation strategies. Poultry Science. 90 :2817–2827

2- **Goodarzi Boroojeni, F.,** A. Mader, F. Knorr, I. Ruhnke, I. Rohe, A. Hafeez, K. Manner and J. Zentek. 2014. The effects of different thermal treatments and organic acids levels on nutrient digestibility in broilers. Poultry Science. 93 (5): 1159-1171.

3- **Goodarzi Boroojeni, F.** W. Vahjen, A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, C. Villodre, K. Männer and J. Zentek. 2014. The effects of different thermal treatments and organic acids levels in feed on microbial composition and activity in gastrointestinal tract of broilers. Poultry Science**.** 93(6):1440–1452.

4- Hafeez, A. A. Mader, F**. Goodarzi Boroojeni,** I. Ruhnke, K. Männer, and J. Zentek. 2014. Impact of thermal and organic acid treatment of feed on apparent absorption and metabolism of minerals and tibia quality in broilers. Poultry Science. 93 (7):1754–1763.

5- Röhe, I. I. Ruhnke, F. Knorr, A. Mader, **F. Goodarzi Boroojeni,** R. Löwe, and J. Zentek. 2014. Effects of grinding method, particle size and physical form of the diet on gastrointestinal morphology and jejunal glucose transport in laying hens. Poultry Science. 93 (8):2060–2068.

6- Ruhnke, I. I. Röhe, F. **Goodarzi Boroojeni, F.** Knorr, A. Mader, A. Hafeez, and J. Zentek. 2014. Feed supplemented with organic acids does not affect starch digestibility, nor intestinal absorptive or secretory function in broiler chickens. Journal of Animal Physiology and Animal Nutrition. Accepted**.**

7- Martin, L., R. Pieper, S. Kröger**, F. Goodarzi Boroojeni,** W. Vahjen, K. Neumann, A.G. van Kessel, and J. Zentek. 2012. Influence of age and *Enterococcus faecium* NCIMB 10415 on development of small intestinal digestive physiology in piglets. Animal Feed Science and Technology. 175: 65– 75

8- Durek, J., A. Ghadiri Khozroughi, A. Fröhling, F. Knorr, A. Mader, **F. Goodarzi Boroojeni,** J. Zentek, D. Knorr, and J. S. Bolling. 2014. Effects of thermally and chemically treated broiler feed on resulting meat composition and parameters related to meat quality. Journal of Agricultural and Food Chemistry. Accepted. In press. http://dx.doi.org/10.1016/j.ifset.2014.05.001

9- Jahanian, R., and **F. Goudarzi.** 2010. Effects of Maternal Factors on Day-old Chick Body Weight and Its Relationship with Weight at Six Weeks of Age in a Commercial Broiler Line. Asian-Australian journal of Animal Science. 23: 302-307

Submitted publications (peer reviewed)

1- Ruhnke, I. I. Röhe, C. Krämer, **F. Goodarzi Boroojeni,** F. Knorr, A. Mader, E. Schultze, A. Hafeez, K. Neumann R. Löwe, and J. Zentek. 2014. The effects of particle size, milling method, and thermal treatment of feed on performance, apparent ileal digestibility and pH of the digesta in laying hens. Poultry Science. Submitted (under review)

2- Hafeez, H. A. Mader, I. Röhe, I. Ruhnke, **F. Goodarzi Boroojeni,** M. Yousaf, K. Maenner and J. Zentek. 2014. The effect of milling method, thermal treatment, and particle size of feed on exterior and interior egg quality in laying hens. Animal Feed Science and Technology. Submitted (under review).

3- Hafeez, H. A. Mader, I. Röhe, I. Ruhnke, **F. Goodarzi Boroojeni,** M. Yousaf, K. Maenner and J. Zentek. 2014. Implication of milling methods, thermal treatment, and particle size of feed in layers on mineral digestibility and retention of minerals in egg contents. Poultry Science. Submitted (under review).

Abstracts in proceedings & participation in conferences

1- **Goodarzi Boroojeni, F.,** R. Vaez Torshizi, and N. E. J. Kashan. 2007. Estimation of direct genetic, maternal genetic and maternal environmental effects for body weights in a commercial broiler line, Hangzhou conference, China.

2- **Goodarzi Boroojeni, F.,** A. Samie, M. A. Edris, A. Mahdavi Damghani, and H. Mahmoudi. 2009. Comparison of Nutritional Characteristics of Three Organic vs. Conventional Millet Species for Poultry Nutrition, Tropentag conference, Hamburg.

3- **Goodarzi Boroojeni, F.** 2012. Using organic and conventionally grown foxtail, millet in broiler diet. 6. Doktorandensymposium & DRS Präsentationsseminar "Biomedical Sciences". Berlin. Germany.

4- **Goodarzi Boroojeni, F.** 2012**.** Replacement of corn in the diet of broiler chicken using foxtail millet produced by two different cultivation strategies. Green Footsteps Award. Bruges. Belgium.

5- **Goodarzi Boroojeni, F.,** F. Knorr, A. Mader and J. Zentek. 2013. Effect of different heat and organic acid treatments as conventional decontamination strategies on feed digestibility and broiler performance. 19th European Symposium on Poultry Nutrition. Potsdam. Germany.

6- **Goodarzi Boroojeni F.,** P. Theobald, H: M. Hafez, K. Männer and J. Zentek. 2013. The effect of Sodium Diformate inclusion on *Salmonella* excretion and organ colonisation of laying hens challenged with *Salmonella*. 19th European Symposium on Poultry Nutrition. Potsdam. Germany.

7- **Goodarzi Boroojeni F.,** S. Keller, D. Parker, H. M. Hafez, K. Männer, and J. Zentek. 2013. The effect of an embedded benzoic acid product inclusion on the performance of broilers challenged with *Salmonella enteritidis* and on ileal lactic acid microflora. 19th European Symposium on Poultry Nutrition. Potsdam. Germany.

8- **Goodarzi Boroojeni, F.,** F. Knorr, A. Mader and J. Zentek. 2013. Effect of different heat treatments and organic acids on feed digestibility in broilers. 17th European Society of Veterinary and Comparative Nutrition congress. Ghent. Belgium.

9- **Goodarzi Boroojeni,** F. W. Vahjen, A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, C. Villodre, K. Männer and J. Zentek. 2014. Effect of different heat treatments and organic acid levels in feed on the gastrointestinal microbiota in broilers. 68th conference of the

Society of Nutrition Physiology (68. Tagung der Gesellschaft für Ernährungsphysiologie). Göttingen. Germany.

10- **Goodarzi Boroojeni, F**., G. Weber, K. Männer, and J. Zentek. 2014. Efficacy of CRINA Poultry Plus on performance and intestinal microbiota in broiler chickens from hatch to 42 days of age. XIVth European Poultry Conference. Stavanger, Norway.

11- **Goodarzi Boroojeni, F**., W. Vahjen, A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, C. Villodre, K. Männer and J. Zentek. 2014. Effect of different heat treatments and organic acid levels in feed on the gastrointestinal microbiota in broilers. XIVth European Poultry Conference. Stavanger, Norway.

12- Abolfathi, M. E., **F. Goodarzi Boroojeni,** J. Porreza, and H. Liaghati. 2010. Comparison of Nutritional Value of Organic V. Conventional Foxtail Millet for Poultry Nutrition, Tropentag conference, Zurich. Switzerland.

13- Mahmoudi, H., **F. Goodarzi Borooejni**, and M. Zohari. 2008. The Impacts of Organic Production on Sustainable Resource Use and Poverty Alleviation in Iran, Tropentag conference, Hohenheim. Stuttgart. Germany.

14- Martin, L., R. Pieper, **F. Goodarzi Borooejni,** W. Vahjen, A. G. van Kessel and J Zentek. 2011. Effects of *Enterococcus faecium* NCIMB 10415 on performance, gene expression, brush border enzyme activity and small intestinal morphology in piglets. SFB 852 Mini symposium "Biology of Nutrition", Berlin. Germany.

15- Martin, L., R. Pieper, **F. Goodarzi Borooejni**, W. Vahjen, K. eumann A. van Kessel and J Zentek. 2011. Influence of *Enterococcus faecium* NCIMB 10415 on performance, small intestine morphology, gene expression and brush border enzyme activity in piglets. International Scientific Conference Probiotics and Prebiotics. Kosice. Slovakia.

16- Mader, A., F. Knorr, **F. Goodarzi Boroojeni,** M. Lahrssen-Wiederholt, H. Schafft and J. Zentek. 2011. Agricultural terrorism in the feed chain. Medical Biodefense Conference. Munich. Germany.

17- Ruhnke, I., I. Röhe. C. Krämer, **F. Goodarzi Boroojeni,** F. Knorr, A. Mader, and J. Zentek. 2013. Effects of feed particle size, heat treatment and milling method on performance, nutrient digestibility, and pH of chyme in laying hens. 19th European Symposium on Poultry Nutrition. Potsdam. Germany.

18- Ruhnke, I., I. Röhe, **F. Goodarzi Boroojeni,** F. Knorr, A. Mader, J. Zentek. 2013. Feed supplemented with 1.5% organic acid had no significant influence on jejunal transporter function in broilers. 17th European Society of Veterinary and Comparative Nutrition congress. Ghent. Belgium.

19- Hafeez, A. A. Mader, **F. Goodarzi Boroojeni**, I. Ruhnke, K. Männer, and J. Zentek. 2014. Impact of thermal and organic acid treatment of feed on apparent absorption and metabolism of minerals and tibia quality in broilers. 68th conference of the Society of Nutrition Physiology (68. Tagung der Gesellschaft für Ernährungsphysiologie). Göttingen. Germany.

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Hiermit erkläre ich an Eides statt, die vorliegende Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben. Die Arbeit ist in dieser Form noch keiner anderen Prüfungsbehörde vorgelegt worden.

Berlin, 9. April 2014 Farshad Goodarzi Boroojeni