Aus dem Institut für Tierernährung
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Effect of different feed treatment strategies on apparent mineral digestibility and retention in broilers and layers and egg quality in laying hens

Inaugural-Dissertation
zur Erlangung des akademischen Doktorgrades
Doctor of Philosophy (Ph.D.)
in "Biomedical Sciences"
an der Freien Universität Berlin

vorgelegt von
Abdul Hafeez
geboren in Haripur, Pakistan
Master of Science aus Peshawar

Berlin 2015
Journal-Nr.: 3783

Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Jürgen Zentek
Zweiter Gutachter:	Univ.-Prof.Dr. Hafez Mohamed Hafez
Dritter Gutachter:	PD Dr. Friederike Stumpff

Deskriptoren (nach CAB-Thesaurus):
broilers, hens, egg quality, poultry feeding, minerals, metals, digestibility, ileum, mineral absorption, bones, feeding programs

Tag der Promotion: 08.06.2015

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <http://dnb.ddb.de> abrufbar.

ISBN: 978-3-86387-608-1
Zugl.: Berlin, Freie Univ., Diss., 2015
Dissertation, Freie Universität Berlin
D188

Choriner Str. 85 - 10119 Berlin
verlag@menschundbuch.de – www.menschundbuch.de

To My Life ***FOZIA NAHEED***

I Was Breathing…
I Met You &
I Started Living…

I wish I could love ***Abdullah Wahab, Asavara Maryam*** *and* ***Saifullah Wahab*** *the same way like…*

My ***Sweet Parents*** *Loved Me…*

Table of Contents

List of tables

List of abbreviations

AA	Amino Acid
AIA	Apparent Ileal Absorption
ATD	Apparent Total Digestibility
AME_N	Apparent Metabolizable Energy adjusted for Nitrogen
ANOVA	ANalysis Of VAriance
BMBF	Bundesministerium für Bildung und Forschung
CP	Crude Protein
DM	Dry Matter
E	Expanding at 130°C
FCR	Feed Conversion Ratio
GfE	Gesellschaft für Ernährungsphysiologie
GLM	Generalized Linear Model
HU	Haugh Unit
IFF	Internationale Forschungsgemeinschaft Futtermitteltechnik e.V.
L	Long term conditioning
LSD	Least Square Differences
MRP	Maillard Reaction Products
NRC	National Research Council
P	Pelleting
SEM	pooled Standard Error of Mean
SileBAT	Projekt, Sicherstellung der Futter- und Lebensmittelwarenkette bei bio- und agro-terroristischen Schadenslagen
SWUSA	Shell Weight per Unit Surface Area

1 INTRODUCTION

Minerals and trace elements are important nutrients in poultry feed by having an impact on different physiological functions and a major role in bone and egg formation in poultry. Especially, rapid broiler growth can negatively affect bone quality (Nestor et al., 1985; Lilburn, 1994; Bessei, 2006; Angel, 2007) and suboptimal bone development can result in deformities, which might lead to lower production output and successive to economic losses (Cook, 2000; Coto et al., 2008). Calcium and phosphorus on their own contribute to over 95 % of the total mineral content (Cook, 2000; Coto et al., 2008). However, the quality of bone depends not only on the mineral quantity but also on the composition of the mineral matrix (Viguet-Carrin et al., 2006). Minerals and trace elements are essential for optimum egg quality (Leeson and Summers, 2001; Mabe et al., 2003), being important components of the egg, especially of the shell (Al-Batshan et al., 1994). Egg quality is not only important for being processible and marketable, but also for breeders. The availability of essential nutrients, including minerals in proper proportion, can result in better embryonic development (Angel, 2007; Dibner et al., 2007; Uni et al., 2012).

Feed hygiene is a problem faced by the poultry industry, since feed ingredients are susceptible to microorganisms during cultivation, storage, transportation, manufacturing and packaging (Crump et al., 2002). Pathogens can be transmitted to poultry and ultimately to humans as consumers of poultry products (Cox et al., 1986). On one hand, the use of thermal treatments can have several positive effects (Fancher et al., 1996; Abdollahi et al., 2013), as heat treatment of feed can reduce microbial load (Mossel et al., 1967b; Cover et al., 1984; Van Immerseel et al., 2009), can enhance feed intake and efficiency (Abdollahi et al., 2013), and can improve the ileal apparent digestibility of nutrients (Abdollahi et al., 2011). On the other hand, the supplementation of organic acids in poultry feed, used for decontamination and to avoid recontamination (Martin and Maris, 2005; Ricke, 2005), can improve feed hygiene (Matlho et al., 1997), can increase the nutritional value of the feed and can have beneficial effects on gastrointestinal function (Jongbloed et al., 2000; Dibner and Buttin, 2002). Broilers are usually fed pellets whereas laying hens are usually offered feed as mash (Amerah et al., 2007). The use of different milling methods can result in production of feed with different particle sizes and shapes (Koch, 2002; Amerah et al., 2007). Laying hens have a preference for feed with a larger particle size (Schiffman, 1968; Portella et al., 1988), which may influence the egg quality due to feed segregation patterns (Tang et al., 2006).

The impact of thermal treatments and acid supplementation of feed on production performance of poultry is well documented. However, their effect on mineral absorption and retention in organs of broilers has not widely been studied. Moreover, the knowledge regarding the effect of feed form and particle size on mineral digestibility, retention in egg components, and egg quality in laying hens is insufficient.

Therefore, the first part of the present PhD thesis emphasizes the impact of thermal treatment (pelleting, long term treatment and expansion) and the inclusion of organic acids in feed on apparent ileal mineral absorption, tibia and liver mineral concentration as well as tibia quality in broilers. Keeping in view the importance of feed particle size and form in layers, the second part of the PhD thesis was performed to investigate the implication of milling methods (roller and hammer mill), thermal treatment (mash vs expansion), and particle size (coarse and fine) in layers feed on mineral digestibility and retention of minerals in egg yolk, albumen and shell. Additionally, the third part of the PhD thesis deals with the effect of milling methods (roller and hammer mill), thermal treatment (mash vs. expansion) and particle size (coarse and fine) in feed on exterior and interior egg quality in laying hens.

2 REVIEW OF LITERATURE

2.1 MINERAL NUTRITION IN BROILERS AND LAYERS

Macro minerals and trace elements have an essential role in different physiological functions, including bone formation. According to their quantity in feed, there are seven macro-minerals and 22 micro-minerals required by at least one animal species (Lukić et al., 2009). The most important minerals, which must be provided in poultry feed for normal body functioning include calcium, phosphorus, sodium, potassium, magnesium, manganese, zinc, iron, copper, chloride, iodine, molybdenum and selenium (Leeson and Summers, 2001).

Rapid broiler growth, an important factor in poultry production, can have a negative impact on skeleton and bone development (Nestor et al., 1985; Lilburn, 1994; Bessei, 2006; Angel, 2007), can lead to metabolic disorders (Brickett et al., 2007) and can exert stress on calcium and phosphorus regulatory system (Bar et al., 2003). For example, Shim et al. (2012) created two subpopulations, slow-growing and fast-growing broilers, based on their growth rate from hatch until six weeks of age, and investigated that tibia density and bone ash values were lower in fast growing broilers than in slow growing broilers of the same strain (Shim et al., 2012). Associated problems in broilers include bone abnormalities, which can result in impaired mobility causing subsequently under-nutrition and dehydration of the animals followed by a high rate of mortality (Fleming, 2008) and economic loss in broiler production (Cook, 2000; Coto et al., 2008). However, reduced skeletal disorders result in higher walking ability leading to an improved bone formation (Reiter and Bessei, 1998). In addition to many other in vivo and post mortem methods, generally the degree of mineralization of the bone matrix can be measured by the parameter of bone breaking strength (Boivin and Meunier, 2002).

Additionally, mineral nutrition is an important aspect for optimal egg production in laying hens. The provision of optimum mineral content in the feed is essential for the production of quality eggs for sale (Leeson and Summers, 2001; Mabe et al., 2003). Minerals are transferred into egg contents through blood, either in ionic form like sodium and potassium or in complex structures with lipoproteins as calcium, iron, and zinc (Larbier and Leclercq, 1992). In addition, the mineral content of egg is important for breeders. The nutrient composition of fertile eggs has a strong influence on nutrient uptake by embryo (Uni et al., 2012). The deficiency of calcium and phosphorus, and copper, manganese, and zinc, in particular, may cause skeletal, immune and cardiovascular system disorders, reduce hatchability and increase embryonic mortality (Angel, 2007; Dibner et al., 2007; Uni et al., 2012).

Minerals and trace elements can be involved in complex interactions with each other and with other nutrients, exhibiting both synergisms and antagonisms (Watts, 1990). Due to the ingredients used in feed production various interactions can take place that may have an influence on mineral digestibility. For example, thermal treatment promotes the development of Maillard products, which is a reaction between amino acids and reducing sugars (van Boekel et al., 2010). Despite the fact that the Maillard reaction products (MRP) may be absorbed from the gastrointestinal tract, they cannot be utilized by the animal (Van Rooijen et al., 2013). Moreover, the consumption of MRP influences osteoclasts (bone resorption) and osteoblasts (bone formation), which might be related with the bone deformities like osteoporosis (Hein, 2006). Nevertheless, it was previously shown that the intake of MRP did not negatively affect calcium bioavailability and bone metabolism in rats (Roncero-Ramos et al., 2013). Beside the interactions between carbohydrates and protein, interactions among minerals are a major cause of variation in mineral availability, influencing the nutritive value of a diet (Bao et al., 2010).

Based on their close association and dependence on each other, in the following some minerals are considered together for their role in bone and egg formation, digestibility, recommended dietary levels and deficiency symptoms. The detailed features of minerals, their interactions, digestibility coefficients and role in body functions have been described in many books including "Nutrition of the Chicken" by Leeson and Summers (2001) and "Nutrition and Feeding of Poultry" by Larbier and Leclercq (1992). Furthermore, requirements of minerals and trace elements in feed of different animal species have been reported in detail by "National Research Council (NRC, 1994)" and "Society of Nutrition Physiology (Gesellschaft für Ernährungsphysiologie), (GfE, 1999)".

2.1.1 Calcium and Phosphorus

The macrominerals calcium and phosphorus are the primary inorganic nutrients, which are considered together for being closely related to each other and constituting the major part of mineral content involved in bone formation in broilers (McDowell, 2003). Calcium is considered as the most important factor for bone and egg formation in layer hens (Lukić et al., 2009). Phosphorus is the focus of research in broilers due to its significant role in bone formation and being an important pollutant of soil and water when inorganic phosphorus sources are fed in high amounts (Lukić et al., 2009).

Calcium and phosphorus contribute to about 95% of the mineral matrix in bones (Rath et al., 2000; Shim et al., 2012). More than 90% of chicken's total calcium is found in bones, where it combines with phosphorus to form calcium-phosphate crystals (or hydroxyapatite) with the molecular formula $Ca_{10}(PO_4)_6(OH)_2$ (Scott et al., 1982). Within certain physiological limits higher levels of calcium and phosphorus in feed increase bone mineral content, bone density, ash content (Onyango et al., 2003; Rao et al., 2006; Coto et al., 2008), and tibia breaking strength (Burnell et al., 1990). Deficiencies of calcium and phosphorus resulted in bone loss (Wilson and Duff, 1991; Hemme et al., 2004), rickets or osteoporosis, growth retardation, and abnormal posture and gait (Leeson and Summers, 2001).

Layers have a high demand of calcium during peak egg production periods (Al-Batshan et al., 1994). The egg shell consists of 90-95% calcium carbonate and on average the egg shell contains 2.3 g of calcium, which is equivalent to about 10% of total calcium of layer skeleton (Lukić et al., 2009). Egg is rich in phosphorus found in the form of phosphoprotein (phosvitin) and phospholipids (Larbier and Leclercq, 1992).

The apparent ileal absorption of calcium in rapidly growing broilers is about 60% in young age which may reduce to about 50% in adult birds. The laying hens may also have up to 50% calcium absorption, particularly in the laying period (Larbier and Leclercq, 1992). Phosphorus, found in both organic and inorganic form has a great variability in digestibility (Rodehutscord et al., 2012). Phosphorus is commonly bound in cereals and in legumes as phytic acid or phytate when in salt form. Additionally, phytate can chelate with trace elements including copper, zinc, manganese and iron, thereby making them less available. Phytate can be hydrolyzed by the phytases, which can be of fungal or bacterial nature. About 60-80% phosphorus found in plant materials is in phytate form (Ravindran et al., 1995). The digestibility of phosphorus bound in phytic acid is 0-50% in young poultry whereas mature hens may have up to 50% digestibility

(Rutherfurd et al., 2002). The availability of inorganic phosphorus is above 90%. For example, monocalcium phosphate is a source of inorganic phosphorus; it contains 98 % available phosphorus (Leeson and Summers, 2001).

The recommended levels of calcium and phosphorus in feed depend on the production type. According to NRC standards, the calcium requirements for immature leghorn type chickens in white egg laying strains ranges from 0.9-2.0% whereas brown egg laying strains require 0.9-1.8% dietary calcium. In white egg layers during egg production periods, the calcium concentration in diet reaches up to 2.71-4.06% whereas in brown egg layers, 3.2-3.6% calcium should be included in feed. The concentration of non-phytate phosphorus for immature leghorn type chickens varies between 0.32-0.40% in white egg laying strains and 0.35-0.40% in brown egg laying strains. In mature leghorn type laying hens, non-phytate phosphorus should be included at 0.21-0.31% in white egg layers diet whereas brown egg layers feed should contain 0.25-0.28% non-phytate phosphorus. Broilers require 0.8-1.0% calcium and 0.30-0.45% non-phytate phosphorus in diet (NRC, 1994). According to GfE (1999), feed for laying hens should contain 2.40-4.35% calcium and 0.27-0.44% non-phytate phosphorus, whereas broilers should be provided with 0.24-1.14% calcium and 0.14-0.78% non-phytate phosphorus in diet.

In addition to absolute levels, minimum ratio of calcium to available phosphorus in poultry diet should be 2.2:1 but should not exceed 2.5:1 in growing chicks. On one hand, a ratio of 3.3:1 resulted in a high incidence of rickets and leg abnormalities and on the other hand, the high level of phosphorus in diet declined egg shell quality due to imbalance with calcium. The plasma phosphorus level increases at night time, however in case of no immediate metabolic need, extra phosphorus is excreted through urine (Leeson and Summers, 2001).

2.1.2 Magnesium

Magnesium constitutes 0.5-0.7% of bone ash and half of the total body magnesium is found in bones. Therefore, bone acts as reservior for magnesium in the body (Leeson and Summers, 2001). Magnesium is important for laying hens and plays an important role in egg shell formation. Out of 25 mg total magnesium found in egg, 2.0 mg is deposited in yolk, 4.3 mg is accumulated in albumen and 18.7 mg is present in shell and shell membranes (Leeson and Summers, 2001). The apparent intestinal absorption of magnesium is up to 60 % (Larbier and Leclercq, 1992).

According to NRC, the recommended magnesium concentration for immature leghorn type chickens in white egg laying strains is 400-600 mg/kg whereas diet of brown egg laying strains should contain 370-570 mg/kg magnesium. In leghorn type laying hens, 420-625 mg/kg dietary magnesium should be included in white egg layers and 50-55 mg/kg in brown egg layers. Broiler diets should contain 600 mg/kg magnesium (NRC, 1994). However, according to recommendations of GfE (1999), a laying hen diet should contain 0.033-0.057% and broiler diets should include 0.01-0.05% magnesium.

The concentration of magnesium in commercial broiler and layer feed is normally sufficient for covering the requirements of birds. Increased concentrations of calcium and phosphorus in diet will increase magnesium requirements. (Leeson and Summers, 2001). A study suggested that 0.9% magnesium fed to broilers was cathartic and increased incidence of leg disorders (Lee and Britton, 1980). In high concentrations, magnesium sulphate ($MgSO_4$) is more toxic than magnesium oxide (MgO) and magnesium chloride (MgCl) (Durlach et al., 2005). Due to the antagonistic relationship between magnesium and calcium, high levels of magnesium negatively affected bone calcification (Atteh and Leeson, 1983). Another study reported that with increased magnesium level (0.2% Mg citrate or 0.2% MgO supplementation) in the diet, calcium to phosphorus metabolism was affected and tibiae from magnesium intoxicated chicks were shortened, thickened, and bowed. Additionally, the percent of tibial ash was greatly reduced (Lee et al., 1980).

2.1.3 Sodium and Potassium

There is a functional synergism between sodium and potassium (Larbier and Leclercq, 1992), therefore both electrolytes are considered together. Sodium and potassium as well as chloride are necessary to maintain a physiological acid-base balance, and their integrated role in osmotic regulations of body fluid is important for regular bone development (Leeson and Summers, 2001).

Within the egg, albumen is the main site for deposition of sodium and potassium. Approximately, 80.4% sodium can be found in albumen as compared to 19.6 % found in yolk. Similarly, albumen contained 64.0% potassium as compared to 36% in the yolk (Uni et al., 2012). The apparent total absorption of sodium was recorded as 43-45%, whereas apparent ileal digestibility of potassium was calculated as 87-90% in broilers (Selle et al., 2009).

According to NRC, both white and brown egg laying strains of immature leghorn type chicken require 0.15% sodium and 0.25% potassium in feed. White egg layer strains of leghorn type laying hens should be provided with 0.13-0.19% dietary sodium and potassium whereas brown egg layer strains require 0.15-0.16% sodium and potassium in feed. Broiler diets should contain 0.12-0.20% sodium and 0.30% potassium (NRC, 1994). According to GfE (1999), feed for laying hens should contain 0.08-0.14% sodium and 0.11-0.17% potassium whereas broiler diet should have 0.03-0.15% sodium and 0.07-0.32% potassium. Diets including soyabean meal are considered as rich source of potassium (approx. 2%) (Ribeiro et al., 2008). A recent study demonstrated that nutritional requirements of potassium in poultry may increase with age. Broilers with 8 to 21, 22 to 42, and 42 to 53 days of age should be provided with 0.628, 0.714, and 0.798% potassium in diet, respectively (Oliveira et al., 2005).

The provision of sodium in excess of 0.35% in the diet can result in higher water intake by the poultry. A sodium level much over 0.5% in the feed influence electrolyte balance and is considered toxic, due to water intoxication (Leeson and Summers, 2001). Electrolyte imbalances result in a number of metabolic disorders. Metabolic acidosis due to feeding of high chloride levels in the diet causes an unusal development of cartilage plug at the growth plate of tibia, which can result in tibial dyschondroplasia. Sodium deficiency in the diet may lead to development of soft bones and reduced egg production (Leeson and Summers, 2001). High potassium concentrations (12.8 g/kg) in soybean meal diet slightly increased the severity of foot pad dermatitits in female turkeys in comparison with low dietary potassium (5.8, 6.2 and11.0 g/kg) on wet litter (28% DM), however no such effects was observed on dry litter (80.7% DM) (Youssef et al., 2011).

2.1.4 Copper

Both copper and iron are closely related due to involvement of copper in the iron metabolism (Leeson and Summers, 2001). Copper is important for optimal bone formation, as it is required for collagen crosslinking and hence provides the basis for physical stability of tendons and articular cartilage (Osphal et al., 1982). With the increase in collagen crosslinking, bone strength increased (Rath et al., 1999). Deficiency of copper may lead to tibial dyschondroplasia (Leeson and Summers, 2001).

The relative availability of copper from different mineral sources including copper sulfate (100%), copper oxide (75%), and copper chloride (70%) is high (Leeson and Summers, 2001). The two inorganic sources of copper used in poultry rations including tribasic copper chloride ($Cu_2(OH)_3Cl$) and copper sulfate pentahydrate ($CuSO_4.5H_2O$) have similar bioavailability (Wang et al., 2014).

The NRC (1994) recommended the inclusion of 4-5 mg/kg copper in diets for both white and brown strains of immature leghorn type chickens, and 8 mg/kg copper in broiler diets. However, no clear recommendations were given about copper inclusion in the feed of laying hens. According to the recommendations of the GfE (1999), feed of laying hens should contain 4-5 mg/kg copper whereas 7 mg/kg copper should be included in broiler feed.

Some previous data show that inclusion of 125-250 mg/kg copper in the diet may improve growth and feed conversion. The supplementation of 375 mg/kg did have no advantage (Wang et al., 2014). At a level of 500-700 mg/kg dietary copper, the growth level of chicks was depressed (Jensen and Maurice, 1979). Copper deficiency in laying hens resulted in the production of eggs with abnormal size and shape, wrinkled and rough textured shells, and an increased shell-less eggs (Baumgartner et al., 1978).

2.1.5 Zinc

Zinc plays an important role in normal bone development. The zinc status of the hen is directly related to activity of carbonic anhydrase (a calcium binding protein), which is essential for egg shell formation. Moreover, the hatchability and embryonic development may adversely be affected due to inadequate intake of dietary zinc (Hudson et al., 2004). In the same context, declined shell quality due to supply of saline water can be counteracted by zinc supplementation in water, as zinc increased the carbonic anhydrase level resulting in less shell defects (Balnave and Zhang, 1993).

The digestibility of zinc sulfate is about 100% and that of zinc oxide is about 44% (Leeson and Summers, 2001). White and brown egg laying strains of immature leghorn type chickens should contain 35-40 and 33-38 mg/kg dietary zinc, respectively. Zinc should be included in feed of white egg laying hens at 29-44 mg/kg whereas brown egg layers should be provided with 39-45 mg/kg zinc in feed. Broiler feed should contain 40 mg/kg zinc (NRC, 1994). According to GfE (1999), dietary zinc requirement is 50 mg/kg in laying hens and 40 mg/kg in broilers.

A corn-soy based poultry feed may contain around 60-80 mg/kg zinc. Phytic acid is a potent chelator to zinc, therefore in presence of phytase, less concentration of supplemental zinc will be required. Zinc concentration over 20,000 mg/kg in diet may have anorexic effects leading to force-molting and reduced feed intake (Leeson and Summers, 2001). Deficiency of zinc in chick diet resulted in a decrease in alkaline phosphatase activity, impaired collagen synthesis and turnover and ultimately tibia deformities (Starcher et al., 1980). Supplementation of zinc to a low calcium diet, which resulted in reduced bone strength, enhanced the negative effect of the calcium deficiency in rats (Kenney and McCoy, 1997).

2.1.6 Manganese

Manganese is involved in skeletal integrity (Gajula et al., 2011) as it is required for glucosyltransferases (Yang and Klimis-Tavantzis, 1998). For optimum egg production and quality, the hen's requirements for critical minerals including manganese should be met (Tang et al., 2006). Dietary manganese influenced the calcification positively and feeding diets low in manganese and calcium concentrations to young layers resulted in lower tibia strength, bone weight, and ash content (Ochrimenko et al., 1992). Addition of manganese in diet decreased the number of cracked eggs and adding manganese with zinc increased shell strength (Essary and Holmes, 1964) and reduced number of broken eggs (Zamani et al., 2005). Manganese sulfate (100%) and manganese oxide (75%) had higher relative availability than manganese carbonate (40%) (Leeson and Summers, 2001).

According to NRC (1994), immature leghorn type chickens should have 30-60 mg/kg dietary manganese in the case of white egg laying strains whereas brown egg laying strains should be provided with 28-56 mg/kg manganese in the diet. The concentration of manganese in white egg layers feed should be 17-25 mg/kg whereas 20-22 mg/kg manganese should be included in feed of brown egg layers. Broilers feed should contain 60 mg/kg manganese (NRC, 1994) and the concentration of 3,000 mg/kg may become toxic (Leeson and Summers, 2001). According to GfE (1999), laying hen diets should contain 50 mg/kg manganese whereas broilers should be provided with 60 mg/kg dietary manganese.

Bone can be used as indicator of manganese status of birds and deficiency of manganese may lead to perosis (Leeson and Summers, 2001). A study on rats fed manganese depleted and manganese-low copper diets showed significantly lower bone density and impaired osteoblast activity (a measure of bone formation) and osteoclast activity (a parameter of bone resorption) (Strause and Saltman, 1987).

2.1.7 Iron

Although iron has no direct role in bone formation, it is mainly stored in the bone marrow (Aoyagi and Baker, 1995) and supplementation of 3.22 % $Fe2(SO4)3$ in layer diet containing 0.9 % iron has shown to impair the phosphorus availability resulting in lower bone ash (Deobald and Elvehjem, 1935). The bone marrow is the best indicator for iron depletion and normalization in case of recovery (Aoyagi and Baker, 1995). Almost all of the yolk iron binds to phosvitin, a phosphoprotein known as an iron-carrier in egg yolk (Ishikawa et al., 2004).

The iron sources available for feed production include highly available forms as ferrous sulfate (100%), ferrous chloride (95%) and ferrous oxide (50%), whereas ferrous carbonate (5%) has a low relative availability (Leeson and Summers, 2001).

NRC (1994) recommended 60-80 mg/kg iron in a diet for white egg laying strains of immature leghorn type chickens and 56-75 mg/kg iron in feed of brown egg laying type strains. The iron level in white egg laying hens diet was recommended as 38-56 mg/kg whereas 45-60 mg/kg iron should be included in feed of brown egg laying hens. Broiler diets should contain 60-80 mg/kg iron (NRC, 1994). According to GfE (1999), both laying hens and broilers should be provided with 100 mg/kg dietary iron. Hematocrit is the main parameter used to assess iron requirements and status in birds (Aoyagi and Baker, 1995). For the maintenance of the normal hematocrit, the laying hen diet should include 35-45 mg/kg iron, however for optimum hatchibility this amount should be as high as 55 mg/kg (Leeson and Summers, 2001).

Furthermore, the recommended level for iron in semi-purified diets based on casein and soy protein concentrate is around 85 mg/kg (Aoyagi and Baker, 1995).

2.2 FEED TREATMENTS AND THEIR IMPACT ON MINERAL DIGESTIBILITY

Various feed treatments are being used by feed industry to ensure balanced, economical and safe diet for poultry. Feed hygiene is an issue, which is associated with production performance, animal health and consequently safety issues for humans consuming chicken meat. This is due to the fact that feed is a potential infection source for animals and therefore, improving feed hygiene is an essential part in safe poultry production. Worldwide effective solutions to improve the feed hygiene are needed that are economically efficacious and have no negative impact on animal welfare, performance and consumer health. For this reason, methods like thermal treatment and acid supplementation are used in industry for feed sanitation purposes. Further, feed particle size and form is an important factor affecting production efficiency, especially in laying hens. Different milling methods including hammer and roller mills may be used to formulate feed of varying size and shape.

2.2.1 Thermal Treatment

Thermal treatment has proved useful in reducing microbial contaminations (Mossel et al., 1967b; Furuta et al., 1980; Jones et al., 1991; Veldman et al., 1995). Depending on the contaminating agent, feed components (Doyle and Mazzotta, 2000), water activity (Mossel et al., 1967b; Liu et al., 1969), and process parameters like temperature (Mossel et al., 1967b), pressure (Jones, 2011), and processing time at a given temperature (Van Schothorst and Brooymans, 1982) affects the efficacy of thermal treatments (Ricke, 2005). For instance pelleting with thermal pre-conditioning, a moderate way of heat treatment, reduced the bacterial load and feed borne pathogens (Mossel et al., 1967b; Cover et al., 1984; Van Immerseel et al., 2009).

Besides improving feed hygiene, thermal treatment like pelleting or expanding affects the diets on various other levels like feed intake, feed efficiency (Abdollahi et al., 2013), ileal apparent digestibility of starch (Abdollahi et al., 2011) and pellet stability (Fancher et al., 1996). Heat treatment was shown to influence bacterial status of the crop (Goodarzi Boroojeni et al., 2014a) and to improve feed conversion (Amerah et al., 2007). Thermal treatment promotes the development of the Maillard reaction, which occurs between amino acids and reducing sugars (van Boekel et al., 2010). High temperature may result in degradation of most heat-labile AA (Papadopoulos, 1989; Svihus and Zimonja, 2011) and provide favorable conditions for Maillard reactions (Mauron, 1981; Cheftel, 1986).

In context of mineral metabolism, especially the effects of thermal treatments on the nutrient digestibility, due to starch gelatinization, protein denaturation and greater accessibility of nutrient components to digestive enzymes are of interest (Abdollahi, 2013). A study reported that phosphorus availability was improved when diets were pelleted, as indicated by higher bone ash (Bayley et al., 1968). Pelleting and crumbling of the diet may reduce bone ash and plasma mineral concentrations in broilers (Kilburn and Edwards, 2004), which might be due to degradation of larger particles into smaller ones during the pelleting process, which could minimize the advantage of larger particle for longer transit time (Amerah et al., 2007). Some other studies revealed that pelleting reduced phytate phosphorus retention in coarse and fine particle size maize diets compared to non-pelleted diets (Kilburn and Edwards, 2001) and that maize showed 19 % and soybean meal had 44 % phytate phosphorus net absorption at terminal ileum of broilers fed diet without microbial phytase supplementation (Rutherfurd et al., 2002). A previous study on broilers indicated that oven drying of fresh maize grains for 24 h resulted in an increased absorption of calcium at 85°C and 95°C as compared to fresh maize. Similarly, phosphorus absorption was improved at 85°C. Furthermore, for both minerals the absorption decreased with a further increase in temperature up to 105°C, however, the effect of heat treatment on absorption of calcium and phosphorus was not clear (Iji et al., 2003).

2.2.2 Acid Supplementation

Another possibility to improve feed hygiene is supplementation of feed with organic acids that are used for decontamination and to avoid recontamination of feed from microbes (Martin and Maris, 2005; Ricke, 2005). For instance *Salmonella* spp. were significantly reduced by 0.2% propionic acid concentration (Matlho et al., 1997).

In general, various organic acids are used in the feed industry, which hold antimicrobial activity, may lower the pH of digesta, improve digestive enzyme and phytase activity, and have the potential to affect gastrointestinal functions beneficially (Jongbloed et al., 2000; Dibner and Buttin, 2002).

There is evidence that the supplementation of organic acids can have an impact on the mineral metabolism in broilers. It was found that citric acid improved apparent total digestibility of calcium, phosphorus and zinc (Brenes et al., 2003) and phosphorus utilization as well as the mineral concentration in tibia (Rafacz-Livingston et al., 2005). Additionally, citric acid enhanced bone strength in broilers by improving the macro mineral digestibility (Islam et al., 2012). Addition of 5.4 % sulfuric acid in poultry and ducks feed increased phosphorus utilization and tibia ash (Capdevielle et al., 1998). Liem et al. (2008) analyzed the effect of several organic acids including citric acid, malic acid, fumaric acid, and ethylenediaminetetraacetic acid (EDTA) on phytate phosphorus hydrolysis and concluded that the reason why some organic acids are effective whereas others are not is not apparent. The effects of organic acids are inconsistent and depending on dosage, buffering capacity of dietary ingredients, and cleanliness of production environment (Dibner and Buttin, 2002).

2.2.3 Milling Method

The use of roller and hammer mills for reduction of the grain particle size is common in poultry feed industry (Koch, 2002; Amerah et al., 2007). Besides the raw material, the energy needed during production has a major impact on the total feed costs and energy saving milling methods such as the roller mill are becoming more used in the feed industry. Diminution of feed is the largest energy cost in layer feed production (Deaton et al., 1989) and the second largest after pelleting in broilers (Reece et al., 1985). The roller mill requires less energy, produces cubic or rectangular shaped (Koch, 2002) and a more uniform sized particles (Amerah et al., 2007) than the hammer mill. The hammer mill is easier to use (Koch, 2002), produces spherical shaped but a greater amount of fine particles (Reece et al., 1985).

2.2.4 Particle Size

The particle size of feed is affected by milling method and processes like expansion, which is critical for nutrient intake and utilization by poultry. In general, a reduction in particle size increases the surface area for enzymatic activity, modifies the physical characteristics of feed and may result in improved animal performance (Waldroup, 1997; Goodband et al., 2002). The coarser grinding of grains to a more uniform particle size may improve the performance of mature poultry due to more gizzard development leading to increased grinding, gut motility and nutrient digestion (Amerah et al., 2007). Particle size distribution in feed affected egg quality and body weight, probably as small particle bound nutrients are less effectively utilized (Tang et al., 2006). Chickens have a preference for larger feed particles (Schiffman, 1968) at all ages (Portella et al., 1988), whereas vitamins and minerals are mainly contained in smaller particles. The particle size is more critical in mash diets as compared to pelleted or crumbled diets, due to selective feed intake of laying hens. Coarse particles require a longer transit time, which may allow higher mineral absorption (Amerah et al., 2007). The utilization of calcium, total phosphorus and phytate phosphorus was higher in broilers fed diets with coarse maize particle size (geometric mean diameter (GMD)=894 µm) as compared to finer particle size (GMD=573 and 484 µm) (Kasim and Edwards, 2000). Moreover, feeding of phosphorus deficient maize diet (5.0 g/kg total phosphorus) in broilers with coarse particle size (GMD=2897 µm) improved bone ash as compared to fine particles size (GMD=869 µm). However bone ash was not affected by particle size when adequate phosphorus diet (7.1 g/kg total phosphorus) was fed (Kilburn and Edwards, 2004).

2.3 EGG QUALITY AND EFFECT OF FEED TREATMENTS ON EGG QUALITY

2.3.1 Importance of Egg Quality in Layers

Egg quality is defined as the characteristics of an egg affecting its acceptability by consumers (Stadelman and Cotterill, 1995) and its technological properties for the production of food products. Furthermore, the beneficial egg quality traits have great importance to poultry breeding industries (Bain, 2005). Therefore, various internal and external egg quality parameters have been defined as important measures for the egg industry.

Egg quality traits of economic importance including weight, size, albumen and yolk content have continuous variability which can be measured to determine egg quality (Islam and Dutta, 2010). The egg shell and interior contents are quality determining factors. The stability or breaking strength of egg shells affect the soundness of the shell and prevents breaking or cracking. Egg weight influences egg quality and is considered as important phenotypic trait (Islam et al., 2001). Due to the fact that hens have a finite capacity to deposit calcium in the shell, larger eggs may have a weaker shell in comparison with smaller eggs (Clunies et al., 1992; Kabir et al., 2014). Interior egg quality is most important from consumer point of view and it starts declining with time once the egg is laid. The yolk percentage is inversely proportional to the albumen percentage due to osmotic pressure gradient as water moves from albumen to yolk with time (Silversides and Budgell, 2004), weakening the vitelline membrane (Saldanha et al., 2010), thereby decreasing firmness and roundness and giving a flattened shape to yolk. Similarly, the presence of a dense albumen layer is indicator of internal egg quality (Stevens, 1995). The ratio of yolk to albumen is important for egg breaking companies, which market egg contents rather than whole egg (Hussein and Harms, 1994). The yolk color is determined by xanthophyll content of the diet and the preference of the consumer for yolk color varies from region to region (Leeson and Summers, 2001).

2.3.2 Determination of Egg Quality

Due to the different preferences of end users, the egg quality has different meanings for consumers, food industry and poultry breeding industry. Therefore, various egg quality parameters have previously been used as indicators of exterior and interior egg quality keeping in view the purpose of egg production.

Parameters which have previously been used to determine the exterior egg quality include egg weight (Parmar et al., 2006; Tang et al., 2006; Lichovnikova, 2007; De Witt et al., 2009; Safaa et al., 2009; Phirinyane et al., 2011), area vs egg weight (Paganelli et al., 1974), shape index (Ehtesham and Chowdhury, 2002; Parmar et al., 2006; Saki et al., 2012), shell weight per unit surface area (SWUSA) (De Witt et al., 2009; Phirinyane et al., 2011; Saki et al., 2012; Safamehr et al., 2013) and stability (Ehtesham and Chowdhury, 2002; Parmar et al., 2006; Lichovnikova, 2007). Some other studies used shell surface area (Ehtesham and Chowdhury, 2002; De Witt et al., 2009; Dudusola, 2009; Phirinyane et al., 2011), shell density (Carter, 1975; Dudusola, 2009), shell thickness, shell weight and relative shell weight (Lichovnikova, 2007; De Witt et al., 2009; Phirinyane et al., 2011; Saki et al., 2012), as well as shell membrane weight (Ehtesham and Chowdhury, 2002) and relative shell membrane weight (Safaa et al., 2009) as indicators of exterior egg quality.

The interior egg quality might be reflected by the measurement of different parameters including air cell and blood spot (Safaa et al., 2009), yolk color (Ehtesham and Chowdhury, 2002; Saki et al., 2012) and Haugh unit (Parmar et al., 2006; Tang et al., 2006; Dudusola, 2009; Safaa et al., 2009; Safamehr et al., 2013; Shim et al., 2013). Other interior egg quality parameters which have previously been used include yolk index (Ehtesham and Chowdhury, 2002; Parmar et al., 2006; Dudusola, 2009; Saki et al., 2012), yolk weight (Ehtesham and Chowdhury, 2002; Parmar et al., 2006; Islam and Dutta, 2010; Phirinyane et al., 2011; Shim et al., 2013), yolk height, yolk width, yolk length, albumen height, albumen width, and albumen length (Ehtesham and Chowdhury, 2002; Phirinyane et al., 2011), albumen weight and albumen index (Ehtesham and Chowdhury, 2002; Parmar et al., 2006; Phirinyane et al., 2011). Furthermore, the albumen to yolk ratio (Hussein and Harms, 1994), % yolk weight (Shim et al., 2013), as well as relative albumen weight (Safaa et al., 2009) may also be used as interior egg quality parameter.

2.3.3 Effect of Feed Treatments on Egg Quality

The comparison between corn based diets milled with hammer or roller mills showed no effects on laying hen performance and egg shell breaking strength (Deaton et al., 1989). Interestingly, layers fed with a barley based diet ground by roller mill had higher egg weight compared to the same diet produced by a hammer mill (Hamilton, 1994). Layers fed a barley based diet ground by roller mill reduced feed intake and egg production as compared to maize and wheat diets, while no differences were observed when a hammer mill was used. Egg quality was not affected by milling methods (P´erez-Bonilla et al., 2014).

Thermal treatment of feed did not affect egg quality parameters including weight, Haugh unit and blood spot (Hamilton and Proudfoot, 1995a). On the other hand, a previous study showed that Leghorn hens receiving a barley based diet flame-roasted at 124°C had eggs with higher Haugh unit scores than hens fed with non-roasted diets (Hamilton, 1994), which might have been resulted by better feed conversion in hens fed with flame-roased diet.

Due to the fact that chickens have a preference for larger feed particles (Schiffman, 1968), the particle size distribution may affect egg quality, as nutrients may not be equally ingested or effectively utilized (Tang et al., 2006). However, coarser particle size increased the feed intake, but seems to have no effect on egg quality and productive performance in young brown layers (Safaa et al., 2009). Another study demonstrated that feed particle size and form, including mash, crumbles, and pellets did not affect egg weight and interior egg quality parameters including Haugh unit and blood spots (Hamilton and Proudfoot, 1995a). Although the fine particle size provides more surface area for enzymatic activity, there is no advantage of reducing particle size below 800 µm in layers (Goodband et al., 2002) and at all ages of bird a particle size of less than 600 µm should be avoided (Waldroup, 1997).

3 AIMS AND OBJECTIVES

The first part of the dissertation (Chapter 4) deals with the impact of feed treatment on mineral absorption and retention in broilers. For this part, the purpose of the study was to evaluate if the widespread aim to improve mineral absorption and subsequently bone quality can be achieved by measures commonly used for improving feed safety. Poor feed hygiene is associated with less production performance as well as high mortality and consequently causes safety issues for humans consuming chicken meat. Thermal processing has been proved useful for decontamination of feed. Organic acids have been used in poultry feeds as preservative agents due to their antimicrobial effects. Improved mineral digestion might be linked with better bone quality. In combination with the inclusion of organic acids in broiler diets, thermal treatment may have an effect on mineral absorption and ultimate retention in bone and liver.

Therefore, the first part of the present dissertation was designed to consider the effect of different thermal treatments including pelleting, long term conditioning at 85ºC for 3 minutes, and expanding at 130ºC for 3-5 seconds without or with 1.5% of a product containing organic acids and their interactions on:

1. Apparent ileal absorption of minerals and trace elements
2. Retention of minerals and trace elements in tibia and tibia quality
3. Retention of minerals and trace elements in the liver

The second part of the dissertation (Chapter 5) focuses on the impact of form, thermal treatment and particle size of feed on mineral absorption and retention in laying hens. Different milling methods produce particles of varying size, shape and uniformity which can affect feed intake, growth rate, feed conversion ratio and laying performance of the laying hen. Furthermore, milling methods and thermal treatment alone and in combination, may affect the absorption of minerals and trace elements and their retention in egg contents as well as egg quality. Therefore, the second part of the dissertation was formulated to investigate the impact of milling methods including roller and hammer mill, thermal treatment (mash vs expansion) and their interaction with coarse and fine particle size of feed in laying hens on:

1. Apparent ileal absorption of minerals and trace elements
2. Apparent total digestibility of minerals and trace elements
3. Retention of minerals and trace elements in yolk, albumen and shell of eggs

The third part of the dissertation (Chapter 6) was performed to discuss the effect of milling methods including roller and hammer mill, thermal treatment (mash vs. expansion) and their interaction with coarse and fine particle size of feed in laying hens on:

1. Exterior egg quality
2. Interior egg quality

4 IMPACT OF THERMAL AND ORGANIC ACID TREATMENT OF FEED ON APPARENT ILEAL MINERAL ABSORPTION, TIBIAL AND LIVER MINERAL CONCENTRATION AND TIBIA QUALITY IN BROILERS

Hafeez, A. Mader, F. Goodarzi Boroojeni, I. Ruhnke, I. Röhe, K. Männer, and J. Zentek[1]

Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Königin-Luise-Str. 49, 14195 Berlin, Germany

2014 Poultry Science 93 (7): 1754–1763
http://dx.doi.org/10.3382/ps.2013-03750

[1] Corresponding author: E-mail: juergen.zentek@fu-berlin.de. Tel: +49 30 838 54245 Fax: +49 30 838 55938.

ABSTRACT Minerals play an important role for growth and bone stability in broilers. Thermal treatment and inclusion of organic acids in feed may affect the mineral absorption and tibial quality in broilers. The study was conducted to investigate the effect of thermal processing of feed including pelleting (P), long-term conditioning at 85°C (L) and expanding at 130°C (E) without and with 1.5 % of an acid mixture containing 64 % formic and 25 % propionic acid on the apparent ileal absorption (AIA) of calcium, phosphorus, magnesium, potassium, sodium, iron, copper, manganese and zinc, their concentrations in liver and tibia as well as various tibial quality parameters in broilers. In total, 480 day-old Cobb broiler chicks were assigned using a completely randomized design with 3 × 2 factorial arrangement. The ileal digesta, liver and tibia were collected at d 35. AIA of calcium and sodium was improved in group E compared to L ($P \leq 0.02$ and $P \leq 0.01$). Group P and E showed higher AIA for potassium than L ($P \leq 0.01$). Bone ash content was increased in group E compared to L ($P \leq 0.04$). The body weight to bone weight ratio was lower and tibial zinc content was higher in group P compared to E ($P \leq 0.05$). Tibial iron content was higher in group L than E ($P \leq 0.03$). Acid addition did not affect AIA, mineral content in tibia or tibial quality parameter. Both, thermal treatment and acid did not affect mineral concentrations in the liver, except an inconsistent interaction effect for dry matter content and sodium ($P \leq 0.03$ and $P \leq 0.04$). In conclusion, long term thermal treatment reduced AIA of some minerals compared to short term thermal treatments, but had no impact on tibia composition. Acid inclusion had no effect on AIA of minerals and tibia quality. Thermal treatment and the use of organic acids can therefore be considered as safe regarding their impact on bone development in broilers.

Key words: feed decontamination, organic acid, pelleting, mineral absorption, tibia

4.1 INTRODUCTION

Macro minerals and trace elements have an essential role in different physiological functions, including bone formation. Rapid broiler growth, an important factor in poultry production, can have a negative impact on skeletal development (Nestor et al., 1985; Lilburn, 1994; Bessei, 2006; Angel, 2007). Bone stability and breaking strength are mainly determined by the degree of mineralization of the bone matrix (Boivin and Meunier, 2002).

Calcium and phosphorus are the primary inorganic nutrients contributing to 95 % of the mineral matrix in bones (Rath et al., 2000; Shim et al., 2012) and about 97 % of total calcium is found in bones (Scott et al., 1982; Leeson and Summers, 2001). Within certain physiological limits higher levels of calcium and phosphorus in feed increase bone mineral content, bone density, and ash content (Onyango et al., 2003; Rao et al., 2006; Coto et al., 2008). Deficiencies of calcium and phosphorus lead to their reduction in bone (Wilson and Duff, 1991; Hemme et al., 2004). Sodium and potassium as well as chloride are necessary to maintain a physiological acid-base balance, which is important for regular bone development (Leeson and Summers, 2001). With increased magnesium level (0.2 % Mg citrate or 0.2 % MgO supplementation in diet), calcium to phosphorus homeostasis was affected and tibiae from chicks showed abnormal development and reduced ash content (Lee et al., 1980).

Within the trace elements, copper is important for optimal bone formation, as it is required for collagen crosslinking (Osphal et al., 1982). With the increase in collagen crosslinking, bone strength increased (Rath et al., 1999). Deficiency of zinc in chick diet resulted in a decrease in alkaline phosphatase activity, impaired collagen synthesis and turnover and ultimately tibia deformities (Starcher et al., 1980). Manganese is involved in skeletal integrity (Gajula et al., 2011) as it is required for glucosyltransferases (Yang and Klimis-Tavantzis, 1998). Dietary manganese influenced the calcification positively and feeding diets low in manganese and calcium concentrations to young layers resulted in lower tibia strength, bone weight, and ash content (Ochrimenko et al., 1992). Iron is mainly stored in the bone marrow (Aoyagi and Baker, 1995) and supplementation of 3.22 % Fe2(SO4)3 in layer diet containing 0.9 % Fe has shown to impair the phosphorus availability resulting in lower bone ash (Deobald and Elvehjem, 1935).

The effects of thermal treatment on mineral absorption, storage and tibial quality have not been widely considered up to now, although mainly positive effects of thermal treatment on microbial contaminations in feed, pellet stability, feed intake, growth, feed conversion ratio, feed efficiency and nutrient digestibility are known (Coelho, 1994; Fancher et al., 1996; Beyer, 2000; Abdollahi et al., 2013). Nevertheless, it could be demonstrated that phosphorus availability improved when diets were pelleted, as indicated by improved bone ash (Bayley et al., 1968).

Various organic acids are used in the feed industry to improve feed hygiene by decontamination by avoiding recontamination (Martin and Maris, 2005; Ricke, 2005), lower the pH of digesta, improve digestive enzyme and phytase activity, and to beneficially affect gastrointestinal functions (Jongbloed et al., 2000; Dibner and Buttin, 2002). Additionally, some data on mineral absorption, storage and tibial quality is available. Citric acid improved phosphorus utilization as well as the mineral concentration in tibia by increasing bone ash (Rafacz-Livingston et al., 2005), affected retention of calcium and zinc, tibial ash as well as tibial phosphorus and calcium content in broilers (Brenes et al., 2003) and enhanced bone strength in broilers by improving total digestibility of calcium, phosphorus and magnesium (Islam et al., 2012).

To our best knowledge, no more data on the impact of thermal and acid treatment of feed on the apparent ileal digestibility and the concentration of minerals in tibia and liver and the tibia quality in broilers, were published.

Therefore, the purpose of the study was to evaluate if apparent ileal absorption, tibial and liver mineral content and bone development can be affected by different levels of thermal processing without or with the use of organic acids and their interaction in broiler feeds.

4.2 MATERIAL AND METHODS

The experiment was conducted under approval of the experimental protocol by the State Office of Health and Social Affairs Berlin (LAGeSo Reg. No. 0113/11).

4.2.1 Animals and Experimental Design

The experiment lasted for 35 days. Day-old male (Cobb) chicks were purchased from a commercial hatchery (Cobb Germany Avimex GmbH, Wiedemar-Wiesenena). Four hundred and eighty chicks were randomly distributed into 48 pens with 10 birds per pen. Each pen had 1.20 m × 1.75 m space. Softwood shaving was used as bedding material for all pens. The temperature of the stable was maintained at 33°C for the first week. From the second week onwards, the temperature was reduced by 3°C per week until the end of experiment. During brooding period, all pens were provided with 24 h light for first 3 days. Light was reduced to 20 h for the next 4 days and 16 h light was provided from day 8 till day 35. Feed and water was provided *ad libitum* throughout the experiment.

During the first 21 days of age, the chicks were provided with starter feed; grower feed was offered during the last 14 days. Diet formulation was done according to recommendations laid down by the German Society of Nutritional Physiology (GfE, 1999). To analyze apparent mineral absorption in the ileum, the grower diet was supplemented with titanium dioxide (TiO_2, Sigma Aldrich, St. Louis, MO, USA) as an indigestible marker at 2 g/kg. Table 1 represents the composition of both, starter and finisher diets. The concentrations of minerals in the grower feed are given in table 2.

The study was conducted using a completely randomized design with a two-factorial arrangement. Feed was treated with three types of thermal processing including steam conditioning at 70°C for 15-20 seconds followed by pelleting (P), long-term conditioning at 85°C for 3 minutes and subsequent pelleting (L) and expansion at 130°C for 3-5 seconds (E) without (0 %) or with 1.5 % supplementation of a commercial product containing 63.75 % formic acid, 25.00 % propionic acid, and 11.25 % water (Lupro-Cid®, BASF SE, Ludwigshafen, Germany). The six different diets, formulated using a 3×2 design, were then randomly assigned to chicks within 48 pens with 8 pens per diet. The procedure of feed production and its technical details were according to Goodarzi Boroojeni et al. (2014).

4.2.2 Feed Production Technology

For primary diets feed components were mixed using a twin-shaft paddle mixer for 3 minutes (Type 300 LTR, Dinnissen B.V., Sevenum, Netherlands). During continuous mixing, Lupro-Cid® was continuously sprayed on the diets containing acids. After mixing, the diets were subjected to the different processing methods.

Pelleting: During short term steam conditioning, the temperature of the feed exiting the conditioner (Type M-Mix, Simon-Heesen B.V., Boxtel, Netherlands) was maintained at 70°C for 15-20 seconds. The conditioned feed was pelleted using a ring die pellet press (Type Monoroll Labor, Simon-Heesen B.V., Boxtel, the Netherlands) with a die channel diameter and length of 3 mm and 60 mm, respectively. The pressed material was cooled on a belt cooler (Fördertechnik GmbH, Mülheim /Ruhr, Germany) to ambient temperature within 15 min.

Long-Term Conditioning and Pelleting: Prior to pressing, diets were pre-conditioned in a twin-screw pre-conditioner (Neuhaus, Delmenhorst, Germany) using steam and subsequently subjected to long-term conditioning in a closed container with a heated jacket. The diets were heated to 85°C for 3 minutes. The conditioned feed was immediately introduced into the pellet press and pelleted as described above.

Expanding: Diets were introduced into an extruder (Type OE 8, AmandusKahl GmbH & Co. KG, Reinbek, Germany) and processed at 130°C. The feed exited the extruder through 3 mm-outlet nozzles and formed strands, which were cut to pellet-shape by rotating blades. The feed was cooled to ambient temperature on a belt cooler (Fördertechnik GmbH, Mülheim/Ruhr, Germany) within 15 minutes. The moisture content was reduced by using preheated air in one of the four segments of the cooler. After production, the pelleted and extruded feed was crumbled using a roller mill (Type A2-E, MIAG, Braunschweig, Germany) with a milling gap of 3 mm to homogenize particles sizes.

4.2.3 Collection of Samples

At the end of experiment at day 35, birds were weighed, stunned, and killed by exsanguination. Carcasses were dissected immediately after slaughtering. For mineral absorption analysis, ileum digesta were collected from six birds, randomly selected from each of the 8 pens of each diet taking the distal 2/3 part of ileum after dissection from Meckel's diverticulum to the ileo–caeco–colic junction. The digesta of birds within one pen were pooled and immediately frozen at -80°C prior to analysis. For analysis of mineral concentration in liver and tibia, 2 birds from 6 pens in each diet group were randomly selected. Livers were removed and cleaned from fat and gall bladder. After weighing, the liver was frozen at -20°C. The left tibiae were removed, de-fleshed and the patella was removed. After cleaning, the tibiae were weighed and frozen (-20°C) until further analysis.

4.2.4 Chemical Analysis

4.2.4.1 Apparent Ileal Absorption

Apparent ileal absorption of minerals was calculated using the following formula:

Apparent Ileal Absorption (%) = 100-((concentration of marker in feed / concentration of marker in ileum) × (concentration of nutrient in ileum / concentration of nutrient in feed) ×100)

4.2.4.2 Tibia Quality Parameters

Tibiae were weighed and length was measured with the help of a caliper. Thicknesses of medial and lateral walls were obtained from the mid-point of the bone. The following formulae were used to calculate robusticity index and tibiotarsal index:

Robusticity index =bone length/cube root of bone weight (Riesenfeld, 1972)

Tibiotarsal index =diaphysis diameter − medullary canal diameter/diaphysis diameter ×100 (Barnet and Nordin, 1960)

Weight of bone in water was measured using electronic semimicro balance (Type 2024MP6, Sartorius GmbH, Göttingen, Germany). Bone density was calculated using the following formula:

Bone density = weight of bone in air / (weight of bone in air – weight of bone in water) × water density at water temperature

The body weight to bone weight ratio was calculated by dividing body weight (g) by bone weight (g).

4.2.4.3 Analysis of Minerals Concentrations

Tibiae were crushed manually into small particles, freeze dried, and defatted. To determine the ash content, tibiae were ashed in a muffle furnace at 600°C for 6 hours. The percent ash was determined with relation to fat free dry matter in tibia. Concentrations of calcium, magnesium, potassium, sodium, iron, copper, manganese, and zinc were determined by atomic absorption spectrometry in an AAS vario6 spectrometer (Analytik Jena, Jena, Germany). Ammonium vanadate/molybdate method was adopted to measure the concentration of phosphorus as described by Gericke and Kurmies (1952).Concentrations of titanium dioxide in feed and ileum digesta were determined according to method described by Short et al. (1996).

Feed, ileum digesta, and liver samples were freeze dried using same procedure like tibia. Dried liver was cut into small pieces using a plastic knife. Dried feed, digesta, and liver were ashed in muffle furnace at 600°C for 6 hours and ash content was calculated in relation to the amount of dry matter in feed, ileum, and liver, respectively. Analysis for mineral concentrations in feed, digesta, and liver were performed in the same way as in tibia.

4.2.5 Statistical Analysis

Data were arranged as a 3 × 2 factorial arrangement including 3 types of thermal processing (P, L, and E) and 2 levels of acid (0 % and 1.5 %). Normal distribution was tested using Shapiro-Wilk. On the basis of the results, normally distributed variables were subjected to ANOVA using the GLM procedure and not normally distributed parameters were analyzed by Kruskal-Wallis test of SPSS 20.0 (SPSS Inc., Chicago, IL). In order to achieve normality, potassium (liver) was log-transformed before analysis. Pearson's correlation method was used for normally distributed parameters. Analyses were performed for main treatment effects and their interactions. Tukey's-b test was used as post-hoc test at $P \leq 0.05$ for grouping of treatment means. Apparent ileal absorption variables were measured on the basis of pen as experimental unit. Bird was used as experimental unit to measure BW, liver and tibia variables.

4.3 RESULTS

Thermal treatment had a significant effect *($P \leq 0.05$)* on the apparent ileal absorption of calcium, potassium, and sodium (Table 3). The absorption of calcium and sodium was higher in the group E compared to group L($P \leq 0.02$ and $P \leq 0.01$, respectively), whereas for the group P, no differences could be found($P > 0.05$). Enhanced apparent potassium absorption was determined in the groups E and P compared to the group L ($P \leq 0.01$). No differences were observed among various thermal treatment groups for phosphorus, magnesium, iron, copper, manganese, and zinc ($P > 0.05$). Acid supplementation and interaction between thermal treatment and acid had no effect ($P > 0.05$) on the apparent ileal absorption of macro and trace elements. Table 4 demonstrates various quality parameters and minerals concentrations in the tibia of broilers at 35 days of age. The ratio between body weight and bone weight was reduced in the group P compared to the group E($P \leq 0.04$), however the group L did not show significant differences. The amount of ash in fat free DM was 55.0 %, 53.7 % and 53.3 % in the groups E, P and L respectively ($P \leq 0.4$). Group L displayed higher iron concentrations compared to group E ($P \leq 0.03$). Pelleting resulted in higher tibia zinc concentration compared to group E ($P \leq 0.01$) and no effect could be found for group L. No differences ($P > 0.05$) were observed among the various treatments groups in bone length, bone weight, tibiotarsal index, robusticity index, and bone density. Dry matter percent and fat free dry matter percent were comparable between the treatment groups. The concentrations of calcium, phosphorus, magnesium, potassium, sodium, copper, and manganese were at similar levels. Acid inclusion had no effect on any of the investigated variables and there was no interaction effect between thermal and acid treatment for any tibia quality parameter or tibia mineral concentration ($P > 0.05$). The concentrations of minerals in the liver were not affected by thermal and acid treatment (Table 5). The interaction of thermal treatments and acid affected dry matter percent in liver ($P \leq 0.03$). The retention of sodium in liver was significantly affected by interaction of thermal treatments and acid ($P \leq 0.05$) which was lower in group E+0 % (2.02±0.07 g/kg DM) compared to groups P+0 %, L+1.5 % and E+1.5 % (2.39±0.13, 2.33±0.07 and 2.29±0.09g/kg DM), respectively($P \leq 0.04$).

4.4 DISCUSSION

The various thermal treatments used in present study are combinations of different defined levels of temperature and duration. Each of the various factors of thermal treatment may have an influence on the investigated parameters. Nevertheless, the different factors of thermal treatment cannot be tested one by one, because the study was designed to compare common thermal treatments used by the industry and the various parameters of common thermal treatments affect each other. The thermal treatments used can be differentiated into two factors: the maximum temperature (°C) and the duration of the feed at the maximum temperature (time). Therefore, both factors have to be taken into account when interpreting the results.

In general the percentages of apparent ileal mineral absorption for the short term treated diets were similar to previous data published, however those for the long term treated diet were partly below previously published ranges of total availability of calcium (68-71 %), phosphorus (44-77 %), magnesium (51-62 %), copper (69-87 %), manganese (48-60 %), and zinc (44-58 %) (Nwokolo and Bragg, 1980; Awyong et al., 1983).

Thermal treatment increased apparent ileal absorption of calcium, potassium, and sodium when the feed was expanded at 130°C for 3-5 seconds compared to pelleting at 80°C for 5 seconds and long term treatment at 85°C for 3 minutes ($P \leq 0.05$). Additionally, apparent ileal absorption values of phosphorus reflect similar impact, although there was no significant change. A previous study on broilers indicated that oven drying of fresh maize grains for 24 h resulted in an increased absorption of calcium at 85°C and 95°C ($P \leq 0.05$) as compared to fresh maize. Similarly, phosphorus absorption was improved at 85°C ($P \leq 0.05$). However, for both minerals the absorption decreased with a further increase in temperature up to 105°C (Iji et al., 2003). Some other studies revealed that pelleting reduced phytate phosphorus retention in coarse and fine particle size maize diets compared to non-pelleted diets (Kilburn and Edwards, 2001) and that maize showed 19% and soybean meal had 44% phytate phosphorus disappearance at ileum (Rutherfurd et al., 2002). According to our knowledge, no further literature on thermal feed treatments is present.

Inclusion of 1.5 % organic acids did not affect the apparent ileal absorption of minerals and trace elements, except a trend for lower absorption of phosphorus in the acid inclusion group ($P \leq 0.08$). Recent study using citric acid in broilers diets reflected that the apparent absorption of calcium and phosphorus was higher by addition of 0.75 % acid, but further addition of acid resulted in a decline of absorption (Islam et al., 2012), the addition of 2 % acid increased calcium, phosphorus, and zinc retention (Brenes et al., 2003) and 4-6 % acid increased the phosphorus utilization without having any effects on the calcium utilization (Boling-Frankenbach et al., 2001). Addition of 5.4 % sulfuric acid in poultry and ducks feed increased phosphorus utilization and tibia ash (Capdevielle et al., 1998). Liem et al. (2008) analyzed the effect of several organic acids including citric acid, malic acid, fumaric acid, andethylenediaminetetraacetic acid(EDTA) on phytate phosphorus hydrolysis and concluded that the reason why some organic acids are effective whereas others are not is not apparent. The effects of organic acids are inconsistent and depending on dosage, buffering capacity of dietary ingredients, and cleanness of buffering environment (Dibner and Buttin, 2002), which may explain the contrast in our finding in comparison with some studies mentioned above.

No interaction effect was observed between acid inclusion and thermal processing. It indicates that the investigated thermal and acid treatment combinations has no effect on mineral absorption in ileum and therefore, can safely be used for decontamination and to avoid recontamination during cooling process of broiler feed formulation to enhance feed hygiene.

The present study showed some interesting results about the effect of thermal treatment on bone quality and tibial mineral contents. We used various parameters to indicate the bone status which have been reported in previous studies including bone breaking strength (Merkley, 1981; Ruff and Hughes, 1985; Park et al., 2003; Kim et al., 2006), bone density (Watkins and Southern, 1992; Onyango et al., 2003; Kim et al., 2006), bone mineral content (Akpe et al., 1987; Onyango et al., 2003; Kim et al., 2006), and bone ash (Garlich et al., 1982; Cheng and Coon, 1990; Park et al., 2003; Shim et al., 2008). In group P, body weight to bone weight ratio was lower ($P \leq 0.04$) which suggests that higher amount of bone content was available in relation to body weight in group P as compared to group E. Due to no observed difference in bone density ($P > 0.05$), it was assumed that difference in this ratio was by chance and has no known biological reason. Our data indicate that in group E, ash content increased ($P \leq 0.05$) when mineral content was enhanced due to a trend for higher calcium ($P \leq 0.1$). With the increase of ash, bone hardness or strength is expected to increase (Bonser and Casinos, 2003). The organic component of bone is important in providing tensile strength and flexibility (Velleman, 2000). Higher zinc content was found in group P ($P \leq 0.01$). The literature suggests that zinc is involved in collagen synthesis and turnover (Starcher et al., 1980), bone development (Ovesen et al., 2001) and integrity of bones (Gajula et al., 2011) whereas copper plays a role in collagen cross linking (Osphal et al., 1982). Interestingly in group L, iron concentration was higher and ash content was lower in comparison with group E, whereas copper concentrations showed a similar trend ($P \leq 0.03$, $P \leq 0.04$ and $P \leq 0.1$ respectively). In general, iron had a negative correlation with ash percent (-0.80) and calcium (-0.33) ($P \leq 0.05$). This indicates that the higher concentration of iron might have reduced mineralization. Our results are in line with literature which suggest that the addition of 3.22% Fe2(SO4)3 having 0.9 % iron as compared to 1.61% Fe2(SO4)3 with 0.45 % iron in layer diet reduced the bone ash from 29.5 % to 25.4 % on fat free dry matter basis. This amount of iron was sufficient to bind one half of the total phosphorus in the ration as FePO4, thereby causing phosphorus deficiency in blood and ultimately severe rickets (Deobald and Elvehjem, 1935).

However, no differences were found for calcium and phosphorus concentrations within this study which is inline with findings of Edwards et al. (1999) who reported that pelleting did not improve the total digestibility of phytate phosphorus. Phosphorus availability was improved by pelleting when corn-soy based mash diets containing no added inorganic phosphate, as indicated by improved bone ash (Bayley et al., 1968). Cereals contain 60-70% of phosphorus in the form of phytic acid which is poorly available (0-50%). In young birds total phosphorus availability from monocalcium phosphate may be as high as 98% (Leeson and Summers, 2001). In the present study diets contained both, phytic acid from the soy and maize and monocalcium phosphate. Therefore, the lack of treatment effects on apparent phosphorus absorption could be explained by the presence of both phytic acid and inorganic phosphorus. Bone density, which can indirectly be used to determine bone strength (Shim et al., 2012) did not differ in present study. Bone length, body weight, robusticity index, tibiotarsal index, dry matter percent, fat free dry matter percent, magnesium, potassium, sodium, and manganese did not show any differences. According to our knowledge, no further previous results are available regarding the effect of feed processing on tibia quality and mineral concentrations in tibia.

Inclusion of organic acids did not affect of the measured tibial quality parameters and mineral concentrations. However, a trend was observed towards a higher tibiotarsal index and lower sodium concentration in 1.5 % acid group. The literature suggests that bone breaking strength, bone density, and dry matter contents were increased by up to 0.75 % dietary citric acid and decreased with increasing acid levels (Islam et al., 2012). Further, tibial ash, calcium, and magnesium decreased numerically in birds consuming a diet with 1.5 % as compared to 0.75 % citric acid (Islam et al., 2012).With an increase of citric acid in diet from 0 - 4 % tibia ash increased linearly (Rafacz-Livingston et al., 2005). Inclusion of citric acid at 3 - 4 % level in broiler feed lead to improved phosphorus utilization and mineral concentration in the tibia (Rafacz-Livingston et al., 2005). Citric acid at 0.5 % level significantly increased tibia ash percentage (Chowdhury et al., 2009). Additionally, the inclusion of citric acid affected calcium and zinc retention, tibia ash, phosphorus, and calcium content in tibia (Brenes et al., 2003). Our results could be interpreted in the context that the effects of organic acids are inconsistent depending on dosage, buffering capacity of dietary ingredients, heterogeneity of gut microbiota and presence of other antimicrobial compounds (Dibner and Buttin, 2002).

Thermal and acid treatment combinations failed to show interaction effects for any of tibial parameters or mineral concentrations ($P>0.05$). Therefore, organic acids at 1.5 % level may safely be used along with various thermal treatments for sanitation of broiler feed.

Our data show that thermal treatment and acid supplementation had no effect on fresh liver weight, ash percentage, and retention of any mineral and trace element in hepatic tissue ($P>0.05$). However, there was an inconsistent interaction between thermal processing and acid treatment for dry matter and sodium ($P\leq0.05$).

In the present study the observed mineral levels in hepatic tissue are in agreement with the literature, which revealed a wide range for some of the minerals. The concentrations reported in different studies are for magnesium (0.644-0.652 g/kg DM), phosphorus (10.7-10.9 g/kg DM), calcium (0.107-0.115 g/kg DM), copper (3.0-15.1 mg/kg DM), zinc (28.0-115 g/kg DM), iron (75.0-634 g/kg DM), and manganese (4.3-11.6 g/kg DM) (Thompson and Weber, 1981; Black et al., 1984; Henry et al., 1987; Skrivan et al., 2005; Bao et al., 2007). However, not only the trace elements were used at various levels in experimental diets for most of these studies but also different methods were used for preparation, digestion and analysis of liver samples (Thompson and Weber, 1981; Korsrud et al., 1985; Henry et al., 1987; Falandysz, 1991; Skrivan et al., 2005; Bao et al., 2007), which might be the reasons for inconsistency in observed mineral values in literature. According to our knowledge, the effect of thermal and acid treatment of feed and their interaction effects on mineral concentrations in liver were not reported in previous studies.

The lack of significant differences in the mineral concentrations of various thermal processing and acid treatment groups in liver might be due to the fact that the impact on apparent ileal absorption was too low and liver is not enough sensitive for indicating mineral retention under these conditions. Previous studies reveal that the tibia is a better indicator for some specific aspects of mineral retention. For example, manganese and zinc accumulate in tibia rather than in liver (Gajula et al., 2011), independently to the organic or inorganic form of manganese supplemented in the diet (Berta et al., 2004).

In conclusion, long term thermal treatment impaired apparent ileal absorption of calcium, potassium, and sodium and resulted in reduced tibial ash content compared to short term thermal treatment. Inclusion of organic acid had no negative effect on apparent ileal absorption and tibial and liver concentrations of minerals and tibia quality. Long and short term thermal treatment, organic acids and their combination seem to have no negative impact on bone development in broilers and may safely be used for sanitation of broiler feed.

4.5 ACKNOWLEDGEMENTS

This work has been funded by the Bundesministerium für Bildung und Forschung (BMBF) Berlin, research grant No. 13N11202, SiLeBAT project. The authors express deep appreciation to P. Wolf from Stiftung Tierärztliche Hochschule Hannover, K.-D. Neumann and R. Löwe from Internationale Forschungsgemeinschaft Futtermitteltechnik e.V. (IFF) Braunschweig-Thune, as well as W. Vahjen, M. Eitinger, P. Huck, K. Topp, L. Ebersbach and I. Bebert from the Institute of Animal Nutrition, Freie Universität Berlin, for their technical support during the animal experiment and laboratory analysis.

4.6 REFERENCES

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.

Akpe, M. P., P. E. Waibel, K. Larntz, A. L. Metz, S. L. Noll, and M. M. Walser. 1987. Phosphorus availability bioassay using bone ash and bone densitometry as response criteria. Poult. Sci. 66:713-720.

Angel, R. 2007. Metabolic disorders: Limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. J. Appl. Poult. Res. 16:138-149.

Aoyagi, S., and D. H. Baker. 1995. Iron requirement of chicks fed a semipurified diet based on casein and soy protein-concentrate. Poultry Sci 74:412-415.

Awyong, L. M., J. S. Sim, and D. B. Bragg. 1983. Mineral availability of corn, barley, wheat, and triticale for the chick. Poult. Sci. 62:659-664.

Bao, Y. M., M. Choct, P. A. Iji, and K. Bruerton. 2007. Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. J. Appl. Poult. Res. 16:448-455.

Barnet, E., and B. Nordin. 1960. The radiological diagnosis of osteoporosis: A new approach. Clin. Radiol. 11:166–169.

Bayley, H. S., J. D. Summers, and S. J. Slinger. 1968. The effect of steam pelleting feed ingredients on chick performance: effect of phosphorus availability, metabolizable energy value and carcass composition. Poult. Sci. 47:1140-1148.

Berta, E., E. Andrasofszky, A. Bersenyi, R. Glavits, A. Gaspardy, and S. G. Fekete. 2004. Effect of inorganic and organic manganese supplementation on the performance and tissue manganese content of broiler chicks. Acta Vet. Hung. 52:199-209.

Bessei, W. 2006. Welfare of broilers: A review. World's poultry science journal 62:455-466.

Beyer, R. S. 2000. The impact of feed milling and manufacturing procedures on nutrient availability and microbial content of poultry feed. Pages 151-162. in California Animal Nutrition Conference, PG Ag Products, , Fresno, CA, USA.

Black, J. R., C. B. Ammerman, P. R. Henry, and R. D. Miles. 1984. Biological availability of manganese sources and effects of high dietary manganese on tissue mineral-composition of broiler-type chicks. Poult. Sci. 63:1999-2006.

Boivin, G., and P. J. Meunier. 2002. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. Calcif. Tissue Int. 70:503–511.

Boling-Frankenbach, S. D., J. L. Snow, C. M. Parsons, and D. H. Baker. 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poult. Sci. 80:783-788.

Bonser, R. H. C., and A. Casinos. 2003. Regional variation in cortical bone properties from broiler fowl - a first look. Br. Poult. Sci. 44:350-354.

Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro, and C. Bravo. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol. 110:201-219.

Capdevielle, M. C., L. E. Hart, J. Goff, and C. G. Scanes. 1998. Aluminum and acid effects on calcium and phosphorus metabolism in young growing chickens (Gallus gallus domesticus) and mallard ducks (Anas platyrhynchos). Arch. Environ. Contam. Toxicol. 35:82-88.

Cheng, T. K., and C. N. Coon. 1990. Sensitivity of various bone parameters of laying hens to different daily calcium intakes. Poult. Sci. 69:2209-2213.

Chowdhury, R., K. M. S. Islam, M. J. Khan, M. R. Karim, M. N. Haque, M. Khatun, and G. M. Pesti. 2009. Effect of citric acid, avilamycin, and their combination on the performance, tibia ash, and immune status of broilers. Poult. Sci. 88:1616-1622.

Coelho, M. B. 1994. Ecological nutrition: A costly or smart move? Feedstuffs 66:13-15.

Coto, C., F. Yan, S. Cerrate, Z. Wang, P. Sacakli, P. Waldroup, J. Halley, C. Wiernusz, and A. Martinez. 2008. Effects of dietary levels of calcium and nonphytate P in broiler starter diets on live performance, bone development, and growth plate conditions in male broilers fed a corn-based diet. Int. J. Poult. Sci. 7:638-645.

Deobald, H. J., and C. A. Elvehjem. 1935. The effect of feeding high amounts of soluble iron and aluminum salts. AM. J. PHYSIOL. 111:118-123.

Dibner, J. 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.

Edwards, H. M., Jr., A. B. Carlos, A. B. Kasim, and R. T. Toledo. 1999. Effects of steam pelleting and extrusion of feed on phytate phosphorus utilization in broiler chickens. Poult. Sci. 78:96-101.

Falandysz, J. 1991. Manganese, copper, zinc, iron, cadmium, mercury and lead in muscle meat, liver and kidneys of poultry, rabbit and sheep slaughtered in the northern part of Poland, 1987. Food Addit. Contam. 8:71-83.

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.

Gajula, S. S., V. K. Chelasani, A. K. Panda, V. L. Mantena, and R. R. Savaram. 2011. Effect of supplemental inorganic Zn and Mn and their interactions on the performance of broiler chicken, mineral bioavailability, and immune response. Biol. Trace Elem. Res. 139:177-187.

Garlich, J., C. Morris, and J. Brake. 1982. External bone volume, ash, and fat-free dry-weight of femurs of laying hens fed diets deficient or adequate in phosphorus. Poult. Sci. 61:1003-1006.

Gericke, S., and B. Kurmies. 1952. Die kolorimetrische Phosphorsäurebestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse. Z. Pflanzenernaehr. Dueng. Bodenkd. 59:235–247.

GfE. 1999. (Gesellschaft fur Ernahrungsphysiologie). Empfehlungen zur Energie- und 485 Nahrstoffversorgung der Legehennen und Masthuhner Broiler. DLG-Verlag, Frankfurt (Main), Germany.

Goodarzi Boroojeni, F., A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, K. Männer, and J. Zentek. 2014b. The effects of different thermal treatments and organic acid levels on nutrient digestibility in broilers. Poult. Sci. 93:1159-1171.

Hemme, A., M. Spark, P. Wolf, H. Paschertz, and J. Kamphues. 2004. Effects of different phosphorus sources in the diet on bone composition and stability (breaking strength) in broilers. J. Anim. Physiol. Anim. Nutr. (Berl). 89:129-133.

Henry, P. R., C. B. Ammerman, D. R. Campbell, and R. D. Miles. 1987. Effect of antibiotics on tissue trace mineral concentration and intestinal-tract weight of broiler chicks. Poult. Sci. 66:1014-1018.

Iji, P. A., K. Khumalo, S. Slippers, and R. M. Gous. 2003. Intestinal function and body growth of broiler chickens on diets based on maize dried at different temperatures and supplemented with a microbial enzyme. Reprod. Nutr. Dev. 43:77-90.

Islam, K. M. S., H. Schaeublin, C. Wenk, M. Wanner, and A. Liesegang. 2012. Effect of dietary citric acid on the performance and mineral metabolism of broiler. J. Anim. Physiol. Anim. Nutr. (Berl). 96:808–817.

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

Kilburn, J., and H. M. Edwards, Jr. 2001. The response of broilers to the feeding of mash or pelleted diets containing maize of varying particle sizes. Br. Poult. Sci. 42:484-492.

Kim, W. K., L. M. Donalson, A. D. Mitchell, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2006. Effects of alfalfa and fructooligosaccharide on molting parameters and bone qualities using dual energy X-ray absorptiometry and conventional bone assays. Poult. Sci. 85:15-20.

Korsrud, G. O., J. B. Meldrum, C. D. Salisbury, B. J. Houlahan, P. W. Saschenbrecker, and F. Tittiger. 1985. Trace-element levels in liver and kidney from cattle, swine and poultry slaughtered in Canada. Can. J. Comp. Med. 49:159-163.

Lee, S. R., W. M. Britton, and G. N. Rowland. 1980. Magnesium toxicity - bone-lesions. Poult. Sci. 59:2403-2411.

Leeson, S., and J. D. Summers. 2001. Nutrition of the chicken. 4th ed. University Books, Guelph, Ontario, Canada.

Liem, A., G. M. Pesti, and H. M. Edwards. 2008. The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. Poult. Sci. 87:689-693.

Lilburn, M. S. 1994. Skeletal growth of commercial poultry species. Poult. Sci. 73:897-903.

Martin, H., and P. Maris. 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.

Merkley, J. W. 1981. A comparison of bone strengths from broilers reared under various conditions in coops and floor pens. Poult. Sci. 60:98-106.

Nestor, K. E., W. L. Bacon, Y. M. Saif, and P. A. Renner. 1985. The influence of genetic increases in shank width on body-weight, walking ability, and reproduction of turkeys. Poult. Sci. 64:2248-2255.

Nwokolo, E., and D. B. Bragg. 1980. Biological availability of minerals in rapeseed meal. Poult. Sci. 59:155-158.

Ochrimenko, C., A. Lemser, G. Richter, U. Krause, and H. Bonsak. 1992. Effect of the manganese content in laying hen feed with different Ca and mineral levels on the egg shell quality and bone mineralization of hens. Arch Tierernahr. 42:25-35.

Onyango, E. M., P. Y. Hester, R. Stroshine, and O. Adeola. 2003. Bone densitometry as an indicator of percentage tibia ash in broiler chicks fed varying dietary calcium and phosphorus levels. Poult. Sci. 82:1787–1791.

Osphal, W., H. Zeronian, M. Ellison, D. Lewis, R. S. Rucker, and R. Riggins. 1982. Role of copper in collagen-linking and its influence on selected mechanical properties of chick bone and tendon. J. Nutr. 12:708–716.

Ovesen, J., B. Moller-Madsen, J. S. Thomsen, G. Danscher, and L. Mosekilde. 2001. The positive effects of zinc on skeletal strength in growing rats. Bone 29:565–570.

Park, S. Y., S. G. Birkhold, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2003. Effect of storage condition on bone breaking strength and bone ash in laying hens at different stages in production cycles. Poult. Sci. 82:1688-1691.

Rafacz-Livingston, K. A., C. Martinez-Amezcua, C. M. Parsons, D. H. Baker, and J. Snow. 2005. Citric acid improves phytate phosphorus utilization in crossbred and commercial broiler chicks. Poult. Sci. 84:1370-1375.

Rao, S. V. R., M. V. L. N. Raju, M. R. Reddy, and P. Pavani. 2006. Interaction between dietary calcium and non-phytate phosphorus levels on growth, bone mineralization and mineral excretion in commercial broilers. Anim. Feed Sci. Technol. 131:133-148.

Rath, N. C., J. M. Balog, W. E. Huff, G. R. Huff, G. B. Kulkarni, and J. F. Tierce. 1999. Comparative differences in the composition and biomechanical properties of tibiae of seven- and seventy-two-week-old male and female broiler breeder chickens. Poult. Sci. 78:1232-1239.

Rath, N. C., G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Factors regulating bone maturity and strength in poultry. Poult. Sci. 79:1024-1032.

Ricke, S. C. 2005. Food safety control in the poultry industry. Woodhead Publishing in Food Science and Technology, Mead GC (Ed.). Cambridge, United Kingdom.

Riesenfeld, A. 1972. Metatarsal robusticity in bipedal rats. Am. J. Phys. Anthropol. 36:229-233.

Ruff, C. R., and B. L. Hughes. 1985. Bone strength of height-restricted broilers as affected by levels of calcium, phosphorus, and manganese. Poult. Sci. 64:1628-1636.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Brit. Poult. Sci. 43:598-606.

Scott, M. L., M. C. Nesheim, and R. J. Young. 1982. Nutrition of the chicken. 3rd ed. M. L. Scott and Associates, Ithaca, New York.

Shim, M. Y., A. B. Karnuah, A. D. Mitchell, N. B. Anthony, G. M. Pesti, and S. E. Aggrey. 2012. The effects of growth rate on leg morphology and tibia breaking strength, mineral density, mineral content, and bone ash in broilers. Poult. Sci. 91:1790–1795.

Shim, M. Y., G. M. Pesti, R. I. Bakalli, and H. M. Edwards. 2008. The effect of breeder age and egg storage time on phosphorus utilization by broiler progeny fed a phosphorus deficiency diet with 1 alpha-OH vitamin D-3. Poult. Sci. 87:1138-1145.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215-221.

Skrivan, M., V. Skrivanova, and M. Marounek. 2005. Effects of dietary zinc, iron, and copper in layer feed on distribution of these elements in eggs, liver, excreta, soil, and herbage. Poult. Sci. 84:1570-1575.

Starcher, B. C., C. H. Hill, and J. G. Madaras. 1980. Effect of zinc-deficiency on bone collagenase and collagen turnover. J. Nutr. 110:2095-2102.

Thompson, A. S., and C. W. Weber. 1981. Effect of dietry fiber sources on tissue mineral level in chicks. Poult. Sci. 60:840-845.

Velleman, S. G. 2000. The role of the extracellular matrix in skeletal development. Poult. Sci. 79:985-989.

Watkins, K. L., and L. L. Southern. 1992. Effect of dietary-sodium zeolite-a and graded-levels of calcium and phosphorus on growth, plasma, and tibia characteristics of chicks. Poult. Sci. 71:1048-1058.

Wilson, S., and S. R. I. Duff. 1991. Effects of vitamin or mineral deficiency on the morphology of medullary bone in laying hens. Res. Vet. Sci. 50:216-221.

Yang, P. Y., and D. J. Klimis-Tavantzis. 1998. Effects of dietary manganese on arterial Glycosaminoglycan metabolism in Sprague-Dawley rats. Biol. Trace Elem. Res. 64:275-288.

4. 1 Ingredients, analyzed nutrient composition (%) and calculated apparent metabolizable energy of experimental starter (1-21-d-old) and grower (21-35-d-old) broilers diets [1]

Acid inclusion levels	0%		1.50%	
	Starter	Grower	Starter	Grower
Ingredient				
Soybean Meal (49% CP)	32.3	20.8	32.8	21.5
Maize	32.0	31.0	29.4	31.0
Wheat	24.8	39.0	25.1	36.5
Soybean Oil	5.95	4.30	6.34	4.65
Limestone	1.46	1.36	1.46	1.36
Monocalcium Phosphate	1.84	1.65	1.84	1.65
Vitamin and Mineral Premix[2]	1.20	1.20	1.20	1.20
Salt	0.10	0.10	0.10	0.10
DL-Methionine	0.18	0.19	0.18	0.19
L-Lysine	0.13	0.13	0.12	0.12
L-Threonine	.	0.06	.	0.05
Lupro-Cid®[3]	0	0	1.50	1.50
Titanium Dioxide[4]	.	0.20	.	0.20
Analyzed Nutrient Composition				
Crude Protein	23.3	19.0	23.1	19.2
Crude Fat	4.11	5.47	4.71	4.93
Crude Fiber	2.80	2.63	2.80	2.75
Calculated Apparent Metabolizable Energy				
AME_N (MJ/kg)	12.6	12.6	12.6	12.6

[1] As-fed basis.

[2] Per kg Premix (MIAVIT GmbH, Essen, Germany): 400,000 IU Vitamin A; 40,000 IU Vitamin D3; 8,000 IU Vitamin E (α-Tocopherol acetate); 300 mg Vitamin K3 (Menadione sodium bisulfate 51%); 250 mg Vitamin B1; 250 mg Vitamin B2; 2500 mg Nicotinic acid; 1,000 mg Vitamin B5 (Pantothenic acid); 400 mg Vitamin B6; 2 mg Vitamin B12; 25 mg Vitamin H (Biotin); 100 mg Folic acid; 80 g Choline chloride; 5,000 mg Zinc (Zinc oxide); 2,000 mg Iron (Iron carbonate); 6,000 mg Manganese (Manganese oxide); 1,200 mg Copper (Copper sulfate); 218.75 mg Iodine (Calcium iodate); 87.5 mg Cobalt (Cobalt carbonate); 35 mg Selenium (Sodium selenite); 130 g Sodium (Sodium chloride); 55 g Magnesium (Magnesium oxide); 74.58 g Calcium (Calcium carbonate)

[3] Lupro-Cid® (BASF SE, Ludwigshafen, Germany): 63.75 % formic acid, 25 % propionic acid and 11.25 % water

[4] $TiO2$ (Sigma Aldrich, St. Louis, MO)

4. 2 Analyzed mineral concentrations in diets

	Starter diet						Grower diet					
Thermal	Pellet 70°C		Long Term 85°C		Expansion 130°C		Pellet 70°C		Long Term 85°C		Expansion 130°C	
Acid	0%	1.50%	0%	1.50%	0%	1.50%	0%	1.50%	0%	1.50%	0%	1.50%
Calcium (g/kg DM)	9.40	8.38	7.99	8.53	8.92	8.97	8.32	7.64	7.93	8.37	8.23	8.51
Phosphorus (g/kg DM)	7.95	8.18	7.97	7.76	8.56	8.28	7.57	7.67	8.00	8.30	8.38	8.75
Magnesium (g/kg DM)	2.10	2.05	1.61	1.86	1.69	1.77	2.49	2.36	2.32	2.46	2.32	2.39
Potassium (g/kg DM)	8.55	8.14	7.66	7.51	7.57	7.74	7.92	7.54	7.51	7.60	7.76	7.86
Sodium (g/kg DM)	2.47	2.05	1.61	1.86	2.14	2.30	2.52	2.43	2.55	2.50	2.42	2.51
Iron (mg/kg DM)	326	373	299	383	292	296	588	555	564	655	616	617
Copper (mg/kg DM)	18.7	25.8	20.9	25.1	16.4	20.1	33.3	31.2	33.8	38.1	32.9	29.4
Manganese (mg/kg DM)	104	110	100	104	93.5	104	111	105	91.6	107	104	103
Zinc (mg/kg DM)	105	115	114	114	93.3	100	89.1	86.2	84.2	95.2	85.4	85.0

4. 3 Effect of thermal and acid treatment of feed on percent ileal absorption of minerals in broilers at d 35[1, 2]

	Thermal			Acid			*P* value		
	Pellet	Long Term	Expand	0	1.50%	SEM	Thermal	Acid	Thermal*Acid
Calcium	55.2^{ab}	45.9^{b}	60.9^{a}	55.5	52.5	2.25	0.02	0.49	0.97
Phosphorus	64.8	62.9	71.3	69.6	63.1	1.87	0.16	0.08	0.73
Magnesium	54.0	47.2	55.2	53.2	51.1	1.93	0.21	0.59	0.62
Potassium	91.2^{a}	88.6^{b}	91.8^{a}	90.7	90.3	0.44	0.01	0.69	0.91
Sodium	52.2^{ab}	41.7^{b}	58.0^{a}	52.8	48.4	2.38	0.01	0.33	0.48
Iron	60.4	49.2	60.6	58.0	55.5	3.13	0.25	0.69	0.22
Copper	67.4	64.9	64.7	67.4	63.8	1.71	0.78	0.30	0.28
Manganese	44.2	36.9	49.0	43.6	43.6	2.32	0.10	0.93	0.62
Zinc	73.5	57.5	70.3	67.7	66.6	3.85	0.23	0.89	0.93

[1] Data are means of 8 replicates per group.

[2] Data within one group were normally distributed.

[a,b] Means with different superscripts in a row differ significantly ($P \leq 0.05$).

4. 4 Effect of thermal and acid treatment of feed on tibia quality and tibial mineral content in broilers at d 35 [1,2]

	Thermal			Acid			*P* value		
	Pellet	Long Term	Expand	0	1.50%	SEM	Thermal	Acid	Thermal*Acid
Body Weight d 35 (g)	1985	2040	2039	2041	2002	6.76	0.92	0.53	0.99
Tibia Length (cm)	8.48	8.38	8.44	8.45	8.41	0.04	0.54	0.63	0.90
Tibia Weight (g)	8.50	8.14	7.76	7.97	8.3	0.13	0.06	0.20	0.42
Body Weight : Bone Weight	238^{b}	253^{ab}	260^{a}	256	245	3.65	0.04	0.12	0.69
Tibiotarsal Index	42.4	42.0	40.9	40.9	42.6	0.45	0.40	0.06	0.64
Robusticity Index	4.14	4.17	4.21	4.19	4.15	0.02	0.19	0.16	0.45
Bone Density (g/cm^3)	1.17	1.15	1.16	1.17	1.16	0.01	0.44	0.39	0.84
Dry Matter (%)	55.4	54.4	54.8	55	54.7	0.29	0.38	0.56	0.76
Fat Free Dry Matter (%)	52.5	51.2	51.2	51.8	51.4	0.38	0.24	0.60	0.33
Ash (%)	53.7^{ab}	53.3^{b}	55.0^{a}	53.9	54.1	0.30	0.04	0.71	0.49
Calcium (g/kg)	170	173	182	177	173	2.46	0.10	0.45	0.27
Phosphorus (g/kg)	94.6	93.5	93.9	94.2	93.8	0.86	0.88	0.83	0.87
Magnesium (g/kg)	4.01	4.01	4.03	4.05	3.98	0.06	0.99	0.56	0.25
Potassium (g/kg)	2.09	2.19	2.06	2.05	2.18	0.06	0.68	0.32	0.98
Sodium (g/kg)	4.66	4.71	4.68	4.81	4.56	0.07	0.96	0.06	0.34
Iron (mg/kg)	128^{ab}	147^{a}	121^{b}	136	128	4.08	0.03	0.31	0.44
Copper (mg/kg)	0.85	0.89	0.67	0.79	0.81	0.04	0.10	0.85	0.38
Manganese (mg/kg)	4.10	4.35	4.54	4.42	4.24	0.11	0.25	0.38	0.61
Zinc (mg/kg)	174^{a}	168^{ab}	160^{b}	168	167	1.94	0.01	0.93	0.48

[1] Data are means of 12 replicates per group, ash, macro minerals and trace elements are related to fat free dry matter.

[2] Data within one group were normally distributed.

^{a,b}Means with different superscripts in a row differ significantly ($P \leq 0.05$).

4. 5 Effect of thermal and acid treatment of feed on the liver mineral concentrations in broilers at d 35 [1,2]

	Thermal			Acid			*P* value		
	Pellet	Long Term	Expand	0	1.50%	SEM	Thermal	Acid	Thermal*Acid
Liver weight (g)	45.2	43.3	43.9	43.6	44.7	0.70	0.54	0.43	0.37
DM (%)	30.1	31.2	30.5	30.8	30.4	0.21	0.07	0.35	0.03
Ash (% of DM)	4.33	4.26	4.34	4.25	4.37	0.04	0.62	0.12	0.14
Calcium (g/kg DM)	0.30	0.29	0.30	0.29	0.29	0.01	0.82	0.89	0.61
Phosphorus (g/kg DM)	8.93	8.83	9.16	9.02	8.93	0.10	0.40	0.67	0.10
Magnesium (g/kg DM)	0.56	0.56	0.55	0.56	0.55	0.01	1.00	0.97	0.45
Potassium (g/kg DM)	15.7	16.2	16.4	15.9	16.2	0.22	0.41	0.52	0.31
Sodium (g/kg DM)	2.27	2.28	2.15	2.22	2.26	0.04	0.34	0.60	0.04
Iron (mg/kg DM)	404	449	472	428	455	15.9	0.23	0.40	0.72
Copper (mg/kg DM)	8.93	9.05	8.47	8.84	8.78	0.17	0.35	0.85	0.57
Manganese (mg/kg DM)	7.36	7.76	7.94	7.65	7.72	0.14	0.25	0.81	0.34
Zinc (mg/kg DM)	55.1	53.6	51.8	53.1	53.9	1.15	0.52	0.73	0.07

[1] Data are means of 12 replicates per group.

[2] Data within one group were normally distributed except for potassium.

5 IMPLICATION OF MILLING METHODS, THERMAL TREATMENT, AND PARTICLE SIZE OF FEED IN LAYERS ON MINERAL DIGESTIBILITY AND RETENTION OF MINERALS IN EGG CONTENTS

A. Hafeez[1], A. Mader, I. Ruhnke, I, Röhe, F. Goodarzi Boroojeni, M. S. Yousaf, K. Männer and J. Zentek

Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Königin-Luise-Str. 49, 14195 Berlin, Germany

2015 Poultry Science 94 (2): 240-248
http://dx.doi.org/10.3382/ps/peu070

[1] Corresponding author: E-mail: hafeezpak@hotmail.com. Tel: +49 30 838 51943 Fax: +49 30 838 55938.

ABSTRACT Feed production with different milling methods, thermal treatment and particle size may influence mineral digestibility and retention in eggs. The present study investigated the impact of roller (R) and hammer (H) mills, mash (M) and expandate (E) with fine (F) and coarse (C) particle sizes, on apparent ileal absorption (AIA) and apparent total digestibility (ATD) and retention of calcium, phosphorus, magnesium, zinc, manganese, copper and iron, in yolk, albumen and shell. A total of 384 hens (Lohmann Brown), 19 weeks old, were assigned using a randomized design with a 2×2×2 factorial arrangement. Eight experimental diets were offered *ad libitum* during the whole experimental period and one week before for diet adaption. The AIA of magnesium, zinc, copper and iron was higher in treatment R in comparison with treatment H ($P<0.01$, $P\leq0.03$, $P<0.01$ and $P<0.01$, respectively). The AIA of magnesium was higher in treatment M than treatment E ($P<0.01$). Due to particle size, the AIA of magnesium was higher in treatment C in comparison with treatment F ($P\leq0.05$). The ATD of copper and iron was higher in treatment R than treatment H ($P<0.01$ and $P\leq0.03$, respectively). The ATD was higher for phosphorus and lower for iron in treatment F than treatment C ($P\leq0.05$ and $P\leq0.02$. respectively). The copper concentration in yolk and albumen was higher in treatment C than treatment F ($P<0.01$ and $P\leq0.03$, respectively). Besides a few overall interactions, the AIA and ATD of copper and manganese were lower in H+M group than R+M group ($P\leq0.05$). The ATD of iron was higher in M+C than M+F group ($P<0.01$) whereas albumen zinc concentration was higher in E+C than E+F group ($P<0.01$). In conclusion, feed produced by hammer mill had negative effect on AIA and ATD for trace elements in particular, but mineral concentrations in egg contents were mostly comparable for all treatments. Therefore, milling methods, thermal treatment and particle sizes used in present study may be used for layer feed formulation without negatively affecting egg quality.

Key words: feed production, poultry, hammer mill, roller mill, expandate

5.1 INTRODUCTION

Mineral nutrition is an important aspect for optimal egg production. In general, minerals and trace elements play major role in vital physiological functions, bone development and egg formation in poultry. The provision of optimum mineral content in the feed is essential for the production of quality eggs (Leeson and Summers, 2001; Mabe et al., 2003). Minerals are transferred into egg contents through blood either in ionic form like sodium and potassium or in complex structures with lipoproteins as calcium, iron and zinc (Larbier and Leclercq, 1992).

The 18 weeks old brown laying hen requires 1.8% calcium in diet which is increased to 3.2-3.6% with start of egg production at 19 weeks of age (NRC, 1994), making this period specifically interesting due to the dynamics of increased egg production, higher calcium intake and the unknown apparent absorption. Furthermore, the layers have a high demand of calcium during peak egg production periods (Al-Batshan et al., 1994). Egg shell consists of 90-95% calcium carbonate and on average the egg shell contains 2.3 g of calcium. Egg is rich in phosphorus found in the form of phosphoprotein (phosvitin) and phospholipids (Larbier and Leclercq, 1992). Magnesium plays an important role in egg shell formation. Out of 25 mg total magnesium found in egg, 18.7 mg is present in shell and shell membranes (Leeson and Summers, 2001).

Dietary trace elements make a significant contribution on egg formation. The zinc status of the hen is directly related to activity of carbonic anhydrase (a calcium binding protein), which is essential for egg shell formation. Moreover, the hatchability and embryonic development may adversely be affected due to inadequate intake of dietary zinc (Hudson et al., 2004). The declined shell quality due to supply of saline water was counteracted by zinc supplementation in water, as zinc increased the carbonic anhydrase level resulting in less shell defects (Leeson and Summers, 2001). For optimum egg production and quality, the hen's requirements for critical minerals including manganese should be met (Tang et al., 2006). Addition of manganese in diet decreased the number of cracked eggs and adding manganese with zinc increased shell strength (Essary and Holmes, 1964) and reduced number of broken eggs (Zamani et al., 2005). The copper deficiency in the laying hen resulted in the production of eggs with abnormal size and shape, wrinkled and rough textured shells, and an increased shell-less eggs (Baumgartner et al., 1978). Almost all of the yolk iron binds to phosvitin, a phosphoprotein known as an iron-carrier in egg yolk (Ishikawa et al., 2004).

In addition to role in egg formation, the mineral content of egg is equally important for breeders. The nutrient composition of fertile eggs has a strong influence on nutrient uptake by embryo (Uni et al., 2012). The deficiency of calcium and phosphorus in general, and copper, manganese and zinc in particular, may cause skeletal, immune and cardiovascular system disorders, reduced hatchability and increased embryonic mortality (Angel, 2007; Dibner et al., 2007; Uni et al., 2012).

The use of roller and hammer mills for reduction of feed particle size is common in poultry feed industry (Koch, 2002; Amerah et al., 2007). The roller mill requires less energy, produces cubic or rectangular shaped (Koch, 2002) and a more uniform sized particles (Amerah et al., 2007) than hammer mill, which is easier to use (Koch, 2002), produces more spherical shaped particles but a greater amount of fines (Reece et al., 1985).

Particle size distribution in feed affected egg quality and body weight, probably as small particle bound nutrients are less effectively utilized (Tang et al., 2006). Chickens have a preference for larger feed particles (Schiffman, 1968) at all ages (Portella et al., 1988), whereas vitamins and minerals are mainly contained in smaller particles. The particle size is more critical in mash diets as compared to pelleted or crumbled diets, due to selective feed intake of birds. Coarse particles require a longer transit time, which may allow higher mineral digestion and absorption (Amerah et al., 2007). The utilization of calcium, total phosphorus and phytate phosphorus was higher in broilers fed diets with coarse maize particle size (Kasim and Edwards, 2000; Kilburn and Edwards, 2001). Moreover, feeding of coarse particle size diet improved bone ash and plasma phosphorus levels in broilers (Carlos and Edwards Jr, 1997; Kilburn and Edwards, 2004).

Thermal treatment is commonly used in feed production to enhance apparent ileal digestibility of nutrients, to improve hygiene status of feed, and for the reduction of anti-nutritional factors (Mossel et al., 1967a; Kilburn and Edwards, 2001; Peisker, 2006; Goodarzi Boroojeni et al., 2014b). Heat treatment was shown to influence bacterial status of the crop (Goodarzi Boroojeni et al., 2014a) and to improve feed conversion (Amerah et al., 2007). In comparison with long term thermal treatment, pelleting and expansion of broiler feed resulted in higher apparent ileal absorption of calcium, potassium and sodium (Hafeez et al., 2014). However, pelleting and crumbling of the diet may reduce bone ash and plasma mineral concentrations in broilers (Kilburn and Edwards, 2004); which might be due to degradation of larger particles into smaller ones during the pelleting process, which could minimize the advantage of larger particle for longer transit time (Amerah et al., 2007).

According to our knowledge, no further data is available regarding the effect of milling method, thermal treatment and particle size of feed on apparent ileal absorption and apparent total digestibility of minerals in layers. Furthermore, their effect on mineral and trace element concentrations in egg contents could also be of interest.

Therefore, the aim of the present study was to investigate the impact of milling methods including roller and hammer mills, thermal treatment including mash vs expansion, and particle size of feed on apparent ileal absorption and apparent total digestibility of minerals, and mineral and trace elements concentrations of yolk, albumen and shell in laying hens.

5.2 MATERIAL AND METHODS

The animal trial was performed according to the Animal Welfare Act of Germany approved by the local state office of occupational health and technical safety (Landesamt für Gesundheit und Soziales, LaGeSo, no. G 0117/11).

5.2.1 Hens and Experimental Design

The experiment lasted for 21 days. Three hundred and eighty four hens (Lohmann Brown) at the age of 19 weeks were used. The hens were obtained from a commercial pullet rearing farm where they were previously fed with mash feed produced with a hammer mill. Hens were kept in cages which consisted of an area for claw abrasion, a perch, and laying nests. Wood shaving was used as bedding material. The hens were divided into 8 experimental groups in 8 repetitions with 6 hens per pen (in total 48 hens per feeding group). The dimensions of the each cage were 2.15 m × 1.40 m, thereby each hen had 0.5 m^2 or 5.4 ft^2 space. Eight experimental diets, produced from one identical basal diet (table 1), meeting the nutritional recommendations by German Society of Nutrition Physiology (GfE, 1999), were offered *ad libitum* during the whole experimental period and one week before for diet adaption. Each diet was supplemented with TiO2 (Sigma Aldrich, St. Louis, MO) as indigestible marker at 2 g/kg to analyze apparent mineral absorption in the ileum and apparent total digestibility in rectum (Short et al., 1996). The experiment was conducted using randomized design with a 3 way factorial arrangement. Experimental diets were formulated using roller mill (R) and hammer mill (H) as mash (M) and expandate (E) with coarse (C) and fine (F) particle size. The 8 different diets formulated using a 2 × 2 × 2 arrangement were then assigned to hens within 64 pens with 8 pen per diet group.

5.2.2 Experimental Diets Production

The experimental diets have been described in previous publication, Ruhnke et al. (2015). In brief, one identical basal experimental diet was produced (Table 1). Two milling types, including a roller mill (Vario-Walzenstuhl, MIAG AG, Bühler GmbH, Braunschweig, Germany) and hammer mill (horizontal rotor hammer mill, Tietjen, Hemdingen, Germany), were used to obtain coarse and fine diets. Defined particle sizes were achieved by using various milling speeds and matrix plates. Coarse feed was defined as feed characterized by discrete mean particle size (dMEAN) above 1.8 mm, while fine diets were characterized by dMEAN below 1.8 mm, based on dry sieving analysis (Wolf et al., 2012). All variants were provided to the hens as expandate (Single Screw Expander OE8, Amandus Kahl GmbH & Co KG, Reinbek, Germany) or mash (Table 2).

5.2.3 Diets for Wet Sieving Analysis

The wet sieving analysis of the experimental diets were performed as described by Ruhnke et al. (2015). In brief, a total of 100 g feed sample was soaked in 1 l water for 1 hour and sieved through nine sieves with different pore sizes (Analysensiebe, Retsch GmbH, Haan, Germany) using 10 l of water for 10 minutes under continuous shaking. The contents of the sieves were dried for 6 hours at 100°C before weighing and dMEAN was calculated as described earlier (Fritz et al., 2012). Table 2 presents the results.

5.2.4 Data for Performance Parameters

Throughout the experimental period, data were collected for performance parameters including laying performance, feed intake, FCR and body weight etc., which is explained in Ruhnke et al. (2015).

5.2.5 Collection of Samples

At the end of experiment at 23 weeks of age, 3 hens per pen were randomly selected and killed after stupefaction by cervical dislocation followed by exsanguination. The digesta from the distal two-thirds of the ileum (without content in the distal 3 cm prior to ileo-cecal junction) and rectum was collected as described by (Kluth et al., 2005; Rezvani et al., 2008), pooled and freeze-dried until analysis for mineral contents. One day before slaughtering, three eggs per pen from 5 out of 8 repetitions for every diet group were randomly collected. After determining the egg quality variables which have been described in Hafeez et al. (2015), the yolk, albumen and shell content of all three eggs from the same pen were pooled and frozen at −20°C until further analysis for DM, ash, mineral and trace elements concentrations.

5.2.6 Apparent Ileal Absorption and Apparent Total Digestibility in Rectum

The apparent absorption of minerals and trace elements in ileum and rectum was calculated using the following formula:

apparent absorption (%) = 100 − [(concentration of marker in feed/concentration of marker in digesta) × (concentration of nutrient in digesta/concentration of nutrient in feed) × 100].

5.2.7 Analysis for Mineral Concentrations

The feed, ileal and rectal digesta, yolk, albumen and shell samples were freeze dried and dry matter content was determined as a ratio of fresh matter weight to dried matter weight. The freeze dried samples were then ashed in a muffle furnace at 600°C for 6 h. The percent ash was determined with relation to DM in respective sample. The method of atomic absorption spectrometry in an AAS Vario 6 spectrometer (Analytik Jena, Jena, Germany) was adopted to determine the concentrations of calcium, magnesium, iron, copper, manganese, and zinc. The concentration of phosphorus was determined with the ammonium vanadate/molybdate method as described by (Gericke and Kurmies, 1952). Concentrations of titanium dioxide in feed, ileum and rectum digesta were determined according to the method described by (Short et al., 1996).

5.2.8 Data Analysis

For statistical analysis, the data were arranged in a three factorial arrangement (2x2x2) where milling methods (roller and hammer), thermal treatments (mash vs expandate), and particle size (coarse and fine) were subjected to ANOVA using GLM procedure. The analyses were performed for main treatment effects and their interactions. Tukey's-b test was used as post-hoc test at $P \leq 0.05$ for grouping of treatment means. The normality was tested using Shapiro-Wilk. For statistical analysis, SPSS 20.0 (SPSS Inc., Chicago, IL) was used. The apparent ileal absorption and apparent total digestibility of minerals and their concentrations in yolk, albumen and shell were measured on the basis of pen as experimental unit.

5.3 RESULTS

In the present study, during the egg sampling week (d 15-21), the percent laying performance was not affected by the roller (89.1±6.64) and hammer mill (91.7±7.73), mash (90.6±5.90) and expandate (90.7±8.35), and the interaction between milling method and thermal treatment (P>0.05). Moreover, feed particle size did not influence the percent laying performance (P>0.05). Feed intake, FCR and body weight were not affected by milling method, thermal treatment and particle size ($P>0.05$). The performance parameters are further discussed in Ruhnke et al. (2015).

Table 2 presents the results of dry and wet sieving analysis. The expansion of feed had a pronounced effect on particle size after wet sieving. The difference between coarse and fine particle size in expansion groups is less pronounced after wet sieving in comparison with mash groups. The impact of milling method, thermal treatment and particle size on apparent ileal absorption of minerals is presented in table 3. The AIA of Mg, Zn, Cu and Fe was higher in treatment R in comparison with treatment H ($P<0.01$, $P\leq0.03$, $P<0.01$ and $P<0.01$, respectively), whereas AIA of Ca, P and Mn was comparable ($P>0.05$). Thermal treatment effected the AIA of Mg which was higher in treatment M than treatment E ($P<0.01$), however other variables were not affected ($P>0.05$). Due to particle size, the AIA of Mg was higher in treatment C in comparison with treatment F ($P\leq0.05$). No effect of particle size was observed on AIA of other minerals and trace elements ($P>0.05$). The interaction between milling method and thermal treatment influenced AIA of only Mn and Cu ($P\leq0.02$ and $P<0.01$, respectively). The AIA of Mn within R+M group (19.7±13.2) was significantly higher than H+M group (-3.79±28.3). The R+E and H+E group (-2.3±21.7 and -8.68±23.4, respectively) had significantly higher interaction effect for AIA of Cu than H+M group (-36.9±37.2) and significantly lower than R+M group (37.5±19.8). Thermal treatment and particle size had an overall interaction effect on the AIA of Cu and Fe ($P<0.02$, $P\leq0.05$, respectively) however no differences were observed between group. Table 4 shows the effect of milling method, thermal treatment and particle size of feed on apparent total digestibility of minerals and trace elements. The ATD of Cu and Fe was higher in treatment R than treatment H ($P<0.01$ and $P\leq0.03$, respectively). The milling method had no influence on ATD of other minerals and trace elements ($P>0.05$). The thermal treatment of feed did not affect ATD for any of the variables ($P>0.05$) except Ca, which was higher in treatment E than treatment M ($P< 0.01$). The feed particle size affected the ATD of P which was higher in treatment F than treatment C ($P\leq0.05$). The ATD of Fe was higher in treatment C in comparison with treatment F ($P\leq0.02$), whereas all other minerals and trace elements were not affected by feed particle size ($P>0.05$). There was an overall interaction effect between milling method and thermal treatment on ATD of Mg and Fe ($P<0.01$ and $P<0.04$, respectively), however no differences were found between group. The ATD for Mn and Cu was affected by interaction between milling methods and thermal treatment ($P<0.01$ and $P<0.01$, respectively). The ATD for Mn was significantly higher in R+M and H+E group (16.2±21.6 and 15.5±13.6, respectively) than H+M group (-18.7±41.0). The R+M group (36.6±19.3) had significantly higher ATD for Cu as compared to H+E, R+E and H+M group (9.47±25.7, -0.44±26.9 and -23.3±41.0, respectively). Furthermore, the ATD value for Cu was significantly higher in H+E group (9.47±25.7) than H+M group (-23.3±41.0). An overall interaction effect between thermal treatment and particle size was found on ATD of Mg and Zn ($P<0.01$ and $P\leq0.05$, respectively), with no differences between groups. The ATD of Fe was affected by interaction of thermal treatment and particle size ($P<0.01$) which was higher in M+C group (26.8±17.6) than M+F group (3.16±27.5). The ATD of P was affected by overall interaction effect of milling method, thermal treatment and particle size ($P<0.03$), however no differences were found between groups. The effects of milling method, thermal treatment and particle size of feed on yolk mineral and trace element concentrations are presented in table 5. The DM and ash content was similar for all treatments ($P>0.05$). The milling method did not affect the egg yolk concentration of any mineral or trace element under consideration ($P>0.05$). Thermal treatment did not show any changes in yolk mineral and trace element levels ($P>0.05$). The particle size influenced Cu concentration in the yolk which was higher in treatment C than treatment F ($P<0.01$). The concentration of Zn was affected by an overall interaction between milling method and particle size ($P<0.04$) with no differences between groups. There were no other interaction effects observed (*P*>0.05). Table 6 shows the response of albumen mineral and trace element concentrations to milling method, thermal processing and feed particle size. There were no differences in DM and ash content due to all treatments (*P*>0.05). The milling method did not affect mineral and trace element concentrations in albumen (*P*>0.05). Due to thermal treatment, the concentration of Mn was higher in treatment M as compared to treatment E (*P*≤0.04), while other variables were not affected (*P*>0.05). The particle size affected albumen concentrations of Cu which was higher in treatment C than treatment F (*P*≤0.03), however the concentrations of other variables under consideration remained unchanged (*P*>0.05). The interaction of thermal treatment and particle size affected the albumen Zn concentration (*P*<0.01) which was higher in E+C group (1.89±1.03) in comparison with E+F group (0.98±0.42). An overall interaction between thermal treatment, milling method and particle size affected albumen Fe concentration (*P*≤0.03) with no differences between groups. The DM, ash content, and mineral and trace element concentrations in egg shell were not affected (*P*>0.05) by milling method, thermal treatment and particle size (table 7). The concentration of Mn in shell was affected by an overall interaction between thermal treatment and particle size (*P*≤0.02) without any differences between groups.

5.4 DISCUSSION

In the present study, milling method influenced some of the egg quality parameters. The yolk index and yolk height were higher and shell membrane weight and percent shell membrane weight were lower in hammer mill treatment as compared to roller mill treatment. Expansion of the feed displayed higher percent shell membrane weight in comparison with mash treatment. The albumen height, shell thickness and shell weight were higher whereas shell density was lower in coarse particle size treatment compared to fine. However most of the economically important indicators for exterior and interior egg quality were comparable among the treatments, which are explained in Hafeez et al. (2015).

The discrete mean (dMEAN) obtained by dry sieving analysis of particle size is similar to macro-structure of feed whereas dMEAN obtained by wet sieving analysis of particle size is similar to microstructure of feed. The macro-structure of the feed is converted to micro-structure in upper part of digestive tract of the bird due to salivary and enzymatic action. Therefore, mash feed may be characterized on the basis of dry sieving analysis whereas expandate feed may be characterized by either dry or wet sieving (Svihus, 2006). Our results indicated that after wet sieving analysis, which involves dissolving feed in water, the differences between coarse and fine particles were less pronounced in expandate groups as compared to mash groups. Therefore, it might be considered that the feed intake may be affected by macro-structure of the feed however, the gut function may be influenced by micro-structure of feed in digestive tract (Svihus, 2006).

The milling methods showed some interesting effects regarding mineral and trace element digestibility. The apparent ileal absorption of magnesium and trace elements including zinc, copper and iron was reduced in the feed ground by the hammer mill as compared to roller mill. Despite of producing irregular, cubic or rectangular shaped particles (Koch, 2002), the roller mill generates a more uniform sized particles in comparison with hammer mill (Amerah et al., 2007), which produces spherical shaped with a greater amount of fine particles (Reece et al., 1985). Due to the fact that the coarser grinding of grains to a more uniform particle size may improve the performance of mature birds due to more gizzard development leading to increased grinding, gut motility and nutrient digestion (Amerah et al., 2007), the production of uniform sized particles by roller mill might have resulted in higher apparent ileal absorption of magnesium and trace elements. A recent study revealed that the particle size distribution affected egg quality and body weight as small particle nutrients were not effectively utilized (Tang et al., 2006). Furthermore, our results indicate that ileal absorption of trace elements reacts more critical than that of minerals to different milling methods. The apparent total digestibility of copper and iron was higher in feed produced by roller mill as compared to hammer mill and other variables remained unchanged. The minerals and trace elements are mostly absorbed in ileum and jejunum (Leeson and Summers, 2001). The apparent total digestibility results show that lower absorption for magnesium and trace elements in ileum was compensated by the absorption in the hind gut. Furthermore, the mineral and trace element concentrations in yolk, albumen and shell were comparable. It suggests that the effect of milling method to lower absorption of magnesium and trace elements was limited to the small intestine, and that the body reserves and absorption in hind gut were sufficient to offset such effects, thereby ensuring the provision of optimum amounts of mineral and trace elements into egg contents.

Thermal treatment of feed did not affect apparent ileal absorption and apparent total digestibility of any of the mineral or trace elements, except the higher apparent total digestibility of calcium and lower apparent ileal absorption of magnesium in birds fed expandate compared to mash feeding. A recent study showed that expansion of broiler diets increased the apparent ileal absorption of calcium of comparison with long term heat treatment, whereas phosphorus, magnesium, zinc, manganese, copper and iron were comparable (Hafeez et al., 2014). Similar data was presented by Iji et al. (2003), revealing that absorption of calcium was increased in broilers by oven drying of fresh maize grains for 24 hours at 85°C and 95°C, however, the phosphorus absorption was also increased at 85°C. In contrast, another study indicated that pelleting of maize diet reduced phytate phosphorus retention (Kilburn and Edwards, 2001). In the present study, the apparent ileal absorption and apparent total digestibility of phosphorus was not affected by thermal treatment. Both organic (corn and wheat) and inorganic (monocalcium phosphate) sources of phosphorus were included in the layer diet. The phosphorus availability in different diet ingredients has substantial variability (Rodehutscord et al., 2012). Cereals contain 60-70% phytic acid, which is 0-50% available, whereas monocalcium phosphate contains 98% available phosphorus (Rutherfurd et al., 2002). Therefore, the presence of both phytic acid and monocalcium phosphate might be the reason for comparable apparent ileal absorption and apparent total digestibility of phosphorus. The concentrations of all minerals and trace elements in yolk, albumen and shell were comparable, except the concentration of albumen manganese, which was higher in hens fed mash compared to those fed expanded feed. Manganese is largely stored in yolk and albumen contains only traces (Leeson and Summers, 2001; Uni et al., 2012). Therefore, the different concentration of manganese in albumen may not have any meaningful physiological consequences on egg quality.

The feed particle size did not affect most of the minerals in egg contents but had some interesting correlations for their apparent ileal absorption and apparent total digestibility. The apparent ileal absorption for magnesium was higher in birds fed with coarse feed particles than fine. Additionally, in this study, the egg shell thickness and shell weight was higher in coarse particle size treatment than fine treatment, which is discussed in Hafeez et al. (2015). Due to the fact that magnesium play an important role in egg shell formation (Leeson and Summers, 2001), the higher apparent ileal digestibility of magnesium with increasing particle size might have resulted in higher shell formation in terms of shell thickness and shell weight. The apparent total digestibility was higher for phosphorus and lower for iron in birds fed with fine particle size than coarse. In general, a reduction in particle size increases the surface area for enzymatic activity, modifies the physical characteristics of feed and may result in improved animal performance (Waldroup, 1997; Goodband et al., 2002). However, some recent studies emphasize the positive effect of coarse feed particles on starch availability (Svihus et al., 2010). Amerah et al. (2007) argued that coarse particles may result in higher mineral digestion and absorption due to possibility of longer transit time. Some other data suggest that coarse maize particles resulted in higher utilization of calcium, total phosphorus and phytate phosphorus in broilers (Kasim and Edwards, 2000; Kilburn and Edwards, 2001). Further findings show that feeding of coarse particle size diets improved bone ash and plasma phosphorus levels in broilers (Carlos and Edwards Jr, 1997; Kilburn and Edwards, 2004).

The interaction between milling methods and thermal treatment resulted in significant differences for apparent ileal absorption and apparent total digestibility of copper and manganese. For copper, the mash feed produced by roller mill resulted in higher apparent ileal absorption and apparent total digestibility than expanded feed and mash feed produced by hammer mill. A similar trend was observed for manganese, where mash feed produced by roller mill resulted in significantly higher apparent ileal absorption and apparent total digestibility compared to hens fed mash feed produced by hammer mill. Therefore, the combination of hammer mill and mash resulted in significantly lower digestibility than roller mill and mash, for both copper and manganese in ileum and rectum. It might be noticed that expansion of feed produced by hammer and roller mill resulted in significantly higher apparent ileal absorption and apparent total digestibility than mash feed obtained from hammer mill, for both copper and manganese. The literature suggests that roller mill produces a more uniform sized particles as compared to hammer mill (Amerah et al., 2007), which produces a greater amount of fine particles (Reece et al., 1985). The particles produced by hammer mill in mash form had more uniformity problems than those, which were subjected to expansion. Furthermore, the particles produced by roller mill in mash and expanded form, had more uniform size than those produced by hammer mill especially in mash form. Due to the fact that more uniform particle size may improve the performance of mature birds (Amerah et al., 2007), the lower apparent ileal absorption and apparent total digestibility for both copper and manganese for interaction effect, was possibly caused by the problem of uniformity of particles produced by hammer mill in mash form, as compared to other combinations of milling type and thermal treatment.

The interaction between thermal treatment and particle size affected some trace elements retention in egg contents. Our results show that coarse particle size in combination with mash form resulted in higher concentration of yolk iron in comparison with fine size with mash form. Similarly, coarse feed in expandate form resulted in higher albumen zinc concentration than fine feed in expandate form. Both interactions show that the coarse particle size in combination with both mash and expandate form resulted in better retention for couple of trace elements in comparison with combinations of fine feed particles with mash and expandate forms. However, due to the fact that interaction between thermal treatment and particle size had no effect on apparent ileal absorption, apparent total digestibility and retention of most of the minerals and trace elements in egg contents, these effects might have no meaningful biological consequences. No further data was reported in literature, to our knowledge, regarding the effect of milling methods, thermal treatments, particle size and their interaction, on mineral digestibility and egg mineral contents.

In general, yolk has higher concentrations of calcium, phosphorus, manganese, iron, copper and zinc as compared to albumen, and shell is important source of calcium and magnesium for their release during embryonic development (Uni et al., 2012). In the present study, out of total mineral concentrations retained in yolk and albumen, the yolk contained approximately 77% calcium, 90% phosphorus, 98% zinc, 94% manganese, 78% copper and 91% iron. Our results regarding ratios of mineral and trace elements contained in yolk and albumen are in agreement with Uni et al. (2012) who reported approximately 83% calcium, 92% phosphorus, 65% zinc, 96% manganese, 77% copper and 88% iron in yolk as compared to albumen.

In conclusion, feed produced by hammer mill had a negative effect on apparent ileal absorption and apparent total digestibility for trace elements in particular, which could be critical due to their major role in various biochemical processes in chicken body. However, the mineral and trace elements concentrations in egg yolk, albumen and shell, the performance parameters and egg quality indicators of economic interest, were mostly comparable for all the treatments. Therefore, any combination of milling methods, thermal treatments and particle sizes used in present study, may be used for feed production of laying hens.

5.5 ACKNOWLEDGEMENTS

The project was financed by the German Federation of Industrial Research Association (Allianz Industrie Forschung- AIF) through the German Federal Ministry of Economics and Technology, project number 16800 N. The authors express deep appreciation to Dr. W. Vahjen from the Institute of Animal Nutrition, Freie Universität Berlin for his valuable inputs and critical review of the manuscript. Special thanks are extended to E. Schulze, M. Eitinger, K. Topp, L. Ebersbach, K. Fiedler and I. Bebert from the Institute of Animal Nutrition, Freie Universität Berlin, for their technical support during the animal experiment and laboratory analysis.

5.6 REFERENCES

Al-Batshan, H. A., S. E. Scheideler, B. L. Black, J. D. Garlich, and K. E. Anderson. 1994. Duodenal calcium uptake, femur ash, and eggshell quality decline with age and increase following molt. Poult. Sci. 73:1590-1596.

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

Angel, R. 2007. Metabolic disorders: limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. J. Appl. Poult. Res. 16:138-149.

Baumgartner, S., D. J. Brown, E. Salevsky Jr, and R. M. Leach Jr. 1978. Copper deficiency in the laying hen. J. Nutr. 108:804-811.

Carlos, A. B., and H. M. Edwards Jr. 1997. Influence of soybean particle size and chlortetracycline on the utilization of phytate phosphorus by broilers. Poult. Sci. 76:suppl.234.

Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J. Appl. Poult. Res. 16:126-137.

Essary, E. O., and C. E. Holmes. 1964. Influence of feeding vitamin C minerals + additives in layer rations on egg production feed conversion shell quality + Haugh Units. Poult. Sci. 43:1316.

Fritz, J., W. J. Streich, A. Schwarm, and M. Clauss. 2012. Condensing results of wet sieving analyses into a single data: a comparison of methods for particle size description. J. Anim. Physiol. Nutr. 96:783-797.

Gericke, S., and B. Kurmies. 1952. Die kolorimetrische Phosphorsäurebestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse. Z. Pflanzenernaehr. Dueng. Bodenkd. 59:235–247.

GfE. 1999. (Gesellschaft fur Ernahrungsphysiologie). Empfehlungen zur Energie- und 485 Nahrstoffversorgung der Legehennen und Masthuhner Broiler. DLG-Verlag, Frankfurt (Main), Germany.

Goodarzi Boroojeni, F., A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, K. Männer, and J. Zentek. 2014a. The effects of different thermal treatments and organic acid levels in feed on microbial composition and activity in gastrointestinal tract of broilers. Poult. Sci. 93:1440-1452.

Goodarzi Boroojeni, F., A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, K. Männer, and J. Zentek. 2014b. The effects of different thermal treatments and organic acid levels on nutrient digestibility in broilers. Poult. Sci. 93:1159-1171.

Goodband, R. D., M. D. Tokach, and J. L. Nelssen. 2002. The effects of diet particle size on animal performance. Kansas State University. May, 2002.:6 pp.

Hafeez, A., A. Mader, F. Goodarzi Boroojeni, I. Ruhnke, I. Röhe, K. Männer, and J. Zentek. 2014. Impact of thermal and organic acid treatment of feed on apparent ileal mineral absorption, tibial and liver mineral concentration and tibia quality in broilers. Poult. Sci. 93:1754-1763.

Hafeez, A., A. Mader, I. Röhe, I. Ruhnke, F. Goodarzi Boroojeni, M. S. Yousaf, K. Männer, and J. Zentek. 2015. Effect of milling method, thermal treatment, and particle size of feed on exterior and interior egg quality in laying hens. Europ.Poult. Sci. 79: ISSN 1612-9199. doi : 10.1399/eps.2015.76.

Hudson, B. P., W. A. Dozier, J. L. Wilson, J. E. Sander, and T. L. Ward. 2004. Reproductive performance and immune status of caged broiler breeder hens provided diets supplemented with either inorganic or organic sources of zinc from hatching to 65 wk of age. J. Appl. Poult. Res. 13:349-359.

Iji, P. A., K. Khumalo, S. Slippers, and R. M. Gous. 2003. Intestinal function and body growth of broiler chickens on diets based on maize dried at different temperatures and supplemented with a microbial enzyme. Reprod. Nutr. Dev. 43:77-90.

Ishikawa, S., Y. Yano, K. Arihara, and M. Itoh. 2004. Egg yolk phosvitin inhibits hydroxyl radical formation from the fenton reaction. Biosci. Biotech. Biochem. 68:1324-1331.

Kasim, A. B., and H. M. Edwards. 2000. Effect of sources of maize and maize particle sizes on the utilization of phytate phosphorus in broiler chicks. Anim. Feed Sci. Technol. 86:15-26.

Kilburn, J., and H. M. Edwards. 2001. The response of broilers to the feeding of mash or pelleted diets containing maize of varying particle sizes. Br. Poult. Sci. 42:484-492.

Kilburn, J., and H. M. Edwards. 2004. The effect of particle size of commercial soybean meal on performance and nutrient utilization of broiler chicks. Poult. Sci. 83:428-432.

Kluth, H., K. Mehlhorn, and M. Rodehutscord. 2005. Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. Arch. Anim. Nutr. 59:271-279.

Koch, K. 2002. Hammermills and rollermills. Kansas State University, May 2002.:8 pp.

Larbier, M., and B. Leclercq. 1992. Nutrition and feeding of poultry. Nottingham University Press, Loughborough, Leicestershire, United Kingdom.

Leeson, S., and J. D. Summers. 2001. Nutrition of the chicken. 4th ed. University Books, Guelph, Ontario, Canada.

Mabe, I., C. Rapp, M. M. Bain, and Y. Nys. 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poult. Sci. 82:1903-1913.

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

NRC. 1994. Nutrient Requirements of Poultry. 9th revised ed. National Academy Press, Washington, D.C.

Peisker, M. 2006. Feed processing–impacts on nutritive value and hygienic status in broiler feeds. Proc. 18th Aust. Poult. Sci. Symp., New South Wales, Australia. 7-16.

Portella, F. J., L. J. Caston, and S. Leeson. 1988. Apparent feed particle-size preference by laying hens. Can. J. Anim. Sci. 68:915-922.

Reece, F. N., B. D. Lott, and 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.

Rezvani, M., H. Kluth, C. Elwert, and M. Rodehutscord. 2008. Effect of ileum segment and protein sources on net disappearance of crude protein and amino acids in laying hens. Br. Poult. Sci. 49:28-36.

Rodehutscord, M., A. Dieckmann, M. Witzig, and Y. Shastak. 2012. A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies. Poult. Sci. 91:965-971.

Ruhnke, I., I. Röhe, C. Krämer, F. Goodarzi Boroojeni, F. Knorr, A. Mader, E. Schulze, A. Hafeez, K. Neumann, R. Löwe, and J. Zentek. 2015. The effects of particle sizes, milling methods, and thermal treatment of feed on performance, apparent ileal digestibility and pH of the digesta in laying hens. Poult. Sci. 94:962-699.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 43:598-606.

Schiffman, H. R. 1968. Texture preference in domestic chick. J. Comp. Physiol. Psych. 66:540-541.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215-221.

Svihus, B. 2006. The role of feed processing on gastrointestinal function and health in poultry. Pages 183–194 in Avian Gut Function in Health and Disease. Vol. 28. G. C. Perry, ed. CAB Int., Wallingford, UK.

Svihus, B., A. Sacranie, V. Denstadli, and M. Choct. 2010. Nutrient utilization and functionality of the anterior digestive tract caused by intermittent feeding and inclusion of whole wheat in diets for broiler chickens. Poult. Sci. 89:2617-2625.

Tang, P., P. H. Patterson, and V. M. Puri. 2006. Effect of feed segregation on the commercial hen and egg quality. J. Appl. Poult. Res. 15:564-573.

Uni, Z., L. Yadgary, and R. Yair. 2012. Nutritional limitations during poultry embryonic development. J. Appl. Poult. Res. 21:175-184.

Waldroup, P. W. 1997. Particle size reduction of cereal grains and its significance in poultry nutrition. Pages 14 pp. in ASA Technical Bulletin Vol. PO34. American Soybean Association, Singapore, Singapore.

Wolf, P., M. Arlinghaus, J. Kamphues, N. Sauer, and R. Mosenthin. 2012. Einfluss der Partikelgröße im Futter auf die Nährstoffverdaulichkeit und Leistung beim Schwein. Übersichten zur Tierernährung 40:21-64.

Zamani, A., H. R. Rahmani, and J. Pourreza. 2005. Effect of different levels of manganese and zinc on performance traits and breaking eggs in laying hens. Pak. J. Biol. Sci. 8:1035-1040.

5. 1 Feed composition and nutrient content of the basal experimental diet

Ingredients	g/kg as fed
Corn	300.8
Wheat	290.6
Soybean meal (42 % CP)	224.8
Calcium carbonate	86.0
Soya oil	44.6
Molasses	30.0
Mineral/Vitamin premix[1]	12.0
Monocalcium phosphate	7.8
Salt	2.0
DL-Methionine	1.0
L-Tryptophane	0.2
Titanium dioxide	0.2
Nutrient content	g/kg dry matter as analyzed
Dry matter	881.9
Crude protein	185.8
Ether extract	59.7
Crude fiber	32.7
Starch	418.5
Crude ash	110.6
Calcium	35.7
Phosphorus	3.54
Magnesium	2.45
Zinc	0.078
Manganese	0.102
Copper	0.018
Iron	0.534
Calculated ME (MJ/kg)	11.4

ME metabolizable energy

[1] Mineral and vitamin premix (Spezialfutter Neuruppin, Neuruppin, Germany) containing per kg premix: 400000 IU vitamin A; 40000 IU vitamin D_3; 8000 mg vitamin E (alpha-tocopherole acetate); 300 mg vitamin K_3; 250 mg vitamin B_1; 250 mg vitamin B_2; 2500 mg nicotinic acid; 400 mg vitamin B_6; 2000 µg vitamin B_{12}; 25000 µg biotin; 1000 mg calcium pantothenate; 100 mg folic acid; 80000 mg choline chloride; 5000 mg zinc (zinc oxide); 2000 mg iron (iron carbonate); 6000 mg manganese (manganese oxide); 1200 mg copper (copper sulfate-pentahydrate); 45 mg iodine (calcium iodate); 30 mg cobalt (cobalt-(II)-sulfate-heptahydrate); 35 mg selenium (sodium selenite); 35 g sodium (sodium chloride); 55 g magnesium (magnesium oxide).

5. 2 Discrete mean (dMEAN) particle size of the eight experimental diets produced by different milling methods, particle sizes and thermal treatment.

Mill	Roller				Hammer			
Particle size	Coarse		Fine		Coarse		Fine	
Thermal	Mash	Expandate	Mash	Expandate	Mash	Expandate	Mash	Expandate
dMEAN after dry sieving analysis (mm)	1.93	2.43	1.28	1.27	2.41	2.15	1.64	1.30
dMEAN after wet sieving analysis (mm)	1.85	0.85	1.14	0.31	2.60	0.93	1.27	0.50

5. 3 Effect of milling method, thermal treatment and particle size of feed on percent apparent ileal absorption of minerals in layers[1]

	Mill		Thermal		Particle Size			*P*-value						
	Roller	Hammer	Mash	Expandate	Coarse	Fine	SEM	Mill	Thermal	Size	Mill× Thermal	Mill× Size	Thermal ×Size	Mill× Thermal×Size
Ca	50	49.1	46.8	52.3	50.9	48.2	2.19	0.85	0.24	0.56	0.39	0.45	0.9	0.35
P	35.2	35.3	35.9	34.6	33.1	37.4	1.39	0.98	0.64	0.14	0.08	0.2	0.9	0.99
Mg	40	32	39.9	32.1	39	33	1.74	0.01	0.01	0.051	0.34	0.25	0.29	0.47
Zn	18.6	8.5	17.1	9.97	15.2	11.9	2.4	0.03	0.13	0.49	0.82	0.27	0.29	0.07
Mn	13.1	2.79	7.97	7.92	8.5	7.39	2.91	0.07	0.99	0.85	0.02	0.39	0.45	0.15
Cu	17.7	-23.8	-0.111	-5.97	1.71	-7.79	4.72	<0.0001	0.37	0.15	<0.0001	0.68	0.02	0.9
Fe	30.3	6.65	22.5	14.5	1.71	-7.79	3.29	0.0002	0.17	0.17	0.64	0.22	0.05	0.78

[1] Data are means of 8 replicates per group.

5. 4 Effect of milling method, thermal treatment and particle size of feed on percent apparent total digestibility of minerals in layers[1]

	Mill		Thermal		Particle Size			P-value						
	Roller	Hammer	Mash	Expandate	Coarse	Fine	SEM	Mill	Thermal	Size	Mill× Thermal	Mill× Size	Thermal ×Size	Mill× Thermal×Size
Ca	52.3	46.1	41.4	57.0	48.8	49.7	2.37	0.16	0.001	0.83	0.61	0.34	0.57	0.16
P	31.1	35.4	32.7	33.8	29.8	36.6	1.71	0.20	0.74	0.05	0.24	0.41	0.28	0.03
Mg	43.8	39.5	40.0	43.2	40.2	43.0	2.65	0.40	0.54	0.59	0.01	0.55	0.01	0.52
Zn	19.4	11.7	14.8	16.2	18.1	12.9	2.79	0.16	0.80	0.35	0.08	0.86	0.051	0.68
Mn	6.50	-1.38	-1.14	6.27	4.00	1.13	3.93	0.29	0.32	0.70	0.0007	0.32	0.84	0.73
Cu	18.1	-6.86	7.08	4.13	4.47	6.74	4.57	0.002	0.69	0.76	<0.0001	0.83	0.06	0.27
Fe	23.2	11.9	14.8	20.3	23.7	11.4	2.79	0.03	0.27	0.02	0.04	0.40	0.01	0.90

[1] Data are means of 8 replicates per group.

5. 5 Effect of milling method, thermal treatment and particle size of feed on yolk minerals in layers[1]

		Mill		Thermal		Particle Size			*P*-value						
		Roller	Hammer	Mash	Expandate	Coarse	Fine	SEM	Mill	Thermal	Size	Mill× Thermal	Mill× Size	Thermal ×Size	Mill× Thermal×Size
DM	%	50.1	50.1	50.1	50.1	50.3	49.9	0.23	0.96	0.88	0.35	0.17	0.91	0.99	0.13
Ash	(% of DM)	3.68	3.62	3.63	3.67	3.62	3.67	0.03	0.36	0.47	0.43	0.47	0.58	0.21	0.36
Ca	g/kg (DM)	2.46	2.56	2.49	2.53	2.49	2.53	0.05	0.27	0.69	0.11	0.16	0.91	0.89	0.37
P	g/kg (DM)	10.0	9.77	9.77	9.95	10.0	9.76	0.09	0.31	0.31	0.27	0.85	0.24	0.92	0.57
Mg	g/kg (DM)	0.18	0.21	0.20	0.19	0.20	0.19	0.01	0.08	0.47	0.81	0.37	0.57	0.63	0.87
Zn	mg/kg (DM)	58.1	61.8	60.5	59.4	60.5	59.5	1.06	0.08	0.60	0.65	0.26	0.04	0.97	0.28
Mn	mg/kg (DM)	2.06	1.94	2.06	1.94	1.87	2.13	0.08	0.44	0.45	0.12	0.19	0.98	0.10	0.43
Cu	mg/kg (DM)	5.25	5.20	5.32	5.13	5.69	4.77	0.15	0.84	0.50	0.003	0.78	0.18	0.51	0.89
Fe	mg/kg (DM)	127	137	133	131	134	130	3.43	0.13	0.82	0.59	0.51	0.14	0.56	0.32

[1] Data are means of 5 replicates per group.

5. 6 Effect of milling method, thermal treatment and particle size of feed on albumen minerals in layers[1]

		Mill		Thermal		Particle Size			*P*-value						
		Roller	Hammer	Mash	Expandate	Coarse	Fine	SEM	Mill	Thermal	Size	Mill× Thermal	Mill× Size	Thermal ×Size	Mill× Thermal×Size
DM	%	13.4	13.6	13.6	13.4	13.5	13.5	0.11	0.24	0.44	0.99	0.60	0.87	0.86	0.58
Ash	(% of DM)	5.86	5.85	5.91	5.79	5.88	5.82	0.04	0.93	0.17	0.55	0.16	0.77	0.10	0.32
Ca	g/kg (DM)	0.76	0.70	0.77	0.69	0.74	0.71	0.05	0.60	0.41	0.79	0.80	0.54	0.33	0.90
P	g/kg (DM)	0.85	0.87	0.86	0.85	0.85	0.86	0.01	0.39	0.47	0.82	0.24	0.26	0.78	0.06
Mg	g/kg (DM)	0.67	0.63	0.65	0.64	0.66	0.64	0.02	0.26	0.84	0.59	0.82	0.45	0.93	0.97
Zn	mg/kg (DM)	1.34	1.44	1.36	1.43	1.50	1.28	0.12	0.65	0.75	0.34	0.67	0.53	0.004	0.58
Mn	mg/kg (DM)	0.21	0.17	0.22	0.16	0.21	0.17	0.01	0.14	0.04	0.12	0.94	0.26	0.46	0.31
Cu	mg/kg (DM)	1.42	1.42	1.31	1.53	1.65	1.19	0.10	0.98	0.29	0.03	0.39	0.32	0.87	0.38
Fe	mg/kg (DM)	12.7	9.36	12.5	9.58	12.6	9.42	1.31	0.19	0.25	0.20	0.76	0.22	0.22	0.03

[1] Data are means of 5 replicates per group.

5. 7 Effect of milling method, thermal treatment and particle size of feed on shell minerals in layers[1]

		Mill		Thermal		Particle Size			*P*-value						
		Roller	Hammer	Mash	Expandate	Coarse	Fine	SEM	Mill	Thermal	Size	Mill× Thermal	Mill× Size	Thermal ×Size	Mill× Thermal×Size
DM	%	87.0	88.6	87.4	88.1	87.4	88.1	0.97	0.45	0.74	0.73	0.91	0.53	0.70	0.98
Ash	(% of DM)	95.9	95.7	95.5	96.0	95.9	95.6	0.14	0.47	0.06	0.29	0.60	0.69	0.48	0.98
Ca	g/kg (DM)	321	328	327	321	325	323	2.00	0.10	0.15	0.73	0.94	0.95	0.69	0.94
P	g/kg (DM)	1.42	1.39	1.46	1.36	1.39	1.43	0.02	0.52	0.053	0.44	0.43	0.51	0.47	0.34
Mg	g/kg (DM)	3.03	3.02	3.01	3.04	2.99	3.07	0.06	0.92	0.79	0.56	0.79	0.23	1.00	0.48
Zn	mg/kg (DM)	3.90	3.31	3.76	3.46	3.78	3.44	0.36	0.43	0.69	0.65	0.26	0.77	0.06	0.96
Mn	mg/kg (DM)	0.26	0.37	0.30	0.33	0.29	0.34	0.04	0.11	0.69	0.55	0.69	0.96	0.02	0.08
Cu	mg/kg (DM)	0.52	0.52	0.50	0.55	0.48	0.56	0.04	0.92	0.50	0.26	0.92	0.15	0.19	0.82
Fe	mg/kg (DM)	1.83	1.53	1.78	1.58	1.70	1.67	0.10	0.16	0.35	0.87	0.64	0.67	0.51	0.22

[1] Data are means of 5 replicates per group.

6 THE EFFECT OF MILLING METHOD, THERMAL TREATMENT, AND PARTICLE SIZE OF FEED ON EXTERIOR AND INTERIOR EGG QUALITY IN LAYING HENS

A. Hafeez, A. Mader, I, Röhe, I. Ruhnke, F. Goodarzi Boroojeni, M. S. Yousaf, K. Männer and J. Zentek[1]

Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Königin-Luise-Str. 49, 14195 Berlin, Germany

2015 European Poultry Science: 79. 2015, ISSN 1612-9199.
http://dx.doi.org/10.1399/eps.2015.76

[1] Corresponding author: E-mail: juergen.zentek@fu-berlin.de. Tel: +49 30 838 54245 Fax: +49 30 838 55938.

You must purchase this part online.

7 GENERAL DISCUSSION AND CONCLUSION

The first part of dissertation was designed to investigate the effect of thermal treatment and acid supplementation of feed on digestibility and retention of minerals in tibia and liver as well as tibia quality in broilers. The results of this study showed some important aspects which have been discussed in chapter 4. In the second part of dissertation, the effect of form and particle size of layer feed on mineral digestibility and retention of minerals in egg contents was evaluated, which revealed some fascinating findings (chapter 5). To investigate the effect of milling method, thermal treatment and particle size in layers on exterior and interior egg quality was the goal for third part of the dissertation. The interesting results from this part have briefly been explained in chapter 6.

7.1 Implication of Feed Treatments in Broilers

Different thermal treatments used in the present trials are common practice used by the industry and consist of different levels of temperature and duration. The investigated parameters might be influenced by temperature and duration, which was not determined individually. Similarly, the organic acid combination used in this study is a well known feed additive. Due to the high number of different organic acid combinations that are available on the market and were previously investigated, data often cannot be compared directly. Due to the fact that the organic acid combination used consists of two different organic acids, it cannot be differentiated which organic acid led to the data obtained or if it was the combination of both. These facts have to be taken into account when interpreting the results.

The supplementation of acids and application of heat treatments were deemed to decontaminate the feed in the broiler trial. The results of the present study on microbial composition and activity in gastrointestinal tract have been discussed (Goodarzi Boroojeni et al., 2014a). The applied thermal treatments and organic acids effectively decontaminated the feed from bacteria which is in agreement with previously published literature (Mossel et al., 1967a; Martin and Maris, 2005; Van Immerseel et al., 2006).

7.1.1 Effect of Thermal Treatment of Feed in Broilers

7.1.1.1 Apparent Ileal Absorption of Minerals

Short term thermal treatments of broiler feed including pelleting and expansion enhanced apparent ileal absorption of some minerals in comparison with long term thermal treatment; however no such effects was observed for the trace elements. Broiler feed treated with expansion processing resulted in higher apparent ileal absorption of calcium and sodium whereas both expansion and pelleting of feed improved apparent ileal absorption of potassium in comparison with long term thermal treatment ($P<0.05$). The same study was conducted to investigate the effect of the expansion treatment at 110 ºC and 0.75% organic acid supplementation in addition to the thermal treatments and organic acid levels used in the present study on nutrient digestibility (Goodarzi Boroojeni et al., 2014b), and microbial composition and activity in gastrointestinal tract in broilers (Goodarzi Boroojeni et al., 2014a), and suggested that apparent ileal digestibility of crude protein and AA except cysteine was lower in long term thermal treatment as compared to short term treatments ($P<0.05$). Furthermore, cell numbers of *Lactobacillus* spp. and lactate concentrations in the ileum were higher in the group fed expanded feed as compared to long-term thermal conditioning. Therefore, our results summarized that long term thermal treatment of broiler feed can have a negative effect in general on absorption of some minerals, digestibility of crude protein and AA, and may also affect the intestinal microbiota. Heat treatment might have resulted in development of Maillard products due to degradation of most heat-labile AA. Moreover, the interactions between minerals and trace elements could have been a reason for differences in ileal mineral absorption.

7.1.1.2 Retention of Minerals in Tibia and Tibia Quality

Most of the tibial mineral and trace element concentrations, and tibia quality parameters were not affected by thermal treatment of broiler feed. Long-term thermal conditioning exhibited lower bone ash and higher tibial iron content than expansion ($P < 0.05$). The body weight to bone weight ratio was lower and tibial zinc content was slightly higher when birds were fed pelleted feed in comparison with expansion ($P \leq 0.05$). Bone quality has been determined by various methods in previous studies. Our study indicated that bone density, calculated as a ratio between weight of bone in water and air, did not differ. Bone density is determined by two factors including number of mineral atoms deposited within the bone matrix and porosity of the bone matrix. Since these two factors are highly related to bone strength, the bone density can indirectly be used to determine bone strength (Shim et al., 2012). Thermal processing did not affect robusticity index in this study, which is another measure of bone strength (Reisenfeld, 1972; Seedor et al., 1991). A low robusticity index is considered as indicator of bone stability (Reisenfeld, 1972; Mutus et al., 2006). Similarly, we did not find any differences for the tibiotarsal index, which is a morphometric method that describes the degree of bone mineralization (Barnet and Nordin, 1960). A high value of tibiotarsal index reflects a high mineralization level of the bone (Mutus et al., 2006). In general, despite a few differences in the apparent ileal absorption of some of minerals, protein and AA, the tibial mineral content and tibia quality in the present study was not affected due to thermal treatment. It may be assumed that the recommended dietary levels of these nutrients were sufficient to cover these minor differences which were limited to ileal digestibility.

7.1.1.3 Retention of Minerals in Liver

Thermal treatments did not affect mineral and trace elements concentrations in broiler liver. The tibia is considered as a better indicator for mineral retention than the liver. For instance, relative retention of manganese and zinc was lower in the liver as compared to the tibia, which indicates a greater response of the tibia for mineral accumulation than the liver (Gajula et al., 2011). The effect of increase in manganese supplementation was better reflected from tibia than liver (Black et al., 1985), independently to the organic or inorganic form of manganese supplemented in diet (Berta et al., 2004). In previous studies, different methods were used for preparation, digestion and analysis of liver samples. Adopting different methodologies could also be the reason for inconsistency in observed mineral values in literature and non-significant observations for impact of heat processing on mineral retention in present study. The method adopted in the present study was practiced by Bao et al. (2007), where ashed liver samples were digested with concentrated HCl (w=37-38%) and analyzed using atomic absorption spectrometry in an AAS vario 6 spectrometer (Analytik Jena, Jena, Germany). Some other researchers used different chemicals and methods including deionized water (Thompson and Weber, 1981), ash less filter paper (Henry et al., 1987), combustion process (Skrivan et al., 2005), 2ml concentrated nitric acid, 5 ml deionized water and 1 ml H_2O_2 (Oana-Mărgărita et al., 2012), concentrated nitric acid (Falandysz, 1991), and 2-3 ml HNO_3 (Korsrud et al., 1985). Therefore, it might be stated that indication of no significant differences in mineral concentrations of various heat processing in liver might be due to the fact that the liver may not reflect the true picture of mineral retention as tibia. Additionally, different processing and digestion procedure adopted for liver samples preparation for analysis with atomic absorption spectrometry could be a reason.

7.1.2 Effect of Organic Acid Supplementation of Feed in Broilers

Organic acid supplementation did not affect apparent ileal absorption of minerals and trace elements, tibia quality, and mineral and trace element concentrations in tibia and liver in broilers. Furthermore, in the same study, the inclusion of organic acids did not affect ileal digestibility of AA and crude protein (Goodarzi Boroojeni et al., 2014b). The *Lactobacillus* spp. and enterobacteria cell numbers were slightly decreased whereas acetate concentrations were moderately increased in ileum due to organic acid supplementation of the feed (Goodarzi Boroojeni et al., 2014a). There might be several reasons why propionic and formic acid supplementation did not show any effect on apparent ileal digestibility of nutrients in the present study. Organic acids are metabolized and absorbed in the upper part of the gastrointestinal tract and may not be significantly effective in the distal part due to insufficient quantities (Hume et al., 1993; Thompson and Hinton, 1997; Van Immerseel et al., 2006). Liem et al. (2008) analyzed the effect of several organic acids including citric acid, malic acid, fumaric acid, and ethylenediaminetetraacetic acid (EDTA) on phytate phosphorus hydrolysis and concluded that the reason why some organic acids are effective whereas others are not is not apparent. Another study demonstrated that the effects of organic acids are inconsistent depending on dosage, buffering capacity of dietary ingredients, heterogeneity of gut microbiota and presence of other antimicrobial compounds (Dibner and Buttin, 2002). Therefore, the absence of effect for supplementation of broiler feed with organic acids may be attributed to the above mentioned reasons.

In practice, broilers are fed with pellets whereas layers are offered mash feed and many studies show that particle size does not influence performance of broilers (Amerah et al., 2007). Feed produced by different milling method has a specific importance for laying hens where size, shape and texture of feed might affect intake and digestive function. Therefore in the present study, the effect of milling method and particle size was determined only in laying hen trials.

Due to a short life span and fast growth rate, broilers are more prone to leg problems (Nestor et al., 1985; Lilburn, 1994; Bessei, 2006; Angel, 2007). Due to the fact that the chicken has a finite capacity to deposit calcium (Clunies et al., 1992; Kabir et al., 2014) in comparison with body weight gain, the calcification of bones might not be compatible resulting in weaker bones. Such bones might not be able to sustain the heavy weight of the broiler, and may lead to bone deformities. Layers are less prone to bone deformities problems due to much slower growth rate and longer life periods, thereby having more bone calcification as compared to broilers. Therefore, bone quality is vital for broiler production and alternatively egg quality in terms of shell strength is critical in laying hens farming. For the reason, in the present study, tibia quality was evaluated in broilers while exterior and interior egg quality was determined in laying hens. Similarly, mineral and trace element concentrations were considered in tibia of broilers and in egg contents of layers.

7.2 Implication of Feed Treatments in Layers

7.2.1 Effect of Thermal Treatment of Feed in Layers

In laying hen trial of this study, apparent ileal absorption of magnesium and albumen manganese content was lower, and apparent total digestibility of calcium and percent shell membrane weight was higher due to expansion of feed in comparison with mash ($P<0.05$). However, for most of the minerals and trace elements, apparent ileal absorption, apparent total digestibility, retention in egg yolk, albumen and shell, and egg quality parameters were comparable. The same study was performed to investigate the effect of grinding method, particle size and physical form of the diet on gastrointestinal morphology and jejunal glucose transport in laying hens (Röhe et al., 2014). The results indicated that hens fed with mash feed had higher proventriculus, gizzard and pancreas weight, longer duodenal and shorter ileal villi, increased duodenal villus height-to-crypt depth ratios and higher glucose rates than hens fed expandate ($P<0.05$). The effect of thermal treatment, milling method and particle size of feed on performance, apparent ileal digestibility and pH of the digesta in layers for the same study was also investigated (Ruhnke et al., 2015), which suggests that apparent ileal digestibility of starch was improved by feeding mash as compared to expandate ($P<0.05$). Thermal treatment did not affect performance parameters, apparent ileal absorption of protein, AA and digesta pH of proventriculus, gizzard, duodenum, jejunum, ileum, cecum and excreta of the laying hens. Expansion may change microstructure and reduce the density of feed particles which ultimately may increase availability of the starch in diet (Armstrong, 1994; Peisker, 1994). Additionally, more gizzard development may result in increased nutrient digestion due to more efficient grinding and increased gut motility (Amerah et al., 2007). Our results suggest that despite of higher gizzard weight in birds receiving mash feed, there was no clear effect on digestibility of nutrients, retention of minerals in egg contents and egg quality. Furthermore, the minor changes observed in digestibility of some of the minerals were not reflected in egg contents; therefore, based on the results of the present study, expansion of feed in comparison with mash is of lower importance in terms of mineral digestibility, egg mineral content and egg quality.

7.2.2 Effect of Milling Method of Feed in Layers

The results of the layer trial indicate that feed produced by roller mill resulted in higher apparent ileal absorption of magnesium, zinc, copper and iron and higher apparent total digestibility of copper and iron in comparison with hammer milled feed ($P<0.05$). Similarly, feed produced by roller mill appeared to be associated with higher shell membrane weight and percent shell membrane weight and lower yolk index and yolk height as compared hammer mill ($P<0.05$). The results of the same study revealed that performance parameters, digestibility of protein and AA, and digesta pH along the gastrointestinal tract were comparable (Ruhnke et al., 2015). Therefore, it may be stated that the feed produced by hammer mill had a negative effect on apparent ileal absorption and apparent total digestibility for trace elements in particular, but mineral concentrations in egg contents were mostly comparable for all treatments. Furthermore, performance parameters, digestibility of nutrients and most of the egg quality parameters of importance were not affected by the milling methods. This implies that concentrations of the trace elements in the feed were sufficient to meet the levels required for optimum egg quality and their concentrations in egg contents including yolk, albumen and shell. Therefore, minor changes observed in trace element concentrations in ileum and rectum were not high enough to influence egg mineral content, egg quality and performance variables in laying hens.

7.2.3 Effect of Particle Size of Feed in Layers

Laying hens fed with coarse feed particles displayed higher apparent ileal absorption of magnesium. Apparent total digestibility was lower for phosphorus and higher for iron in birds fed with coarse sized particles ($P<0.05$). Moreover, copper concentration in yolk and albumen, shell thickness, shell weight and albumen height was higher whereas shell density was lower in eggs produced by hens fed with coarse feed particles ($P<0.05$). The findings of the same study published in other manuscripts revealed that feeding coarse sized particles resulted in higher gizzard weight (Röhe et al., 2014) and improved ileal starch digestibility in comparison with fine feed particles, however performance parameters, protein and AA digestibility and digesta pH of different compartments of digestive tract remained unchanged (Ruhnke et al., 2015). The feed used in the present study comprised of maize, wheat and sorghum as major ingredients. Coarser grinding of grains to a more uniform particle size may improve the performance of mature birds (Amerah et al., 2007) while using different ingredients including maize (Reece et al., 1985; Proudfoot and Hulan, 1989; Hamilton and Proudfoot, 1995b) sorghum (Nir et al., 1990) and wheat (Nir et al., 1995; Amerah et al., 2007). Such impact may have resulted due to more gizzard development leading to increased grinding, gut motility and nutrient digestion (Amerah et al., 2007). Therefore, minor changes observed in some of the minerals and trace elements as well as a few egg quality parameters in the present study might have resulted from gizzard development in hens receiving coarse feed particles. Moreover, due to the fact that minor changes observed in the ileal and total digestibility of the minerals were not visible in egg contents and most of the egg quality parameters of importance were not affected, it may be stated that minor effect of particle size on mineral digestibility and egg quality in the present study was not that pronounced.

7.2.4 Interaction Effects of Feed Treatments in Layers

Our results indicated some interaction effects among feed treatments under study. Feed in mash form in combination with roller mill resulted in higher apparent ileal absorption of copper and manganese than mash feed produced by hammer mill. The combination of coarse particle size with both mash and expandate showed higher retention for yolk iron and albumen zinc in comparison with fine particle size feed as mash and expandate. In general, digestibility for most of the minerals, their retention in egg contents and egg quality was not affected by interactive effects of milling methods, thermal treatments and feed particle size. Therefore, these interaction affects seem to be incidental and may not have any meaningful biological consequences.

7.3 CONCLUSION

In conclusion, the findings of the present dissertation suggest that long term thermal treatment can have a negative effect on apparent ileal absorption of some minerals in comparison with pelleting and expansion in broilers, however mineral concentrations and quality of tibia were similar for applied thermal treatments. Organic acid supplementation did not affect apparent ileal absorption and tibial retention of minerals as well as tibia quality. Liver mineral concentrations were not affected by thermal treatment and acid supplementation in broilers. Therefore, application of thermal treatment with organic acid supplementation used in present study could be a practical strategy for sanitation of broiler feed. However, the impact of long-term thermal treatment on apparent ileal absorption of minerals is of particular interest, and need further investigation in terms of its effect on digestive and absorptive processes in broilers.

In laying hens trial, milling method influenced the apparent ileal absorption and apparent total digestibility of trace elements in particular, however the negative impact of hammer mill was limited and was not observed in egg contents including yolk, albumen and shell, as well as for egg quality. Expansion of feed did not affect apparent ileal absorption and apparent total digestibility of most of the minerals and trace elements, their retention in egg contents, and egg quality in comparison with mash feed. The effects shown by feed particle size on apparent ileal absorption and apparent total digestibility of some minerals, and egg quality parameters had limited importance. Therefore, layer feed may be produced by using any milling method, thermal treatment and particle size used in present study without any marked effect on mineral digestibility, retention of minerals in egg content and egg quality. However, the feed production cost should be taken into account while formulating layer rations using various feed production strategies. Furthermore, the effect of hammer mill on trace element digestibility offers an interesting perspective for further investigation on its implication on other biochemical processes in laying hens.

8 SUMMARY

Effect of different feed treatment strategies on apparent mineral digestibility and retention in broilers and layers and egg quality in laying hens

Minerals play a vital role in bone and egg formation. Rapid broiler growth may lead to bone deformities resulting in suffering for the animals, lower production performance and economic losses. Similarly, inadequate mineral deposition, especially of calcium in the egg, may end up in weaker shelled eggs, thus adversely affecting egg quality. Such insufficient mineral supply may cause a higher incidence of egg breakage and ultimately reduce layer productivity and profitability. The poultry industry uses various techniques to produce feed with high quality and maximum sanitation. Long and short term thermal treatments and acid supplementation of feed are considered as efficient strategies to achieve high feed safety standards. However, the effects of these treatments on mineral digestibility are not well documented. Broilers are usually fed with pellets having least differences in particle size and shape. The feed form and particle size is of specific interest in laying hens, which are offered feed either as mash or expandate. The effect of particle size and feed form in layers has previously been studied, however their impact on mineral digestibility and retention in egg contents as well as egg quality was not considered in detail. Keeping in view the importance of various feed treatment strategies distinctly in broilers and layers, the investigations presented in this thesis were designed in two different studies.

The first study was conducted to investigate the effect of thermal processing of feed including pelleting (P), long-term conditioning at 85°C (L) and expanding at 130°C (E) without or with 1.5 % of an acid mixture containing 64 % formic and 25 % propionic acid on the apparent ileal absorption of calcium, phosphorus, magnesium, potassium, sodium, iron, copper, manganese and zinc, their concentrations in liver and tibia as well as various tibial quality parameters in broilers. In total, 480 day-old Cobb broiler chicks were assigned using a completely randomized design with a 3 × 2 factorial arrangement. The ileal digesta, liver and tibia were collected at d 35. AIA of calcium and sodium was improved in group E compared to L. Group P and E showed higher AIA for potassium than L. Bone ash content was increased in group E compared to L. The body weight to bone weight ratio was lower and tibial zinc content was higher in group P compared to E. Tibial iron content was higher in group L than E. Acid addition did not affect AIA, mineral content in tibia or tibial quality parameter. Both thermal treatment and acid did not affect mineral concentrations in the liver, except an inconsistent interaction effect for dry matter content and sodium.

The second study dealt with the impact of roller (R) and hammer (H) mills, mash (M) and expandate (E) with fine (F) and coarse (C) particle sizes, on apparent ileal absorption (AIA) and apparent total digestibility (ATD), egg quality and retention of calcium, phosphorus, magnesium, zinc, manganese, copper and iron, in yolk, albumen and shell. A total of 384 hens (Lohmann Brown), 19 weeks old, were assigned using a randomized design with a $2\times2\times2$ factorial arrangement. Eight experimental diets were offered *ad libitum* during the whole experimental period and one week before for diet adaption. At 23 weeks of age, digesta from the ileum and rectum and eggs were collected. AIA of magnesium, zinc, copper and iron was higher in treatment R in comparison with treatment H. The ATD of copper and iron was higher in treatment R than treatment H. The shell membrane weight and percent shell membrane weight was lower whereas yolk index and yolk height were higher in treatment H as compared to treatment R. The AIA of magnesium was higher in treatment M than treatment E and in treatment C than treatment F. Thermal treatments displayed higher percent shell membrane weight in treatment E than treatment M. The ATD was higher for phosphorus and lower for iron in treatment F than treatment C. The copper concentration in

yolk and albumen was higher in treatment C than treatment F. The shell thickness, shell weight and albumen height were higher and shell density was lower in treatment C as compared to treatment F.

In conclusion, for both broiler and layer studies, the minor impact of feed treatments was limited to ileal and total digestibility of minerals in general and trace elements in particular. The retention of minerals and trace elements in tibia, liver and egg contents as well as quality of tibia and egg was not altered at most. It may be assumed that mineral concentrations in the feed were sufficient to compensate minor differences in digestibility induced by various feed treatments used in the present study. Therefore, different thermal treatments, acid supplementation, milling method and particle sizes used in the present study seem to be of lower importance for feed production regarding optimized tibia and egg quality and therefore may be used for broiler and layer feed formulation.

9 ZUSAMMENFASSUNG

Der Effekt verschiedener Behandlungsverfahren von Futtermitteln auf die scheinbare Verdaulichkeit und Retention von Mengen- und Spurenelementen in Broilern und Legehennen sowie die Eiqualität

Mengen- und Spurenelemente spielen eine entscheidende Rolle in der Knochen- und Eischalenbildung. Schnelles Wachstum von Broilern kann zu Knochendeformationen führen, welche leidensrelevant sind und einen verminderten Fleischertrag und ökonomische Verluste zur Folge haben. Auf ähnliche Weise kann ein veränderter Mineralstoffwechsel (insbesondere der von Kalzium) eine verminderte Eischalenqualität verursachen, welche wiederum die Eiqualität beeinträchtigt. Das Auftreten von Brucheiern kann die Produktivität und Rentabilität der Legehennenhaltung verringern. Die Mischfutterindustrie setzt verschiedenste Techniken ein, um qualitativ hochwertiges und hygienisch einwandfreies Futter zu produzieren. Sowohl Kurz- und Langzeithitzebehandlung als auch der Einsatz von organischen Säuren dienen als effiziente Maβnahmen, um hohe hygienische Standards und eine optimale Futtermittelsicherheit zu gewährleisten. Der Einfluss dieser Methoden auf die Mineralstoffverfügbarkeit wurde bisher jedoch nicht eingehend untersucht. Broiler werden üblicherweise mit Pellets gefüttert, welche eine einheitliche Partikelgröβe und -form gewährleisten. Die Darreichungsform des Futters und die Partikelgröβe sind insbesondere bei Legehennen von Interesse, die ihr Futter vorwiegend in Schrotform, teils auch als Expandat erhalten. Der Effekt der Partikelgröβe und Darreichungsform bei Legehennen auf die scheinbare Absorption und Retention von Mengen- und Spurenelementen und die daraus resultierende Eischalenqualität wurde bisher jedoch noch nicht untersucht. Aufgrund der unterschiedlichen Fragestellungen zur Futtermittelproduktion für Broiler bzw. Legehennen wurden die Untersuchungen dieser Dissertation in zwei verschiedenen Studiendesigns durchgeführt.

Der erste Versuch wurde durchgeführt, um den Effekt der Hitzebehandlung des Futters in Form von Pelletierung (P), Langzeitkonditionierung bei 85°C (L) sowie Expandierung bei 130°C (E) mit oder ohne dem Zusatz von 1,5% eines Produkts mit organischen Säuren auf die scheinbare ileale Mineralstoffverdaulichkeit bei Broilern zu erfassen. Als organische Säuren kam ein Produkt aus Ameisensäure und Propionsäure zum Einsatz. Die in diesem Versuch erfassten Parameter beinhalteten die scheinbare ileale Absorption von Kalzium, Phosphor, Magnesium, Kalium, Natrium, Eisen, Kupfer, Magnesium und Zink, die Konzentration dieser Elemente in der Leber und Tibia sowie die Evaluierung verschiedenster Parameter zur Darstellung der Tibiaqualität. 480 Cobb-Eintagsküken wurden randomisiert einem 3×2 faktoriellen Versuchsdesign zugeordnet. Der Inhalt des Ileums, die Leber und Tibia wurden am 35. Lebenstag beprobt. Die scheinbare ileale Absorption von Kalzium und Natrium war bei Broilern der Gruppe E höher als in Broilern der Gruppe L. Verglichen zur Broilern der Gruppe L wiederum wiesen die Broiler der Gruppen P und E eine höhere scheinbare ileale Absorption für Kalium auf. Der Mineralstoffgehalt der Tibia war in Broilern der Gruppe E höher als in Broilern der Gruppe L. Das Verhältnis Körper-/Tibiamasse war geringer und die Zinkkonzentration höher in Broilern der Gruppe P verglichen zu Broilern der Gruppe E. Der Eisengehalt der Tibia war höher in Broilern der Gruppe L verglichen zur Gruppe E. Der Einsatz von organischen Säuren beeinflusste weder die scheinbare ileale Mineralstoffabsorption noch den Mineralstoffgehalt der Tibia oder die Tibiaqualität. Weder die Hitzebehandlung des Futters noch der Einsatz von organischen Säuren hatten einen Einfluss auf die Mineralstoffkonzentration der Leber. Ausgenommen hiervon war eine Interaktion hinsichtlich des Natriumgehalts.

Der zweite Versuch wurde durchgeführt, um den Einfluss einer Zerkleinerung mittels Walzenstuhl (W) und Hammermühle (H), einem schrotförmigen (S) und expandiertem (E) Futter sowie feiner (F) und grober (C) Futterzerkleinerung auf den Mineralstoffwechsel der Legehennen zu erfassen. Die scheinbare ileale und Gesamttraktabsorption, Parameter der Eiqualität und die Retention von Kalzium, Phosphor, Magnesium, Kupfer und Eisen im Eidotter, Eiklar und in der Eischale wurden erfasst. Insgesamt waren 384 Legehennen der Rasse Lohmann Brown Gegenstand dieser Studie. Im Alter von 19 Wochen wurden diese randomisiert einem 2×2×2 faktoriellen Arrangement zugeordnet. Die acht Versuchsfutter wurden während der einwöchigen Adaptionsperiode sowie der dreiwöchigen Versuchsdauer *ad libitum* verabreicht. Im Alter von 23 Wochen wurden die Hennen beprobt und der Darminhalt von Ileum und Rektum entnommen. Die scheinbare ileale Absorption von Magnesium, Zink, Kupfer und Eisen von Hennen der Gruppe W war höher verglichen zu Hennen der Gruppe H. Sowohl das absolute als auch das prozentuale Kutikulagewicht war geringer in Eiern von Hennen der Gruppe H verglichen zu Gruppe W. Weiterhin wiesen die Eier von Hennen der Gruppe H einen signifikant höheren Eigelbindex und eine gröβere absolute Eigelbhöhe auf als Eier von Hennen der Gruppe W. Hennen der Gruppe S bzw. C hatten eine erhöhte scheinbare ileale Absorption von Magnesium als Hennen der Gruppe E bzw. F. Die Hitzebehandlung des Futters ging bei Eiern der Hennen der Gruppe E mit einem signifikant höheren prozentualen Kutikulagewicht einher als bei Eiern der Gruppe S. Die scheinbare Nettoabsorption von Phosphor war höher und die von Eisen geringer bei Hennen der Gruppe F verglichen zu Hennen der Gruppe C. Verglichen mit Gruppe F konnte im Eidotter und Eiklar von Hennen der Gruppe C mehr Kupfer nachgewiesen werden. Verglichen mit der Gruppe F waren die Eischalendicke, das Eischalengewicht und die Höhe des Eiklar höher als in den Eiern der Gruppe C. Die Eischalendichte der Eier von Gruppe C war wiederum geringer als die der Gruppe F.

Zusammenfassend ergibt sich aus den Daten, dass sowohl bei Broilern als auch bei Legehennen der Einfluss der Futterbehandlung auf die ileale- und gesamte scheinbare Absorption von Mengen- und Spurenelementen vernachlässigbar ist. Die Retention der Mineralstoffe in Tibia, Leber und Eiprodukten sowie die Qualität der Tibiae und der Eier wurden nicht nachhaltig beeinflusst. Es kann daher vermutet werden, dass der Mineralstoffgehalt im Versuchsfutter ausreichend war, um eventuelle kleinere Beeinflussungen durch die Futterbehandlung auszugleichen. Der Effekt der Hitzebehandlung, des Zusatzes organischer Säuren, unterschiedlicher Mahlmethoden sowie der Partikelgröβe scheint daher von vernachlässigbarer Bedeutung zu sein unter den Aspekten einer optimalen Skelettmineralisation und Eischalenqualität.

10 REFERENCES

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. V. 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.

Al-Batshan, H. A., S. E. Scheideler, B. L. Black, J. D. Garlich, and K. E. Anderson. 1994. Duodenal calcium uptake, femur ash, and eggshell quality decline with age and increase following molt. Poult. Sci. 73:1590-1596.

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

Angel, R. 2007. Metabolic disorders: limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. J. Appl. Poult. Res. 16:138-149.

Aoyagi, S., and D. H. Baker. 1995. Iron requirement of chicks fed a semipurified diet based on casein and soy protein-concentrate. Poult. Sci. 74:412-415.

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

Atteh, J. O., and S. Leeson. 1983. Influence of increasing dietary calcium and magnesium levels on performance, mineral metabolism, and egg mineral content of laying hens. Poult. Sci. 62:1261-1268.

Bain, M. M. 2005. Recent advances in the assessment of eggshell quality and their future application. World's Poult. Sci. J. 61:268-277.

Balnave, D., and D. Zhang. 1993. Responses of Laying Hens on Saline Drinking-Water to Dietary Supplementation with Various Zinc-Compounds. Poult. Sci. 72:603-606.

Bao, Y. M., M. Choct, P. A. Iji, and K. Bruerton. 2007. Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. J. Appl. Poult. Res. 16:448-455.

Bao, Y. M., M. Choct, P. A. Iji, and K. Bruerton. 2010. Trace mineral interactions in broiler chicken diets. Br. Poult. Sci. 51:109-117.

Bar, A., D. Shinder, S. Yosefi, E. Vax, and I. Plavnik. 2003. Metabolism and requirements for calcium and phosphorus in the fast-growing chicken as affected by age. Br. J. Nutr. 89:51-60.

Barnet, E., and B. Nordin. 1960. The radiological diagnosis of osteoporosis: A new approach. Clin. Radiol. 11:166–169.

Baumgartner, S., D. J. Brown, E. Salevsky Jr, and R. M. Leach Jr. 1978. Copper deficiency in the laying hen. J. Nutr. 108:804-811.

Bayley, H. S., J. D. Summers, and S. J. Slinger. 1968. The effect of steam pelleting feed ingredients on chick performance: effect of phosphorus availability, metabolizable energy value and carcass composition. Poult. Sci. 47:1140-1148.

Berta, E., E. Andrasofszky, A. Bersenyi, R. Glavits, A. Gaspardy, and S. G. Fekete. 2004. Effect of inorganic and organic manganese supplementation on the performance and tissue manganese content of broiler chicks. Acta Vet. Hung. 52:199-209.

Bessei, W. 2006. Welfare of broilers: A review. Worlds Poult. Sci.J. 62:455-466.

Black, J. R., C. B. Ammerman, P. R. Henry, and R. D. Miles. 1985. Effect of dietary manganese and age on tissue trace mineral-composition of broiler-type chicks as a bioassay of manganese sources. Poult. Sci. 64:688-693.

Boivin, G., and P. J. Meunier. 2002. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. Calcif. Tissue Int. 70:503–511.

Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro, and C. Bravo. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol. 110:201-219.

Brickett, K. E., J. P. Dahiya, H. L. Classen, C. B. Annett, and S. Gomis. 2007. The impact of nutrient density, feed form, and photoperiod on the walking ability and skeletal quality of broiler chickens. Poult. Sci. 86:2117-2125.

Burnell, T. W., G. L. Cromwell, and T. S. Stahly. 1990. Effects of particle-size on the biological availability of calcium and phosphorus in defluorinated phosphate for chicks. Poult. Sci. 69:1110-1117.

Capdevielle, M. C., L. E. Hart, J. Goff, and C. G. Scanes. 1998. Aluminum and acid effects on calcium and phosphorus metabolism in young growing chickens (Gallus gallus domesticus) and mallard ducks (Anas platyrhynchos). Arch. Environ. Contam. Toxicol. 35:82-88.

Carter, T. C. 1975. Hens egg - estimation of shell superficial area and egg volume, using measurements of fresh egg weight and shell length and breadth alone or in combination. Br. Poult. Sci. 16:541-543.

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

Clunies, M., D. Parks, and S. Leeson. 1992. Calcium and phosphorus metabolism and eggshell thickness in laying hens producing thick or thin shells. Poult. Sci. 71:490-498.

Cook, M. E. 2000. Skeletal deformities and their causes: Introduction. Poult. Sci. 79:982–984.

Coto, C., F. Yan, S. Cerrate, Z. Wang, P. Sacakli, P. Waldroup, J. Halley, C. Wiernusz, and A. Martinez. 2008. Effects of dietary levels of calcium and nonphytate P in broiler starter diets on live performance, bone development, and growth plate conditions in male broilers fed a corn-based diet. Int. J. Poult. Sci. 7:638-645.

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. Proc. International Symposium on Salmonella, New Orleans, USA.

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.

De Witt, F. H., N. P. Kuleile, H. J. Van Der Merwe, and M. D. Fair. 2009. Effect of limestone particle size on egg production and eggshell quality of hens during late production. S. Afric. J. Anim. Sci. 39.

Deaton, J. W., B. D. Lott, and J. D. Simmons. 1989. Hammer mill versus roller mill grinding of corn for commercial egg layers. Poult. Sci. 68:1342-1344.

Deobald, H. J., and C. A. Elvehjem. 1935. The effect of feeding high amounts of soluble iron and aluminum salts. Am. J. Physiol. 111:118-123.

Dibner, J. 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.

Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J. Appl. Poult. Res. 16:126-137.

Doyle, M. E., and A. S. Mazzotta. 2000. Review of studies on the thermal resistance of salmonellae. J. Food Prot. 63:779-795.

Dudusola, I. O. 2009. Effects of storage methods and length of storage on some quality parameters of Japanese quail eggs. Tropicultura 27:45-48.

Durlach, J., A. Guiet-Bara, N. Pagès, P. Bac, and M. Bara. 2005. Magnesium chloride or magnesium sulfate: a genuine question. Magnesium Res. 18:187-192.

Ehtesham, A., and S. D. Chowdhury. 2002. Responses of laying chickens to diets formulated by following different feeding standards. Pak. J. Nutr. 1:127-131.

Essary, E. O., and C. E. Holmes. 1964. Influence of feeding vitamin C minerals + additives in layer rations on egg production feed conversion shell quality + Haugh Units. Poult. Sci. 43:1316.

Falandysz, J. 1991. Manganese, copper, zinc, iron, cadmium, mercury and lead in muscle meat, liver and kidneys of poultry, rabbit and sheep slaughtered in the northern part of Poland, 1987. Food Addit. Contam. 8:71-83.

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.

Fleming, R. H. 2008. Nutritional factors affecting poultry bone health. Proc. Nutr. Soc. 67:177-183.

Furuta, K., S. Morimoto, and S. Sato. 1980. Bacterial-Contamination in Feed Ingredients, Formulated Chicken Feed and Reduction of Viable Bacteria by Pelleting. Lab. Anim. 14:221-224.

Gajula, S. S., V. K. Chelasani, A. K. Panda, V. L. Mantena, and R. R. Savaram. 2011. Effect of supplemental inorganic Zn and Mn and their interactions on the performance of broiler chicken, mineral bioavailability, and immune response. Biol. Trace Elem. Res. 139:177-187.

GfE. 1999. (Gesellschaft fur Ernahrungsphysiologie). Empfehlungen zur Energie- und 485 Nahrstoffversorgung der Legehennen und Masthuhner Broiler. DLG-Verlag, Frankfurt (Main), Germany.

Goodarzi Boroojeni, F., A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, K. Männer, and J. Zentek. 2014a. The effects of different thermal treatments and organic acid levels in feed on microbial composition and activity in gastrointestinal tract of broilers. Poult. Sci. 93:1440-1452.

Goodarzi Boroojeni, F., A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, K. Männer, and J. Zentek. 2014b. The effects of different thermal treatments and organic acid levels on nutrient digestibility in broilers. Poult. Sci. 93:1159-1171.

Goodband, R. D., M. D. Tokach, and J. L. Nelssen. 2002. The effects of diet particle size on animal performance. Kansas State University. May, 2002.:6 pp.

Hamilton, R. M. G. 1994. Effect of flame roasting of dietary grains on the performance of leghorn hens. Can. J. Anim. Sci. 74:341-346.

Hamilton, R. M. G., and F. G. Proudfoot. 1995a. Effects of ingredient particle-size and feed form on the performance of leghorn hens. Can. J. Anim. Sci. 75:109-114.

Hamilton, R. M. G., and F. G. Proudfoot. 1995b. Ingredient particle-size and feed texture - effects on the performance of broiler-chickens. Anim. Feed Sci. Technol. 51:203-210.

Hein, G. E. 2006. Glycation endproducts in osteoporosis - Is there a pathophysiologic importance? Clin. Chim. Acta 371:32-36.

Hemme, A., M. Spark, P. Wolf, H. Paschertz, and J. Kamphues. 2004. Effects of different phosphorus sources in the diet on bone composition and stability (breaking strength) in broilers. J. Anim. Physiol. Anim. Nutr. (Berl). 89:129-133.

Henry, P. R., C. B. Ammerman, D. R. Campbell, and R. D. Miles. 1987. Effect of antibiotics on tissue trace mineral concentration and intestinal-tract weight of broiler chicks. Poult. Sci. 66:1014-1018.

Hudson, B. P., W. A. Dozier, J. L. Wilson, J. E. Sander, and T. L. Ward. 2004. Reproductive performance and immune status of caged broiler breeder hens provided diets supplemented with either inorganic or organic sources of zinc from hatching to 65 wk of age. J. Appl. Poult. Res. 13:349-359.

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

Hussein, S. M., and R. H. Harms. 1994. Effect of amino acid deficiencies on yolk: albumen ratio in hen eggs. J. Appl. Poult. Res. 3:362-366.

Iji, P. A., K. Khumalo, S. Slippers, and R. M. Gous. 2003. Intestinal function and body growth of broiler chickens on diets based on maize dried at different temperatures and supplemented with a microbial enzyme. Reprod. Nutr. Dev. 43:77-90.

Ishikawa, S., Y. Yano, K. Arihara, and M. Itoh. 2004. Egg yolk phosvitin inhibits hydroxyl radical formation from the fenton reaction. Biosci. Biotech. Biochem. 68:1324-1331.

Islam, K. M. S., H. Schaeublin, C. Wenk, M. Wanner, and A. Liesegang. 2012. Effect of dietary citric acid on the performance and mineral metabolism of broiler. J. Anim. Physiol. Anim. Nutr. (Berl). 96:808–817.

Islam, M. A., S. M. Bulbul, G. Seeland, and A. B. M. M. Islam. 2001. Egg quality of different chicken genotypes in summer and winter. Pak. J. Biol. Sci. 4:1411-1414.

Islam, M. S., and R. K. Dutta. 2010. Egg quality traits of indigenous, exotic and crossbred chickens (Gallus domesticus L.) in Rajshahi, Bangladesh. J. Life Earth Sci. 5:63-67.

Jensen, L. S., and D. V. Maurice. 1979. Influence of sulfur amino acids on copper toxicity in chicks. J. Nutr. 109:91-97.

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

Jones, F. T., R. C. Axtell, D. V. Rives, S. E. Scheideler, F. R. Tarver, R. L. Walker, and M. J. Wineland. 1991. A Survey of Salmonella Contamination in Modern Broiler Production. J. Food Prot. 54:502-507.

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

Kabir, M., R. O. Sulaiman, R. K. Idris, S. B. Abdu, O. M. Daudu, S. M. Yashim, M. R. Hassan, H. Y. Adamu, N. M. Eche, and T. S. Olugbemi. 2014. Effects of strain, age and the interrelationships between external and internal qualities of eggs in two strains of layer chickens in northern Guinea Savannah zone of Nigeria. Iran. J. Appl. Anim. Sci. 4:179-184.

Kasim, A. B., and H. M. Edwards. 2000. Effect of sources of maize and maize particle sizes on the utilization of phytate phosphorus in broiler chicks. Anim. Feed Sci. Technol. 86:15-26.

Kenney, M. A., and H. McCoy. 1997. Adding Zinc Reduces Bone Strength of Rats Fed a Low-Calcium Diet. Biol. Trace Elem. Res. 58.

Kilburn, J., and H. M. Edwards. 2004. The effect of particle size of commercial soybean meal on performance and nutrient utilization of broiler chicks. Poult. Sci. 83:428-432.

Kilburn, J., and H. M. Edwards, Jr. 2001. The response of broilers to the feeding of mash or pelleted diets containing maize of varying particle sizes. Br. Poult. Sci. 42:484-492.

Koch, K. 2002. Hammermills and rollermills. Kansas State University, May 2002.:8 pp.

Korsrud, G. O., J. B. Meldrum, C. D. Salisbury, B. J. Houlahan, P. W. Saschenbrecker, and F. Tittiger. 1985. Trace-element levels in liver and kidney from cattle, swine and poultry slaughtered in Canada. Can. J. Comp. Med. 49:159-163.

Larbier, M., and B. Leclercq. 1992. Nutrition and feeding of poultry. Nottingham University Press, Loughborough, Leicestershire, United Kingdom.

Lee, S., and W. M. Britton. 1980. Magnesium toxicity - effect on phosphorus utilization by broiler chicks. Poult. Sci. 59:1989-1994.

Lee, S. R., W. M. Britton, and G. N. Rowland. 1980. Magnesium toxicity - bone-lesions. Poult. Sci. 59:2403-2411.

Leeson, S., and J. D. Summers. 2001. Nutrition of the chicken. 4th ed. University Books, Guelph, Ontario, Canada.

Lichovnikova, M. 2007. The effect of dietary calcium source, concentration and particle size on calcium retention, eggshell quality and overall calcium requirement in laying hens. Br. Poult. Sci. 48:71-75.

Liem, A., G. M. Pesti, and H. M. Edwards. 2008. The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. Poult. Sci. 87:689-693.

Lilburn, M. S. 1994. Skeletal growth of commercial poultry species. Poult. Sci. 73:897-903.

Liu, T. S., G. H. Snoeyenb, and V. L. Carlson. 1969. Thermal resistance of salmonella senftenberg 775w in dry animal feeds. Avian Dis. 13:611-631.

Lukić, M., Z. Pavlovski, and Z. Škrbić. 2009. Mineral nutrition of modern poultry genotypes. Biotechnology in Animal Husbandry 25:399-409.

Mabe, I., C. Rapp, M. M. Bain, and Y. Nys. 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poult. Sci. 82:1903-1913.

Martin, H., and P. Maris. 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.

Matlho, G., S. Himathongkham, H. Riemann, and P. Kass. 1997. Destruction of Salmonella enteritidis in poultry feed by combination of heat and propionic acid. Avian Dis. 41:58-61.

Mauron, J. 1981. The Maillard reaction in food; a critical review from the nutritional standpoint. Prog. Food Nutr. Sci. 5:5-35.

McDowell, L. R. 2003. Minerals in animal and human nutrition. Elsevier Science B.V., Amsterdam, Netherlands.

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

Mossel, D. A. A., M. Vanschot, and E. H. Kampelma. 1967b. Comparative study on decontamination of mixed feeds by radicidation and by pelletisation. J. Sci. Food Agric. 18:362-367.

Mutus, R., N. Kocabagli, M. Alp, N. Acar, M. Eren, and S. S. Gezen. 2006. The effect of dietary probiotic supplementation on tibial bone characteristics and strength in broilers. Poult. Sci. 85:1621-1625.

Nestor, K. E., W. L. Bacon, Y. M. Saif, and P. A. Renner. 1985. The influence of genetic increases in shank width on body-weight, walking ability, and reproduction of turkeys. Poult. Sci. 64:2248-2255.

Nir, I., R. Hillel, I. Ptichi, and G. Shefet. 1995. Effect of particle-size on performance .3. grinding pelleting interactions. Poult. Sci. 74:771-783.

Nir, I., J. P. Melcion, and M. Picard. 1990. Effect of particle-size of sorghum grains on feed-intake and performance of young broilers. Poult. Sci. 69:2177-2184.

NRC. 1994. Nutrient Requirements of Poultry. 9th revised ed. National Academy Press, Washington, D.C.

Oana-Mărgărita, G., Ţ. Cristina, R. George-Bogdănel, M. Elena, B. Florica, and M. Manuella. 2012. Assesement of mineral nutrients, heavy metals and pesticides in poultry liver using inductively coupled plasma-mass spectrometry and gas chromatography-mass spectrometry. Scientific Works. C Series. Veterinary Medicine LVIII:174-183.

Ochrimenko, C., A. Lemser, G. Richter, U. Krause, and H. Bonsak. 1992. Effect of the manganese content in laying hen feed with different Ca and mineral levels on the egg shell quality and bone mineralization of hens. Arch Tierernahr. 42:25-35.

Oliveira, J. E., L. F. T. Albino, H. S. Rostagno, L. E. Páez, and D. C. O. Carvalho. 2005. Dietary levels of potassium for broiler chickens. Braz. J. Poult. Sci. 7:33-37.

Onyango, E. M., P. Y. Hester, R. Stroshine, and O. Adeola. 2003. Bone densitometry as an indicator of percentage tibia ash in broiler chicks fed varying dietary calcium and phosphorus levels. Poult. Sci. 82:1787–1791.

Osphal, W., H. Zeronian, M. Ellison, D. Lewis, R. S. Rucker, and R. Riggins. 1982. Role of copper in collagen-linking and its influence on selected mechanical properties of chick bone and tendon. J. Nutr. 12:708–716.

P´erez-Bonilla, A., M. Frikha, R. P. L´azaro, and G. G. Mateos. 2014. Type of grinding of the main cereal of the diet affects production of brown egg-laying hens. Anim. Feed Sci. Technol. 194:121-130.

Paganelli, C. V., A. Olszowka, and A. Ar. 1974. Avian egg - surface-area, volume, and density. Condor 76:319-325.

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

Parmar, S. N. S., M. S. Thakur, S. S. Tomar, and P. V. A. Pillai. 2006. Evaluation of egg quality traits in indigenous Kadaknath breed of poultry. Livest. Res. Rural Dev. 18:18-25.

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

Phirinyane, T. B., J. C. Moreki, H. J. van der Merwe, and J. P. Hayes. 2011. Effect of dietary different ratios of coarse and fine limestone particles on egg production and eggshell quality of laying hens at peak production. Res. Opin. Anim. Vet. Sci. 1:334-338.

Portella, F. J., L. J. Caston, and S. Leeson. 1988. Apparent feed particle-size preference by laying hens. Can. J. Anim. Sci. 68:915-922.

Proudfoot, F. G., and H. W. Hulan. 1989. Feed texture effects on the performance of roaster chickens. Can. J. Anim. Sci. 69:801-807.

Rafacz-Livingston, K. A., C. Martinez-Amezcua, C. M. Parsons, D. H. Baker, and J. Snow. 2005. Citric acid improves phytate phosphorus utilization in crossbred and commercial broiler chicks. Poult. Sci. 84:1370-1375.

Rao, S. V. R., M. V. L. N. Raju, M. R. Reddy, and P. Pavani. 2006. Interaction between dietary calcium and non-phytate phosphorus levels on growth, bone mineralization and mineral excretion in commercial broilers. Anim. Feed Sci. Technol. 131:133-148.

Rath, N. C., J. M. Balog, W. E. Huff, G. R. Huff, G. B. Kulkarni, and J. F. Tierce. 1999. Comparative differences in the composition and biomechanical properties of tibiae of seven- and seventy-two-week-old male and female broiler breeder chickens. Poult. Sci. 78:1232-1239.

Rath, N. C., G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Factors regulating bone maturity and strength in poultry. Poult. Sci. 79:1024-1032.

Ravindran, V. B. W. K. E., W. L. Bryden, and E. T. Kornegay. 1995. Phytates: occurrence, bioavailability and implications in poultry nutrition. Avian Poult. Biol. Rev.

Reece, F. N., B. D. Lott, and 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.

Reisenfeld, A. 1972. Metatarsal robusticity in bipedal rats. Am.J. Phys. Anthropol. 40:229-234.

Reiter, K., and W. Bessei. 1998. Effect of locomotor activity on bone development and leg disorders in broilers. Arch. Geflugelkd. 62:247-253.

Ribeiro, A. M. L., A. M. Kessler, T. H. Viola, I. C. M. Silva, L. Rubin, M. Raber, C. Pinheiro, and L. F. Lecznieski. 2008. Nutritional interaction of methionine sources and sodium and potassium levels on broiler performance under Brazilian summer conditions. J. Appl. Poult. Res. 17:69-78.

Ricke, S. C. 2005. Food safety control in the poultry industry. Woodhead Publishing in Food Science and Technology, Mead GC (Ed.). Cambridge, United Kingdom.

Rodehutscord, M., A. Dieckmann, M. Witzig, and Y. Shastak. 2012. A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies. Poult. Sci. 91:965-971.

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. Poult. Sci. 93:2060-2068.

Roncero-Ramos, I., C. Delgado-Andrade, A. Haro, B. Ruiz-Roca, F. J. Morales, and M. P. Navarro. 2013. Effects of dietary bread crust Maillard reaction products on calcium and bone metabolism in rats. Amino Acids 44:1409-1418.

Ruhnke, I., I. Röhe, C. Krämer, F. Goodarzi Boroojeni, F. Knorr, A. Mader, E. Schulze, A. Hafeez, K. Neumann, R. Löwe, and J. Zentek. 2015. The effects of particle sizes, milling methods, and thermal treatment of feed on performance, apparent ileal digestibility and pH of the digesta in laying hens. Poult. Sci. 94:692-699.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 43:598-606.

Safaa, H. M., E. Jiménez-Moreno, D. G. Valencia, M. Frikha, M. P. Serrano, and G. G. Mateos. 2009. Effect of main cereal of the diet and particle size of the cereal on productive performance and egg quality of brown egg-laying hens in early phase of production. Poult. Sci. 88:608-614.

Safamehr, A., S. Hedatyati, and M. H. Shahir. 2013. The effects of dietary calcium sources and vitamin D_3 on egg quality and performance in laying hens. Iran. J. Appl. Anim. Sci. 3:167-175.

Saki, A. A., P. Zamani, M. Rahmati, and H. Mahmoudi. 2012. The effect of cage density on laying hen performance, egg quality, and excreta minerals. J. Appl. Poult. Res. 21:467-475.

Saldanha, E. S. P. B., E. A. Garcia, C. C. Pizzolante, H. C. Goncalves, A. B. G. Faittarone, A. S. Sechinato, and C. Lagana. 2010. Effects of dietary trace mineral sources and levels fed to layers in their second laying cycle on the quality of eggs stored at different temperatures and for different periods. Braz. J. Poult. Sci. 12:223-231.

Schiffman, H. R. 1968. Texture preference in domestic chick. J. Comp. Physiol. Psych. 66:540-541.

Scott, M. L., M. C. Nesheim, and R. J. Young. 1982. Nutrition of the chicken. 3rd ed. M. L. Scott and Associates, Ithaca, New York.

Seedor, J. G., H. A. Quartuccio, and D. D. Thompson. 1991. The bisphosphonate alendronate (Mk-217) inhibits bone loss due to ovariectomy in rats. J. Bone Miner. Res. 6:339-346.

Selle, P. H., V. Ravindran, and G. G. Partridge. 2009. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention and growth performance in wheat-based broiler diets. Anim. Feed Sci. Technol. 153:303-313.

Shim, M. Y., A. B. Karnuah, A. D. Mitchell, N. B. Anthony, G. M. Pesti, and S. E. Aggrey. 2012. The effects of growth rate on leg morphology and tibia breaking strength, mineral density, mineral content, and bone ash in broilers. Poult. Sci. 91:1790–1795.

Shim, M. Y., E. Song, L. Billard, S. E. Aggrey, G. M. Pesti, and P. Sodsee. 2013. Effects of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. Poult. Sci. 92:2687-2696.

Silversides, F. G., and K. L. Budgell. 2004. The relationships among measures of albumen height, pH, and whipping volume. Poult. Sci. 83:1619-1623.

Skrivan, M., V. Skrivanova, and M. Marounek. 2005. Effects of dietary zinc, iron, and copper in layer feed on distribution of these elements in eggs, liver, excreta, soil, and herbage. Poult. Sci. 84:1570-1575.

Stadelman, W. J., and O. J. Cotterill. 1995. Quality identification of shell eggs. in Egg Science and Technology. 4th ed. The Haworth Press, Binghamton, New York 13904-1580, USA.

Starcher, B. C., C. H. Hill, and J. G. Madaras. 1980. Effect of zinc-deficiency on bone collagenase and collagen turnover. J. Nutr. 110:2095-2102.

Stevens, L. 1995. Egg proteins: what are their functions? Sci. Prog. 79:65-87.

Strause, L., and P. Saltman. 1987. Role of Manganese in Bone Metabolism. ACS Symp. Ser. 354:46-55.

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

Tang, P., P. H. Patterson, and V. M. Puri. 2006. Effect of feed segregation on the commercial hen and egg quality. J. Appl. Poult. Res. 15:564-573.

Thompson, A. S., and C. W. Weber. 1981. Effect of dietry fiber sources on tissue mineral level in chicks. Poult. Sci. 60:840-845.

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.

Uni, Z., L. Yadgary, and R. Yair. 2012. Nutritional limitations during poultry embryonic development. J. Appl. Poult. Res. 21:175-184.

van Boekel, M., V. Fogliano, N. Pellegrini, C. Stanton, G. Scholz, S. Lalljie, V. Somoza, D. Knorr, P. R. Jasti, and G. Eisenbrand. 2010. A review on the beneficial aspects of food processing. Mol. Nutr. Food Res. 54:1215–1247.

Van Immerseel, F., L. De Zutter, K. Houf, F. Pasmans, F. Haesebrouck, and R. Ducatelle. 2009. Strategies to control Salmonella in the broiler production chain. Worlds Poult. Sci. J. 65:367–391.

Van Immerseel, F., J. Russell, M. Flythe, I. Gantois, L. Timbermont, F. Pasmans, F. Haesebrouck, 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.

Van Rooijen, C., G. Bosch, A. F. B. Van der Poel, P. A. Wierenga, L. Alexander, and W. H. Hendriks. 2013. The Maillard reaction and pet food processing: effects on nutritive value and pet health. Nutr. Res. Rev. 26:130-148.

Van Schothorst, M., and A. W. M. Brooymans. 1982. Handbook of nutritive value of processed food. Animal Feedstuffs. Miloslav Rechcigl Jr., ed., CRC Press, Boca Raton, FL.

Veldman, A., H. A. Vahl, G. J. Borggreve, and D. C. Fuller. 1995. A Survey of the Incidence of Salmonella Species and Enterobacteriaceae in Poultry Feeds and Feed Components. Vet. Rec. 136:169-172.

Viguet-Carrin, S., P. Garnero, and P. D. Delmas. 2006. The role of collagen in bone strength. Osteoporos. Int. 17:319-336.

Waldroup, P. W. 1997. Particle size reduction of cereal grains and its significance in poultry nutrition. Pages 14 pp. in ASA Technical Bulletin Vol. PO34. American Soybean Association, Singapore, Singapore.

Wang, H., C. Zhang, Y. Mi, and M. T. Kidd. 2014. Copper and lysine amino acid density responses in commercial broilers. J. Appl. Poult. Res. 23:470-477.

Watts, D. L. 1990. Nutrient Interrelationships Minerals—Vitamins—Endocrines. J. Ortho. Mol. Med. 5:11-19.

Wilson, S., and S. R. I. Duff. 1991. Effects of vitamin or mineral deficiency on the morphology of medullary bone in laying hens. Res. Vet. Sci. 50:216-221.

Yang, P. Y., and D. J. Klimis-Tavantzis. 1998. Effects of dietary manganese on arterial Glycosaminoglycan metabolism in Sprague-Dawley rats. Biol. Trace Elem. Res. 64:275-288.

Youssef, I. M. I., A. Beineke, K. Rohn, and J. Kamphues. 2011. Effects of high dietary levels of soybean meal and its constituents (potassium, oligosaccharides) on foot pad dermatitis in growing turkeys housed on dry and wet litter. Arch. Anim. Nutr. 65:148-162.

Zamani, A., H. R. Rahmani, and J. Pourreza. 2005. Effect of different levels of manganese and zinc on performance traits and breaking eggs in laying hens. Pak. J. Biol. Sci. 8:1035-1040.

Publications List

- **Hafeez**, **A.,** A. Mader, F. Goodarzi Boroojeni, I. Ruhnke, I. Röhe, K. Männer, and J. Zentek. 2014. Impact of thermal and organic acid treatment of feed on apparent ileal mineral absorption, tibial and liver mineral concentration, and tibia quality in broilers. Poult. Sci. 93:1754–1763.

- **Hafeez**, **A.,** A. Mader, I. Ruhnke, I, Röhe, F. Goodarzi Boroojeni, M. S. Yousaf, K. Männer, and J. Zentek. 2015. Implication of milling methods, thermal treatment, and particle size of feed in layers on mineral digestibility and retention of minerals in egg contents. Poult. Sci. 94: 240-248.

- **Hafeez**, **A.,** A. Mader, I. Ruhnke, I, Röhe, F. Goodarzi Boroojeni, M. S. Yousaf, K. Männer, and J. Zentek. 2015. The effect of milling method, thermal treatment, and particle size of feed on exterior and interior egg quality in laying hens. Europ. Poult. Sci. 79. 2015, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart.
  doi : 10.1399/eps.2015.76.

- Goodarzi Boroojeni, F., W. Vahjen, A. Mader, F. Knorr, I. Ruhnke, I. Röhe, **A. Hafeez**, K. Männer, and J. Zentek. 2014. The effects of different thermal treatments and organic acid levels on nutrient digestibility in broilers. Poult. Sci. 93:1159–1171.

- 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 acid levels in feed on microbial composition and activity in gastrointestinal tract of broilers. Poult. Sci. 93:1440–1452.

- Ruhnke, I., I. Röhe, F. Goodarzi Boroojeni, F. Knorr, A. Mader, **A. Hafeez**, and J. Zentek. 2015. Feed supplemented with organic acids does not affect starch digestibility, nor intestinal absorptive or secretory function in broiler chickens. J. Anim. Physiol. Anim. Nutr. 99 (Suppl.1):29-35

- Ruhnke, I., I Röhe, C. Krämer, F.Goodarzi Boroojeni, F. Knorr, A. Mader, E. Schulze, **A. Hafeez**, R. Löwe and J. Zentek. 2015. Effect of feed particle size, milling method, and heat treatment on performance, apparent ileal digestibility and pH of the digesta in laying hens. Poult. Sci. 94:692-699.

Abstracts in proceedings & participation in conferences

- **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.

- **Hafeez**, **A.,** A. Mader, I. Ruhnke, I. Röhe, F. Goodarzi Boroojeni, E. Schulze, K. Männer and J. Zentek. 2014. The effect of feed particle size, milling method, and thermal treatment, on interior and exterior egg quality in laying hens. 18th Congress of the European Society of Veterinary and Comparative Nutrition. Utrecht, The Netherlands.

- **Hafeez**, **A.,** A. Mader, I. Röhe, I. Ruhnke, F. Goodarzi Boroojeni, M. S. Yousaf, K. Männer and J. Zentek. 2014. The impact of milling methods and thermal treatment of feed on apparent ileal absorption, apparent total digestibility and retention of minerals in egg contents in laying hens. 30th Annual Meeting of Society of Minerals and trace elements, 2014- Interactions of trace elements / studies in animal models. (30. Jahrestagung der GMS, 2014 Gesellschaft für Mineralstoffe und Spurenelemente e.V- Interaktionen von Spurenelementen / Untersuchungen im Tiermodell.). Freising-Weihenstephan, Germany.

- **Hafeez**, **A.,** A. Mader, F. Goodarzi Boroojeni, I. Ruhnke, I. Röhe, K. Männer, and J. Zentek. 2014. Impact of thermal and organic acid treatment of feed on apparent ileal mineral absorption and tibial and liver mineral concentration in broilers. SFB Workshop 2014, Microbial Hazards in the Feed Chain. Berlin, Germany.

- **Hafeez, A**., A. Mader, I. Ruhnke, I. Röhe, F. Goodarzi Boroojeni, M. S. Yousaf, K. Männer, and J. Zentek. 2015. Effect of milling methods, thermal treatment, and particle size of feed in layers on prececal and total tract digestibility as well as on trace element content of eggs. 69th conference of the Society of Nutrition Physiology (69. Tagung der Gesellschaft für Ernährungsphysiologie). Göttingen. Germany.

- 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.

- 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.

- 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. 18th Congress of the European Society of Veterinary and Comparative Nutrition. Utrecht, The Netherlands.

- Ruhnke, I., I. Röhe, F. Goodarzi Boroojeni, F. Knorr, A. Mader, **A. Hafeez** and J. Zentek. 2014. Bacterial metabolites in the crop, ileum and caecum depend on milling method, thermal treatment and particle size of feed in laying hens. 18th Congress of the European Society of Veterinary and Comparative Nutrition. Utrecht, The Netherlands.

- 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 nutrient digestibility and gut microbiota in broilers. SFB Workshop 2014, Microbial Hazards in the Feed Chain. Berlin, Germany.

- 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 acid levels in feed on bacterial composition and activity in gastrointestinal tract of broilers. XVI International Symposium Feed Technology. Novi Sad, Serbia.

- Goodarzi Boroojeni, F., Vahjen, W., Mader, A., Knorr, F., Ruhnke, I., Röhe, I., **Hafeez, A**., Villodre, C., Männer, K. und Zentek, J. 2014. The effect of different thermal treatments and organic acid levels on nutrient digestibility in broilers. XVI International Symposium Feed Technology. Novi Sad, Serbia.

- Mader, A., Goodarzi Boroojeni, F., Knorr, F., Ruhnke, I., Röhe, I., **Hafeez, A**., Männer, K., Vahjen, W. und Zentek, J. 2014. Ernährungsphysiologische Untersuchungen von dekontaminierten Futtermitteln, 14. Fachtagung Fleisch- und Geflügelhygiene, Berlin, Germany.

Acknowledgements

I have no words for the true reflection of my feelings of gratitude for untiring efforts, keen interest and continued support of Prof. Dr. Jürgen Zentek throughout my PhD studies. I always found him a nice, noble and humble character adorned with enormous patience and sympathetic approach. I could never have achieved this milestone without his guidance, support and encouragement. His belief in me has been the source of inspiration which stirred up my thirst for knowledge and excellence and enabled me to complete my task in a graceful way. His in depth knowledge, fully developed skills and well balanced approach, and curiosity and inquisitiveness towards scientific matters make him a role model for me. I can proudly say that I am lucky to have such a nice, visionary, learned, supportive, encouraging, humble and sympathetic person as my PhD supervisor. I don't know about others, but if I am given a chance to choose my advisor a hundred times, I will have only one choice, Prof. Zentek, an epitome of goodness…

For his continued support and guidance during my stay at the institute, I am very thankful to Prof. Dr. Klaus Männer. I learnt a lot from him especially for his excellent skills in management of animal trials and thorough knowledge regarding poultry nutrition.

I am indebted to thank and appreciate the support and efforts of Dr. Wilfried Vahjen. I learnt a lot of laboratory procedures and data analysis techniques from him, which would guide me like a lighthouse throughout my career. I acknowledge his patience, sincerity, wisdom and scientific approach coupled with selfless attitude. He provided me with valuable suggestions and reviewed the manuscripts critically, which enabled me to produce research papers with high scientific standards.

I am wholeheartedly grateful to considerable contribution of Dr. Anneluise Mader during my studies. Her valuable inputs, fruitful discussions and worthy guidance was the driving force to achieve my goal in a better way. I learnt innumerable things from her and admire her scientific, professional and personal support. Her expertise and understanding was adorable and I express my respect and sincere gratitude for her continued support.

This study would have not accomplished without help, guidance and encouragement of Isabelle Ruhnke. I am much thankful for her support and sympathetic approach regarding profound personal matters. Special thanks are extended to Farshad Goodarzi Boroojeni, Ping Liu, Heike Vodde, Erin Schulze, Ilen Röhe, Fanny Knorr, Muhammad Shahbaz Yousaf and Ibrahim Youssef for their support, encouragement and motivation. I am grateful to Marita Eitinger, Luisa Eberbach, Katharina Topp, Annet Kriesten, Petra Huck, Kristie Fiedler, Ines Bebert, Rainer Jokiel, A. Kannegießer, I. Walther, and M. Modrow for their help, and technical support in laboratory analysis and animal trials. It would be a sheer injustice not to thank Higher Education Commission of Pakistan (HEC) for funding my PhD studies in Germany. Thanks are also due to Deutscher Akademischer Austaush Dienst (DAAD) for constant help and guidance. Special thanks to BMBF (Bundesministerium für Bildung und Forschung, Berlin, Germany, Research grant 13N11202 – SileBAT project) and AIF (Allianz Industrie Forschung, project number 16800 N) for financing animal experiments.

Last but not the least, I would have not done anything without consistent help, motivation, love and prayers of my loving parents, brother and sisters. The love, care, sacrifice, trust and encouragement of my loving wife did not let me distract from my goal. I could not hug my cute children for long, but I hope that accomplishment of this milestone will make them proud and pay them off for their whole life. I breathe for them and for me, success means nothing without them. Thanks to all of my family members and friends for being there and making my life so special.

Declaration

I hereby declare that this dissertation is my own work and has not previously been submitted anywhere for any award. Where other sources of information have been used they have been acknowledged.

Berlin, 10.02.2015 Abdul Hafeez