

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

**Studies on the Impact of Acidified Feed and Feed Processing on
Digestive Physiology in Broiler Chickens and Laying Hens**

Inaugural-Dissertation
zur Erlangung des akademischen Doktorgrades
philosophiae doctor (Ph.D.)
in "Biomedical Science"
an der Freien Universität Berlin

vorgelegt von
Dr. Isabelle Ruhnke, geb. Stahl
Tierärztin aus Siegburg

Berlin 2014

Journal Nr.: 3699

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: Univ.-Prof. Dr. Jörg Aschenbach

Deskriptoren (nach CAB-Thesaurus):

acids, digestive tract, feeding, fowls, hens, nutrition, physiology

Tag der Promotion: 30.04.2014

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: 978-3-86387-478-0

Zugl.: Berlin, Freie Univ., Diss., 2014

Dissertation, Freie Universität Berlin

D 188

Dieses Werk ist urheberrechtlich geschützt.

Alle Rechte, auch die der Übersetzung, des Nachdruckes und der Vervielfältigung des Buches, oder Teilen daraus, vorbehalten. Kein Teil des Werkes darf ohne schriftliche Genehmigung des Verlages in irgendeiner Form reproduziert oder unter Verwendung elektronischer Systeme verarbeitet, vervielfältigt oder verbreitet werden.

Die Wiedergabe von Gebrauchsnamen, Warenbezeichnungen, usw. in diesem Werk berechtigt auch ohne besondere Kennzeichnung nicht zu der Annahme, dass solche Namen im Sinne der Warenzeichen- und Markenschutz-Gesetzgebung als frei zu betrachten wären und daher von jedermann benutzt werden dürfen.

This document is protected by copyright law.

No part of this document may be reproduced in any form by any means without prior written authorization of the publisher.

Alle Rechte vorbehalten | all rights reserved

© Mensch und Buch Verlag 2014

Choriner Str. 85 - 10119 Berlin

verlag@menschundbuch.de – www.menschundbuch.de

Dedicated to Jonathan, Philippe, and Marlene

In beloved memory of

Oma Leni

Table of Contents

List of Abbreviations	7
1. Introduction	9
1.1. Aim of the Study and Objectives	10
2. Literature Review	11
2.1. The Impact of Feed on Digestive Physiology in Poultry	11
2.2. The Characterization of Feed Particle Size	12
2.3. Grinding of Feed	15
2.3.1. Roller Mill	15
2.3.2. Hammer Mill	17
2.3.3. Disc Mill	18
2.3.4. Wedge-Shaped Disc Mill	19
2.4. Thermal Treatment of Feed	20
2.4.1. Pelleting	20
2.4.2. Expanding	20
2.4.3. Extruding	20
2.5. Effect of Particle Size and Thermal Treatment of Feed on Poultry	21
2.6. Use of Organic Acids in Poultry Nutrition	22
2.7. Characterizing Intestinal Nutrient Transport by the Ussing Chamber	23
3. Part I: Method for the Preparation of Mucosal Flaps from the Jejunum of Laying Hens for Transporter Studies in Ussing Chambers	25
3.1. Materials and Methods	25
3.1.1. Study Population	25
3.1.2. Sampling and Mounting Jejunal Mucosa into the Net-Supported Ussing Chamber	25
3.1.3. Electrical Measurements	27
3.1.4. Experimental Design	27
3.1.5. Histologic Evaluation	27
3.1.6. Data Analysis	27
3.2. Results	28
3.2.1. Electrical Measurements	28
3.2.2. Absorptive and Secretory Function	28
3.2.3. Histologic Evaluation	28
4. Part II: The Impact of Organic Acid in Feed on Starch Digestibility, Jejunal Conductance, Glucose Transporter Expression, as well as Glucose and Chloride Transporter Function in Broiler Chicken	30
4.1. Materials and Methods	30
4.1.1. Birds and Housing	30
4.1.2. Sampling and Analysis	32
4.1.2.1. Evaluation of Apparent Ileal Starch Digestibility	32
4.1.2.2. Expression of Glucose Transporters SGLT-1 and GLUT-2 using qPCR	32
4.1.2.3. Ex Vivo Measurements in Ussing Chambers	33

4.1.2.4. Data Analysis	34
4.2. Results	34
5. Part III: Effect of Feed Particle Size, Milling Method, and Heat Treatment n Performance, Apparent Ileal Digestibility and pH of the Digesta in Laying Hens.....	35
5.1. Materials and Methods.....	35
5.1.1. Experimental Diets	35
5.1.2. Birds and Housing.....	38
5.1.3. Sampling and Analysis.....	38
5.1.3.1. Diets	38
5.1.3.2. Performance Parameters	38
5.1.3.3. Apparent Ileal Nutrient Digestibility	38
5.1.3.4. pH Measurements.....	39
5.1.3.5. Data Analysis	39
5.2. Results	39
6. Discussion	47
6.1. Assessment of the Ussing Chamber Method (Part I).....	47
6.2. Evaluating the Impact of Acidified Feed on Intestinal Physiology of Broiler Chickens (Part II)	48
6.3. Investigating the Impact of Feed Processing on Intestinal Physiology of Laying Hens (Part III)	50
6.4. Conclusion	53
7. Summary.....	54
8. Zusammenfassung	56
9. References	58
10. Appendix.....	72
10.1. Legend of Figures	72
10.1. Legend of Tables	72
11. List of Publications	73
12. Acknowledgements	75
13. Selbstständigkeitserklärung	77

List of Abbreviations

AA	Amino acid
A_T^2	Annealing temperature
ATPase	Adenosine tri phosphatase
cDNA	Complementary deoxyribonucleic acid
cMEAN	Continuous mean particle size
cMED	Continuous median particle size
CP	Crude protein
cT	Cycle threshold
DM	Dry matter
dMEAN	Discrete mean particle size
dMED	Discrete median particle size
DNA	Deoxyribonucleic acid
EE	Ether extract
G	Gravitational acceleration
g	Gram
GADPH	Glyceraldehyde 3-phosphate dehydrogenase
GLUT	Glucose transporter
GMD	Geometric mean diameter
G_t	Transepithelial conductance
I	Current
I_{sc}	Short circuit current
IU	International unit
kWh/t	Kilowatt hours/ton
l	Liter
LaGeSo	Landesamt für Gesundheit und Soziales (State Office of Health and Social Affairs, Berlin)
M	Mol
MOF	Module of fineness
mosmol	Milliosmol
mPa	Millipascal
mRNA	Messenger RNA
mS	Millisiemens
n	Number
OS	Organic substance
p	P-value
PCR	Polymerase chain reaction
pH	Decimal logarithm of the reciprocal hydrogen ion activity in solution
qPCR	Quantitative polymerase chain reaction
R	Resistance
Reg no	Registration number
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SD	Standard deviation
SEM	Standard error of the mean
SGLT-1	Sodium-linked glucose transporter 1

1. Introduction

The digestive tract of poultry is, compared to mammals, relatively short. The duration time of feed passing the intestinal tract takes approximately four to six hours, while the duration of feed digestion in mammals requires at least twelve hours (Larbier and Leclercq, 1994; Hetland and Svihus, 2001; Engelhardt and Breves, 2009). The fast passage of feed through the gastrointestinal tract demands for very efficient nutrient digestion and absorption. For example, the apparent ileal digestibility of starch varies between 93 and 99% (Weurding et al., 2001; Lee et al., 2011). The physiology of this highly efficient digestive system is challenged in today's animal production systems. Broiler chickens can be raised within 32 days in order to produce meat for human consumption. Therefore, the immature intestinal tract of young chickens is forced to digest high amounts of nutrients within a limited period of time. The fragility of this highly challenged intestinal system is of practical relevance to the livestock producers. Infections with intestinal bacterial pathogens result frequently in animal losses and require the use of antibiotics. Therefore, modification of feed is commonly used to support the health status of the flock. For example, compacting and thermal treatment of feed (e.g. pelleting) is a common method to reduce bacterial load of the feed and to maintain its gut health. Another approach is the use of organic acids as feed additives. Acidification of feed is commonly used in the feed industry, as it is known to preserve feed but also to improve weight gain, as well as the feed conversion ratio (Denli et al., 2003; Hernandez et al., 2006; Paul et al., 2007). However, the cause for those beneficial effects and its impact on the intestinal physiology of the chickens remains unknown.

The use of the laying hen starts at the age of 18 weeks and lasts until the age of approximately 68 weeks. Laying hens are raised slower, resulting in an average body weight of approximately 1800 g when starting the laying period (depending on the genetic line of the bird). The structure of the feed (particle size, particle size distribution as influenced by the milling method and the use of thermal treatment) is known to affect the performance, the gastrointestinal organ weight, the pancreatic enzyme activity, and the microbial content of broiler chickens. The impact of feed structure on the digestive physiology of laying hens has not been extensively studied to date.

Since cereals are the predominant energy source for chickens, starch digestibility and glucose transport is of highest impact to the chicken digestive system. The glucose uptake in the small intestine is predominantly transporter mediated (Kellett 2001). While up to 13 glucose transporters (GLUT) and 3 electrogenic sodium-glucose co-transporter (SGLT) could be isolated in various organs of mammals, the glucose uptake in the intestine of the avian species is primarily explained by SGLT-1 and GLUT-2 transporters (Takata, 1996; Thorens and Joost, 2001; Humphrey et al., 2004; Drozdowski and Thompson, 2006).

A high amylase activity and a large resorptive surface of the small intestine are held responsible for the efficient starch digestibility (Moran, 1985).

The Ussing chamber method has been proven to be a valuable method for the evaluation of intestinal ion transport. While this method is well established for the investigation of ruminants and monogastric mammals, the intestinal tract in chickens has only rarely been assessed (Amat et al., 1999; Li et al., 2004; Awad et al., 2007; Clarke, 2009; Collins et al., 2007; Lennernas, 2007; Neirinckx et al., 2011).

1.1. Aim of the Study and Objectives

Feed has a major impact on the digestive physiology of chickens. In practice, acidification of feed, as well as particle size, particle size distribution and the milling method are of relevance in poultry nutrition. However, the specific impact of these parameters on the physiology of the intestinal tract of chicken has not been widely studied.

Therefore, the aim of this study was:

- (I) to modify the Ussing chamber method for reliable assessment of transport physiology of the intestinal mucosa of poultry
- (II) to evaluate the impact of acidified feed on apparent ileal digestibility of starch, the glucose transporter expression, and physiologic intestinal transport function in broiler chickens
- (III) to investigate the impact of the milling method, thermal treatment, and particle size of feed on performance, apparent ileal digestibility, and pH of digesta in laying hens.

2. Literature Review

2.1. The Impact of Feed on Digestive Physiology in Poultry

The major function of the gastrointestinal tract is the interplay of digestion and absorption of nutrients, enabled by mechanical, chemical, and enzymatic dissociation and subsequent nutrient uptake. The continuous and direct contact of the digestive tract with feed and the environment highlights the function of the gastrointestinal tract as barrier against external influences (Yegani and Korver, 2008; Choct, 2009). Feed modulates the intestinal microbiota and subsequently the immune system of the host, it determines the development of the villi and epithelial surfaces, subsequently its gene expression as well as its endocrine regulation (Kraehenbuhl & Neutra, 1992; Dahlke et al., 2003; Williams et al., 2008; Amerah et al., 2009; Betscher et al., 2010). Subsequently, factors affecting the health status of the digestive system will affect animal health. The feed ingredients and feed structure including the structure of the feed before and after thermal treatment are affecting gut development. Intestinal weight, intestinal villus length and crypt depth can be modified by these parameters and intestinal resistance against pathogens as indicated in reduced pathogen colonization and delayed pathogen spreading was shown after the ingestion of coarser feed particles (Bjerrum et al., 2005; Huang et al., 2006; Santos et al., 2008; Moen et al., 2012).

Compared to mammals, the digestive tract of avians is characterized by its relatively short length (Larbier and Leclercq, 1994; Engelhard and Breves, 2009). Amongst other peculiarities, the digestive tract of the avian species is absent of teeth, developed a crop for storage and moisturizing of the feed, two stomachs (proventriculus and gizzard), as well as two caeca.

Especially the grinding mechanism of the gizzard are responsible for the impact of coarse feed structure on improved barrier function, improved nutrient digestibility, and improved animal performance (Svihus and Hetland 2001; Svihus et al., 2006; Williams et al., 2008; Denstadli et al., 2010). Gut development requires physical stimulation by dietary fiber and suitable feed particle size (Hetland et al., 2003; Svihus et al., 2004b). Several studies determined a linkage between coarse feed structure and stimulation of the gizzard development as evaluated by organ mass and concurrent stimulation of the pancreatic mass (Engberg et al., 2002; Peron et al., 2005; Williams et al., 2008; Svihus, 2011). Higher gizzard and pancreas weight is accompanied by increased bile acid secretion, and increased enzymatic activity of the pancreatic amylase (Engberg et al., 2004; Svihus et al., 2004a). Coarse particles in feed improve apparent digestibility of starch (Svihus and Hetland 2001; Svihus et al., 2004b). With starch being the predominant energy source, these findings made feed structure of high interest to poultry nutrition. The natural demand of avians for gizzard stimulating diets can be observed by the uptake in grit stones, wood shavings, or other litter material when chickens were fed with finely ground feed (Hetland et al., 2005).

However, dietary fiber and feed structure do not only promote the development of intestinal organs, both also affect animal behavior (Vilarino et al., 1996; Hartini et al., 2002; Hetland et al., 2003; VanKrimpen et al., 2008). For example, chickens obtaining mash feed spend more time on feed ingestion, resulting in fewer stereotypes and less aggressive behavior (Vilarino et al., 1996). Reduced feather pecking was shown in laying hens fed with coarsely ground diets, diets of low energy, and diets with increased contents of non-starch polysaccharides (VanKrimpen et al., 2008).

2.2. The Characterization of Feed Particle Size

In order to characterize the impact of feed particle size on digestive physiology, standardized evaluation of feed particle size is essential.

Several approaches can be used. Due to the three dimensional nature, the single particle is characterized by its specific surface and shape. The sum of particles in a feed or digesta sample can be characterized by its uniformity and particle size distribution. According to the experimental approach and the quality of the sample various methods of evaluation, mathematical descriptions, and comparisons have been developed.

For the evaluation of particles, methods including laser fractioning, mass spectrometry, imaging technique and sieve analysis can be used (Heidenreich and Sundermeier, 1996; Hetland et al., 2002). These methods are usually capable of analyzing wet or dry particle sizes varying between 0.02 - 2000 μM . The optic measurements are based on the fact that particles which are hit by a light reflect that light in a specific angle depending on their size. Additionally, the intensity of the reflected light provides information about the particle volume. These computer operated laser assisted systems are predominantly used in medical and industrial applications. Feed particle distribution is commonly determined by sieve analysis (Shaver et al., 1988; Giger-Reverdin, 2000; Logan and Sannson, 2000; Fritz et al., 2011).

Sieve analysis is performed by stacking several sieves of various mesh sizes regarding their descending mesh size on top of each other. The feed sample is rinsed through the sieves under continuous shaking. The fractions on top of each sieve are determined and serve for further description of the particle size distribution. Depending on the question addressed, the quality of the sample, or simply the method of the lab, various variants of sieving analysis have been described. For example, mesh size of the sieves may vary between 10 μm and 125 mm, the volume of feed samples may be between 10 g and 25 kg, the shaking intensity can be determined between 1 G and 15 G, the movement of the shaking might be strict horizontally or horizontal-vertically, and the duration of the sieving can vary between a few seconds or several hours. Varying those parameters results in different results obtained from the same sample (Wolf et al., 2012). Especially with pelleted feed, the originate structure that affects the gastrointestinal tract is of interest. Therefore, wet sieving analysis is often performed in those samples (Svihus et al., 2004b). Additionally to the variables that were mentioned above, in wet sieving analysis the amount of water that is used for soaking the feed, the duration of soaking the feed, the amount of liquid that is used for rinsing the suspension through the sieves, the water pressure that is used to rinse the feed through the sieves, the time for drying the sieved contents, as well as the temperature for drying the feed contents may be altered. While the American Society of Agricultural and Biological Engineers introduced a standardized analyzing system for feed samples, the lack of a standardized method for feed sieving performance is considered as major disadvantage for interstudy comparisons (ASAE 1983; ASABE 2008).

In order to describe and interpret the complex information of the sample fraction retained by every sieve, the results may be expressed in frequency plots (describing the percentage of feed obtained at each given mesh size), cumulative curves (adding up the various sample sizes), or the comparison of samples obtained above to a defined threshold. However, for comparison of feed groups or for correlation of results to particle size, reducing the information obtained from analysis to a single characteristic value is crucial, even though it is leaving the information about the homogeneity of the sample behind. For example, a homogenous sample that results in evenly distributed particle fractions on every sieve will result in the

same average particle size compared to feed that has a very large amount of coarse particles and a very high amount of small particles, but no medium-sized particles.

Various mathematical approaches have been developed in order to characterize data obtained from sieving analysis. Some of the most common methods are the

- Module Of Fineness (MOF). This approach is based on multiplying the proportion of particles retained on the sieve with lowest mesh size with 1, the proportion of particles retained on the sieve with a mesh size larger than the smallest mesh size with 2, the one on the next larger with 3, then adding up all these data (Poppi et al., 1980). While the resulting values have no unit, studies can only be compared when using exactly the same set of sieves with identical mesh sizes (Poppi et al., 1980; Okamoto, 1997; Clauss et al., 2002).

$$\text{MOF} = \sum_{i=1}^n p(i) * i$$

For example, four sieves with mesh sizes of 6, 2, 0.5, and 0.125 mm were used for feed analysis. After sieving, the dry mass of the particles obtained on top of each sieve was 25, 30, 35, and 10 g. Therefore, the particle proportion (p) was 0.25, 0.30, 0.35, and 0.10. Multiplying the values with 4, 3, 2, or 1 results in the following numbers: 0.25 x 4 = **1**; 0.3 x 3 = **0.90**; 0.35 x 2 = **0.7**; 0.1 x 1 = **0.1**. Adding up all these sums results in the value of 2.7, which can be used for comparison (Poppi et al., 1980; Okamoto, 1997; Clauss et al., 2002). While all the resulting values have no unit, studies can only be compared when using exactly the same set of sieves with identical mesh sizes.

- Discrete Mean Particle Size (dMEAN). This method is based on the sums of the arithmetic mean weight of particles that are obtained between the sieves collecting the particle fraction and the sieve above that particle fraction.

$$\text{dMEAN} = \sum_{i=1}^n p(i) * \frac{S(i+1) + S(i)}{2}$$

For example, four sieves with mesh sizes (S) of 6, 2, 0.5, and 0.125 mm were used for feed analysis. After sieving, the dry mass of the particles obtained on top of each sieve was 25, 30, 35, and 10 g. Therefore, the particle proportion (p) was 0.25, 0.30, 0.35, and 0.10. Now multiplying these proportions with the average sieve mesh sizes of the sieve below and above the feed mass is performed. In order to obtain a value of the feed mass above the sieve with the largest mesh size, the coarsest particle on top of the largest sieve was determined and serves for calculation of the first value. For the present example, the largest particle obtained on the 6 mm sieve was 8 mm. Therefore, the following calculation is performed: 0.25 x (8 mm + 6 mm)/2 = 0.25 x 7 = **1.75 mm**; 0.3 x (6 mm+2 mm)/2 = 0.3 x 4 = **1.2 mm**; 0.35 x (2 mm + 0.5 mm)/2 = 0.35 x 1.25 = **0.4375 mm**; 0.1 x (0.5 mm + 0.125 mm)/2 = 0.1 x 0.3125 = **0.03125 mm**. Adding up all these sums will result in a dMEAN of 3.42 mm.

2. Literature Review

- Discrete Median Particle Size (dMED). The median particle size can be obtained by summarizing the median weight of the cumulative proportion of particles obtained between two sieve sizes.

$$dMED = S(i - 1) + (S(i) - S(i - 1)) * \frac{pcum(i - 1) - 0.5}{pcum(i - 1) - pcum(i)}$$

For example, four sieves with mesh sizes (S) of S(4) = 6 mm, S(3) = 2 mm, S(2) = 0.5 mm, and S(1) = 0.125 mm were used for the feed analysis. After sieving, the dry mass of the particles obtained on the sieves was 25, 30, 35, and 10 g. Therefore, the particle proportion p(i) is p(4) = 0.25, p(3) = 0.3, p(2) = 0.35, and p(1) = 0.1 and the cumulative proportions pcum(4) = **0.25**; pcum(3) = **0.55**, pcum(2) = 0.9, and pcum(1) = 1. By inserting those results in the equation, the following term is calculated: dMED = 2+(6-2)x(0.55-0.5)/(0.55-0.25)=2.667.

- Continuous Median Particle Size (cMED). This value is obtained by plotting the values obtained from dMED expressed as cumulative curve against a regular scale or a log scale that reflects the mesh sizes of the sieves. The calculation of cMED requires a special software.
- Continuous Mean Particle Size (cMEAN). This value is obtained by plotting the values obtained from dMEAN expressed as cumulative curve against a regular scale or a log scale that reflects the mesh sizes of the sieves. The calculation of cMEAN requires a special software program.

Fritz et al. (2011) analyzed 580 fecal samples of various species, including mammals, reptiles, and birds by wet sieving and calculated MOF, dMEAN, dMED, cMEAN and cMEAD for comparison. The authors used nine sieves to determine the fractions of particle sizes. Additionally, they simulated the use of different sieve series (only six sieves, only odd-numbered sieves, only even-numbered sieves) and calculated MOF, dMEAN, dMED, cMEAN and cMEAD for comparison. Their results indicated, that dMEAN, dMED, cMEAN and cMED values are more suitable to compare values obtained from the experiments using different sets of sieves than MOF. In agreement with that study, Steuer et al. (2010) showed a good correlation of dMEAN compared to cMEAN for a broad range of particle sizes, cMED and dMED overestimate the large particles in samples of high proportions of very large particles. This observation was most likely due to the asymmetry in the particle size distribution. Additionally, irregularities were noted with large particles, therefore this measure appears less suitable for the particle size range. A major advantage of cMEAN compared to MOF or cMED, was the lesser susceptibility to the influence of sieve sets used as the results were most consistent using all nine sieves, only odd-numbered sieves or only even-numbered sieves. Even more, dMEAN values showed an accurate fit with the cMEAN values that varied only slightly when different sieve sets were compared. One further advantage of dMEAN is the simple calculation from the original sieving data without specialized curve fitting software. While dMEAN is considered as a specific variant of the cMEAN calculation, its predominant difference is the assumption of particle size distribution of particles obtained from the particular sieves. For dMEAN, by definition, a normal distribution of particle sizes on the sieve is assumed. However, an asymmetric particle size distribution might be more

common in practice. This discrepancy becomes evident when focusing on the lower/higher particle size range, where cMEAN differs from dMEAN. Therefore, dMEAN and cMEAN appear to be useful parameters for particle size estimation. Due to the practicability of calculating dMEAN and its consistent results when using various mesh sizes for sieving, dMEAN was proposed for the description of the feed particles size (Fritz et al., 2011).

2.3. Grinding of Feed

Grinding of poultry feed is performed for various reasons. Smaller and therefore more homogeneous particles prevent demixing of the diet and an uniform and balanced nutritional support of the animals (Axe, 1995; Koch, 1996; Löwe, 2013). Breaking up the macrostructure of the grains enables a more efficient digestion of nutrients by the intestinal enzymes due to the increased surface area as well as the enhanced access through the cracked hull. Furthermore, if thermal treatment (pelleting, expanding, extruding) of the diet is warranted due to various reasons (hygienization of the feed, increased energy density of the feed, reduced demixing of the diet), grinding is mandatory due to technical aspects. Grinding improves the adhesive functions of the ingredients and subsequently the pellet stability (Goihl, 1995; Behnke, 2001; Löwe, 2007). The feed ingredients and the feed composition determine the resulting particle size at a given grinding intensity and milling method (Audet, 1995). While primary grinding of the feed is accomplished by various milling methods, secondary diminution of the feed results due to successional technologies such as thermal treatment or crumbling (Svihus et al., 2004; Wolf et al., 2012).

Grinding feed is energy-consuming and the choice of diminution technologies can have a substantial impact in this regard (Wondra et al., 1995). In order to reduce the amount of energy needed for feed production, innovative energy-saving milling methods have been developed (Hoffmann et al., 2011). However, the energy consumption of a mill is not only determined by the milling type, but depends also on the way of installation, the set up of the mill, the material of the mill components, and other factors (Audet, 1995). The use of the hammer mill is still common, however, alternative milling methods such as the roller mill or the wedge-shaped disc mill are becoming more customary in practice (Hoffmann et al. 2011). The milling technology affects not only feed particle size but also the particle shape (Reece et al., 1985; Koch 1996). Therefore, the use of milling methods may have an impact on the intestinal health status of chickens.

2.3.1. Roller Mill

The roller mill is designed of two horizontal cylinders that rotate with various speeds against each other (figure 1). The feed is rinsed through the gap between these cylinders. Depending on the rotation speeds, the width of the milling gap, the diameters of the cylinders, the length of the cylinders, and the surface of the cylinders, the feed is exposed to various squeezing and shearing forces. Particles obtained by the roller mill are cubically and irregularly shaped (Koch, 1996). The use of this milling method is characterized by its relatively low operating energy (approximately 3 kWh/t) and its diminished operating noise (Koch, 1996). The use of the roller mill results in a relatively uniform particle size distribution with subsequently reduced fractures of small particles that were exposed to relatively low temperatures (e.g. room temperature) (Koch, 1996; Löwe, 2011). A disadvantage of this milling technique may be the fractions of unprocessed small grains that rinse through the milling gap without any treatment (Douglas et al., 1990).

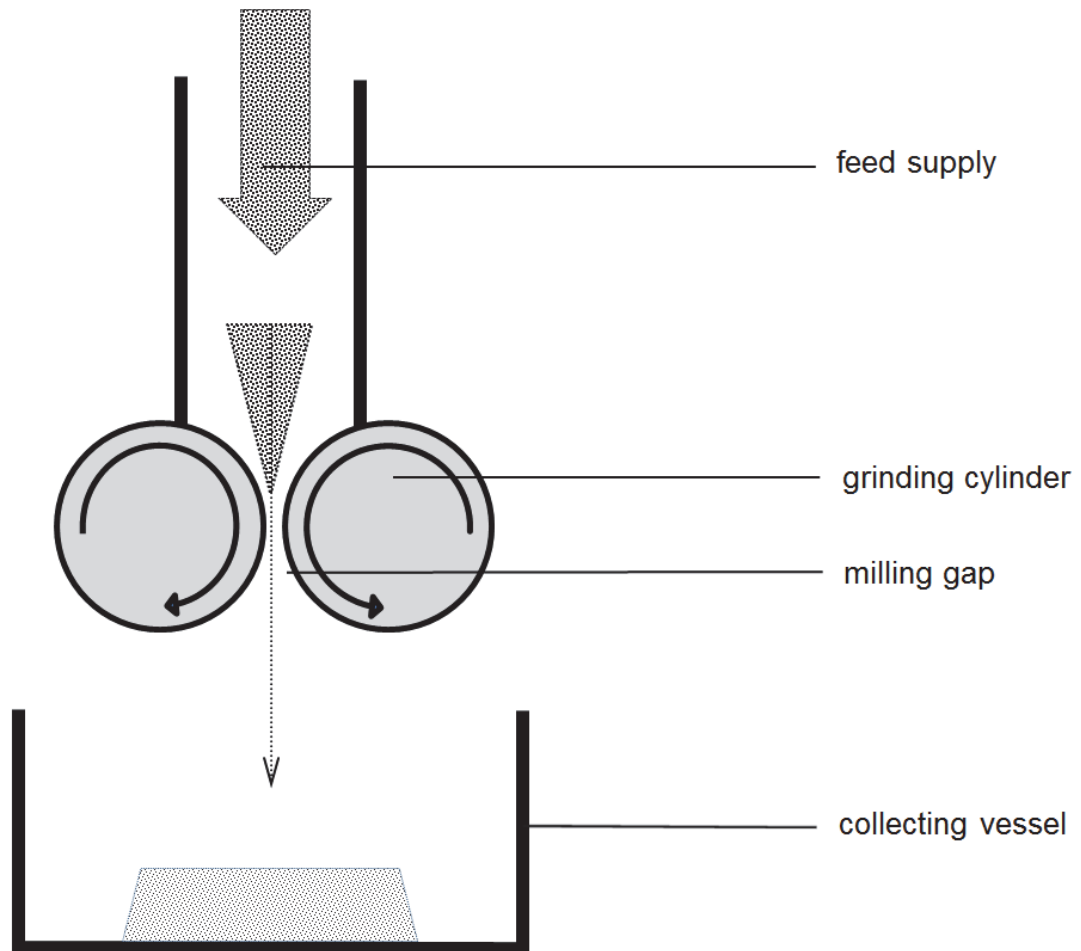


Figure 1. Roller mill. Feed is processed by two grinding cylindrical rolls. The milling gap between the rolls can be adjusted and determines the squeezing forces applied to the particles. The various speeds of the cylinders determine the shearing forces that are applied to the grains.

2.3.2. Hammer Mill

The hammer mill is designed of a hollow round metal jacked equipped with a centrally located rotor with several hammers being attached to the rotor (figure 2). The feed is rinsed towards the rotating hammers and leaves the system through a sieve on the outer surface. Therefore, only particles that are small enough to pass the pore sizes of the sieve are enabled to leave the system. The impingement demolition of the feed results in a broad spectrum of particle fractions and the liberation of intracellular substances, such as fat (Svihus et al., 2004b; Wolf et al., 2012). The particles obtained from this grinding method are characterized by spherical form and furbished surface (Reece et al., 1985; Koch, 1996). Hammer mills are capable to grind fibrous material. Therefore, the hammer mill is considered as method of choice when cereals rich on spelt, such as oats or barley need to be processed (Koch, 1996). The energy required for feed processing is dependent on the number of hammers, the shape of the hammers, the rotation speed, the area of the die, the numbers and shape of the die holes, the feed ingredients and the feed composition (Amad, 2001; Löwe and Mohrig, 2013). In general, the hammer mill is characterized by its relatively high energy consumption (approximately 5-15 kWh/t) and dust pollution. The advantages include its universal application to various feed components, as well as its relatively low acquisition costs, easy operation and maintenance (Audet, 1995; Koch, 1996).

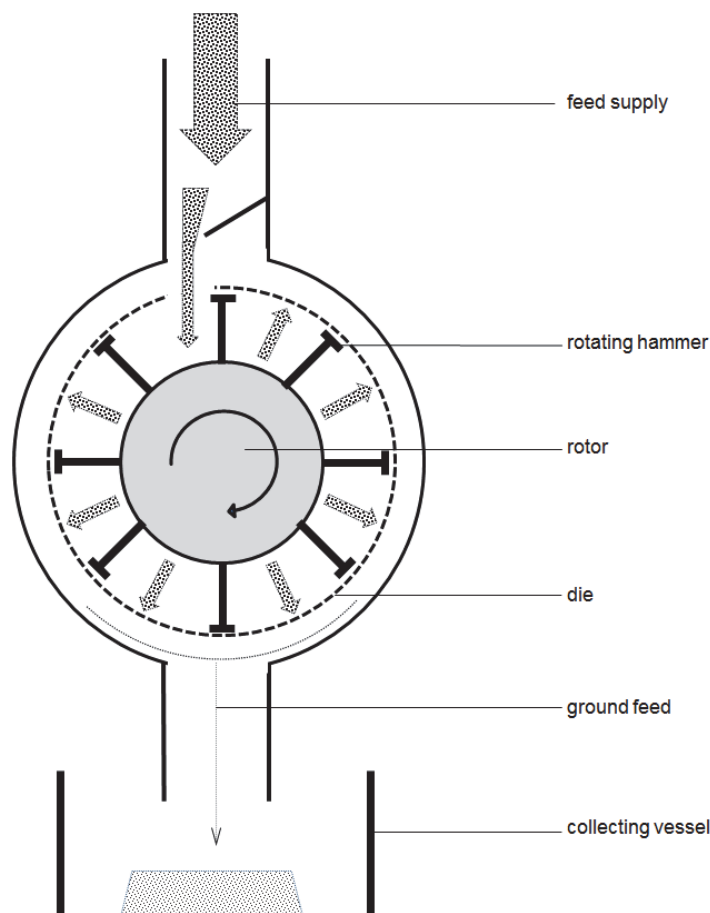


Figure 2. Hammer mill. Feed is supplied into a hollow metal jacked, in which rotating hammers force the grains to leave through the surrounding die. Due to the impingement demolition, fibrous ingredients can be grinded.

2.3.3. Disc Mill

The disc mill is designed of two horizontally oriented structured metal discs where the feed is distributed in between (figure 3). While one disc is fixed, the second one rotates providing the grinding activity. The gap between the discs can be adjusted by the operator, additionally the form and size of the discs, and the speed of the rotating disc can be varied. At slow rotation speed, shearing and pressure are the predominant forces affecting the feed, at high rotation shearing and punching are the predominant forces. The advantage of this milling method includes the obtainment of coarse particles without high fractions of small particle sizes and its relatively low operating energy (Löwe and Mohrig, 2013).

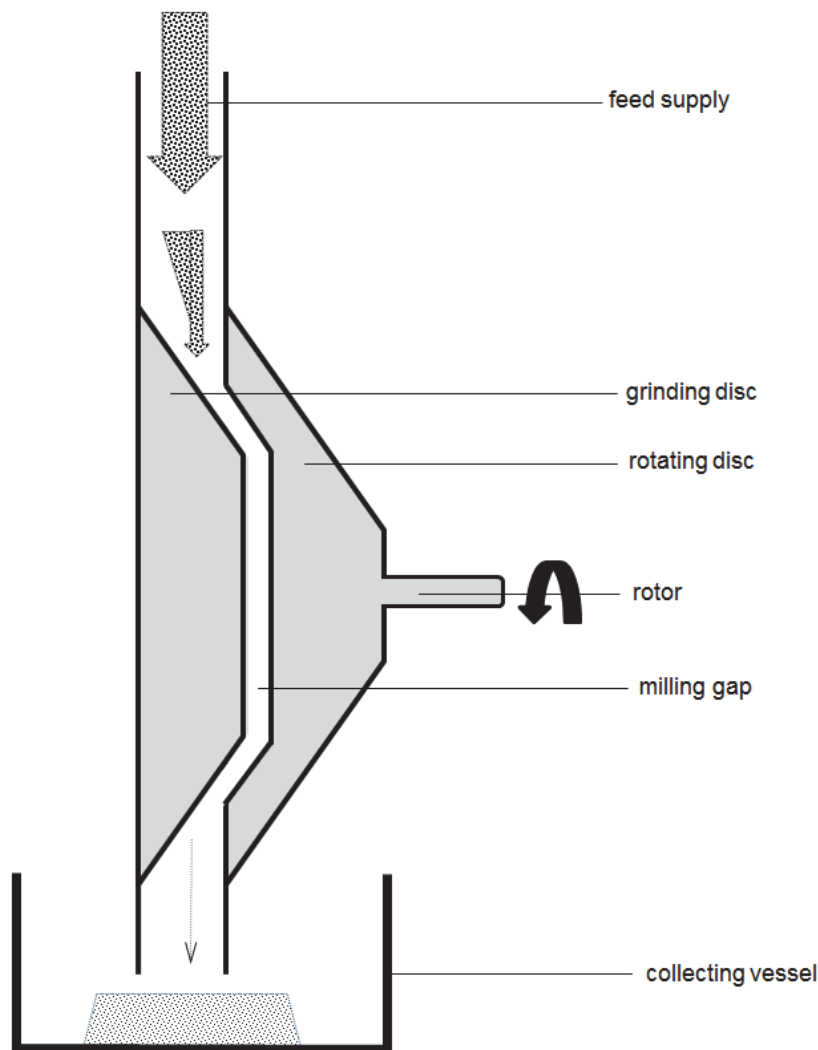


Figure 3. Disc mill. Disc mills can vary in shape and surface structure of the discs. Usually one disc rotates while the complementary one is immobilized.

2.3.4. Wedge-Shaped Disc Mill

The wedge-shaped disc mill is characterized by two horizontal cylinders that rotate with various speeds against each other. In contrast to the roller mill, the cylinders consist of wedge shape discs that not only extend the milling gap but also serve as cutting blades (figure 4). Therefore, a major advantage is a low operating energy (<3 kWh/t) that is comparable to the roller mill, but depending on the profile of the wedge discs, the angles of the wedge discs, the curvature of the wedge discs, the rotation speeds, and the milling gap, the wedge-shaped disc mill is also capable of grinding spelt materials (Hoffmann et al., 2011).

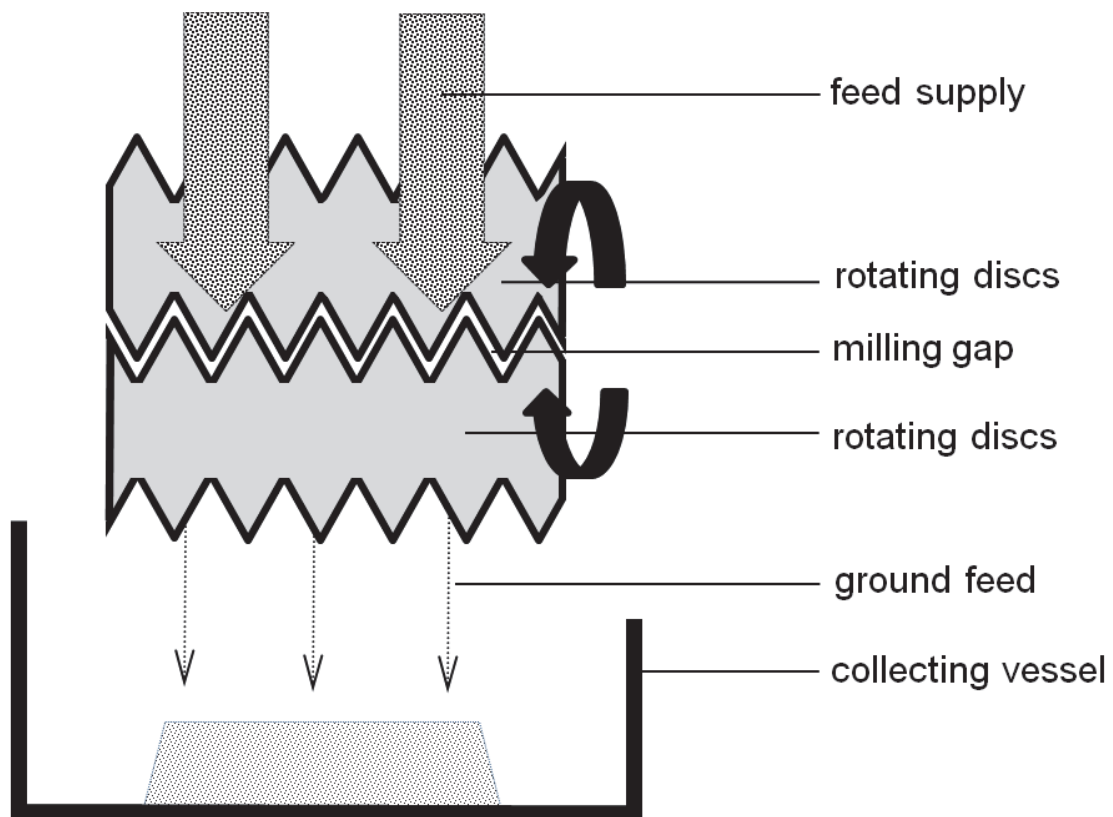


Figure 4. Wedge-shaped disc mill. Feed is distributed between two rolling cylinders, which are wedge-shaped. Therefore, the milling gap is prolonged compared to the roller mill.

2.4. Thermal Treatment of Feed

Commonly, poultry are fed with a complete diet composed of all ingredients required for a balanced animal nutrition. However, the macrostructure of the feed varies depending on the production type. While feed for laying hens is commonly provided in mash form, broiler feed is mostly provided in pellets. Several factors (particle size, particle number, particle shape, flowability, moisture content, etc.) affect demixing of a diet, it can hardly be prevented in mash feed (Axe, 1995; Löwe and Mohrig, 2013). Chickens receiving an unbalanced diet exhibit poor performance (Behnke, 2002).

Therefore, the beneficial effects of compacting on the uniformity of the mixture are of great impact to the poultry industry. Furthermore, thermal treatment of the feed reduces selective feed ingestion, results in thermal modification of starch and proteins, reduces microbial contamination and decreases feed wastage (Mossel et al., 1967; Fancher et al., 1996; Behnke 2001; Peisker, 2006).

2.4.1. Pelleting

Pelleting of feed can be described as mechanical process, where small particles are agglomerating depending on the moisture, heat, and pressure applied. Commonly, steam pressure is applied to the mash feed (conditioning) and dozed into the heated pelleting chamber. In the pelleting chamber, the feed is forced to leave the pelleting press through a metal die. The resulting threads can be cut to various length and are finally cooled and dried. Pelleting results due to mechanical interlocking, diffusion, and adsorption of the microstructure (Behnke, 2001). Therefore, the pellet quality can be affected by diet formulation. The pellet quality is especially determined by the protein, fat, and wheat content of the feed, the grinding intensity (particle size) of the feed, the grinding method (particle size distribution, particle shape) of the feed, the application of steam conditioning, as well as the cooling process after pelleting (Goihl, 1995; Briggs et al., 1999; Fairfield, 2003). Usually 3 to 6% moisture is added to the mash during conditioning at temperatures that can range from 60 to 90°C for several seconds (Fairfield, 2003). Long term conditioning can last 20 minutes or more (Fairfield, 2003). Extremely high temperatures can also develop during pellet formation due to friction with the die walls as the conditioned mash is forced through die openings (Fairfield, 2003). The physical advantages of pelleting include the ease of handling, reduced ingredient segregation, reduced feed wastage, and increased energy density (Behnke, 2001).

2.4.2. Expanding

Similar to the pelleting machine, the expander forces pre-conditioned mash feed through a die of various sizes. However, the end-temperature in the feed at the end of the expanding chamber is higher and reaches up to 130°C. The feed is subjected to a short treatment time with a maximum of 3 seconds. The structure of the resulting final product breaks up when leaving the expander. Therefore, expandate is characterized by a porous surface and lower density, compared to firmly pressed pellets (Heidenreich and Michaelsen, 1995; Thomas, 1997). While the impact on feed hygiene is non-controversial, the consequences for nutrient digestibility are less clear and will be described in detail in section “2.5. Effect of Particle Size and Thermal Treatment of Feed on Poultry” (Thomas, 1997; Peisker, 2006; Lundblad et al., 2011).

2.4.3. Extruding

Similar to pelleting and expanding, the extruder forces the conditioned mash feed through a die of various sizes. However, higher levels of moisture, pressure, and heat are used for extruding, which results in feed of a high density, pressed into firm structures (Fancher et al., 1996; Heidenreich and Michaelsen, 2001). Therefore, this

method is relatively expensive and primarily used in the pet food or fish feed industry. Depending on the number of screws, single-, double-, or multiple-screw extruders can be used. Double screw extruders are designed of two parallel or anti-parallel running screws. Depending on the different speeds, the shape, diameters and distance of the augers, the feed is transported with various speed and exposed to various mixing and cutting forces (Jämlich, 2010). The use of these extruders is primarily of importance in the food industry and is capable of the production of specialized items, such as highly viscous foods (e.g. chewing gums, fatty products). Multiple screw extruders serve the same purpose, however, the processing speed is increased by the amount of screws used.

2.5. Effect of Particle Size and Thermal Treatment of Feed on Poultry

In order to ensure optimized nutrition of poultry, it is not only important to provide the correct amount of ingredients, but also to secure the intake of structured feed (Yegani and Korver, 2008). The structure of compound feed has an impact on animal health and performance (Maxwell et al., 1970; Villarino et al., 1996; VanKrimpen et al., 2008; Yegani and Korver, 2008; Grosse Liesner et al., 2009; Betscher et al., 2010). Chicken discriminate their feed intake by the size, color, and form of the feed. Larger feed particles are preferred and more rapidly ingested (Nir et al., 1994; Yasar, 2003; Amerah et al., 2004). Feed with high fractions of small particle sizes (<500 µm) results in decreased feed intake, increased feed selection, dust pollution, increased feed waste and may result in malnutrition of animals (Safaa et al, 2009). Additionally, the impact of feed particle size on chicken behavior has been proven. Hens fed with coarse particles exhibit less aggressive and stereotype behavior (Villarino et al., 1996; Hartini et al., 2002; VanKrimpen et al., 2008).

The impact of feed particle size on the performance and nutrient digestibility of laying hens has not been widely studied. Safaa et al. (2009) investigated the effect of particle size on performance parameters and egg quality in 960 laying hens. The only effect that could be detected was an increased feed intake of hens that were fed with larger feed particles. In contrast, for broilers specific particle sizes are recommended (Nir et al., 1994; Denstadli et al., 2010). It has been proposed, that at least 20% of the cereal particles should be larger than 1.5-2.0 mm (Svihus, 2011). Chickens are well known for their high ability of digesting coarse feed (intact maize and wheat particles, particles ≥ 3.1 mm) (Dozier et al., 2006; Lundblad, 2009; Rougiere et al., 2009).

Even though study results differ, there is increasing evidence supporting the use of coarse feed particles. Several studies demonstrated the positive impact of coarser particle size on performance and ileal nutrient digestibility in broilers (Leibetseder, 1987; Amad, 2001; Amerah et al., 2007b; Svihus et al., 2010). Additionally, the impact of coarse particle size is considered beneficial for the intestinal development and intestinal microbiota of the animals, and thereby on product safety (Engberg et al., 2002; Bjerrum et al., 2005; Santos et al., 2008; Moen et al., 2010). For example, coarse feed particles are essential for gizzard development and reduce intestinal colonization of pathogens (Engberg et al., 2002; Bjerrum et al., 2005; Santos et al., 2008; Moen et al., 2010). A well-developed intestinal barrier is essential for the ability of the animal to resist pathogens and its concurrent diseases. Coarse feed particles affect intestinal development and are held responsible for reduced *Salmonella* spp. and *Clostridium* spp. concentrations in the intestine and reduced pathogen spreading (Bjerrum et al., 2005; Huang et al., 2006; Santos et al., 2008; Yegani and Korver, 2008; Moen et al., 2010).

Thermal treatments are commonly used in broiler feed production in order to reduce antinutritive factors (e.g. trypsin inhibitors), and to increase the apparent ileal nutrient digestibility (Kilburn and Edwards, 2001; Jia and Slominski, 2010). Since pelleting or expanding prevents demixing of the diets, a reduced feed selection by the animals results in an optimized feed conversion ratio compared to mash feed (Amerah et al., 2008). Therefore, an improved performance can be expected when broiler chickens are given pelleted diets (Hamilton and Proudfoot, 1995). In order to reduce energy requirements for grinding and pelleting, 35% of the corn could be added after pelleting without adversely affecting growth performance of broilers (Dozier et al., 2006).

However, when comparing feed processed by various thermal treatments including pelleting, expanding, and extruding for broiler chickens, hydro-thermal processing increased total apparent starch digestibility, but due to reduced feed intake of the expanded and in particular of the extruded diets, only pelleting improved growth rate and feed utilization (Lundblad et al., 2011). The reduced bulk density of expanded and extruded feed was held responsible for these effects. Additionally, extruded feed had the highest extract viscosity, while starch gelatinization was improved (Lundblad et al., 2011).

A possible disadvantage of thermal treatment of feed is the potential negative impact on protein utilization. While moderate temperatures and short term treatment showed no effects on amino acid availability, intensive thermal treatment significantly reduced amino acid availability (Panigrahi et al., 1996; Dänicke et al., 1998; Mavromichalis and Baker, 2000; Amezcua and Parsons, 2007). Autoclaving of diets for 40-60 min at 121°C and 105 kPa reduced amino acid digestibility which subsequently led to lower growth performance of the animals (Achinewhu and Hewitt, 1979; Fernandez et al., 1994).

Due to the more mature digestive system of laying hens, transferring data obtained from studies in broiler chickens to laying hens seems questionable. Additionally, the combination of milling methods with thermal treatment and its possible interactions has not been studied in laying hens.

2.6. Use of Organic Acids in Poultry Nutrition

Acidification of feed is known to preserve feed but also to improve weight gain of animals as well as the feed conversion ratio (Denli et al., 2003; Hernandez et al., 2006; Paul et al., 2007). The cause for the beneficial effects remains controversial. One proposed mode of action of organic acids in the intestinal tract is based on their antimicrobial activity (Roy et al., 2002; Chaveerach et al., 2002). Acidification of feed reduces unfavorable bacterial contamination, resulting in a positive impact on animal health and improved carcass hygiene (Izat et al., 1990; Thompson and Hinton, 1997). Organic acids are able to enter the microbial cells in their undissociated form by diffusion as well as carrier-mediated. In the microbial cell, the acids dissociate leading to an intracellular accumulation of hydrogen ions and the respective anions which subsequently impair the bacterial metabolism (Ricke, 2003). Even though the antibacterial mechanisms of organic acids are not completely understood, it has been proposed, that the active export of the accumulated hydrogen ions results in depletion of the cellular adenosine triphosphate levels (Davidson, 2001). Additionally, the accumulation of hydrogen ions may have a direct toxic effect on the bacterial organisms, resulting in damage of the bacterial walls and a negative impact on macromolecular synthesis (Russell, 1992; Alakoni et al., 2000; Davidson, 2001). Another hypothesis includes the interference of organic acids with membrane proteins, which uncouples electron transport and subsequently reduces ATP

production (Davidson, 2001). Furthermore, the use of organic acids enhances microbial phytase activity due to its low pH optimum (Jongbloed et al., 2000; Dibner and Buttin, 2002; Liem et al., 2008). Additionally, the use of organic acids in feed results in an increased pancreatic enzyme secretion which is mediated by the accumulation of hydrogen ions and the involvement of secretin release (Harada et al., 1986; Dibner and Buttin, 2002). It is also known that organic acids in chicken feed can affect epithelial cell proliferation and results in enhanced villus height (Hernandez et al., 2007). A direct stimulation of cell proliferation (especially of butyric acid, acetic acid and propionic acid) is hypothesized (Sakata, 1987; Dibner and Buttin, 2002; Hernandez et al., 2007). Additionally, indirect cell proliferation due to the influence on the intestinal microbiota is also considered (Hernandez et al., 2007). An increased epithelial surface might be accompanied by an increased number of intestinal nutrient transporters and may lead to higher nutrient transport capacity. Thirteen glucose transporters (GLUT) and three electrogenic sodium-glucose co-transporters (SGLT) could be isolated in various organs of mammals. The glucose uptake in the intestine of avian species seems to be primarily provided by the apical SGLT-1 and the basolateral GLUT-2 transporters (Takata 1996; Thorens and Joost, 2001; Humphrey et al., 2004; Drozdowski and Thomson, 2006).

To our knowledge, it has not been investigated whether the dietary inclusion of organic acids affects the nutrient absorption capacity or the reactivity of the intestinal epithelium in the upper digestive tract of chickens.

2.7. Characterizing Intestinal Nutrient Transport by the Ussing Chamber

The Ussing chamber technique enables qualitative and quantitative evaluation of absorption and secretion of ions and nutrients that are taken up in electrogenic co-transport with ions (Stevens 1964; Wright, 1993). Furthermore, the use of the Ussing chamber technique enables the evaluation of intestinal conductance and short circuit current, which can serve as parameters for tissue integrity and barrier function (Ruhnke et al., 2014).

The assessment of mucosal transport processes using the Ussing chamber technique is a standard method to study the intestinal function and ion transport in many species (Amat et al., 1999; Li et al., 2004; Awad et al., 2007; Collins et al., 2007; Lennernas, 2007; Clarke, 2009; Neirinckx et al., 2011).

In detail, heated and oxygenated buffer reservoirs provide physiologic support to an epithelial membrane (e.g. intestinal tissue). Applying an electrical potential difference to the membrane allows for the determination of the current that is generated by the membrane due to active ion transportation. The electrical measurements of voltage and current allow for the determination of the membrane resistance and subsequently its conductance. Additionally, application of chemical compounds such as glucose, phloridzin, a selective inhibitor of SGLT-1, histamine or carbachol (both are mediators of chloride secretion), can be used in order to evaluate functional tissue traits (Ruhnke et al., 2013).

The tissue preparation for Ussing chamber experiments is crucial, since the mucosa of the intestine needs to be removed from the *muscularis mucosae* and/or the underlying *serosa* ("stripping") (Clarke, 2009). Preparation techniques vary between the species and the age of the animal, since development of the intestinal layers differ. Additionally, the definition of the technical term "stripping" varies amongst the authors. While some authors (Yunus et al., 2011) consider stripping as the removal of the *serosa* and the *lamina muscularis mucosae*, others use this term for any manipulative removal of an intestinal layer, such as the *tunica serosa* (Awad et al., 2007).

2. Literature Review

Additionally, buffer compositions vary within the experiments. In Ussing chamber experiments performed with poultry intestine, buffers have not been widely studied for their optimized composition. However, the benefit of glutamine on the gastrointestinal function of poultry and mammals has been shown (Smith and Wilmore, 1990; Watford and Wu, 2005). Glutamine is a precursor for many metabolic functions such as the synthesis of amino acids, proteins and nucleotides as well as essential metabolic pathways such as anaerobic glycolysis (Fleming et al., 1997; Newsholme et al., 2003). Amongst other effects, the absence of glutamine in long term parenteral nutrition is associated with atrophy of the intestinal mucosal cells in humans (McCauley et al., 1996). In broilers, enhanced levels of glutamine in feed improved growth performance, gastrointestinal development and also humoral immune response (Bartell and Batal, 2007). It also increased heat stress tolerance of broilers as evaluated by growth performance and meat quality (Dai et al., 2009).

3. Part I: Method for the Preparation of Mucosal Flaps from the Jejunum of Laying Hens for Transporter Studies in Ussing Chambers

(This part of the thesis, as well as the discussion of this part (pp. 47) was published in the Archives of Animal Nutrition: Ruhnke I, Röhe I, Meyer W, Kröger S, Neumann K, Zentek J. (2013). Method for the preparation of mucosal flaps from jejunum of laying hens for transporter studies in Ussing chambers. *Archives of Animal Nutrition*, 67 (2): 161-168).

You have to buy this part.

<http://dx.doi.org/10.1080/1745039X.2013.776328>

4. Part II: The Impact of Organic Acid in Feed on Starch Digestibility, Jejunal Conductance, Glucose Transporter Expression, as well as Glucose and Chloride Transporter Function in Broiler Chicken

(This part of the thesis, as well as the discussion of this part (pp. 48) was accepted to the Journal of Animal Physiology and Animal Nutrition: Ruhnke I, Röhe I, Goodarzi Boroojeni F, Knorr F, Mader A, Zentek J. Feed supplemented with organic acids does not affect starch digestibility, nor intestinal absorptive or secretory function in broiler chickens).

You have to buy this part. The text will be published soon in the journal.

[http://www.onlinelibrary.wiley.com/journal/10.1111/\(ISSN\)1439-0396](http://www.onlinelibrary.wiley.com/journal/10.1111/(ISSN)1439-0396)

5. Part III: Effect of Feed Particle Size, Milling Method, and Thermal Treatment on Performance, Apparent Ileal Digestibility and pH of the Digesta in Laying Hens

(This part of the thesis, as well as the discussion of this part (pp. 50) was submitted to British Poultry Science: Ruhnke J, Röhe I, Krämer C, Goodarzi Boroojeni F, Knorr F, Mader A, Hafeez A, Neumann K, Löwe R, Zentek J. Effect of feed particle size, milling method, and thermal treatment on performance, apparent ileal digestibility, and pH of the digesta in laying hens).

You have to buy this part. The text will be published soon in the journal.

<http://www.ps.oxfordjournals.org>

6. Discussion

6.1. Assessment of the Ussing Chamber Method (Part I)

In order to determine the influences of acidified feed on transport physiology, the Ussing chamber method was optimized for studying the transporter function of chicken intestine.

The results of part I of this thesis indicate that the use of net-supported tissue preparation allows a reliable assessment of electrogenic transport processes of stripped jejunal mucosa in laying hens. Conductance of stripped jejunum from laying hens in this study ranged from 4.7 to 20.3 mS/cm². These values are comparable to previously reported data for stripped ileum from laying hens and broilers (mean values varying between 3.16 mS/cm² and 60.6 mS/cm²) (Amat et al., 1999; Awad et al., 2005; Rehman et al., 2006; Rehman et al., 2009). The impact of the collection and stripping procedure on the variability of tissue resistance has been reported and may also have played a role in our experiments (Stevens, 1964). The duration of our experiments varied between 30-45 minutes and was relatively short. In contrast, Ussing chamber experiments in other species are usually performed for up to 180 minutes (Clarke, 2009).

The histological evaluation (figure 6) of the samples used in this study indicated an evenly dissected *muscularis mucosae* and an intact mucosal architecture, visualizing the suitable tissue separation by the stripping method. Physiologic variations of tissue conductance might be caused by individual alterations in tissue stretching, tight junction expression, individual thickness of the intestinal mucosa, or varying thickness of the different intestinal regions. However, mean basal tissue conductance of both groups was not significantly different. Additionally, when comparing basal I_{sc} values between samples of the control group to those exposed to glutamine, no statistical significant difference could be detected ($p = 0.580$).

All stripped jejunum samples from both, control and glutamine group responded to the absorptive stimulus and its inhibitor (glucose and phloridzin). This indicates that the mucosal SGLT-1 transporter in laying hens was involved in the glucose uptake. Such findings are supported by previous studies which identified the jejunum as main site of carrier mediated glucose transport in chicken (Ferrer et al., 1994; Awad et al., 2007). The ability of the samples of both groups to maintain functioning glucose transport *ex vivo* indicates the ability of physiologic response and therefore the quality of the prepared tissue samples. A functional response could also be observed when testing phloridzin and carbachol. Mean ΔI_{sc} did not differ significantly when comparing the control group and the glutamine group ($p = 0.712$, $p = 0.450$). Therefore, it can be concluded that the results of this study indicate no beneficial effect of 5 mM glutamine on mucosal transporter function. A competitive inhibition between glucose and some individual amino acids in the rat intestine was described (Vinardell et al., 1990). These mechanisms include a theory of "competition for energy" that hypothesizes, that the energy of the enterocyte is not sufficient to transport both substrates at maximal level, a mechanical interaction of the substrates at the brush border membrane, and a competition due to the limited capability of the sodium-potassium ATPase in order to obtain the ion gradient necessary for electrogenic nutrient uptake (Vinardell et al., 1990). To our knowledge, studies in chickens regarding the influence of glutamine on glucose uptake have not been performed to date.

Electrophysiological reactions of all samples exposed to carbachol, a potent inducer of chloride secretion in the intestine, varied between 94.0% in the control group and 87.0% in the glutamine group. It has been demonstrated that the application of carbachol increases the short circuit current of stripped jejunum from laying hens, suggesting a stimulation of electrogenic chloride secretion (Yunus et al., 2010). The velocity until maximum I_{sc} reached was shorter compared to the response to glucose and phloridzin (table 1). Compared to the number of responding samples exposed to compounds affecting absorptive transporter function (glucose and phloridzin: 100%), a decreased number of responding samples was observed after application of carbachol (94 % and 87 %). This reduced response indicates that some samples were unable to maintain physiologic secretory function, probably as the experimental conditions might have damaged the tissue integrity. While carbachol was the third reagent added in sequence to the tissue, the samples might have been too long maintained under *ex vivo* conditions to maintain sustained physiologic function. The diminished response in both groups indicates a short life span of the tissue. Especially in stripped epithelia the capacity of the tissue to provide the enterocytes with nutrients and energy is limited. In order to prolong the life span in the Ussing chamber of enterocytes of the stripped jejunum from laying hens, further studies with additionally modified buffers are warranted. Despite these possible limitations, the magnitude of the responses to glucose and carbachol observed in this study was higher than observed formerly (Yunus et al., 2010).

Histologic evaluation showed no evidence of damage from stripping on the enterocytes of the intestinal mucosa (figure 6). However, changes of tissue structure, in particular edematous phenomena and villus shortening were observed after incubation in the Ussing chamber (figure 6a). The evaluation of the histologic structure of tissue that has been in the Ussing chamber remains challenging. While being in the buffer solution, the cell connectivity becomes fragile subsequently predisposing for edema and a loss of membrane integrity caused by fixation chemicals. In conclusion, part I of this thesis demonstrates the feasibility of using a net supported Ussing chamber for the evaluation of transport physiology in isolated mucosa from laying hens. The stripped jejunum from laying hens can successfully be obtained and reliably transferred into the Ussing chamber, when handled as described.

6.2. Evaluating the Impact of Acidified Feed on Intestinal Physiology of Broiler Chickens (Part II)

The second part of this thesis focused on the impact of acidified feed on the intestinal function of broiler chickens. In detail, the effects on apparent ileal starch digestibility, glucose transporter expression, and physiologic intestinal transport function were determined.

The results of the present study indicate no influence of 1.5 % organic acids in feed on the measured traits. Additionally, the performance (body weight, feed intake, feed conversion ratio) of the animals was not affected by the use of 1.5 % organic acids in the diet; these data is reported in a different publication (Goodarzi Boroojeni et al., 2014b).

The application of organic acids as feed additives has a long history of use and both, positive or indifferent results have been obtained in broilers. The benefits of using propionic and formic acid on broiler performance, nutrient digestibility and the intestinal microbiota have been shown in some previous studies (Izat et al., 1990; Hernandez et al., 2005). Acid concentrations between 0.15-3 % were tested in former studies (Dibner and Buttin, 2002). An impact of organic acids on the intestinal microbiota could frequently be observed when used at concentrations between 0.5-

1.5 % in the feed (Roy et al., 2002). The use of 1.25 % propionic acid could suppress the growth of bacteria associated with turkey poult enteritis and mortality syndrome most effectively (Roy et al., 2002). On the other hand, formic acid and propionic acid concentrations at 2% had adverse effects including decreased feed intake and reduced body weight gain in broilers (doVale et al., 2004; Isabel and Santos, 2009).

Various studies investigated the effect of organic acids in the intestinal tract. The highest concentrations of organic acids as well as strongest impact on the intestinal microbiota are known to occur in the proximal intestine of broilers (Hume et al., 1993; Thompson & Hinton, 1997). Supplemented acids can usually be detected in the crop and stomach, but the concentrations are decreasing rapidly in the upper intestinal tract (Hume et al., 1993; Thompson & Hinton, 1997). In broilers of this study, the use of organic acids was of relevance to the intestinal microbiota, affecting the composition and the metabolic activity of the intestinal microbiota mainly in the upper digestive tract (Goodarzi Boroojeni et al., 2014a *in press*). Similar to the results obtained in the present thesis, no effect of organic acids on nutrient digestibility (ileal protein digestibility) could be observed (Goodarzi Boroojeni et al., 2014b).

In spite of the rapid disappearance, effects of added organic acids on the microbiota of the small and large intestine were detected (Rehman et al., 2007; Wang et al., 2010). Therefore, direct effects of organic acids might be relevant in the upper small intestine, while indirect “downstream” effects by microbial interaction might be present also in the more distal parts of the gut.

The current Ussing chamber study was performed with jejunal samples, as the jejunum is considered as main site of nutrient absorption. The results indicate no influence of acid supplementation on the apparent ileal starch digestibility. Therefore, the effect and mode of action of organic acids on broiler performance, mainly improved feed conversion and higher weight gain, seems to be related to other interfering factors, including feed composition, hygiene and animal health status. Favourable effects have been shown including increased intestinal length, increased villus height and the modulation of the intestinal microbiota (Denli et al., 2003; Hernandez et al., 2006; Paul et al., 2007). Organic acids might affect epithelial cell proliferation and mucosal cell turnover which affect villous height. A consequence of increased epithelial surface might be an increased intestinal transport capacity. In the present study, no differences in glucose transporter expression and glucose transport capacity could be observed between chickens of the control and the treatment group. Since grain starch is the traditional and most important source of carbohydrates in poultry nutrition, a high glucose uptake is the prerequisite for dietary tolerance and performance. Organic acids can increase the height of intestinal villi which is assumed to be related to the proposed microbial effects (Paul et al., 2007). Bacterial concentration (lactic acid bacteria and coliforms) in the ileum and caecum has been reduced by diets containing 0.5-1.5% of fumaric and sorbic acids (Pirgozliev et al., 2008). Therefore the intestinal mucosal barrier may be maintained more efficiently, and the invasion of pathogens that would subsequently lead to chronic inflammation, reduced villus height and decreased absorptive capacity may be efficiently prevented (Podolski, 1993; Paul et al., 2007). Broilers fed with antibiotics showed an increased number of villi in the duodenum and jejunum, greater villus height and enhanced depth compared to broilers fed without antibiotics (Miles et al., 2006).

Other investigators found a thinner *tunica muscularis mucosae* of the ileum in chicken fed with organic acids, antibiotic feed additives were also able to reduce the relative intestinal weight by the reduction of muscularis thickness in jejunum and ileum (Henry et al., 1987; Gunal et al., 2006; Miles et al., 2006). Similar to antibiotics, feed supplemented with probiotics and organic acids resulted in a numerically lower thickness of the ileal *tunica muscularis mucosae* in 21 and 42 day old broilers (Miles et al., 2006).

Organic acids have been shown to reduce mortality in experimentally infected chickens and to decrease the cell count of *Escherichia coli*, *Salmonella* spp. and coliforms in the small intestine (Izat et al., 1990; Sun et al., 2005; Gunal et al., 2006; Van Immerseel et al., 2006). As pathogens can affect the gastrointestinal barrier function, the use of organic acids might have an impact on tissue physiology. In broiler chickens suffering from enteritis, organic acids used at concentrations of 1.25 and 2.5% significantly reduced colonization of various Enterobacteriaceae resulting in significantly reduced mortality (Roy et al., 2002). Speculatively, the physiological effects of organic acids might therefore depend on the general health status of broiler chickens.

In conclusion, the addition of organic acids to broiler feed had no impact on small intestinal function under the experimental conditions using healthy broilers. The ability of organic acids to sustain physiologic digestive function under different hygienic conditions or in the presence of gastrointestinal pathogens is of high interest and to our knowledge has not been characterized to date.

6.3. Investigating the Impact of Feed Processing on Intestinal Physiology of Laying Hens (Part III)

Part III of this thesis was performed in order to investigate the impact of the milling method, thermal treatment and particle sizes of feed on performance, apparent ileal digestibility, and pH of digesta in laying hens.

The present study shows that the various diets produced by different milling methods and treated with heat had only minor effect on the performance of the animals. The positive impact of feeding mash (and subsequently smaller particle sizes) on feed intake observed in week one and two were limited to this time period. Since the chickens were obtained from a commercial hatchery, hens were fed mash feed before assignment to this study. The reduced intake of expanded feed might be caused by the sudden change of feed form. The adaption period of one week might not have been sufficient to fully adapt the hens on the new housing conditions. Long-term studies by other investigators found no influence of feed confectioning on animal performance. However, one study observed that feed intake correlated with higher particle sizes (Safaa et al., 2009). In broilers, the effect of coarse feed particles on intestinal physiology was described and included higher gizzard weight and improved animal performance. These findings have resulted in recommendations for feed particle sizes (Nir et al., 1994; Denstadli et al., 2010). One recommendation is, that at least 20 % of the cereal particles should be larger than 1.5-2.0 mm (Svihus et al., 2011). In broilers, pelleting and thermal treatment of feed is used frequently. The pellet structure provides a higher energy density compared to mash feed and broilers fed with pellets are known to consume more feed resulting in an increased body weight (Svihus and Hetland, 2001). Laying hens increase energy intake with pelleted feed resulting in luxury consumption and higher body weight gain (Safaa et al., 2009). Only one study could demonstrate an impact of the milling method on the feed intake (Amad, 2001). The investigators showed that 27 day old broilers fed with particles obtained with a roller mill had a lower feed intake than broilers fed with feed of the same particle size obtained with a hammer mill. The underlying cause of feed structure influencing feed intake was assumed to be the effects on energy density when further feed processing such as pelleting is performed (Amad, 2001).

The positive correlation of apparent ileal digestibility of starch with dMEAN analyzed by wet sieving is in agreement with previous observations that showed that

starch digestibility was improved by feeding coarse particles (Svihus et al., 2001; Svihus et al., 2004a; Denstadli et al., 2010). In broilers, coarse feed particles are known to stimulate gizzard activity, resulting in larger amounts of small particles of the digesta in the small intestine (Hetland et al., 2002). In contrast, smaller feed particles lead to minor stimulation of the gizzard activity, which consequently increased larger particle fractions in the digesta (Hetland et al., 2002; Amerah et al., 2007a; Amerah et al., 2008;). In contrast to fine particles, the intake of coarse feed particles increases not only the gizzard weight but also the pancreas mass, indicating increased digestive activity and enzyme secretory capacity (Williams et al., 2008). However, studies regarding the effect of particle size on starch digestibility in broilers revealed equivocal results. Kilburn and Edwards (2001) compared feed based on corn with an average particle size of 1 or 3.5 mm. Under these conditions, ileal starch digestibility was significantly increased in feed with smaller particle sizes. Peron et al. (2005) observed similar effects when feeding wheat based diets with particle sizes of 380 μm GMD and 955 μm GMD (dry sieving analysis). The influence of the milling method on starch digestibility was evident in one study: feed produced with a roller mill increased starch digestibility compared to feed obtained with a hammer mill (Svihus et al., 2004). In the present study, we could not observe milling effects on starch digestibility. It has been demonstrated, that not only the particle sizes, but also the use of whole grain instead of crushed grain seems to have an impact on the apparent starch digestibility. Several studies have been performed in broilers demonstrating that feeding whole grains subsequently resulted in higher grinding intensity of the ingesta in the gizzard compared to feed with processed grains (Svihus and Hetland 2001; Hetland et al., 2002; Svihus et al., 2004a; Svihus et al., 2010).

Starch digestibility is additionally determined by other factors, including digesta viscosity. Non-starch polysaccharides are mainly responsible for the impact of different cereals on the viscosity of the digesta (Choct and Anison, 1990). The viscosity of intact wheat decreased the ileal digesta viscosity in one study (Yasar, 2003), other investigators found a significant increase in ileal viscosity when intact or coarse wheat was fed compared to fine particle sizes (Engberg et al., 2004). In the present study, viscosity of feed was analyzed. Feed containing coarse particle sizes indicated by higher dMEAN obtained by wet sieving analysis had a lower extract viscosity compared to feed variants comprised of finer particles (table 7). This might be an additional explanatory factor why coarse feed with lower viscosity improves starch availability and subsequently higher starch digestibility. However, the viscosity of the digesta of birds from the present study was not determined. Additionally, the effect on the ileal viscosity is also related to the dietary ingredients. A high content of soluble non-starch polysaccharides is considered as most important antinutritional factor. Varieties of wheat and barley are known to increase gut viscosity, subsequently leading to negative effects on body weight gain and feed conversion (Peisker, 2006). In the present study, the diets were based on wheat and corn. Corn, in contrast to other cereals, is considered as a nutrient source that does not increase the digesta viscosity (Carre' et al., 2013). Therefore, effects on starch digestibility might have been more pronounced in diets based on cereals having more non-starch polysaccharides. Digesta viscosity is also influenced by the intake of heat-treated feed (Sun et al., 2013). Depending on the processing conditions (duration, water saturation, temperature), thermal treatment of feed is known to affect starch gelatinization and might be the reason why the digestibility of starch was decreased in chickens fed with the expanded diet in this study. Starch digestibility was improved in previous studies using feed produced with a roller mill compared with feed produced with a hammer mill (Svihus et al., 2004). Higher apparent ileal digestibility of proteins and of the essential amino acids threonine, methionine and cysteine was

observed in broilers fed with feed produced with a roller mill compared to broilers fed with feed produced with a hammer mill (Amad et al., 2001). This finding may be explained by obtaining different particle size distributions and particle shapes from the various milling methods. The spectrum of particle sizes influences not only the feed digestibility by endogenous enzymes, but also the pellet stability, which subsequently affects feed intake and the feed conversion ratio (Briggs et al., 1999; Fairfield, 2003; Svihus et al., 2004). Similar to starch, the protein digestibility is not only influenced by particle size rather than by particle size in combination with the ingredients used in the diet. For example, coarse maize particles induced an improved ileal amino acid digestibility compared to fine maize particles, while soybean meal was more efficiently utilized as fine particles (Ganzer et al., 2007). In the present study, the apparent ileal digestibility of crude protein was not affected by the milling methods, thermal treatments or particle sizes.

The ileal digestibility of fat is also known to be influenced by feed particle size. In a study with broilers, fine particles significantly increased the ileal fat digestibility by 5% (Jia and Slominski, 2010). These observations have been confirmed by other investigators (Stolle et al., 2011). An intense milling process resulting in higher release of cellular fat content that would otherwise be preserved by cell wall fraction of the grains can be responsible for these effects. The results of this thesis are in agreement with the previous studies, as apparent ileal digestibility of ether extract correlated negatively with increased dMEAN obtained by wet sieving analysis.

The results of this thesis indicate, that thermal treatments had only occasionally effects on pH of digesta. While the effect of smaller feed particles on the pH of the digesta has been described in the literature, contrary findings were observed in the present study based on the positive correlation of dMEAN to increasing pH of the digesta (Nir et al., 1994; Huang et al., 2006). In general, the acidification of feed and intestinal content is considered to be beneficial as it may reduce colonization of pathogens (Engberg et al., 2002; Engberg et al., 2004; Bjerrum et al., 2005). Comparing the pH of the gizzard contents of hens in this study (pH 4-5) to the pH of the gizzard content of hens from previous studies, similar values could be obtained (Mirzaie et al., 2012). The high buffering capacity of the calcium carbonate added to the diets as required for laying hens may be held responsible for the relatively high pH observed in laying hens and the inconsistent ability to be influenced by coarse feed particle size.

Comparing the results of various studies previously investigated to data obtained from the present study remains challenging in many ways. Sieve analysis is a common method for investigating particle size distribution (Ehle, 1984; Giger-Reverdin, 2000). However, no standardized method for feed particle size analysis is validated to date. The lack of a standardized method challenges the comparison of particle sizes of feed or digesta between studies and experiments from different investigators. For broilers, feed is heat treated which results in conglomerated particles of various sizes forming a larger pellet. As a consequence, effects of feed particle size on the physiological digestive function should be related closer to particle sizes obtained by wet sieving analysis. Results obtained from the wet sieving analysis uncovered an additional shearing of the particles caused by the extrusion process resulting in lower dMEAN values for the expanded feed variants (table 7). In order to avoid subjective classification of feed in "fine" and "coarse" particles, we decided to correlate the results to a discrete value characterized by dMEAN. However, the use of dMEAN reduces the complex information of particle size distribution to a single value and could have been evaded by characterizing the feed using frequency plots or cumulative curves of particle sizes. Each milling method results in a characteristic spectrum of particle size distributions and particle shapes.

By obtaining feed of similar particle sizes by different milling methods, we tried to overcome the disadvantage using characteristic dMEAN values.

The laying hens used in this study were also evaluated for ileal glucose uptake via Ussing chamber experiments, as well as for their changes in intestinal organ weight and intestinal microstructure (Röhe, 2014; Röhe et al., 2014 - *in press*). The results of the present study correlate to the concurrent investigations: For example, while this thesis revealed a beneficial impact of coarse particle size on starch digestibility, Röhe et al., demonstrated enhanced intestinal glucose uptake in those animals. Furthermore, the access to coarse feed particles significantly increased gizzard and pancreatic organ weight (Röhe et al., 2014 - *in press*). Therefore, one may assume, that the beneficial impact of coarse particles on the intestinal barrier function may be comparable to the effects observed in broiler chickens (Huang et al., 2006; Williams et al., 2008; Svihus et al., 2011).

In conclusion, the results of part III of this thesis indicate, that unconventional energy-saving milling methods may be used for the feed production of laying hens without affecting animal performance. As mash feed with inherently coarser structure improved the apparent ileal starch digestibility, further studies regarding the impact feed particle size on the gastrointestinal physiology of laying hens are warranted.

6.4. Conclusion

In conclusion, the Ussing chamber technique was successfully modified for the specific conditions in the poultry intestine, which allowed the evaluation of the effects of acidified feed in broiler chickens. The use of 1.5 % organic acids in broiler chickens had no negative impact on intestinal barrier function, starch digestibility or glucose uptake, and can be considered as safe. In laying hens, the use of coarse particle sizes was beneficial for ileal starch digestibility, whereas the milling method had only minor impact on the parameters investigated. Therefore, unconventional milling methods may be used without negatively impacting laying performance and the digestive function of laying hens.

7. Summary

Summary of the PhD thesis “**Studies on the Impact of Acidified Feed and Feed Processing on Digestive Physiology in Broiler Chickens and Laying Hens**”.

The intestinal tract of chickens is characterized by efficient nutrient digestion. Disturbances of this fragile system results frequently in reduced animal health and welfare and subsequently increased use of antibiotics. Feed additives and various feed processing technologies can be used in order to affect intestinal function of the chicken. The goal of this study was to study the impact of acidification and thermal treatment of feed, the use of various feed particle sizes, and the impact of milling methods on intestinal physiology of poultry.

The aims of this thesis were:

- I) to adapt the Ussing chamber method for reliable assessment of transport physiology of the intestinal mucosa of poultry,
- II) to evaluate the impact of acidified feed on apparent ileal digestibility of starch, the glucose transporter expression, and intestinal transport function in broiler chickens,
- III) to investigate the impact of the milling method, thermal treatment and particle size of feed on performance, apparent ileal nutrient digestibility, and pH of digesta in laying hens.

Part I was performed by stripping the tunica mucosa from a total of 104 jejunal samples from 10 hens and using a net device for stabilizing support. In order to investigate the benefit of glutamine, 50 samples were maintained with a conventional buffer (group 1), and 54 samples were maintained with additional 5 mM glutamine (group 2). Mean short circuit current (ΔI_{sc}) of samples exposed to 10 mM glucose in group 1 and group 2 was $17.0 \pm 2.47 \mu A/cm^2$ and $14.6 \pm 1.54 \mu A/cm^2$ ($p = 0.836$), of samples exposed to 100 μM phloridzin $-13.3 \pm 1.63 \mu A/cm^2$, and $-11.8 \pm 1.22 \mu A/cm^2$ ($p = 0.712$) and of samples exposed to 100 μM carbachol $4.7 \pm 0.70 \mu A/cm^2$, and $3.7 \pm 0.43 \mu A/cm^2$ ($p = 0.450$), respectively. While glutamine had no beneficial effect on tissue response, the method described here allowed for reliable investigation of the intestine.

Part II of this thesis was performed by feeding broilers a standard diet without or with 1.5 % Lupro-Cid[®] (64 % formic acid, 25 % propionic acid, 11 % water) for 32-35 days. Apparent ileal starch digestibility was determined using 0.2 % titanium dioxide as indigestible marker. Intestinal transporter function was analyzed in Ussing chamber experiments where jejunal samples were sequentially exposed to 10 mM glucose, 100 μM phloridzin, 100 μM histamine, and 100 μM carbachol. Additionally, gene expressions of SGLT-1 and GLUT-2 transporters were analyzed using qPCR analysis. Apparent ileal starch digestibility of the control group ($97.5 \pm 0.35 \%$) and the acid-treated group ($97.0 \pm 0.59 \%$) did not differ significantly ($p = 0.674$). Tissue conductance of intestinal samples obtained from the control group and the acid-treated group was $10.6 \pm 0.68 mS/cm^2$, and $9.4 \pm 0.80 mS/cm^2$, respectively ($p = 0.147$). The mean short circuit currents (ΔI_{sc}) of the samples exposed to glucose, phloridzin, histamine, and carbachol did not differ ($p = 0.209$, $p = 0.296$, $p = 0.498$, and $p = 0.640$, respectively). Additionally, no differences in the expression of SGLT-1 and GLUT-2 could be observed ($p = 0.942$, $p = 0.431$).

Part III of this thesis was performed using twelve variants of a complete diet that were produced by four different milling techniques including hammer mill, roller mill,

disc mill, and a wedge-shaped disc mill. Coarse feed particles were obtained from all mills, additional fine feed particles were obtained from the hammer mill and the roller mill. Each of this six feed variants was offered as mash or expandate, resulting in twelve treatments in total. Laying hens at 20 weeks of age were kept for three weeks in eight replicates with six hens per feeding group. Performance parameters (feed consumption, laying performance, feed conversion ratio) and apparent ileal digestibility of crude protein, amino acids, ether extract, and starch were determined. The pH of the digesta was determined along the gastrointestinal tract. In week 1 and 2 of the experiment, feed intake was significantly increased in hens fed with mash feed compared to hens fed expanded feed ($p = 0.003$ and $p = 0.023$). The apparent ileal digestibility of starch was improved ($p < 0.001$) by feeding mash and correlated positively to increased particle size (p -linear = 0.001; p -quadratic = 0.006; $r = 0.363$). The apparent ileal digestibility of ether extract correlated negatively to increased particle sizes (p -linear = 0.003; p -quadratic = 0.225; $r = -0.311$). The pH of the ileum content (p -linear = 0.044; p -quadratic = 0.146; $r = 0.212$) and feces (p -linear = 0.010; p -quadratic = 0.016; $r = 0.267$) correlated with increased particle sizes.

Based on the results of this thesis, organic acids had no impact on ileal starch digestibility and intestinal nutrient uptake. Therefore, the investigation of the mode of action needs further studies. Feeding coarse particle sizes improved ileal starch digestibility. Unconventional, energy saving milling methods enabling coarse particle sizes of feed may be used without negative impact on laying performance and the digestive function of laying hens.

8. Zusammenfassung

Zusammenfassung der PhD-Arbeit „**Studien über den Einfluss von organischen Säuren sowie der Futtermittelverarbeitung auf verdauungsphysiologische Parameter bei Broilern und Legehennen**“.

Der Verdauungstrakt des Geflügels zeichnet sich durch eine effiziente Nährstoffnutzung aus. Durch Störungen kommt es zu unerwünschten Konsequenzen für die Tiergesundheit und das Wohlbefinden, sie können den Einsatz von Antibiotika erforderlich machen. Futteradditive und technologische Verfahren können Einfluss auf den Gesundheitsstatus von Hühnern nehmen. In vorliegender Dissertation sollte die Verwendung von organischen Säuren, der Hitzebehandlung des Futters, die Darreichung verschiedener Futterpartikelgrößen, sowie der Einsatz von verschiedenen Mahlmethode zur Futterherstellung untersucht werden. Der Einfluss dieser Maßnahmen auf die Physiologie des Magen-Darm-Traktes des Geflügels ist bisher nicht umfassend untersucht worden.

Das Ziel dieser Arbeit war daher:

- I) die Ussingkammermethode zur Charakterisierung von Absorptions- und Sekretionsprozessen im Geflügeldarm zu verbessern,
- II) den Einfluss von organischen Säuren als Futteradditive auf die ileale Stärkeverdaulichkeit, die intestinale Expression von Glukosetransportern sowie die jejunale Transport- und Barrierefunktion zu erfassen,
- III) die Bedeutung verschiedener Mahlmethode, der Hitzebehandlung des Futters sowie der Futterpartikelgröße auf die Leistung, die scheinbare ileale Nährstoffverdaulichkeit und den pH der Digesta bei Legehennen zu bestimmen.

Teil I der Fragestellung wurde durchgeführt, indem die Tunica Mucosa von insgesamt 104 Jejunumproben aus 10 Legehennen mit einem Netz stabilisiert und in die Ussingkammer verbracht wurden. Während 50 Proben mit einem konventionellen Puffer behandelt wurden (Gruppe 1), wurde 54 Proben zusätzlich 5 mM Glutamin zugesetzt (Gruppe 2). Nach Zugabe von 10 mM Glukose betrug die durchschnittliche Höhe des Kurzschlussstromes (ΔI_{sc}) der Gruppe 1 $17,0 \pm 2,47 \mu A/cm^2$ und der Gruppe 2 $14,6 \pm 1,54 \mu A/cm^2$ ($p = 0,836$). Nach Zugabe von 100 μM Phloridzin betrug der ΔI_{sc} $-13,3 \pm 1,63 \mu A/cm^2$ bzw. $-11,8 \pm 1,22 \mu A/cm^2$ ($p = 0,712$), nach Zugabe von 100 μM Carbachol $4,7 \pm 0,70 \mu A/cm^2$ bzw. $3,7 \pm 0,43 \mu A/cm^2$ ($p = 0,450$).

Teil II der Dissertation wurde durchgeführt, indem Masthähnchen einer Kontrollgruppe mit Masthähnchen einer Versuchsgruppe verglichen wurden. Die Tiere der Versuchsgruppe hatten während der gesamten Aufzucht von 32-35 Tagen 1,5 % Lupro-Cid® (64 % Ameisensäure, 25 % Propionsäure, 11 % Wasser) über das Futter aufgenommen. Die ileale Stärkeverdaulichkeit wurde mittels 0,2 % Titandioxid ermittelt. Die jejunale transmembranale Transporterfunktion wurde mittels Ussingkammermethode bestimmt, indem die Darmproben nacheinander mit 10 mM Glukose, 100 μM Phloridzin, 100 μM Histamin und 100 μM Carbachol behandelt wurden. Zusätzlich wurde die Genexpression der Glukosetransporter SGLT-1 und GLUT-2 mittels qPCR-Analyse bestimmt. Die scheinbare ileale Stärkeverdaulichkeit der Kontrollgruppe betrug ($97,5 \pm 0,35 \%$), die der Säuregruppe ($96,0 \pm 0,59 \%$). Der Unterschied war statistisch nicht signifikant ($p = 0,674$). Die mittlere ($\pm SEM$) Leitfähigkeit des Gewebes betrug für die Kontroll- und Säuregruppe $10,6 \pm 0,68 mS/cm^2$, bzw. $9,4 \pm 0,80 mS/cm^2$ ($p = 0,147$). Die mittleren Kurzschlussströme (ΔI_{sc})

der Gruppen nach Behandlung mit Glucose, Phloridzin, Histamin und Carbachol waren statistisch nicht signifikant unterschiedlich ($p = 0,209$, $p = 0,296$, $p = 0,498$ und $p = 0,640$). Weiterhin konnten keine Unterschiede in der relativen Genexpression der SGLT-1 und GLUT-2 Transporter nachgewiesen werden ($p = 0,942$, $p = 0,431$).

Teil III der Dissertation wurde ausgeführt, indem zwölf Varianten derselben Futterrezeptur mit vier Mahlmethode (Hammermühle, Walzenstuhl, Scheibenmühle, Keilscheibenzerkleinerer) hergestellt wurden. Die Mühlen wurden so eingesetzt, dass relativ grobe Partikelgrößen hergestellt wurden. Zusätzlich wurden mittels Hammermühle und Walzenstuhl feine Futterpartikel gefertigt. Jede der sechs Varianten wurde entweder als Mehl oder als Expandat verfüttert, wodurch insgesamt 12 Versuchsgruppen entstanden.

Legehennen im Alter von 20 Wochen wurden für jeweils drei Wochen mit dem Versuchsfutter gefüttert. Dazu wurden in acht Versuchsdurchgängen je sechs Hennen/Gruppe gehalten. Neben den Leistungsdaten (Futteraufnahme, Legeleistung, Futteraufwand) wurde die scheinbare ileale Verdaulichkeit von Rohprotein, Aminosäuren, Rohfett und Stärke sowie der pH-Wert der Digesta in verschiedenen Abschnitten des Magen-Darm-Traktes ermittelt. Während der ersten beiden Versuchswoche war die Futteraufnahme der Hühner bei Gabe des mehlartigen Futters höher verglichen mit den Hühnern, die expandiertes Futter erhielten ($p = 0,003$; $p = 0,023$). Die scheinbare ileale Verdaulichkeit der Stärke war bei den Hühnern, die mehlartiges Futter erhielten, gegenüber Expandat erhöht ($p < 0,001$) und korrelierte positiv mit der Partikelgröße des Futters (p -linear = $0,001$; p -quadratisch = $0,006$; $r = 0,363$). Die scheinbare ileale Verdaulichkeit von Rohfett korrelierte hingegen negativ mit der Partikelgröße (p -linear = $0,003$; p -quadrat = $0,225$; $r = -0,311$). Auch der pH-Wert des Ileuminhaltes (p -linear = $0,044$; p -quadrat = $0,146$; $r = 0,212$) und der Exkremete (p -linear = $0,010$; p -quadratisch = $0,016$; $p = 0,267$) korrelierte positiv mit der Partikelgröße.

Als Schlussfolgerung ergibt sich aus den durchgeführten Studien, dass hinsichtlich der in der Literatur beschriebenen positiven Wirkungen organischer Säuren als Futterzusatzstoffe hinsichtlich der physiologischen Mechanismen weiterer Untersuchungsbedarf besteht, da sich unter den vorliegenden experimentellen Bedingungen kein Effekt auf die Stärkeverdaulichkeit und die intestinale Nährstofftransportfunktion abzeichnete. Es konnte bestätigt werden, dass eine gröbere Futterstruktur unabhängig von der Zerkleinerungsmethode günstige Effekte auf die scheinbare Stärkeverdaulichkeit bei Legehennen hatte. Insofern sollten innovative energiesparende Mahlverfahren weiter entwickelt werden, die gleichzeitig eine gröbere Partikelstruktur ermöglichen. Die ernährungsphysiologischen Konsequenzen sollten bei technologischen Weiterentwicklungen überprüft werden.

9. References

Achinewhu SC, Hewitt D. (1979). Assessment of the nutritional quality of proteins: the use of ileal digestibilities of amino acids as measures of their availabilities. *British Journal of Nutrition*; **41**(3): 559-571.

Alakomi HL, Skyttä E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology*; **66**(5): 2001-2005.

Amad AA. (2001). Zum Einfluss unterschiedlicher Behandlungsverfahren und Zusatzstoffe auf ernährungsphysiologische Parameter und Leistung wachsender Broiler nach Verabreichung weizenbetonter Futtermischungen. [German] *Dissertation; Georg-August-Universität Göttingen Germany*.

Amat C, Piqueras JA, Planas JM, Moreto M. (1999). Electrical properties of the intestinal mucosa of the chicken and the effects of luminal glucose. *Poultry Science*; **78**(8): 1126-1131.

Amerah AM, Ravindran V, Lentle RG, Thomas DG. (2007a). Feed particle size: Implications on the digestion and performance of poultry. *World's Poultry Science Journal*; **63**: 439-453.

Amerah AM, Ravindran V, Lentle RG, Thomas DG. (2007b). Influence of feed particle size and feed form on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters. *Poultry Science*; **86**(12): 2615-2623.

Amerah AM, Ravindran V, Lentle RG, Thomas DG. (2008). Influence of feed particle size on the performance, energy utilization, digestive tract development and digesta parameters of broiler starters fed wheat- and corn-based diets. *Poultry Science*; **87**(11): 2320-2328.

Amerah AM, Ravindran V, Lentle RG. (2009). Influence of insoluble fiber and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. *British Poultry Science*; **50**(3): 366-375.

Amezcuca CM, Parsons CM. (2007). Effect of increased heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles. *Poultry Science*; **86**(2): 331-337.

ASABE. (2008). Method of determining and expressing fineness of feed materials by sieving. American Society of Agricultural and Biologicals Engineers Standard S319.4. *Yearbook of Standards, American Society of Agricultural and Biologicals Engineers, St. Joseph, MO, USA*.

ASAE. (1983). Method of determining and expressing fineness of feed materials by sieving. American Society of Agricultural and Biologicals Engineers Standard S319.2. *Yearbook of Standards, American Society of Agricultural Engineers, St. Joseph, MO, USA*.

- Audet L. (1995). Emerging feed mill technology: keeping competitive. *Journal of Animal Feed Science and Technology*; **53**: 157-170.
- Awad WA, Rehman H, Bohm J, Razzazi-Fazeli E, Zentek J. (2005). Effects of luminal deoxynivalenol and L-proline on electrophysiological parameters in the jejunums of laying hens. *Poultry Science*; **84**(6): 928-932.
- Awad WA, Razzazi-Fazeli E, Bohm J, Zentek J. (2007). Influence of deoxynivalenol on the D-glucose transport across the isolated epithelium of different intestinal segments of laying hens. *Journal of Animal Physiology and Animal Nutrition*; **91**(5-6): 175-180.
- Axe DE. (1995). Factors affecting uniformity of a mix. *Animal Feed Science and Technology*; **53**(2): 211-220.
- Bartell SM, Batal AB. (2007). The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract, and humoral immune response of broilers. *Poultry Science*; **86**(9): 1940-1947.
- Behnke KC. (2001). Factors influencing pellet quality. *Feed Technology*; **5**(4): 19-22.
- Behnke KC, Beyer RS. (2002). Effect of feed processing on broiler performance. *VIII. Seminar on Poultry Production and Pathology, Santiago, Chile*.
- Betscher S, Beineke A, Schönfeld L, Kamphues J. (2010). Effects of diet's physical form (grinding intensity; meal/pellets) on morphological and histological parameters (e.g. ratio of neutral to acid mucins) of the gastrointestinal tract in weaned piglets. *Livestock Science*; **134**(1-3): 149-151.
- Bjerrum L, Pedersen K, Engberg RM. (2005). The influence of whole wheat feeding on Salmonella infection and gut flora composition in broilers. *Avian Diseases*; **49**(1): 9-15.
- Briggs JL, Maier DE, Watkins BA, Behnke KC. (1999). Effect of ingredients and processing parameters on pellet quality. *Poultry Science*; **78**(10): 1464-1471.
- Brunner E, Domhof S, Langer F. (2002). Nonparametric analysis of longitudinal data in factorial experiments, Wiley, New York.
- Carre´ B, Lessire M, Juin H. (2013). Prediction of metabolizable energy value of broiler diets and water excretion from dietary chemical analyses. *Animal*; **7**(8): 1246-1258.
- Chaveerach P, Keuzenkamp DA, Urlings HAP, Lipman LJ, van Knapen F. (2002). *In vitro* study on the effect of organic acids on campylobacter jejuni/coli populations in mixtures of water and feed. *Poultry Sciences*; **81**(5): 621-628.
- Choct M. (2009). Managing gut health through nutrition. *British Poultry Science*; **50**(1): 9-15.
- Choct M, Annison G. (1990). Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science*; **31**(4): 811-821.

- Clarke LL. (2009). A guide to Ussing chamber studies of mouse intestine. *American Journal of Physiology Gastrointestinal and Liver Physiology*; **296**(6): G1151-1166.
- Clauss M, Lechner-Doll M, Streich WJ. (2002). Faecal particle size distribution in captive wild ruminants: an approach to the browser/grazer-dichotomy from the other end. *Oecologia* **131**: 343-349.
- Collins CB, McGrath J, Baird AW, Campion DP. (2007). Effect of mast cell degranulation on chicken ileal ion transport in vitro. *Poultry Science*; **86**(5): 843-849.
- Dänicke S, Kracht W, Jeroch H, Zachmann R, Heidenreich E, Löwe R. (1998). Effect of different technical treatments of rapeseed on the feed value for broilers and laying hens. *Archives of Animal Nutrition*; **51**(1): 53-62.
- Dahlke F, Ribero AML, Kessler AM, Lima AR, Malonka A. (2003). Effects of corn particle size and physical form of the diet on the gastrointestinal structures of broiler chickens. *Brazilian Journal of Poultry Science*; **5**(1): 61-67.
- Dai SF, Wang LK, Wen AY, Wang LX, Jin GM. (2009). Dietary glutamine supplementation improves growth performance, meat quality and color stability of broilers under heat stress. *British Poultry Science*; **50**(3): 333-340.
- Daveby YD, Razdan A, Aman P. (1998). Effect of particle size and enzyme supplementation of diets based on dehulled peas on the nutritive value for broiler chickens. *Animal Feed Science and Technology*; **74**(3): 229-239.
- Davidson PM. (2001). Chemical Preservatives and natural antimicrobial compounds. In: *Food microbiology-Fundamentals and Frontiers*; 2nd ed. Doyle ED, Beuchat LR, Montville TJ; American Society for Microbiology, Washington, DC: 593-627.
- Denli M, Okan F, Çelik K. (2003). Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. *Pakistan Journal of Nutrition*; **2**(2): 89-91.
- Denstadli V, Balance S, Knutsen SH, Westereng B, Svihus B. (2010). Influence of graded levels of brewers dried grains on pellet quality and performance in broiler chickens. *Poultry Science*; **89**(12): 2640-2645.
- Dibner JJ, Buttin P. (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *The Journal of Applied Poultry Research*; **11**(4): 453-463.
- Ding MX, Yuan ZH, Wang YL, Zhu HL, Fan SX. (2006). Olaquinox and cyadox stimulate growth and decrease intestinal mucosal immunity of piglets orally inoculated with *Escherichia coli*. *Journal of Animal Physiology and Animal Nutrition*; **90**(5-6): 238-243.
- Douglas JH, Sullivan TW, Bond PL, Struwe FJ, Baier JG, Robeson LG. (1990). Influence of grinding, rolling, and pelleting on the nutritional value of grain sorghums and yellow corn for broilers. *Poultry Science*; **69**(12): 2150-2156.

- doVale MM, Menten FJM, deMorais SCD, deAlmeida Brainer MM. (2004). Mixture of formic and propionic acid as additives in broiler feeds. *Scientia Agricola* (Piracicaba, Brazilia); **61**(4): 371-375.
- Dozier WA, Behnke KC, Kidd MT, Branton SL. (2006). Effects of the addition of roller mill ground corn to pelleted feed on pelleting parameters, broiler performance, and intestinal strength research. *Journal of Applied Poultry Research*; **15**(2): 236-244.
- Drozdowski LA, Thomson AB. (2006). Intestinal sugar transport. *World Journal of Gastroenterology*; **12**(11): 1657-1670.
- Ehle FR. (1984). Influence of particle size on determination of fibrous feed components. *Journal of Dairy Science*; **67**(7): 1482-1488.
- Engberg RM, Hedemann MS, Jensen BB. (2002). The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British Poultry Science*; **43**(4): 569-579.
- Engberg RM, Hedemann MS, Steinfeldt S, Jensen BB. (2004). Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poultry Science*; **83**(6): 925-938.
- Engelhardt W, Breves G. (2009). Chymuspassage und Verweilzeit, Passage von Futter durch den Magen-Darm-Kanal. [German] *Physiologie der Haustiere*, 3. Auflage, Enke-Verlag, Stuttgart: 377, 449.
- Fancher BI, Rollins D, Trimbee B. (1996). Feed processing using the annular gap expander and its impact on poultry performance. *The Journal of Applied Poultry Research*; **5**(4): 386-394.
- Fairfield DA. (2003). Pelleting for profit-Part 1. *National Grain and Feed Association: Feed and Feed Digest*; **54**(6): 1-5.
- Fernandez SR, Zhang Y, Parsons CM. (1994). Effect of overheating on the nutritional quality of cottonseed meal. *Poultry Science*; **73**(10):1563-71.
- Ferrer R, Gil M, Moreto M, Oliveras M, Planas JM. (1994). Hexose-transport across the apical and basolateral membrane of enterocytes from different regions of the chicken intestine. *Pflügers Archive*; **426**(1-2): 83-88.
- Fleming SE, Zambell KL, Fitch MD. (1997). Glucose and glutamine provide similar proportions of energy to mucosal cells of rat small intestine. *American Journal of Physiology, Gastrointestinal and Liver Physiology*; **273**(4): G968-G978.
- Fritz J., Steich WJ, Schwarm A, Clauss M. (2011). Condensing results of wet sieving analyses into a single data: a comparison of methods for particle size description. *Journal of Animal Physiology and Animal Nutrition*; **95**(5): 783-797.
- Ganzer C, Kluth H, Rodehutscord M. (2007). Effect of particle size on praececal digestibility on amino acids from maize and soybean meal in broilers. *Proceedings of the 16th European Symposium of Poultry Nutrition*; 459-462.

GfE. (1999). Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler) [German]; *Frankfurt/Main, DLG-Verlag*.

Giger-Reverdin S. (2000). Characterization of feedstuffs for ruminants using some physical parameters. *Animal Feed Science and Technology*; **86**(1): 53-69.

Gilbert ER, Li H, Emmerson DA, Webb Jr KE, Wong EA. (2007). Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. *Poultry Science*; **86**(8): 1739-1753.

Goihl J. (1995). Effect of mill type, particle size on finishing pigs growth evaluated. *Feedstuffs*; **67**: 13-17.

Goodarzi Borojoni F, Vahjen W, Mader A, Knorr F, Ruhnke I, Röhe I, Hafeez A, Villodre C, Männer K, Zentek J. (2014a). Effect of different thermal treatments and organic acid levels in feed on microbial composition and activity in gastrointestinal tract of broilers. *Poultry Science- in press*.

Goodarzi Borojoni F, Mader A, Knorr F, Ruhnke I, Röhe I, Hafeez A, Männer K, Zentek J. (2014b). The effects of different thermal treatments and organic acid levels on nutrient digestibility in broilers. *Poultry Science*; **93**(5): 1159-1171.

Grosse Liesner V, Taube V, Leonhard-Marek S, Beinecke A, Kamphues J. (2009). Integrity of gastric mucosa in reared piglets- effects of physical form of diets (meal/pellets), pre-processing grinding (coarse/fine) and addition of lignocellulose (0/2.5%). *Journal of Animal Physiology and Animal Nutrition*; **93**(3): 373-380.

Gunal M, Yayli G, Kaya O, Karahan N, Sulak O. (2006). The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *International Journal of Poultry Science*; **5**: 149-155.

Hamilton RMG, Proudfoot FG. (1995). Ingredient particle size and feed texture: effects on the performance of broiler chickens. *Animal Feed Science and Technology*; **51**(3): 203-210.

Harada E, Niiyama M, Syuto B. (1986). Comparison of pancreatic exocrine secretion via endogenous secretin by intestinal infusion of hydrochloric acid and monocarboxylic acid in anesthetized piglets. *The Japanese Journal of Physiology*, **36**(5): 843-856.

Hartini S, Choct M, Hinch G, Nolan JV. (2002). Effect of diet composition and feed form on the behavior of ISA brown laying hens. *Proceedings of the Australian Poultry Science Symposium*; **4**: 104-110.

Heidenreich E, Michaelsen T. (1995). Extrudieren und Expandieren für die Mischfutterherstellung. [German] *Mühle und Mischfuttertechnik*; **132**: 794-798.

Heidenreich E, Michaelsen T. (2001). Mash pasteurization and drying: principles and application. In: *Advances in Nutritional Technology. Proceedings of the 1st World Feed Conference*; 97-108.

- Heidenreich E, Sundermeier T. (1996). On-line determination of particle size distribution during the manufacture of compound feed. *Particle and Particle System Characterization*; **13**: 309-312.
- Henry PR, Ammerman CB, Campbell DR, Miles RD. (1987). The effects of antibiotics on tissue trace mineral concentration and intestinal tract weight of broiler chicks. *Poultry Science*; **66**(6): 1014-1018.
- Hernández H, Garcíá V, Madrid J, Orengo J, Catalá P, Megías MD. (2006). Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. *British Poultry Science*; **47**(1): 50-56.
- Hetland H, Svihus B. (2001). Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British Poultry Science*; **42**(3): 354-361.
- Hetland H, Svihus B, Choct M. (2005). Role of insoluble fiber on gizzard activity in layers. *Journal of Applied Poultry Science Research*; **14**: 38-46.
- Hetland H, Svihus B, Krogdahl A. (2003). Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *British Poultry Science*; **44**(2): 275-282.
- Hetland H, Svihus B, Olaisen V. (2002). Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *British Poultry Science*; **43**(3): 416-423.
- Hoffmann T, Füll C, Hempel H. (2011). Energiesparendes Zerkleinern von Getreide mit einem Keilscheibenzerkleinerer. [German] *Chemie Ingenieur Technik*; **83**(5): 699-703.
- Huang DS, Li DF, Xing JJ, Ma YX, Li ZJ, Lv SQ. (2006). Effects of feed particle size and feed form on survival of *Salmonella Typhimurium* in the alimentary tract and *Salmonella Typhimurium* reduction in growing broilers. *Poultry Science*; **85**(5): 831-836.
- Hume ME, Corrier DE, Ivie GW, Deloach JR. (1993). Metabolism of ¹⁴C propionic acid in broiler chicks. *Poultry Science*; **72**(5): 786-793.
- Humphrey BD, Stephensen CB, Calvert CC, Klasing KC. (2004). Glucose and cationic amino acid transporter expression in growing chickens (*Gallus gallus domesticus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*; **138**(4): 515-525.
- Isabel B, Santos Y. (2009). Effects of dietary organic acids and essential oils on growth performance and carcass characteristics of broiler chickens. *The Journal of Applied Poultry Research*; **18**(3): 472-476.
- Izat AL, Tidwell NM, Thomas RA, Reiber MA, Adams MH, Colberg M, Waldroup PW. (1990). Effects of a buffered propionic acid in diets on the performance of broiler chickens and on the microflora of the intestine and carcass. *Poultry Science*; **69**(5): 818-826.

Jämlich K. (2010). Herstellung von Extrudaten als Grundlage für Snackprodukte und gemahlene Zwieback. [German] *Bachelorarbeit; Hochschule Neubrandenburg, University of Applied Sciences, Neubrandenburg, Germany.*

Janardhana V, Broadway MM, Bruce MP, Lowenthal JW, Geier MS, Hughes RJ, Bean AG. (2009). Prebiotics modulate immune responses in the gut-associated lymphoid tissue of chickens. *The Journal of Nutrition*; **139**(7): 1404-1409.

Jia W, Slominski BA. (2010). Means to improve the nutritive value of flaxseed for broiler chickens: The effect of particle size, enzyme addition, and pelleting. *Poultry Science*; **89**(2): 261-269.

Jongbloed AW, Mroz Z, Van der Weij-Jongbloed R, Kemme PA. (2000). The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. *Livestock Production Science*, **67**(1): 113-122.

Kellett GL. (2001). The facilitated component of intestinal glucose absorption. *The Journal of Physiology*; **531**(3): 585-595.

Kilburn J, Edwards HM. (2001). The response of broilers to the feeding of mash or pelleted diets containing maize of varying particle sizes. *British Poultry Science*; **42**(4): 484-492.

Kluth H, Mehlhorn K, Rodehutschort M. (2005). Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. *Archives of Animal Nutrition*; **59**(4): 271-279.

Koch K. (2002). Hammer mills and roller mills, MF-2048. *Feed Manufacturing*; Department of Grain, Science and Industry, Kansas State University: 1-8.

Kraehenbuhl JP, Neutra MR. (1992). Molecular and cellular basis of immune protection of mucosal surfaces. *Physiological Reviews*; **72**(4): 853-879.

Larbier M, Leclercq B. (1994). Nutrition and feeding of poultry. *Ed Julian Wiseman. Nottingham University Press.*

Lee TT, Huang YF, Chiang CC, Chung TK, Chiou PW, Yu B. (2011). Starch characteristics and their influences on in vitro and pig prececal starch digestion. *Journal of Agricultural Food Chemistry*; **59**(13): 7353-7359.

Leibetseder J. (1987). Über die Bedeutung der Mahlfeinheit und Pelletgröße für Futteraufnahme, Verdaulichkeit und Gesundheitsstatus bei Schwein und Geflügel. [German] *Übersichten zur Tierernährung*; **15**: 135-152.

Lennernäs H. (2007). Animal data: the contributions of the Ussing Chamber and perfusion systems to predicting human oral drug delivery in vivo. *Advanced Drug Delivery Reviews*; **59**(11): 1103-1120.

Li H, Sheppard DN, Hug MJ. (2004). Transepithelial electrical measurements with the Ussing chamber. *Journal of Cystic Fibrosis*; **3**(2): 123-126.

Li YP, Bang DD, Handberg KJ, Jorgensen PH, Zhang MF. (2005). Evaluation of the suitability of six host genes as internal control in real-time RT-PCR assays in chicken

- embryo cell cultures infected with infectious bursal disease virus. *Veterinary Microbiology*; **110**(3): 155-165.
- Liem A, Pesti GM, Edwards HM. (2008). The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. *Poultry Science*, **87**(4): 689-693.
- Löwe R. (2007). Judging pellet stability as part of pellet quality. *Forschungsinstitut Futtermitteltechnik (IFF)*; 1-8.
- Löwe R. (2011). Technological aspects of feedstuff comminuting and compacting. *Proceedings of the Society of Nutrition Physiology*; **20**: 147-149.
- Löwe R, Mohrig V. (2013). Verbesserung der technologischen und ernährungsphysiologischen Eigenschaften von Legehennenfutter. [German] *Schlussbericht des Forschungsinstitut Futtermitteltechnik der IFF und des Instituts für Tierernährung der Freien Universität Berlin*; 24-33.
- Logan M, Sanson GD. (2000). A new technique for measuring size distributions of fine ingesta/digesta particles. *Wildlife Research*; **27**(2): 191-194.
- Lundblad, K. (2009). Effect of diet conditioning on physical and nutritional quality of feed for pigs and chickens. *PhD-Thesis; Norwegian University of Life Sciences, Ås, Norway*.
- Lundblad KK, Issa S, Hancock JD, Behnke KC, McKinney LJ, Alavi S, Prestløy E, Fledderus J, Sørensen M. (2011). Effects of steam conditioning at low and high temperature, expander conditioning and extruder processing prior to pelleting on growth performance and nutrient digestibility in nursery pigs and broiler chickens. *Animal Feed Science and Technology*; **169**(3-4): 208-217.
- Manzanilla EG, Nofrarias M, Anguita M, Castillo M, Perez JF, Martin-Orúe SM, Kamel C, Gasa J. (2006). Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. *Journal of Animal Sciences*; **84**(10): 2743-2751.
- Mavromichalis I, Baker DH. (2000). Effects of pelleting and storage of a complex nursery pig diet on lysine bioavailability. *Journal of Animal Sciences*; **78**(2): 341-347.
- Maxwell CV, Reimann EM, Hoekstra WG, Kowalczyk T, Benevenga NJ, Grummer RH. (1970). Effect of dietary particle size on lesion development and on the contents of various regions of the swine stomach. *Journal of Animal Science*; **30**(6): 911-922.
- McCauley RD, Heel KA, Christiansen KJ, Hall JC. (1996). The effect of minimum luminal nutrition on bacterial translocation and atrophy of the jejunum during parenteral nutrition. *Journal of Gastroenterology and Hepatology*; **11**(1): 65-70.
- Miles RD, Butcher GD, Henry PR, Littell RC. (2006). Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. *Poultry Science*; **85**(3):476-485.
- Mirzaie S, Zaghari M, Aminzadeh S, Shivazad M, Mateos GG. (2012). Effect of wheat inclusion and xylanase supplementation of the diet on productive performance,

nutrient retention, and endogenous intestinal enzyme activity of laying hens. *Poultry Science*; **91**(2): 413-425.

Moen B, Rudi K, Svihus B, Skanseng B. (2012). Reduced spread of *Campylobacter jejuni* in broiler chickens by stimulating the bird's natural barriers. *Journal of Applied Microbiology*; **113**: 1176-1183.

Moran ET Jr. (1985). Digestion and absorption in fowl and events through perinatal development. *Journal of Nutrition*; **115**(5): 665-674.

Mossel DAA, Van Schothorst M, Kampelmacher EH. (1967). Comparative study on decontamination of mixed feeds by radication and by pelletisation. *Journal of the Science of Food and Agriculture*; **18**(8): 362-367.

Naumann K, Bassler R. (2004). Methodenbuch Band III: Die chemische Untersuchung von Futtermitteln. Melsungen [German]: Neumann-Neudamm.

Neirinckx E, Vervaeet C, Michiels J, DeSmet S, VandenBroeck W, Remon JP, DeBacker P, Croubles S. (2011). Feasibility of the Ussing chamber technique for the determination of in vitro jejunal permeability of passively absorbed compounds in different animal species. *Journal of Veterinary Pharmacology Therapy*; **34**(3): 290-297.

Newsholme P, Procopio J, Lima MMR, Pithon-Curi TC, Curi R. (2003). Glutamine and glutamate - their central role in cell metabolism and function. *Cell Biochemistry and Function*; **21**(1): 1-9.

Nir I, Hillel R, Shefet G, Nitsan Z. (1994). Effect of grain particle size on performance. 2. Grain texture interactions. *Poultry Science*; **73**(6): 781-791.

Okamoto M. (1997). Comparison of particle size in the feces of various herbivores. *Journal of the Rakune Gakuen University (Natural Sciences)* **22**: 151-153.

Panigrahi S, Oguntona EB, Roberts BR. (1996). Effects of oven-drying tubers of two high-protein sweet potato varieties at different temperatures on their feeding value in broilers. *British Poultry Science*; **37**(1): 173-188.

Paul SK, Halder G, Mondal MK, Samanta G. (2007). Effect of organic acid salt on the performance and gut health of broiler chicken. *Journal of Poultry Science*; **44**(4): 389-395.

Peisker M. (2006). Feed processing - impacts on nutritive value and hygienic status in broiler feeds. *Australian Poultry Science Scientific Symposium*; **18**: 7-16.

Peron A, Bastianelli D, Oury FX, Gomez J, Carre´ B. (2005). Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed on a pelleted diet. *British Poultry Science*; **46**(2): 223-230.

Pfaffl MW, Horgan GW, Dempfle L. (2002). Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleoide Acids Research*; **30**(9): e36.

- Podolsky DK. (1993). Regulations of intestinal epithelial proliferation: a few answers, many questions. *American Journal of Physiology: Gastrointestinal and Liver Physiology*; **264**(2): G179-G186.
- Poppi DP, Norton BW, Minson DJ, Hendricksen RE. (1980). The validity of the critical size theory for particles leaving the rumen. *The Journal of Agricultural Science*; **94**(2): 275-280.
- Reece FN, Lott BD, Deaton JW. (1985). The effects of feed form, grinding method, energy level, and gender on broiler performance in moderate (21°C) environment. *Poultry Science* **64**(10): 1834-1939.
- Rehman H, Awad WA, Lindner I, Hess M, Zentek J. (2006). Clostridium perfringens alpha toxin affects electrophysiological properties of isolated jejunal mucosa of laying hens. *Poultry Science*; **85**(2): 1298-1302.
- Rehman H, Ijaz A, Specht A, Dill D, Hellweg P, Männer K, Zentek J. (2009). In vitro effects of alpha toxin from clostridium perfringens on the electrophysiological parameters of jejunal tissues from laying hens preincubated with inulin and N-acetyl-L-cysteine. *Poultry Science*; **88**(1): 199-204.
- Rehman HU, Vahjen W, Awad AW, Zentek J. (2007). Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Archives of Animal Nutrition*; **61**(5): 319-335.
- Rezvani M, Kluth H, Elwert C, Rodehutsord M. (2007). Effect of ileum segment and protein sources on net disappearance of crude protein and amino acids in laying hens. *British Poultry Science*, **49**(1): 28-36.
- Ricke SC. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science*; **82**(4):632-639.
- Röhe I. (2014). Effects of feed structure on animal performance, gastrointestinal morphology, gut-associated lymphoid tissue and jejunal glucose transport in laying hens. [German] *Dissertation Freie Universität Berlin Germany*.
- Röhe I, Ruhnke I, Knorr F, Mader A, Goodarzi Borojani F, Löwe R, Zentek J. (2014). Effects of grinding method, particle size, and physical form of the diet on gastrointestinal morphology and jejunal glucose transport in laying hens. *Poultry Science- in press*.
- Rougiere N, Gomez J, Mignon-Grasteau S, Carre` B. (2009). Effects of diet particle size on digestive parameters in D+ and D- genetic chicken lines selected for divergent digestion efficiency. *Poultry Science*; **88**(6): 1206-1215.
- Roy RD, Edens FW, Parkhurst CR, Queshi MA, Havenstein GB. (2002). Influence of a propionic acid feed additive on performance of turkey poults with experimentally induced poult enteritis and mortality syndrome. *Poultry Science*; **81**(7):951-957.
- Ruhnke I, deBiasio JV, Suchodolski JS, Newman SJ, Musch MW, Steiner JM. (2014). Evaluation of endoscopically obtained duodenal biopsy samples from cats and dogs in an adapter-modified Ussing chamber. *Journal of Veterinary Science; in press*.

Ruhnke I, Röhe I, Meyer W, Kröger S, Neumann K, Zentek J. (2013). Method for the preparation of mucosal flaps from the jejunum of laying hens for transporter studies in Ussing chambers. *Archives of Animal Nutrition*; **67**(2):161-168.

Russell JB. (1992). Another explanation for the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. *Journal of Applied Bacteriology*; **73**: 363-370.

Sacranie A, Svihus B, Denstadli V, Moen B, Iji PA, Choct M. (2012). The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. *Poultry Science*; **91**(3): 693-700.

Safaa HM, Jiminez-Moreno E, Valencia DG, Frikha M, Serrano MP, Mateos GG. (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. *Poultry Science*; **88**(3): 608-614.

Sakata, T. (1987). Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *British Journal of Nutrition*; **58**(1): 95-103.

Santos FBO, Sheldon BW, Santos AA, Ferket PR. (2008). Influence of housing system, grain type, and particle size on *Salmonella* colonization and shedding of broilers fed triticale or corn-soybean meal diets. *Poultry Science*; **87**(3): 405-420.

Shaver RD, Nytes AJ, Satter LD, Jorgensen NA. (1988). Influence of feed intake, forage physical form, and forage fiber content on particle size of masticated forage, ruminal digesta, and feces of dairy cows. *Journal of Dairy Science*; **71**(6): 1566-1572.

Short FJ, Gorton P, Wiseman J, Boorman KN. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology*; **59**(4): 215-221.

Smith RJ, Wilmore DW. (1990). Glutamine nutrition and requirements. *Journal of Parenteral and Enteral Nutrition*; **14**(4): S94-S99.

Steuer P, Clauss M, Südekum KH, Hatt JM, Silinski S, Klomburg S, Zimmermann W, Fickel J, Streich WJ, Hummel J. (2010). Comparative investigations on digestion in grazing (*Ceratotherium simum*) and browsing (*Diceros bicornis*) rhinoceroses. *Compendium of Biochemistry and Physiology Part A: Molecular Integrative Physiology*; **156**(4): 380-388.

Stevens CE. (1964). Transport of sodium and chloride by the isolated rumen epithelium. *American Journal of Physiology-Legacy Content*; **206**(5): 1099-1105.

Stolle H, Bank S, Radko D, Günter R, Kamphues J. (2011). Concentrations of starch and fat in excreta of fattening turkeys acting as indicators for digestive capacity and/or its limitations. *Proceedings of the Society of Nutrition Physiology*; **20**: 45.

Sun Q, Dai L, Nan C, Xiong L. (2013). Effect of heat treatment on physicochemical and morphological properties of wheat starch and xylitol mixture. *Food Chemistry*; **143**: 54-59.

- Sun X, McElroy A, Webb Jr KE, Sefton AE, Novak C. (2005). Broiler performance and intestinal alterations when fed drug-free diets. *Poultry Science*; **84**(8):1294-1302.
- Svihus B. (2006). The role of feed processing on gastrointestinal function and health in poultry. *Avian gut function in health and disease*. G. C. Perry. Wallingford, UK, Cabi: 183-194.
- Svihus B. (2011). The gizzard: function, influence of diet structure and effects on nutrient availability. *World's Poultry Science Journal*; **67**: 207-224.
- Svihus B, Hetland H. (2001). Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. *British Poultry Science*; **42**(5): 633-637.
- Svihus B, Juvik E, Hetland H, Krogdahl A. (2004). Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. *British Poultry Science*; **45**(1): 55-60.
- Svihus B, Klovstad KH, Perez V, Zimonja O, Sahlström S, Schüller RB, Jeksrud WK, Prestlokken E. (2004). Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. *Animal Feed Science and Technology*; **117**(3): 281-293.
- Svihus B, Sacranie A, Denstadli V, Choct M. (2010). Nutrient utilization and functionality of the anterior digestive tract caused by intermittent feeding and inclusion of whole wheat in diets for broiler chickens. *Poultry Science*; **89**(12): 2617-2625.
- Takata, K. (1996). Glucose transporters in the transepithelial transport of glucose. *Journal of Electronic Microscopy (Tokyo)*; **45**(4): 275-284.
- Thomas M, VanZuilichem DJ, VanderPoel AFB. (1997). Physical quality of pelleted animal feed. 2. Contribution of processes and its conditions. *Animal Feed Science Technology*; **64**(2): 173-192.
- Thompson JL, Hinton M. (1997). Antibacterial activity of formic and propionic acids in the diet of hens on salmonellas in the crop. *British Poultry Science*; **38**(1): 59-65.
- Thorens HG, Joost B. (2001). The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members. *Molecular Membrane Biology*; **18**(4): 247-256.
- Valencia DG, Serrano MP, Lazaro R, Jimenez-Moreno E, Mateos GG. (2009). Influence of micronization (fine grinding) of soya bean meal and full-fat soya bean on the ileal digestibility of amino acids for broilers. *Animal Feed Science and Technology*; **150**(3-4): 238-248.
- VanDeGinste J, Deschrijver R. (1998). Expansion and pelleting of starter, grower and finisher diets for pigs: effects on nitrogen retention, ileal and total tract digestibility of protein, phosphorus, and calcium and in vitro protein quality. *Animal Feed Science and Technology*; **72**(3): 303-314.

- VanImmerseel F, Russell J, Flythe M, Gantois I, Timbermont L, Pasmans F, Haesebrouck F, Ducatelle R. (2006). The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficiency. *Avian Pathology*; **35**(3): 182-188.
- VanKrimpen MM, Kwakkel RP, Van der Peet-Schwering CM, DenHartog LA, Verstegen MW. (2008). Low dietary energy concentration, high nonstarch polysaccharide concentration, and coarse particle sizes of nonstarch polysaccharides affect the behavior of feather-pecking-prone laying hens. *Poultry Science*; **87**(3): 485-496.
- Vilarino M, Picard ML, Melcion JP, Faure JW. (1996). Behavioural adaption of laying hens to dilution of diets under mash and pellet form. *British Poultry Science*; **37**(5): 895-907.
- Vinardell MP. (1990). Mutual inhibition of sugars and amino-acid intestinal-absorption. *Comparative Biochemistry and Physiology Part A: Physiology*; **95**(1): 17-21.
- Wang JP, Lee JH, Yoo JS, Cho JH, Kim HJ, Kim IH. (2010). Effects of phenyllactic acid on growth performance, intestinal microbiota, relative organ weight, blood characteristics, and meat quality of broiler chicks. *Poultry Science*; **89**(7):1549-1555.
- Watford M, Wu GY. (2005). Glutamine metabolism in uricotelic species: Variation in skeletal muscle glutamine synthetase, glutaminase, glutamine levels and rates of protein synthesis. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*; **140**(4): 607-614.
- Weurding RE, Veldman A, Veen WA, VanDerAar PJ, Verstegen MW. (2001). Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. *Journal of Nutrition*; **131**(9): 2329-2335.
- Williams J, Mallet S, Leconte M, Lessire M, Gabriel I. (2008). The effects of fructo-oligosaccharides of whole wheat on the performance and digestive tract of broiler chickens. *British Poultry Science*; **49**(3): 329-339.
- Wolf P, Arlinghaus M, Kamphues J, Sauer N, Mosenthin R. (2012). Impact of feed particle size on nutrition digestibility and performance in pigs. *Übersicht der Tierernährung*; **40**: 21-64.
- Wondra KJ, Hancock JD, Behnke KC, Stark CR. (1995a). Effects of mill type and particle size uniformity on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. *Journal of Animal Science*; **73**(9): 2564-2573.
- Wondra KJ, Hancock JD, Behnke KC, Hines RH, Stark CR. (1995b). Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. *Journal of Animal Science*; **73**(3):757-63.
- Wright EM. (1993). The intestinal Na⁺/glucose cotransporter. *Annual Review of Physiology*; **55**(1): 575-589.

Yasar S. (2003). Performance, gut size and ileal digesta viscosity of broiler chickens fed with a whole wheat added diet and diets with different wheat particle sizes. *International Journal of Poultry Science*; **2**(1): 75-82.

Yegani M, Korver DR. (2008). Factors Affecting Intestinal Health in Poultry. *Poultry Science*; **87**(10): 2052-2063.

Yunus AW, Awad WA, Kröger S, Zentek J, Bohm J. (2010). In vitro aflatoxin B₁ exposure decreases response to carbamylcholine in the jejunal epithelium of broilers. *Poultry Science*; **89**(7): 1372-1378.

Yunus AW, Awad WA, Kröger S, Zentek J, Bohm J. (2011). Dose-dependent increase and decrease in active glucose uptake in jejunal epithelium of broilers after acute exposure to ethanol. *Alcohol*; **45**(4): 411-414.

Zareie M, Johnson-Henry K, Juri J, Yang PC, Ngan BY, McKay DM, Soderolm JD, Perdue MH, Sherman PM. (2006). Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut*; **55**(11): 1553–1560.

10. Appendix

10.1. Legend of Figures

Figure 1:	Roller mill	16
Figure 2:	Hammer mill.....	17
Figure 3:	Disc mill.....	18
Figure 4:	Wedge-shaped disc mill.....	19
Figure 5:	Stripping the jejunum of a laying hen and transfer onto the net supported Ussing chamber cylinders.....	26
Figure 6:	Micrographs of stripped jejunum from laying hens	29

10.2. Legend of Tables

Table 1:	Response of tissue samples exposed to various chemical compounds.....	28
Table 2:	Feed composition and nutrient content of the experimental starter and grower diets.....	31
Table 3:	Information on primers used in this study	33
Table 4:	Characteristic values of the control and acid group at the beginning of the experiment	34
Table 5:	Response of short current circuit of tissue samples (ΔI_{sc}) exposed to various chemical compounds.....	34
Table 6:	Feed composition and nutrient content of the experimental diet	36
Table 7:	Energy consumption for feed production, discrete mean particle size (dMEAN) after dry and wet sieving analysis and extract viscosity of the twelve experimental diets.....	37
Table 8:	Performance of the laying hens during the experimental period.....	41
Table 9:	Statistical analysis of performance parameters regarding thermal treatment, milling method and correlation with dMEAN analysed by wet sieving analysis.....	42
Table 10:	Mean values of apparent ileal digestibility (%) for laying hens after being fed twelve different experimental feed variants.....	43
Table 11:	Statistical analysis of apparent ileal digestibility and correlation with dMEAN analysed by wet sieving analysis	44
Table 12:	pH of digesta of laying hens fed the experimental diets.....	45
Table 13:	Statistical analysis of pH values obtained from the digesta and correlation with dMEAN analysed by wet sieving analysis	46

11. List of Publications

Publications (peer reviewed):

Ruhnke I, Röhe I, Meyer W, Kröger S, Neumann K, Zentek J. (2013). Method for the preparation of mucosal flaps from jejunum of laying hens for transporter studies in Ussing chambers. *Archives of Animal Nutrition*, 67 (2): 161-168.

Goodarzi Borojeni F, Mader A, Knorr F, Ruhnke I, Röhe I, Hafeez A, Männer K, Zentek J. Effect of different thermal treatments and organic acid levels on nutrient digestibility in broilers. *Poultry Science*, 93(5): 1159-1171.

Publications in press:

Röhe I, Ruhnke I, Knorr F, Mader A, Goodarzi Borojeni F, Löwe R, Zentek J. Effects of grinding method, particle size, and physical form of the diet on gastrointestinal morphology and jejunal glucose transport in laying hens. *Poultry Science*

Goodarzi Borojeni F, Vahjen W, Mader A, Knorr F, Ruhnke I, Röhe I, Hafeez A, Villodre C, Männer K, Zentek J. Effect of different thermal treatments and organic acid levels in feed on microbial composition and activity in gastrointestinal tract of broilers. *Poultry Science*

Hafeez A, Mader A, Vahjen W, Goodarzi Borojeni F, Ruhnke I, Männer K, Zentek J. Impact of thermal and organic acid treatment of feed on apparent absorption and metabolism of minerals and tibia quality in broilers. *Poultry Science*

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

Publication under review:

Ruhnke I, Röhe I, Krämer C, Goodarzi Borojeni F, Knorr F, Mader A, Hafeez A, Neumann K, Löwe R, Zentek J. Effect of feed particle size, milling method, and thermal treatment on performance, apparent ileal digestibility, and pH of the digesta in laying hens. *British Poultry Science*

Abstracts in proceedings and participation in conferences:

Ruhnke I, Röhe I, Goodarzi Borojeni F, Knorr F, Mader A, Zentek J. Feed supplemented with 1.5% organic acid had no significant influence on jejunal transporter function in broilers. *17th ESVCN Congress, Ghent, Belgium, 2013. Congress proceedings, 21.*

11. List of Publications

Ruhnke I, Röhe I, Krämer C, Goodarzi Boroojeni F, Knorr F, Mader A, Zentek J. Effects of feed particle size, heat treatment and milling method on performance, nutrient digestibility, and pH of chyme in laying hens. *19th European Symposium on Poultry Nutrition, Potsdam, Germany, 2013. Congress proceedings, 158.*

Röhe I, Ruhnke I, Löwe R, Zentek J. Effects of grinding method, particle size, and physical form of the diet on the glucose transport of the isolated epithelium of the jejunum in laying hens. *67th Meeting of the Society of Nutrition Physiology, Göttingen, Germany, 2013. Proceedings of the Society of Nutrition Physiology, 22: 158.*

Ruhnke I, Röhe I, Meyer W, Kröger S, Neumann K, Zentek J. Method for the preparation of mucosal flaps from jejunum of laying hens for transporter studies in Ussing chambers. *16th ESVCN Congress, Bydgoszcz, Poland, 2012. Congress proceedings, 112.*

12. Acknowledgements

Professor Zentek:

Ich bedanke mich von ganzem Herzen für Ihre zielgerichtete und konsequente Unterstützung, Ihr Grundvertrauen, die Möglichkeit zum eigenverantwortlichen Arbeiten, Ihr fachliches Wissen, die herrlich produktiven zielstrebigem persönlichen Gespräche, kurzfristige Terminverfügbarkeiten, die offene, ehrliche und hochwertige wissenschaftliche Zusammenarbeit. Ich durfte an meine eigenen Grenzen stoßen und daran arbeiten- DANKE! Ohne Sie wäre ich nicht die, die ich jetzt bin.

Professor Aschenbach, Professor Hafez:

Ein herzliches Dankeschön an Sie beide für eine professionelle und reibungslose Betreuung! Ihre hilfreichen Kommentare und Anmerkungen sowie Ihr ehrliches Interesse und die Freude über Fortschritte haben mich immer wieder motiviert! Es war mir eine Ehre.

Professor Männer:

Seit nunmehr fast 9 Jahren durfte ich Ihre Unterstützung genießen! Ob Wochenende oder Schweinestall, Sie haben immer ein offenes Ohr und die treffende Antwort parat! Ich bedanke mich für das stetige Willkommen in „Ihrem“ Bürotrakt, für unzählige Hühnertransporte, dem Blick für das Wesentliche, die Achtung vor dem Mittelgang, Futterrationen, Humor, und Zutrauen, den Kaffeeduft am frühen Morgen- dass Sie so sind wie Sie sind!

Dr. Konrad Neumann:

Ich bedanke mich für Ihre stetige Begleitung, Erklärungen, Auswertungen und Überprüfungen! Für die Beantwortung schier unlösbarer Fragen und Ihre Geduld. Für das Ei des Kolumbus!

Wilfried:

Gedankt sein Dir für Deine Organisation und Unterstützung. Für unzählige geduldige Freitag Morgende, TA-Verfügbarkeiten und high speed emails voller Statistikweisheit. Das war alles keinesfalls selbstverständlich!

Ilen, Farshad, Fanny, Anneluise, Abdul Hafez, Erin und Carolin:

My deepest gratitude to the „chicken task-force“ for organizing and collecting samples of 576+960 birds, several thousand eggs, more than tenthousand data points, and so much more! Without you this project would not have been possible!

Luisa, Katharina, Nadine, Annett, Marita, Petra und Kirstie:

Für die unzähligen unglaublich wichtigen 5-Minuten-Besprechungen, das Schokoladenlager, die Dreisatznachhilfe, die Exceltricks, die Liebe zum Detail und zum Puffer, die Bildschirmschonereinhörner, und nicht zuletzt für die wundersame Probenaufbereitung sage ich „Danke“ von Herzen! Danke für Eure Leidenschaft zum Mitdenken, Vorrausdenken, Nachdenken. Für Eure unzähligen Stunden, die in diesem Projekt stecken und dafür, dass ihr das Leben lebenswert macht!

12. Acknowledgements

Corinna, Ines, Chris und Sandra:

Futter mischen, Hühner wiegen, Ställe kärchern, Kürbis kochen, Schweine hüten
Äpfelbäume schütteln, Hunde flicken, Couchsurfen, Lakritze essen - mit Euch kann
man einfach Pferde stehen! Ein hoch auf die weltbesten „Nachbarn“ die man sich
wünschen kann! DANKE!

Rainer und Susi:

Um fünfe Früh beginnt der Trubel/ der Abwasch rauscht, lauter Jubel/ ist die Susi
schon am spülen/ um im Alltag los zu wühlen/ und fliegt was rum, ist was defekt/
dann gibt's auch schon mal einen Sekt/ den Laden haltet Ihr in Schuss/ und
manchmal gibt's auch einen Kuss/ geplatztes Rohr im Wäägeraum/ der Rainer
kommt, Ihr seid ein Traum/ Ihr seid des Institutes Kern/ drum hab ich Euch
besonders gern!

Jördis, Kerstin, Tini, Linda, Jenny, Alex, Tanja, Patricia, Viola, Anne, und Danja:

Dafür, dass Ihr mich immer noch nicht aufgegeben habt! Für die einsamen Nächte im
Labor, die zweisam durchtanzten Morgengrauen, die gruppendedynamischen
durchklebten WM-Abende der Probenvorbereitung, Fachpostschokolade und Eure
jahrelange Freundschaft, die viel zu viele erschöpft-verschlafende Abende aushalten
musste! Danke für Euer Verständnis für meinen Arbeitswahn! Auf dass die Party
niemals Ende ;-)

Beate, Jule und Norman:

Danke für Eure ständige, großzügige und liebevolle Unterstützung! Euer Verständnis
und die Liebe, die Ihr mir und dieser unserer Lebensart entgegenbringt! Ihr seid
einmalig!

Flo: Für immer und Dich!

13. Selbstständigkeitserklärung

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

Berlin, 27. Januar 2014

Isabelle Ruhnke