The effects of particle size, milling method, and thermal treatment of feed on performance, apparent ileal digestibility, and pH of the digesta in laying hens

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ABSTRACT Various milling methods result in different particle size distributions and, in combination with mash and thermal treatment (expandate) of the feed, may have an impact on nutrient digestibility, pH of the digesta and subsequently the performance of an animal. Since this aspect has not been widely considered in laying hens, the objective of the present study was to investigate the effects of milling method, expansion, and particle size of feed on performance, apparent ileal nutrient digestibility, and pH of digesta in laying hens. Twelve variants of the same diet were produced. Four different milling techniques (hammer mill, roller mill, disc mill, and wedge-shaped disc mill) were used to grind the feed cereals. Coarse feed was obtained from all four mills. Additionally, fine feed was obtained from the hammer mill and the roller mill. Each of the six feed variants was offered as mash or expandate, resulting in a total of 12 treatments. The duration of the experimental period was 21 days. A total of 576 layers, each 19 weeks of age, were used in eight replicates. The statistical analysis for the four milling methods and two thermal treatments was performed using a 4 × 2 factorial arrangement. The effect of particle size was investigated using a 2 × 2 × 2 factorial arrangement including the coarse and fine particle sizes that were produced with the hammer mill and the roller mill as well as the mash and expandate. The animal performance and the pH of the digesta were not affected by the treatments. Ileal digestibility of starch was significantly improved by feeding mash compared to expandate ($P = 0.013$) and by feeding coarse compared to fine feed ($P = 0.028$). Based on this study, the tested milling methods can be used for the production of feed for laying hens without affecting performance and digestibility of nutrients.

Key words: chicken, feed technology, hammer mill, poultry, roller mill

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INTRODUCTION

Producing feed is energy consuming, and grinding technology can have a substantial impact on electric power consumption. In order to reduce the amount of energy needed for feed production, energy saving milling methods have been developed (Hoffmann et al., 2011). Milling technology affects feed particle size and feed particle shape. In order to ensure optimized nutrition of poultry, it is considered important to provide a balanced intake of structured feed (Yegani and Korver, 2008), as this can have an impact on animal health and performance (Amerah et al., 2009; Betscher et al., 2010; Grosse Liesner et al., 2009; VanKrimpen et al., 2008). Broiler and layer chickens discriminate their feed intake by the size, color, and form of the feed. Larger feed particles are preferred and more rapidly ingested (Amerah et al., 2007; Nir et al., 1994; Yasar, 2003). Feed with high fractions of small particle sizes (<500 μm) result in decreased feed intake, increased feed selection, dust pollution, and feed wastage, and may result in malnutrition of animals (Safaa et al., 2009).

The impact of feed particle size on the nutrient digestibility of laying hens has not been widely studied. Hamilton and Proudfoot (1994) and Safaa et al. (2009) investigated the effect of particle size on performance parameters and egg quality. The only effect that could be detected was an increased feed intake in hens that were fed larger feed particles. In contrast to laying hens, specific particle sizes are recommended for broilers (Denstadli et al., 2010; Nir et al., 1994). It has been proposed that at least 20% of the cereal particles should be larger than 1.5 to 2.0 mm (Svihus, 2011). Thermal treatments are commonly used in broiler feed production in order to increase the hygienic status of the feed, and to increase the apparent ileal nutrient digestibility (Jia and Slominski, 2010; Kilburn and Edwards, 2001).

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Since pelleting or expanding prevents a separation of ingredients in the diets, optimised feed intake of a balanced diet can be maintained compared to mash feed (Amerah et al., 2008). An improved feed conversion and body weight can be expected when broiler chickens are given pelleted diets (Hamilton and Proudfoot, 1995).

However, when comparing feed processed by various heat treatments including pelleting, expansion, and extrusion for broiler chickens, all 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, compared to expanded feed, extruded feed had the highest extract viscosity, while starch gelatinization was improved (Lundblad et al., 2011).

Due to the more mature digestive system of laying hens, transferring data obtained from studies in broiler chickens to laying hens seems questionable. Additionally, to our knowledge, the effects of combinations of milling methods (disc and wedge-shaped mills) and thermal treatments (such as expanding) on layer performance, ileal digestibility and pH of the digesta has not been studied in laying hens. The effect of feed manufacturing may be beneficial for nutrient utilization in young laying hens and subsequently impact laying performance.

Therefore, the aim of this study was to investigate the impact of four different milling methods, expansion and particle size on performance, apparent ileal digestibility, and pH of the digesta in laying hens.

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

Ingredients	g/kg as fed
Corn	300.8
Wheat	290.6
Soybean meal (42% CP[1])	224.8
Calcium carbonate	86.0
Soya oil	44.6
Molasses	30.0
Mineral/Vitamin premix[3]	12.0
Monocalcium phosphate	7.8
Salt	2.0
DL-Methionine	1.0
L-Tryptophan	0.2
Titanium dioxide	0.2
Nutrient content	g/kg dry matter as analyzed
Dry matter	881.9
Crude protein	185.8
Ether extract	59.7
Crude fiber	32.7
Starch	418.5
Crude ash	110.6
Calcium	35.7
Phosphorus	3.54
Sodium	1.2
Calculated ME[2] (MJ/kg)	11.4

[1] CP crude protein.

[2] ME metabolizable energy.

[3] Mineral and Vitamin Premix (Spezialfutter Neuruppin, Germany) containing per kg premix: 400,000 IU vitamin A; 40,000 IU vitamin D3; 8000 mg vitamin E (alpha-tocopherole acetate); 300 mg vitamin K3; 250 mg vitamin B1; 250 mg vitamin B2; 2500 mg nicotinic acid; 400 mg vitamin B6; 2000 μg vitamin B12; 25,000 μg biotin; 1000 mg calcium pantothenate; 100 mg folic acid; 80,000 mg choline chloride; 5000 mg zinc (zinc oxide); 2000 mg iron (iron carbonate); 6000 mg manganese (manganese oxide); 1200 mg copper (copper sulfate-pentahydrate); 45 mg iodine (calcium iodate); 30 mg cobalt (cobalt- (II)-sulfate-heptahydrate); 35 mg selenium (sodium selenite); 35 g sodium (sodium chloride); 55 g magnesium (magnesium oxide).

MATERIALS AND METHODS

Animal experiments were conducted in accordance with the Animal Welfare Act of Germany approved by the local state office of occupational health and technical safety (Landesamt für Gesundheit und Soziales, LaGeSo, no. G 0117/11).

Experimental Diets

One identical basal experimental diet was produced (Table 1). Four milling methods, including a roller mill with one pair of rollers (Vario-Walzenstuhl Modell C, MIAG AG, Bühler GmbH, Braunschweig, Germany), a hammer mill (horizontal rotor hammer mill, Tietjen, Hemdingen, Germany), a disc mill (SK 5000, SKIOLD A/S, Saeby, Denmark), and a wedge-shaped disc mill (ATB Potsdam, Leibnitz Institut für Agrartechnik, Potsdam, Germany, PTW Technologies GmbH, Lollar, Germany) were used to grind the cereals of the diet. Defined particle sizes were achieved by using various milling speeds and matrix plates when grinding the feed components. Based on the literature and technical capabilities, coarse feed was defined as feed characterized by discrete mean particle size (**dMEAN**) above 1.8 mm, while fine diets were characterized by dMEAN below 1.8 mm based on dry sieving analysis (Table 2) (Wolf et al., 2012). Coarse diets were produced by all four milling methods, while fine diets were produced by the roller mill and the hammer mill only. After grinding the main cereals, all components of the final diet were mixed (Zweiwellenpaddelmischer, Dinnissen BV, Sevenum, The Netherlands) and half of the respective amounts were processed as expandate to be provided to the hens (Single Screw Expander OE8, Amandus Kahl GmbH & Co KG, Reinbek, Germany). The expansion temperature was 115 to 117°C for less than 3 seconds at 2 bar steam saturation.

Animals and Housing

Five hundred and sixty-seven hens (Lohmann brown) were obtained from a commercial pullet-rearing farm at the age of 19 weeks. Prior to this study, the pullets were fed mash feed produced with a hammer mill. After arrival, hens were adapted to the experimental diet for one week. Cages were equipped with wood shaving litter, an area for claw abrasion, a perch, and laying

Table 2. Discrete mean particle size (dMEAN) after dry and wet sieving analysis of the twelve experimental diets.

	Roller mill				Hammer mill				Disc mill		Wedge-shaped disc mill	
	Coarse particles		Fine particles		Coarse particles		Fine particles		Coarse particles		Coarse particles	
	Mash	Expandate	Mash	Expandate	Mash	Expandate	Mash	Expandate	Mash	Expandate	Mash	Expandate
dMEAN[1] after dry sieving analysis	1.93	2.43	1.28	1.27	2.41	2.15	1.64	1.30	2.12	2.80	2.15	2.66
dMEAN[1] after wet sieving analysis	1.85	0.85	1.15	0.31	2.60	0.93	1.27	0.50	1.45	0.82	1.26	0.68

[1] dMEAN = discrete mean particle size.

nests. For the duration of the 21-day experimental period, the animals were fed ad libitum with one of the twelve diets, meeting the recommendations for laying hens (GfE, 1999). The hens were killed at 23 weeks of age by cervical dislocation followed by exsanguination.

The experiment was performed in eight replicates, using 8 cages per treatment. Each cage with six hens was considered as an experimental unit. In total 96 cages with six hens per cage were used.

Sampling and Analysis

Performance parameters The health status of the hens was monitored daily. The body weight of the individual hens was recorded at the beginning of the experiment, weekly, and on the day of slaughter. Feed intake was recorded weekly and on the day of slaughter. The eggs from each group were counted and the individual egg weight was determined daily. Feed conversion ratio (FCR) was calculated as follows:

$$\frac{\text{Feed intake per hen per week}}{((\text{egg weight} \times \text{laying performance})/100 + \text{weight gain per hen per week})}$$

After 21 days, weekly data were averaged per pen for statistical analysis.

Apparent ileal nutrient digestibility Three hens per cage were used for collecting digesta in order to analyze the apparent ileal nutrient digestibility. The intestinal content of the distal two-thirds of the ileum (not including the content localized in the distal 3 cm prior to the ileocaecal junction) was sampled (Kluth et al., 2005; Revazvani et al., 2007). The digesta samples were pooled and freeze-dried for further analysis. One pooled sample of each replicate was used for statistical analysis, resulting in eight samples for each of the twelve groups. Weende analysis was performed to determine dry matter (**DM**), crude protein (**CP**) and ether extract (**EE**) (Naumann and Bassler, 2004). Titanium dioxide (0.2%) was implemented as an indigestible marker and determined as previously described (Short et al., 1996). Starch was determined using a standard procedure (Naumann and Bassler, 2004). A commercially available enzymatic test was used to determine starch content (Stärke UV-Test, R-Biopharm, Darmstadt, Germany). Amino acids were analyzed using a Biochrom 20 Amino Acid Analyzer (Amersham Pharmacia Biotech, Amersham, UK) after hydrolysis of 0.5 g dried digesta in 25 ml 6 M HCl at 110°C for 24 hours. After cooling on ice, 20 ml of 8 M NaOH was added and the pH adjusted to 2.2. For sulphur-containing amino acids, 30% H_2O_2 and 88% formic acid was incubated for 1 hour at 30°C, cooled, and 5 ml of that solution added to the 0.5 g sample. Afterwards, the samples were transferred into an ice bath for 24 hours and oxidation was stopped by adding 0.9 g sodium disulfide. Immediately before chromatography, all samples were filtered through a cellulose-acetate membrane (25 mm syringe filter with 0.45 μm cellulose acetate membrane, VWR International, Radnor PA).

pH measurements Five birds from each pen were used for digesta pH measurements. Immediately after sampling, the pH of the digesta obtained from the following sections was measured including crop, proventriculus, gizzard, duodenum, jejunum, ileum, caecum, and excreta. For statistical analysis, the mean values per pen were used.

Data Analysis

The experimental unit was defined as one pen. The effects of the four milling methods (considering only coarse feed particles) and the thermal treatment (mash vs. expandate) of feed were investigated using a 4 × 2 factorial arrangement. The effect of the particle size was investigated using a 2 × 2 × 2 factorial arrangement which included coarse and fine particles produced by the roller and the hammer mill and fed as mash or expandate. For all statistical analyses, IBM SPSS Statistics Version 21 was used (IBM, Chicago, IL, USA). Statistically significant differences were set at $P < 0.05$.

RESULTS

Wet sieving analysis of the feed revealed a strong impact of expanding on particle size; expanded feed had lower dMEAN values compared to the mash feed produced under the same milling conditions (Table 2). Expanded feed also had higher viscosity compared to mash feed.

Table 3. Performance of the laying hens during the last week (day 15 to 21) of the experimental period in respect to milling method (M), thermal treatment (T), and particle size (P).

	Milling method[1]				Thermal treatment		*P*-value		
	Roller mill	Hammer mill	Disc mill	Wedge-shaped disc mill	Mash	Expandate	M[2]	T[3]	M X T
Body weight at day 21(g)	1709	1703	1693	1680	1696	1696	0.592	0.988	0.250
Feed intake (g)	116	115	110	118	114	115	0.298	0.781	0.281
Weight gain per week (g)	40.1	32.7	21.4	16.7	29.1	26.4	0.112	0.718	0.832
Egg weight (g)	54.1	54.4	55.2	54.5	54.7	54.8	0.482	0.872	0.528
Laying performance (%)	89.1	91.7	89.5	92.2	90.6	90.7	0.561	0.946	0.882
FCR[4]	2.19	2.10	2.10	2.24	2.14	2.18	0.260	0.515	0.859

[1] Analysis of the effect of the milling method included only the coarsely ground feed particles of roller mill and hammer mill.

[2] M = milling method.

[3] T = thermal treatment.

[4] FCR = feed conversion ratio.

Table 4. Analysis of the effect of coarse and fine particle size (P) depending on milling method (M; roller and hammer mill only) and thermal treatment (T) on the performance of the laying hens during the last week (day 15–21) of the experimental period.

	Milling method		Thermal treatment		Particle size[1]		*P*-value						
	Roller mill	Hammer mill	Mash	Expandate	Coarse	Fine	M[2]	T[3]	P[4]	M X T	M X P	P X T	M X T X P
Body weight at day 21(g)	1715	1705	1711	1710	1706	1715	0.523	0.942	0.575	0.083	0.789	0.359	0.475
Feed intake (g)	117	115	115	116	116	116	0.572	0.624	0.913	0.318	0.871	0.191	0.912
Weigh gain per week (g)	42.7	34.3	41.5	35.5	36.4	40.6	0.240	0.401	0.561	0.968	0.884	0.479	0.292
Egg weight (g)	54.5	54.6	54.4	54.6	54.5	54.6	0.884	0.718	0.895	0.662	0.155	0.179	0.687
Laying performance (%)	90.0	92.4	92.2	90.2	90.4	92.0	0.210	0.266	0.401	0.770	0.868	0.343	0.992
FCR[5]	2.13	2.10	2.06	2.17	2.14	2.09	0.598	0.068	0.314	0.290	0.276	0.558	0.290

[1] Effect of the variation of fine and coarse particle size (P) of feed produced by roller mill (cleft size 0.75 and 1.0 mm for fine particles or 2.0 mm and 3.25 mm for coarse particles for wheat and maize), and hammer mill (sieve size 5 and 6 mm for fine particles or no sieve for coarse particles for wheat and maize).

[2] M = milling method.

[3] T = thermal treatment.

[4] P = particle size.

[5] FCR = feed conversion ratio.

After feeding the experimental diets for 21 days, neither body weight, nor feed intake, weight gain, egg weight, laying performance or feed conversion ratio was affected by the milling method, heat treatment, or particle size. Results are displayed in Tables 3 and 4.

Ileal digestibility of starch was significantly increased in hens fed with mash compared to expandate (97.3% vs. 95.9%, respectively; $P = 0.013$). In addition, ileal starch digestibility was significantly increased in hens that consumed feed with coarse particles compared to finely ground feed (96.9% vs. 95.7%, respectively; $P = 0.028$). Interaction between particle size and thermal treatment could be observed for starch digestibility (mean values of hens fed with fine particles of expandate was 94.5% compared to mean values of 97.0% for hens fed with coarse mash). Apart from that, neither the milling method, nor the heat treatment or the particle size had any effect on ileal nutrient digestibility (Table 5 and 6).

The effect of experimental treatments on pH measurements of the digesta obtained from the hens is shown in Tables 7 and 8. Neither milling method nor heat treatment or particle size had an effect on the pH of the digesta (Tables 7 and 8). Interactions of particle size and thermal treatment could be observed in pH of gizzard content, ileum content, and excreta.

DISCUSSION

Feed Intake and Performance

The present study shows that the various diets produced by different milling methods, thermal treatments, and particle sizes had no effect on the performance of young laying hens. Long-term studies by other investigators found a significantly positive impact of mashed diets compared to crumbled or pelleted diets on laying performance (Hamilton and Proudfoot, 1994). In addition, one study observed that feed intake was increased with larger particle size (Safaa et al., 2009). In feed for broilers, thermal treatment such as pelleting is applied frequently. The pellet structure provides a higher bulk density compared to mash feed and broilers fed pellets are known to consume more feed resulting in an increased body weight (Svihus and Hetland, 2001). In laying hens, increased energy intake with pelleted feed resulted in enhanced consumption and higher body weight gain (Safaa et al., 2009).

Apparent Ileal Nutrient Digestibility

Improved apparent ileal digestibility of starch was found when birds were fed coarse particle size and

Table 5. Apparent ileal nutrient digestibility (%) with respect to milling method (M), thermal treatment (T), and particle size (P) in the laying hens.

	Milling method[1]				Thermal treatment		*P*-value		
	Roller mill	Hammer mill	Disc mill	Wedge-shaped disc mill	Mash	Expandate	M[2]	T[3]	M X T
Ether Extract	94.1	92.8	94.6	97.3	93.7	95.7	0.102	0.109	0.447
Starch	96.7	97.1	95.9	96.8	97.3	95.9	0.377	0.013	0.205
Crude Protein	69.3	68.1	72.9	70.6	70.1	70.4	0.113	0.842	0.881
Alanine	65.2	66.0	71.2	68.3	68.4	67.0	0.377	0.599	0.643
Arginine	78.9	80.2	80.1	80.2	80.0	79.7	0.962	0.912	0.495
Aspartic acid	65.8	66.8	68.3	67.1	67.0	67.0	0.943	0.999	0.427
Cysteine	58.1	57.3	58.9	60.3	58.1	59.2	0.936	0.772	0.815
Glutamic acid	78.2	77.9	79.4	78.8	79.1	78.0	0.904	0.488	0.600
Glycine	54.5	55.0	60.1	55.4	55.6	56.9	0.561	0.670	0.471
Histidine	62.5	62.5	65.0	65.4	63.5	64.3	0.832	0.788	0.710
Isoleucine	68.4	70.9	72.6	71.8	70.7	71.1	0.800	0.901	0.262
Leucine	72.9	74.1	76.4	74.3	74.8	74.0	0.713	0.707	0.553
Lysine	67.7	69.7	74.1	72.3	70.7	71.3	0.281	0.804	0.538
Methionine	80.5	80.7	79.8	82.5	81.4	80.4	0.791	0.619	0.716
Phenylalanine	41.9	44.6	48.9	46.6	44.9	46.1	0.750	0.782	0.686
Proline	74.7	76.1	77.5	75.7	76.5	75.6	0.741	0.602	0.855
Serine	63.5	65.4	66.8	64.8	65.5	64.8	0.880	0.806	0.390
Threonine	52.9	55.6	58.9	57.2	56.6	55.8	0.726	0.831	0.434
Tyrosine	71.3	72.3	75.1	73.8	73.1	73.1	0.674	0.983	0.726
Valine	60.3	62.4	65.7	63.5	63.3	62.7	0.696	0.848	0.301

[1] Analysis of the effect of the milling method included only the coarsely ground feed particles of roller mill and hammer mill.

[2] M = milling method.

[3] T = thermal treatment.

Table 6. Analysis of the effect of coarse and fine particle size (P) depending on milling method (M; roller and hammer mill only) and thermal treatment (T) on apparent ileal nutrient digestibility (%) in laying hens.

	Milling method		Thermal treatment		Particle size[1]		*P*-value						
	Roller mill	Hammer mill	Mash	Expandate	Coarse	Fine	M[2]	T[3]	P[4]	M X T	M X P	P X T	M X T X P
Ether Extract	94.8	94.3	93.9	95.1	93.4	95.6	0.613	0.298	0.057	0.318	0.569	0.346	0.440
Starch	96.2	96.4	96.9	95.6	96.9	95.7	0.693	0.016	0.028	0.626	0.644	0.044	0.995
Crude Protein	70.8	68.4	70.6	68.6	68.7	70.5	0.097	0.170	0.188	0.404	0.383	0.116	0.799
Alanine	65.5	64.0	65.9	63.7	65.6	63.9	0.596	0.429	0.542	0.823	0.418	0.795	0.744
Arginine	80.1	78.7	79.8	79.0	79.6	79.3	0.474	0.676	0.889	0.666	0.177	0.761	0.590
Aspartic acid	66.9	64.8	66.9	64.8	66.3	65.4	0.408	0.430	0.724	0.684	0.227	0.846	0.507
Cysteine	57.7	55.5	57.1	56.1	57.7	55.5	0.527	0.777	0.534	0.721	0.687	0.732	0.752
Glutamic acid	78.2	76.7	77.9	77.0	78.0	76.9	0.272	0.508	0.420	0.569	0.357	0.521	0.861
Glycine	55.9	53.1	55.1	53.9	54.7	54.2	0.350	0.691	0.858	0.905	0.271	0.785	0.928
Histidine	64.1	59.6	62.3	61.5	62.5	61.3	0.125	0.780	0.663	0.835	0.129	0.607	0.561
Isoleucine	69.6	68.0	68.9	68.7	69.7	68.0	0.646	0.938	0.621	0.924	0.237	0.960	0.856
Leucine	73.3	71.7	73.3	71.7	73.5	71.6	0.484	0.484	0.416	0.936	0.226	0.906	0.916
Lysine	71.5	68.4	71.2	68.7	68.7	71.2	0.227	0.330	0.323	0.807	0.052	0.335	0.676
Methionine	80.1	79.0	80.1	79.1	80.6	78.6	0.545	0.590	0.278	0.619	0.481	0.706	0.856
Phenylalanine	44.0	40.4	44.2	40.2	43.3	41.2	0.419	0.385	0.643	0.931	0.168	0.301	0.877
Proline	75.0	74.2	75.5	73.7	75.4	73.8	0.659	0.297	0.336	0.967	0.207	0.706	0.912
Serine	64.1	62.8	64.9	62.0	64.5	62.5	0.641	0.285	0.464	0.901	0.246	0.768	0.529
Threonine	55.1	52.2	55.2	52.0	54.3	53.0	0.442	0.386	0.733	0.942	0.139	0.658	0.645
Tyrosine	71.7	70.4	71.2	71.0	71.8	70.4	0.594	0.908	0.563	0.800	0.353	0.763	0.768
Valine	62.4	59.9	62.0	60.3	61.4	60.9	0.461	0.606	0.885	0.994	0.182	0.798	0.930

[1] Effect of the variation of fine and coarse particle size (P) of feed produced by roller mill (cleft size 0.75 and 1.0 mm for fine particles or 2.0 mm and 3.25 mm for coarse particles for wheat and maize), and hammer mill (sieve size 5 and 6 mm for fine particles or no sieve for coarse particles for wheat and maize).

[2] M = milling method.

[3] T = thermal treatment.

[4] P = particle size.

mashed feed. This is in agreement with previous observations (Denstadli et al., 2010; Svihus and Hetland, 2001; Svihus et al., 2004a). In broilers, coarse feed particles are known to stimulate gizzard activity, resulting in larger numbers of small particles in the digesta in the small intestine compared to broilers fed with fine feed particles (Hetland et al., 2002). The intake of coarse feed particles increases not only the gizzard weight but also the pancreas mass, indicating increased 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

Table 7. pH of digesta with respect to milling method (M), thermal treatment (T), and particle size (P) in laying hens.

	Milling method[1]				Thermal treatment		*P*-value		
	Roller mill	Hammer mill	Disc mill	Wedge-shaped disc mill	Mash	Expandate	M[2]	T[3]	M X T
Proventriculus	4.79	5.00	4.75	5.02	4.86	4.92	0.721	0.516	0.223
Gizzard	4.17	4.19	4.12	4.31	4.22	4.17	0.619	0.586	0.635
Duodenum	6.02	5.93	5.93	5.91	6.02	5.87	0.106	0.821	0.591
Jejunum	6.17	5.94	6.11	6.09	6.07	6.09	0.799	0.273	0.695
Ileum	7.48	7.56	7.51	7.47	7.52	7.49	0.609	0.774	0.150
Cecum	7.00	7.00	7.04	6.89	6.93	7.03	0.215	0.656	0.961
Excreta	6.97	6.96	7.09	7.11	7.10	6.97	0.308	0.770	0.443

[1] Analysis of the effect of the milling method included only the coarsely ground feed particles of roller mill and hammer mill.

[2] M = milling method.

[3] T = thermal treatment.

Table 8. Analysis of the effect of coarse and fine particle size (P) depending on milling method (M; roller and hammer mill only) and thermal treatment (T) on of the pH values in the digesta in laying hens.

	Milling method		Thermal treatment		Particle size[1]		*P*-value						
	Roller mill	Hammer mill	Mash	Expandate	Coarse	Fine	M[2]	T[3]	P[4]	M X T	M X P	P X T	M X T X P
Proventriculus	4.93	4.86	4.77	5.01	4.90	4.89	0.727	0.213	0.981	0.899	0.149	0.746	0.564
Gizzard	4.21	4.36	4.22	4.34	4.18	4.38	0.163	0.301	0.063	0.932	0.222	0.049	0.503
Duodenum	5.95	5.91	6.00	5.87	5.97	5.89	0.739	0.247	0.430	0.979	0.654	0.704	0.298
Jejunum	6.16	6.04	6.12	6.08	6.06	6.15	0.193	0.632	0.302	0.580	0.218	0.886	0.840
Ileum	7.38	7.56	7.51	7.43	7.52	7.41	0.014	0.253	0.136	0.058	0.161	0.047	0.663
Caecum	7.00	6.96	6.95	7.02	7.00	6.97	0.629	0.361	0.712	0.336	0.632	0.702	0.355
Excreta	6.94	6.96	7.18	6.72	6.97	6.93	0.834	0.000	0.789	0.857	0.803	0.050	0.181

[1] Effect of the variation of fine and coarse particle size (P) of feed produced by roller mill (cleft size 0.75 and 1.0 mm for fine particles or 2.0 mm and 3.25 mm for coarse particles for wheat and maize), and hammer mill (sieve size 5 and 6 mm for fine particles or no sieve for coarse particles for wheat and maize).

[2] M = milling method.

[3] T = thermal treatment.

[4] P = particle size.

3.5 mm. Under these conditions, ileal starch digestibility was significantly increased by smaller particle sizes. Peron et al. (2005) observed similar effects when feeding wheat-based diets with higher particle sizes. Feed produced with a roller mill increased starch digestibility compared to feed obtained with a hammer mill (Svihus et al., 2004b). Higher apparent ileal digestibility of proteins and of the essential amino acids including threonine, methionine, and cysteine was observed in broilers consuming feed produced with a roller mill as opposed to a hammer mill (Amad, 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 (Briggs et al., 1999; Fairfield, 2003; Svihus et al., 2004b).

While heat treatment of feed results in a secondary reduction of particle size (Table 2), this might explain the improved starch digestibility in mash diets compared to expandate, as well as the interaction between particle size and thermal treatment.

A possible disadvantage of heat treatment of feed is the potential negative impact on protein utilization. Moderate temperatures and short-term treatment showed no effects on amino acid availability, but intensive heat treatment significantly reduced amino acid availability (Amezucua and Parsons, 2007; Dänicke et al., 1998; Mavromichalis and Baker, 2000; Panigrahi et al., 1996). In the present study, the apparent ileal digestibility of crude protein was not affected by the milling methods, heat treatments, or particle sizes.

pH Measurements

The results indicate that the heat treatments as well as the milling methods had no effect on the pH of the digesta. While the effect of smaller feed particles in raising the pH of the digesta has been described in the literature, such obervations could not be confirmed in this study (Nir et al., 1994, Huang et al., 2006). The pH of the gizzard contents in the present study (Table 5) is comparable with literature as reported by Mirzaie et al., 2012. The high buffering capacity of the calcium carbonate added as mineral salt to the diets, according to the requirements of laying hens, might have been responsible for the relatively high digesta pH and the lack of effects by coarse feed particle size. Investigating the interaction of particle size and thermal treatment demonstrates that feeding hens with fine expandate results in higher pH compared to coarse expandate, coarse mash, or fine mash. In contrast, fine expandate decreases the pH of digesta in the lower gastrointestinal tract (ileum and excreta), which may be explained by reduced stimulation of the gizzard activity and, hence, a larger proportion of coarse particles in the lower gastrointestinal tract.

Comparing the results of studies on particle size 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 has been validated to date. Results obtained from the wet sieving analysis indicate the effects of additional shearing of the particles caused by the extrusion process resulting in lower dMEAN values for the expanded feed variants (Table 2). Wet sieving analysis demonstrated the effect of expanding on particle size; compared to the mash form of the diet, the equivalent expandate always contained finer particles as indicated by a reduced dMEAN.

While this study revealed a moderately positive impact of coarse particles and mash feed on starch digestibility, enhanced intestinal glucose uptake was demonstrated in animals fed mash feed compared to expandate (Röhe et al., 2014). Furthermore, feeding of coarse feed particles significantly increased gizzard and pancreatic organ weight (Röhe et al., 2014). Therefore, one may assume that the beneficial impact of coarse particles on the intestinal function of laying hens may be comparable to the effects observed in broiler chickens (Huang et al., 2006; Svihus et al., 2010; Williams et al., 2008).

CONCLUSION

Mash feed with coarser structure improved the apparent ileal starch digestibility, while expansion had no beneficial effect under the experimental conditions. Tested milling methods can be used for the feed production of laying hens without affecting animal performance.

Conflict of Interest Statement

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REFERENCES

Amad, A. A. 2001. Zum Einfluss unterschiedlicher Behandlungsverfahren und Zusatzstoffe auf ernährungsphysiologische Parameter und Leistung wachsender Broiler nach Verabreichung weizenbetonter Futtermischungen. [German] Diss. Georg-August-Universität Göttingen.

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

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

Amerah, A. M., V. Ravindran, and R. G. Lentle. 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Br. Poult. Sci. 50:366–375.

Amezuca, C. M., and C. M. Parsons. 2007. Effect of increased heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles. Poult. Sci. 86(2):331–337.

Betscher, S., A. Beineke, L. Schönfeld, and J. Kamphues. 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. Livest. Sci. 134:149–151.

Briggs, J. L., D. E. Maier, B. A. Watkins, and K. C. Behnke KC. 1999. Effect of ingredients and processing parameters on pellet quality. Poult. Sci. 78(10):1464–1471.

Dänicke, S., W. Kracht, H. Jeroch, R. Zachmann, E. Heidenreich, and R. Löwe. 1998. Effect of different technical treatments of rapeseed on the feed value for broilers and laying hens. Arch. Anim. Nutr. 51:53–62.

Denstadli, V., S. Balance, S. H. Knutsen, B. Westereng, and B. Svihus. 2010. Influence of graded levels of brewers dried grains on pellet quality and performance in broiler chickens. Poult. Sci. 89:2640–2645.

Ehle, F. R. 1984. Influence of particle size on determination of fibrous feed components. J. Dairy Sci. 67:1482–1488.

Fairfield, D. A. 2003. Pelleting for profit-Part 1. National Grain and Feed Association: Feed and Feed Digest. 54:1–5.

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

Giger-Reverdin, S. 2000. Characterization of feedstuffs for ruminants using some physical parameters. Anim. Feed Sci. Technol. 86:53–69.

Grosse Liesner, V., V. Taube, S. Leonhard-Marek, A. Beinecke, and J. Kamphues. 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%). J. Anim. Physiol. Anim. Nutr. 93:373–380.

Hamilton, R. M. G., and F. G. Proudfoot. 1994. Effects of ingredient particle size and feed form on the performance of Leghorn hens. Can. J. Anim. Sci. 75:109–114.

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

Hetland, H., B. Svihus, and V. Olaisen. 2002. Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. Brit. Poul. Sci. 43(3):416–423.

Hoffmann, T., C. Fürl, and H. Hempel. 2011. Energiesparendes Zerkleinern von Getreide mit einem Keilscheibenzerkleinerer[German]. Chemie Ingenieur Technik 83(5):699–703.

Huang, D. S., D. F. Li, J. J. Xing, Y. X. Ma, Z. J. Li, and S. Q. Lu. 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. Poult. Sci. 85:831–836.

Jia, W., and B. A. Slominski. 2010. Means to improve the nutritive value of flaxseed for broiler chickens: The effect of particle size, enzyme addition, and pelleting. Poult. Sci. 89:261–269.

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

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

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

Mavromichalis, I., and D. H. Baker. 2000. Effects of pelleting and storage of a complex nursery pig diet on lysine bioavailability. J. Anim. Sci. 78:341–347.

Mirzaie, S., M. Zaghari, S. Aminzadeh, M. Shivazad, and G. Mateos. 2012. Effects of wheat inclusion and xylanase supplementation of the diet on productive performance, nutrient retention, and endogenous intestinal enzyme activity of laying hens. Poult. Sci. 91(2):413–425.

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

Nir, I., R. Hilley, G. Shefet, and Z. Nitsan. 1994. Effect of grain particle size on performance. 2. Grain texture interactions. Poult. Sci. 73:781–791.

Panigrahi, S., E. B Oguntona, and B. R. Roberts. 1996. Effects of oven-drying tubers of two high-protein sweet potato varieties at different temperatures on their feeding value in broilers. Br. Poult. Sci. 37:173–188.

Peron, A., D. Bastianelli, F.-X. Oury, J. Gomez, and B. Carre. 2005. Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed on a pelleted diet. Br. Poult. Sci. 46:223–230.

Rezvani, M., H. Kluth, C. Elwert, and M. Rodehutscord. 2007. Effect of ileum segment and protein sources on net disappearance of crude protein and amino acids in laying hens. Brit. Poult. Sci. 49(1):28–36.

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

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

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

Svihus, B. 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. World's Poult. Sci. J. 67:207–224.

Svihus, B., and H. Hetland. 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. Br. Poult. Sci. 42:633–637.

Svihus, B., E. Juvik, H. Hetland, and A. Krogdahl. 2004a. Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. Br. Poult. Sci. 45: 55–60.

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

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

VanKrimpen, M. M, R. P. Kwakkel, C. M. Van der Beet-Schwering, L. A. DenHartog, and M. W. Verstegen. 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. Poul. Sci. 87:485–496.

Williams, J., S. Mallet, M. Leconte, M. Lessire, and I. Gabriel. 2008. The effects of fructo-oligosaccharides of whole wheat on the performance and digestive tract of broiler chickens. Br. Poult. Sci. 2:75–82.

Wolf, P., M. Arlinghaus, J. Kamphues, N. Sauer, and R. Modsenthin. 2012. Impact of feed particle size on nutrition digestibility and performance in pigs. Übers. Tierern. 40:21–64.

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. Int. J. Poult. Sci. 2:75–82.

Yegani, M., and D. R. Korver. 2008. Factors affecting intestinal health in poultry. Poult. Sci. 87:2052–2063.