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Different particle sizes of laying hens diets and it's effect on blood biochemical parameters, ileal digesta viscosity and nitrogen retention

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Abstract: The aim of this work was to evaluate the effect of different particle sizes of hens diets on blood biochemical parameters, ileal digesta viscosity and nitrogen retention. In the experimental part, the effects of different physical structure were investigated on two groups of laying hens of the Bovans Brown hybrid from 76 to 80 weeks of age. A finely ground mash diet [geometric mean diameter (GMD), 632 μm] and a coarsely ground mash diet (GMD, 1 258 μm) with the equal nutritional content were used. In the experiment, the particle sizes of the feed mixtures were analysed and compared with the particle size of unaccepted feed residues using a feed separator. Furthermore, feed consumption, live weight of laying hens, blood biochemical parameters, digestive viscosity and nitrogen retention coefficient were assessed. The study revealed that the particle sizes of hens' diets significantly influenced the nitrogen retention coefficient, with higher values observed in the finely ground diet as compared to the coarsely ground diet (30.3 vs 24.0%; $P < 0.05$). However, no significant differences were observed in feed intake, live weight, blood biochemical parameters, or digesta viscosity between the dietary groups ($P > 0.05$). This finding highlights the potential of diet structure optimisation to improve nutrient utilisation efficiency, which is particularly relevant for reducing nitrogen excretion and its environmental impact. These novel insights provide a foundation for further research on the effects of feed structure on productivity and organ health.

Keywords: chromium oxide; geometric mean diameter; ileal viscosity; nutrition; poultry

The physical structure of compound feeds is determined by the size and shape of the particles of these compounds. Size can be defined as the average particle size distribution of the individual

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feed components or more simply as the fineness of feed grinding (Amerah et al. 2007). Davis et al. (1951) used the general terms "fine, medium and coarse" to describe the particle size. The specific size distribution according to the designation was described by Wolf et al. (2012) as coarse >1.4 mm; medium 0.8–1.4 mm; fine 0.4–0.8 mm and very fine <0.4 mm.

The average particle size is given as the geometric mean diameter (GMD; also referred to as dgw), and the uniformity of the particle size is described as the geometric standard deviation (GSD; also noted as Sgw), with lower GSD representing higher particle uniformity (Nir et al. 1994). Discrete mean (dMEAN) defines the mean particle size as the so-called discrete weighted average. This means that, in contrast to the simple arithmetic diameter of the particles of the feed mixture, it also considers their amount captured on the individual sieves. This quantity allows to characterise the structure of the feed mixture by a single number (Wolf et al. 2012).

The different feed particle size of diet affects poultry performance, such as growth (Amerah et al. 2007) or feed intake (Safaa et al. 2009). A well-chosen structure supports the development of the gizzard (Svihus 2011), duodenal weight (Gabriel et al. 2003), liver weight and small intestine length (Ege et al. 2019). Not only for this, but also for other reasons, the feed structure is also considered one of the main strategies in poultry nutrition (Svihus 2011). The ability to digest and utilise nutrients is then closely related to the viscosity of the digestion (Yasar 2003) and the associated digestibility of nutrients (Liu et al. 2015). It is generally presumed that finer grinding increases the surface area of the substrate, and thus its availability for enzymatic digestion, thereby stimulating higher secretion of digestive juices (Yokhana et al. 2016). However, the coarser parts of the feed also influence digestion due to better permeability of the digestive juices through the digesta (Lentle 2005). Therefore, it is important to find the optimal balance between these factions. The aim of present study was to evaluate the effects of different particle sizes of hen diets on blood biochemical parameters, digesta viscosity and nitrogen retention. Present study, primarily focused on the mentioned three factors, the performance was not monitored in this experiment. Furthermore, the study was conducted on laying hens after their peak egg production period, when productivity is already declining.

MATERIAL AND METHODS

Animals and diets

The animal procedures were reviewed and approved by the Animal Care Committee of Mendel University in Brno and by the Ministry of Education, Youth and Sports (MSMT-22771/2019-4).

The trial was carried out with 16 Bovans Brown hens from 77th to 80th weeks of age. The trial lasted for 21 days. A preparatory period was carried out for 7 days (from 76th week of the hens' age) and the balance period lasted for 21 days. Animals were divided by body mass into two groups with 4 rep-

Table 1. Composition and chemical analysis of experimental diets (g/kg)

Components	Fine diet	Coarse diet
Maize	330	330
Wheat	330	330
Soybean meal	193.8	193.8
$CaCO_3$	74.1	74.1
Rapeseed oil	31.7	31.7
Premix*	30.0	30.0
Monocalcium phosphate	5.0	5.0
Cr_2O_3	3.0	3.0
L-Lysine	1.4	1.4
DL-Methionine	1.0	1.0
Analysed nutrient content (as fed)		
Dry matter (g/kg)	880	880
ME_N by calculation (MJ/kg)	11.5	11.5
Crude protein (g/kg)	161	164
Ether extract (g/kg)	44.6	44.7
Crude fibre (g/kg)	18.0	18.4
Ash (g/kg)	117	125
Calcium (g/kg)	35.6	33.6
Total phosphorus (g/kg)	62.2	65.4

*Premix contains (per kg): L-Lysine 0.390 g; DL-Methionine 1.35 g; calcium 8.85 g; phosphorus 2.01 g; copper 9.00 mg; zinc 54.0 mg; iron 60 mg; manganese 72.0 mg; iodine 0.900 mg; selenium 0.24 mg; retinol 9 900 international units (IU); calciferol 3 000 IU; tocopherol 15.0 mg; thiamine 1.20 mg; riboflavin 3.60 mg; pyridoxin 1.62 mg; cobalamin 12.0 mg; biotin 0.090 mg; folic acid 0.900 mg; niacinamide 12.6 mg; calcium pantothenate 7.50 mg; choline chloride 180 mg; butylated hydroxyanisole 0.300 mg; butylhydroxytoluene 1.50 mg; ethoxyquin 3.00 mg

ME_N – apparent metabolisable energy (by calculation)

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licates, i.e. there were 8 hens per treatment. The Coarse experimental group was fed a diet containing coarser particles (wheat and maize were ground on a roller mill). The Fine experimental group was fed a diet containing finer particles (wheat and maize were ground on a hammer mill with a 3-mm sieve). The rations were calculated as isonitrogenous and isoenergic. The non-pelleted diets were offered to hens. The hens had free access to feed and water. Table 1 shows the composition and chemical analysis of the diets. Particle size distribution and geometric mean diameter, geometric standard deviation and discrete mean particle size of feed components in the fine and coarse diets are shown in Tables 2 and 3. The feeds were ground to the required particle size and collected. Samples were systematically taken using a quartering method to minimise sampling bias. The collected samples were subjected to particle size analysis using a standardised sieve set. The chemical composition of nutrient content of the diets was determined for dry matter, crude protein, ether extract, crude fibre, and ash according to Commission regulation (EC) 152/2009 (European Commission 2009). All hens

Table 2. Determined particle size distribution (percentage of retained particles on sieves) and geometric mean diameter, geometric standard deviation and discrete mean particle size of feed components

Particle size	*n*	Sieve openings sizes (µm)						GMD (µm)	GSD (µm)	dMEAN (mm)
		<300	300	1 000	1 500	2 000	3 000			
Ground wheat										
Fine	6	16.4[a]	36.2[a]	31.0[a]	13.5[b]	3.4[b]	0.8[b]	684[b]	694[b]	1.64[b]
SE		0.38	0.48	0.44	0.37	0.11	0.02	9.15	6.62	0.01
Coarse		5.81[b]	15.4[b]	14.1[b]	23.2[a]	41.9[a]	1.06[a]	1 703[a]	1 197[a]	3.27[a]
SE	6	0.44	0.90	0.27	0.28	1.15	0.11	320	20.9	0.43
P-value		0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00
Ground maize										
Fine	6	13.0[a]	57.2[a]	21.2[a]	7.09[a]	1.09[b]	0.00[b]	590[b]	472[b]	1.36[b]
SE		1.64	1.75	0.18	0.24	0.06	0.00	14.1	7.86	0.02
Coarse		0.62[b]	2.12[b]	2.47[b]	3.69[b]	25.8[a]	65.7[a]	2 838[a]	1 217[a]	4.82[a]
SE	6	0.06	0.23	0.21	0.20	0.26	0.88	28.9	36.7	0.03
P-value		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Soybean extracted meal										
As is	6	14.6	54.1	23.1	6.20	2.18	0.24	591	508	1.40
SE		0.58	0.32	0.48	0.21	0.09	0.05	9.20	3.93	0.01

[a,b]Different letters in one column for each component mean statistically significant differences ($P < 0.05$)
dMEAN = discrete mean; GMD = geometric mean diameter; GSD = geometric standard deviation; SE = standard error

Table 3. Determined particle size distribution (percentage of retained particles on sieves) and geometric mean diameter, geometric standard deviation and discrete mean particle size of fine and coarse diet

Diets	*n*	Sieve openings (µm)						GMD (µm)	GSD (µm)	dMEAN (mm)
		<300	300	1 000	1 500	2 000	3 000			
Fine diet	7	13.3[a]	51.8[a]	23.0[a]	7.94[b]	3.54[b]	0.70[b]	632[b]	555[b]	1.49[b]
SE		1.05	0.64	0.47	0.16	0.15	0.09	11.8	5.66	0.01
Coarse diet		8.79[b]	22.9[b]	12.5[b]	11.0[a]	26.9[a]	18.1[a]	1 258[a]	1 426[a]	2.91[a]
SE	7	0.49	0.51	0.13	0.05	0.38	0.47	20.3	17.8	0.03
P-value		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a,b]Different letters in one column mean statistically significant differences ($P < 0.05$)
dMEAN = discrete mean; GMD = geometric mean diameter; GSD = geometric standard deviation; SE = standard error

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were housed in the balance cages in temperature maintained room at approximately 24 °C. The room had controlled ventilation and lighting (16L : 8D). Health status was evaluated daily and live weight of hens was measured regularly during the trial.

All experimental animals were individually weighted at 76, 78 and 80 week of age. Excreta samples were collected at regular time intervals during two balance periods, each consisting of three consecutive days, resulting in a total of six replicates. In total, 96 samples were collected from both groups. The samples were placed in Petri dishes and subsequently stored in a freezing chamber at –20 °C until lyophilisation and further analysis. The experiment was terminated at 80th week of the hens' age. At the end of the experiment, hens were weighed and slaughtered by decapitation. At the same time, blood was collected for further biochemical analysis. The entire digestive tracts were removed and fresh digesta was obtained from the distal part of the ileum to determine viscosity.

The particle size of the feed ingredients used (wheat and corn) varied significantly (Table 2) to meet the criteria for fine and coarse forms. The structure of the soybean extracted meal could not be modified otherwise. The respective feed mixtures were composed of the fine and coarse components, which are shown in Table 3. The fine and coarse feed mixtures showed a significant difference, as indicated by the GMD value, which was considerably higher for the coarse feed mixture.

Blood biochemical parameters

Hens' blood was collected into heparinised tubes and centrifuged for 10 min at 3 000 rpm during 2 h after the collection. The separated blood plasma was frozen (–20 °C) until biochemical examination. The following parameters were determined using standardised biochemical methods using Erba Lachema (Czech Republic) commercial sets on the Ellipse automatic biochemical analyser (AMS Spa, Italy) in blood plasma samples (n = 8): enzyme activity AST – aspartate aminotransferase (AST/GOT 500); GGT – gamma-glutamyltransferase (GGT 250); ALT – alanine aminotransferases (ALT/GPT 500); ALP – alkaline phosphatase (ALP AMP 500) and LD – lactate dehydrogenase (LDH-L 100); creatine kinase – CK (CK – 100, No. 10004494). As other markers of hepatic metabolism, fat and nitrogen metabolism, as well as kidney functions, were determined concentrations of the total bilirubin – Bili (BIL T JG 350); glucose – GLU (GOD/POD, GLU 500); cholesterol (CHOL 250); TG – triglycerides (TG 250); uric acid (UA 500); TP – total protein (TP 500); albumin (Alb 500); creatinine – Creat (CREA 500). The globulin content was calculated (TP minus albumin). The concentration of urea was determined using commercial sets by Randox, United Kingdom (Urea, UR 107).

Measurement of ileal digesta viscosity

The fresh digesta (from each hen) was removed from the distal part of the ileum to determine viscosity according to Yasar (1999). An insufficient sample quantity was obtained from two hens; therefore, only six samples were included in the final analysis of digesta viscosity. The digesta was collected in tubes and then centrifuged for 10 min at 3 000 rpm. The resulting supernatant was pipetted into Eppendorf tubes. The samples were analysed for dynamic viscosity on an RST rheometer (Brookfield, MA, USA) at a constant shear strain rate of 50 s^{-1} with a standard cone-plate geometric arrangement (RCT-50-2; $\alpha = 2°$), including a temperature duplicator system. The measurement was performed in 10 replicates at 40 °C and the sample volume was 1.2 ml.

Chromium oxide determination

The excreta were lyophilised and then homogenised (ground to pass a 1-mm sieve) before analyses. Chromium oxide (Cr_2O_3) content was then determined in the feed and faeces, as well. The principle is that the chromium content is determined by titration after oxidation to dichromate. A 1 g sample (to 3 decimal places) of feed or faeces was weighed into a porcelain crucible, which was then burned in a muffle furnace at 550 ± 20 °C for 4 hours. The resulting ash was melted on burner with 2–3 g of melting mixture ($KClO_3$ + Na_2CO_3; 4 : 1, respectively). After cooling, the crucible with the melt was poured into the beaker with hot distilled water. It was then covered with a watch glass and leached for 30 min while heating. The contents of the beaker were quantitatively transferred to a 100 ml volumetric flask after cooling, made up to the mark, mixed and filtered through a thick

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filter paper. For the titration, 50 ml of filtrate were pipetted off, 10 ml of potassium iodide (30% solution) and 5 ml of 25% sulfuric acid solution were added. A few drops of dissolved starch (2% solution) were added as an indicator. The solution was stirred and titrated with a standard solution of sodium thiosulfate (0.1 N) until the colour changed. Each sample was performed duplicate. The proportion of chromium oxide was calculated from the measured consumption of thiosulphate.

Equation (1) = chromium oxide content (X) g/kg in sample was calculated as:

$$X = (cv \times F \times V_0 \times 2.533)/(V_1 \times w) \quad (1)$$

where:

cv – consumption of a standard volumetric solution of thiosulphate;
F – volumetric solution factor;
V_0 – leachate volume (100 ml);
V_1 – pipetted volume;
w – sample weight in g.

The dry matter and nitrogen content in feed and excreta were determined according to the Commission regulation (EC) 152/2009 (European Commission 2009).

The nitrogen retention was calculated as follows:

$$100 - [(\%\ Cr_2O_3 \text{ in the diet} \times \%\ \text{nutrient in the excreta content}) / (\%\ Cr_2O_3 \text{ in the excreta content} \times \%\ \text{nutrient in the diet})] \times 100 \quad (2)$$

Sieve analysis of feed and leftovers

Feeds, coarse and fine diets and appropriate leftovers were analysed using a Retsch AS 200 Control sieve set (Germany). The feed, feed mixtures and leftovers were separated by a cascade of 5 sieves with square holes of different sizes: sieve 1 = 3.0 mm; sieve 2 = 2.0 mm; sieve 3 = 1.5 mm; sieve 4 = 1.0 mm; sieve 5 = 0.3 mm. The separation time was 10 min, and the amplitude was set at 1.80 mm/g.

After shaking, the sieves with separated samples were weighed and the weight of each was recorded. After subtracting the weight of the empty sieves, the weight of the particles adhering to the individual sieves (including the bottom dish) was determined. Subsequently, the GSD, GMD and dMEAN were calculated (American Society of Agricultural and Biological Engineers 2008 and Fritz et al. 2012, respectively).

Statistical analysis

The data were processed by Microsoft Excel (USA) and TIBCO Statistica v12.0 (USA). The Shapiro-Wilk W test was used to test the normality of the data distribution.

Extreme values were excluded to ensure that the data set adhered to a normal distribution model.

Of the 96 analysed samples for chromium dioxide determination, only 82 faecal samples were included in the final evaluation after the exclusion of extreme values based on statistical assessment.

The results are presented as mean and standard error (SE) for determination of particle size distribution or standard error of the mean (SEM). Students' t-test was used to determine the differences between groups and $P < 0.05$ was regarded as a statistically significant difference.

RESULTS

Particle sizes of feeds, diets and unaccepted feed residues

Table 3 shows that the coarse diet contained a significantly higher proportion of 1.5–3 mm particles, compared to the fine diet, in which more than 50% of the sample weight adhered to the 0.3 mm sieve. This corresponds to a significant difference ($P < 0.05$) between GMD, GSD and dMEAN values of fine and coarse feed mixtures.

As in the case of the experimental diets, a significant difference in the monitored parameters between the fine and coarse groups ($P < 0.05$) was demonstrated in the unaccepted residues of the feed mixtures. However, no significant difference was observed ($P > 0.05$) comparing coarse residues with the coarse mixture. On the other hand, fine diet compared to fine feed residues showed significant difference ($P < 0.05$). Similar results for diets and unaccepted feed residues were also observed for the weight distribution of the individual fractions of these mixtures on the separator sieves, as shown in Table 4.

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Table 4. Comparison of feed particle size distribution (percentage of retained particles on sieves) and geometric mean diameter, geometric standard deviation and discrete mean between approtiate diets and feed residues

Particle size	*n*	Openings (μm)						GMD (μm)	GSD (μm)	dMEAN (mm)
		<300	300	1 000	1 500	2 000	3 000			
Diets × feed residues										
Fine diet	7	13.3[a]	51.8[b]	23.0	7.94[a]	3.54[a]	0.70[a]	632	555[a]	1.49[a]
SE		1.05	0.64	0.47	0.16	0.15	0.09	11.8	5.66	0.01
Fine residues		9.92[b]	60.1[a]	21.5	6.09[b]	2.05[b]	0.27[b]	626	465[b]	1.41[b]
SE	24	0.54	0.93	0.55	0.38	0.23	0.03	10.3	9.89	0.02
P-value		0.01	0.00	0.16	0.02	0.00	0.00	0.76	0.00	0.03
Coarse diet	7	8.79	22.9	12.5	11.0	26.9	18.1	1 258	1 426	2.91
SE		0.49	0.51	0.13	0.05	0.38	0.47	20.3	17.8	0.03
Coarse residues		10.0	24.9	13.0	11.6	30.0	18.3	1 264	1 441	3.10
SE	23	0.82	2.56	1.01	0.82	2.79	1.31	34.3	35.8	0.21
P-value		0.42	0.67	0.8	0.69	0.54	0.94	0.93	0.83	0.63

[a,b]Different letters in one column mean statistically significant differences ($P < 0.05$)

dMEAN = discrete mean; GMD = geometric mean diameter; GSD = geometric standard deviation; SE = standard error

Table 5. Mean feed consumption per trial

Diet	*n*	Average feed consumption per trial (g)	Average feed consumption per hen and day (g)
Fine diet	8	2 412	115
Coarse diet	8	2 555	122
SEM		102	5.09
P-value		0.52	0.52

SEM = standard error of the mean

Table 6. Live weight (g) of hens during trial

Diet	*n*	76 weeks of life	78 weeks of life	80 weeks of life
Fine diet	8	1 918	1 865	1 768
Coarse diet	8	1 933	1 829	1 848
SEM	–	46.7	66.0	47.7
P-value	–	0.87	0.79	0.42

SEM = standard error of the mean

Feed consumption and live weight

Feed consumption in laying hens fed the fine mixture did not significantly differ from laying hens fed the coarser mixture ($P > 0.05$), as shown in Table 5. Laying hens fed the fine diet had only a slightly lower average daily feed intake compared to laying hens fed the coarse diet. Similarly, there were no differences in the live weights of laying hens at 76, 78 and 80 week of age, as shown in Table 6. Interestingly, in both the fine and coarse groups, there was a gradual decrease in weight during the experiment, which lasted throughout the experiment in the fine group, while the coarse group experienced a slight increase in mean weight again after 78th week of age.

Blood biochemical parameters

Based on blood biochemical analysis, no statistically significant differences were found ($P > 0.05$) in any of the monitored parameters in respective groups of laying hens. This finding suggests that different feed particle size does not affect biochemical blood parameters. Detailed results can be found in Table 7.

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Table 7. Blood biochemical parameters

Parametre	Fine diet	Coarse diet	SEM	*P*-value
	n = 8	*n* = 8		
AST (µkat/l)	3.47	3.42	0.19	0.89
GGT (µkat/l)	0.15	0.13	0.04	0.85
ALP (µkat/l)	8.21	10.9	1.51	0.39
ALT (µkat/l)	0.43	0.45	0.04	0.87
LD (µkat/l)	8.65	9.42	0.71	0.61
CK (µkat/l)	59.0	67.4	8.65	0.65
TBili (µmol/l)	3.36	2.76	0.56	0.61
Glu (mmol/l)	12.2	12.5	0.24	0.55
TG (mmol/l)	4.85	5.68	0.55	0.47
Chol (mmol/l)	3.85	4.56	0.41	0.41
TP (g/l)	53.3	51.9	1.52	0.64
Alb (g/l)	20.6	22.1	0.50	0.14
Glob (g/l)	32.7	29.8	1.46	0.33
Alb/Glob	0.66	0.76	0.04	0.18
UA (µmol/l)	426	396	29.10	0.61
Urea (mmol/l)	1.31	0.74	0.19	0.14
Creat (µmol/l)	25.4	20.8	2.04	0.27

Alb = albumins; Alb/Glob = albumins/globulins; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; creat = creatinine; GGT = gamma-glutamyltransferase; Glob = globulins; Glu = glucose; Chol = cholesterol; LD = lactate dehydrogenase; *n* = number of hens; SEM = standard error of the mean; TBili = total bilirubin; TG = triacylglycerols; TP = total protein; UA = uric acid

Table 8. Ileum digesta viscosity

Diet	*n*	Ileum digesta viscosity (mPa·s)
Fine diet	6	4.08
Coarse diet	6	4.49
SEM	–	0.31
P-value	–	0.54

SEM = standard error of the mean

Ileal digesta viscosity

There was no statistically significant difference ($P > 0.05$) in the viscosity of the digestion between the groups of laying hens fed fine and coarse feed mixtures, as shown in Table 8.

Nitrogen retention

Nitrogen retention was significantly higher ($P < 0.05$) in the group of laying hens fed the fine mixture compared to the group of laying hens fed the coarse mixture (Table 9).

Table 9. Nitrogen retention coefficient

Diet	*n*	Nitrogen retention (%)
Fine diet	41	30.7[a]
Coarse diet	41	24.0[b]
SEM	–	1.34
P-value	–	0.01

[a,b]Different letters in one column are statistically different $P < 0.05$

SEM = standard error of the mean

DISCUSSION

Particle sizes of feed mixtures and feed residues

Studies describing the different feed particle sizes in diet mention many ways to describe and distinguish these feed mixtures. In the present study, a total of four methods were used to describe and distinguish them.

The first method was the mass resolution of the individual fractions on the separator sieve system. Our results were consistent with the characteristics

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reported by Wolf et al. (2012), which concerned both feed mixtures and unaccepted feed residues. The dMEAN, GMD and GSD values were calculated by means of this method.

The discrete mean of the diets used in present study approximately corresponded to the experiment of Hafeez et al. (2015). This diameter considers not only the size diameter of the particles retained on the individual sieves according to their roughness, but also the weight fraction of the individual particle fractions retained on these sieves. As mentioned by Fritz et al. (2012), the calculation of dMEAN in similar analyses is not often used. This may be due to the fact that the obtained results are relatively distorted by the discrete way of method, which does not consider the scattering of particle size between sieves, but is affected by the different sieve screen size of the sieves themselves. The largest parts, compared to the smaller ones, even if they were in a large weight predominance, can distort the result. Particles retained to the finest sieves can thus be "backed up" by the larger ones, despite their significant share. In the presented study, therefore, this value is rather an additional parameter.

The more generally used value for describing the structure of the diets and the average particle size relative to the mass representation of the individual fractions is the geometric mean diameter. With the GMD, there is no tendency of the data to adapt to higher values, which are observed with a lower frequency, so the obtained values are also more accurate. The results obtained by GMD values in our experiment are approximately identical to the experiments of other authors who studied the effects of fine and coarser particle structure on poultry performance (Reece et al. 1985: 814 µm vs 1 343 µm; Nir et al. 1990: 574 µm vs 905 µm; Lott et al. 1992: 679 µm vs 1 196 µm; Ege et al. 2019: 707 vs 1 096 µm).

The geometric standard deviation, in this case relative to the mass, determines the extent to which the particle size values deviate from their mean size. In the case of a lower GSD value, the feed particles acquire a more homogeneous character, and conversely, with a higher GSD value, larger mutual differences are observed – the mixture is more heterogeneous. Based on the findings of present study, it can be assumed that the separation, sorting and preference of some fractions of the coarse diet compared to other fractions in feed residues of the coarse diet was almost non-existent and their intake by laying hens was not affected. Some authors mention the preference for larger particles in feed mixtures for poultry (Nir et al. 1994) which, in our study, has been proven in the group fed the fine diet.

In the feed residues from the fine diet, a smaller proportion of particles larger than 1 500 µm was found compared to the fine diet. This means that diet was separate by hens, as evidenced by the reduced value of dMEAN (Table 3). The higher proportion of particles 300–1 000 µm in the fine feed residues means that the hens tried to select coarser particles from a fine mixture. The difference in the fraction smaller than 300 µm (between the fine diet and the fine feed residues) could be due to the spraying of finer particles (vitamin–mineral supplement etc.) because the feed mixture was fed in non-pelleted form. This phenomenon can be prevented by feed pelleting, which we would focus on in future work.

Feed consumption and live weight

According to the Bovans Brown technological manual, the average feed consumption per laying hen at the observed age should be 116 g per day. In present study, feed consumption for laying hens fed the fine diet was slightly lower, while for laying hens fed the coarse diet it was higher. Although there was no statistically significant difference between groups in our results ($P > 0.05$), Safaa et al. (2009) observed a higher feed intake in laying hens fed coarsely ground cereals in their experiment. Nir et al. (1990) and Amerah et al. (2007) reported similar findings. In general, it can be stated that poultry will be more willing to accept feed mixtures with a coarse feed particle size. In addition, some recent research confirms that the relative weights of the gizzard and pancreas increase during the intake of coarser mixtures (Ege et al. 2019; Novotny et al. 2023), which has a positive effect on intestinal motility (Ferket 2000).

Technological manual for Bovans Brown states that laying hens from 76 to 80 weeks of age should reach a weight of 1 993–1 996 g. In our experiment, these results were not achieved, but the gradual weight loss in laying hens at this age could be due to a slower adaptation to the experimental environment, which persisted until the balance period. The slight weight gain in laying hens fed a coarse

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mixture from 78th week of age is supported by experiments performed by Reece et al. (1985), Nir et al. (1990), Amerah et al. (2007) and others. Thus, it can be stated that the use of coarser particles in compound feeds can more effectively support not only the willingness to eat, but also positively affect the live weight of animals.

Blood biochemical parameters

The results of the biochemical analysis of blood in present study are consistent with findings of Rezaeipour and Gazani (2014), who did not show a significant difference in values for blood serum parameters in broilers fed a fine and a coarse diet. But it was affected by shaping; the content of triacylglycerols and VLDL (very low-density lipoproteins) in unshaped diets was significantly lower than in the moulded mixture. On the other hand, Novotny et al. (2023) found that the use of finely ground particles in the feed increased the level of gamma-glutamyl transferase and decreased the level of urea. This could indicate adverse changes in the liver of broiler chickens. The trial of Amoozmehr et al. (2023), however, even in the case of shaped vs unshaped diet, did not show significant differences in blood parameters of broiler parent stock pullets. This was probably due to the fact that the nutritional composition of the feed has a greater effect on the biochemical parameters of blood, rather than the feed particle size of the diet. This is proved, for example, by the experiments of Alagawany et al. (2014), Ndazigaruye et al. (2019) and other authors.

Ileal digesta viscosity

The viscosity of the chyme is an important parameter in the evaluation of animal nutrition. Increased digestive viscosity can cause several problems, such as reducing digestive tract passage (Denbow 2015), minimising mixing of intestinal content (Ward 1996), enzyme substrate binding (Almirall et al. 1995), and adverse changes of the microbial population in digestive tract (Ward 1996). Although the results of the present study did not show significant changes in the digesta viscosity of laying hens, the results of other studies vary. For example, Yasar (2003) found that poultry fed a fine wheat-based mixture had a high digestive viscosity compared to those fed medium or coarse wheat particles in feed mixtures. Moreover, the composition of the feed and especially the higher proportion of non-starch polysaccharides (NSPs), which increase the digesta viscosity, play an important role, as confirmed by Lee et al. (2010) or Hejdysz et al. (2018).

Nitrogen (N) retention

It is generally believed that finer grinding feed increases the surface area of the substrate availability for enzymatic digestion, which stimulates higher secretion of digestive juices, and thus facilitates retention (Hetland et al. 2002; Yokhana et al. 2016). Based on our findings, it can be stated that hens fed with finely ground diet achieved better nitrogen retention than hens fed with coarse diet. This can be caused by the fact mentioned above. Similar values of nitrogen retention in the experiment with laying hens were also found by Meluzzi et al. (2001), who observed a gradual decrease in nitrogen retention (48.9% in the 40th week of laying period with a gradually declining trend). On the other hand, Ege et al. (2019) did not notice significant differences in nitrogen retention, when feeding coarse or fine diets. The increase in the nitrogen retention coefficient was observed mainly in experiments with pelleted feed mixtures, as evidenced by Zelenka (2003) in an experiment on broiler chickens. Extrusion of feed mixtures also improved the retention of nitrogen and other nutrients, as demonstrated by the experiment of Lichovnikova et al. (2004).

It was found that the fine structure of the diet in present study led to a higher nitrogen retention, but at the same time, there was no effect (or trend) on any other monitored parameters. It can be noted that this is a primary study and for more detailed results it is necessary to repeat the experiment with a larger number of animals and extend the selection of monitored parameters. In future research, we will focus on the histology and morphometry of the digestive tract, as well as performance parameters.

Additionally, we plan to investigate the pelleted feed mixtures and the impact of its particles on both digestive tract health and overall performance. Regarding economic issues, higher N retention should be considered in relation to animal

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performance as well as blood biochemical parameters (protein metabolism). Therefore, at this point, it is difficult to say whether the effect of higher N retention can somehow manifest itself economically in egg production. In the same way, it is still impossible to say whether it is profitable to produce compound feeds with a fine or coarse structure. In addition, Novotny et al. (2023) found that the use of coarse feed particle size in the diet of broiler chickens positively affected gizzard weight, as well as the height and crypt depth of small intestinal villi, thereby increasing the surface area available for nutrient digestion.

CONCLUSION

In the present study with laying hens after their laying peak period, it was found that the structure of the feed mixture influenced the nitrogen retention coefficient, with higher values observed in the finely ground diet.

Although the hens consumed more of the coarse diet, this did not affect ($P > 0.05$) the differences in overall feed intake or other monitored parameters. This preference for coarser feed particles is particularly important during the life of pullets and the early stages of laying in hens, where it has been shown that coarser particles have a more significant effect on the development of individual sections of the digestive tract, particularly the gizzard. Therefore, in older laying hens with fully developed digestive tracts, our results suggest a gradual change in diet structure due to better nutrient retention in fine diets. Present study serves as a pilot investigation, and its findings should be further explored through a more comprehensive study focusing on the effects of feed structure on productivity and organ health, particularly the small intestine.

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Conflict of interest

The authors declare no conflict of interest.

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