British Poultry Science

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/cbps20

Feed form and perch design do not interact to production performance, gastrointestinal tract traits, behaviour and welfare of laying hens reared in enriched cages

B. Tarım, Ş. E. Demirtaş, M. Bozkurt, A. Ö. Üstündağ, O. Eray, O. Ahlat, H. Ö. Bayır, H. Akşit, S. Kamanlı, S. Özkan, S. Yalçın & A. E. Tüzün

To cite this article: B. Tarım, Ş. E. Demirtaş, M. Bozkurt, A. Ö. Üstündağ, O. Eray, O. Ahlat, H. Ö. Bayır, H. Akşit, S. Kamanlı, S. Özkan, S. Yalçın & A. E. Tüzün (19 Feb 2024): Feed form and perch design do not interact to production performance, gastrointestinal tract traits, behaviour and welfare of laying hens reared in enriched cages, British Poultry Science, DOI: 10.1080/00071668.2024.2308278

To link to this article: https://doi.org/10.1080/00071668.2024.2308278

BRITISH POULTRY SCIENCE
https://doi.org/10.1080/00071668.2024.2308278

Taylor & Francis
Taylor & Francis Group

Feed form and perch design do not interact to production performance, gastrointestinal tract traits, behaviour and welfare of laying hens reared in enriched cages

B. Tarım[a], Ş. E. Demirtaş[a], M. Bozkurt[b], A. Ö. Üstündağ[b], O. Eray[a], O. Ahlat[c], H. Ö. Bayır[a], H. Akşit[d], S. Kamanlı[e], S. Özkan[f], S. Yalçın[f] and A. E. Tüzün[g]

[a]Department of Breeding, Poultry Research Institute, Ankara, Turkey; [b]Department of Animal Science, Faculty of Agriculture, Aydın Adnan Menderes University, Aydın, Turkey; [c]Department of Pathology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey; [d]Department of Biochemistry, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey; [e]Department of Animal Science, Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Turkey; [f]Department of Animal Science, Faculty of Agriculture, Ege University, İzmir, Turkey; [g]Koçarlı Vocational School, Aydın Adnan Menderes University, Aydın, Turkey

ABSTRACT

1. It was hypothesised that perch material and design may affect utility and maintenance energy demand in laying hens, affecting their feed form preferences and daily feed consumption. Accordingly, perch design and feed form on hen performance, gastrointestinal tract functions and some behavioural and welfare-related traits were studied in laying hens (ATAK-S) reared in enriched colony cages from 24 to 40 weeks of age.
2. The experiment was a 2 × 2 factorial investigating two perch materials and design (circular steel or mushroom-shaped plastic) and feed form (mash or crumble). A total of 396 hens were randomly assigned to one of the four treatment groups with nine replicates each (11 birds per replicate).
3. Except for feeding behaviour and prevalence of foot pad dermatitis at 40 weeks of age, the modification of the perch design did not have a significant effect on the traits examined. Mushroom-shaped plastic perches reduced feeding behaviour ($p < 0.01$) and the incidence of foot pad dermatitis at 40 weeks of age ($p < 0.001$).
4. Performance traits were not affected by feed form. Intake, final body weight and FCR for crumble-fed laying hens were greater than those fed mash ($p < 0.01$).
5. Hens fed mash had higher ($p < 0.01$) relative gizzard weights along with lower ($p < 0.05$) pH values, pancreatic chymotrypsin, amylase and lipase activities ($p < 0.05$), and duodenal absorption surface areas ($p < 0.01$). Ultimately, this gave higher protein digestibility ($p < 0.05$) compared to those receiving crumble.
6. In conclusion, in enriched cage rearing systems, mashed feed was preferred over crumble to efficiently maintain productive performance. Compared to circular steel, plastic mushroom-shaped perches were associated with better footpad health and welfare.

ARTICLE HISTORY
Received 16 May 2023
Accepted 20 December 2023

KEYWORDS
Mash; crumble; perch design; hens; performance

Introduction

Over the decades, there has been an important shift away from conventional battery cages to enriched colony cages as a result of growing public concern about the welfare and behaviour problems with laying hens reared in battery cages. Although this is still being debated in some countries such as Italy, Spain, Serbia, Turkey, Brazil and China, other countries, including the Netherlands, UK, the U.S.A. and Germany, have converted from cages to aviary or free range system (Konkol, Popiela, and Korczynski 2020; Mench, Summer, and Rosen-Molin 2011; Pickel, Schrader, and Scholz 2011; Tactacan et al. 2009). Accordingly, a large body of research has examined hens' responses to new rearing systems in terms of welfare, productivity and health concerns (Chargo et al. 2019; Hetland et al. 2003; Konkol, Popiela, and Korczynski 2020; Pohle and Cheng 2009; Tactacan et al. 2009). When compared to conditions in conventional cages, the additional space offered to laying hens in enriched colony cages, such as perches, nest and scratching pads, might stimulate a greater variety of locomotor activity, which in turn, could increase hens' maintenance energy requirements, and, ultimately, daily feed consumption (Carey, Kuo, and Anderson 1995; Preisingir 2000). Perches are regarded as the most essential enrichment in colony cages (Hester et al. 2013; K. Liu et al. 2018).

Various perch design features influence their use, such as shape (Pickel, Schrader, and Scholz 2011; Struelens et al. 2009) and material (Appleby, Smith, and Hughes 1992; Pickel, Scholz, and Schrader 2010) shape and material (Skanberg, Nielsen, and Keeling 2021). Perches made from circular steel pipes with smooth and slippery surfaces have poor grip quality, preventing hens from wrapping their toes around in a locked grip (Struelens et al. 2009). Hence, their feet may slip backward and forward, resulting in extra activity to maintain stability during perching (Duncan, Appleby, and Hughes 1992). However, there is a lack of information regarding the preference of birds and their balance on perches. Recently, Skanberg et al. (2021) compared successful and problematic attempts of laying hen chicks to land on different types of perches including rope, round wood and flat wood perches differing in sizes. They reported that chicks had more successful landings on wide ropes (4.5 cm) and both narrow (1.5 × 1.5 cm) and wide flat (6.7 × 0.8 cm)

CONTACT M. Bozkurt mbozkurt@adu.edu.tr Department of Animal Science, Faculty of Agriculture, Aydın Adnan Menderes University, Çakmar - Koçarlı, Aydın 09700, Turkey

perches. However, similar rates of successful or problematic landing attempts were reported for round perches that had a diameter of 3.5 cm (wide) or 1.5 cm (narrow). Therefore, it has been suggested that round perch shapes may negatively affect balance of the chicks as compared to rope and flat wood perches. In an earlier study, Scott and MacAngus (2004) used wood, metal and poly-vinyl-chloride perches and reported that laying hens slipped more on metal and poly-vinyl-chloride than on wooden ones, but only when the perches were clean. However, there was no difference between the perch types when the perches got covered with manure.

Despite these concerns with using steel perches, they are still the choice of enriched cage manufacturers and laying hen breeders worldwide owing to durability, usefulness and cage hygiene concerns (Appleby, Smith, and Hughes 1992; Oester 1994). However, owing to their wider and slightly sloping surface compared to smoother steel pipe, it has been assumed that mushroom-shaped plastic perches assist the hens when grasping the perch (Pickel, Scholz, and Schrader 2010; Scholz, Kjaerand, and Schrad 2014). This enables hens to be less active and calmer when roosting, while providing additional health benefits on foot and keel bone (Struelens et al. 2009; Tauson 2002; Tauson and Abrahamsson 1994). Mushroom-shaped plastic perches and round metal perches are usually preferred in enriched cages (EFSA 2015). Perch usage has been found to be associated with reduced footpad lesions, aggression and stress with positive impact on welfare of the birds (Bist et al. 2023). Although perching behaviour improved bone mineralisation, it is accompanied by a higher incidence of keel bone abnormalities, including fractures and deviations (Hester et al. 2013; Tauson and Abrahamsson 1994, 1996). Kappeli et al. (2011) reported a higher KBD incidence in rubber-coated metal perches in comparison to plastic perches. Circular shaped perches are associated with the lower peak force on the footpad but higher peak force on the keel bone, compared to sharp edged perches (Pickel, Schrader, and Scholz 2011). Therefore, perch design and material are important in minimising these welfare problems.

The feed form holds a critical role in determining feed intake, development of digestive organs and the health and performance of poultry. However, compared to broiler chickens, feed form remains an under-researched area in the nutrition of laying hens. It is noticeable that, as recently reported by several studies (Bozkurt et al. 2020; Kandasami et al. 2023; Wan et al. 2022), almost all of the data corresponding to feed structure-induced changes in the health, welfare and productivity of laying hens have been generated by studies carried out in conventional cages. No previous study has investigated whether changes in perch design can stimulate hens to increase feed intake by increasing their energy demand. Additionally, it is unclear to what extent feed form may influence the magnitude of this effect in satisfying the preference for additional energy demand.

Collectively, it has been assumed that increased motor activity, including attempts for successive perching on steel perches, affects energy requirement and feed consumption in hens. The existing evidence shows that laying hens select larger feed particles instead of finer ones, due to their innate feeding behaviour. In line with this, the feed in mash form was selected less than the pellet sticks and crumbled pellets (Ege et al. 2019; Koçer et al. 2016; Röhe et al. 2014). It was hypothesised that hens using steel perches will consume more feed with a preferable selection for the crumbled pellets than those perching on the plastic perches, suggesting interactions between feed form and perch design on feed intake, digestive functions and performance traits. Thus, the aim of the present study was to examine the influence of feed form (mash and crumbles) and perch design (circular steel perches and mushroom-shaped plastic perches) on feed intake behaviour, productive performance and gastrointestinal tract traits, including digestibility in hens during peak production.

Materials and methods

Birds, housing, and experimental design

The protocol for the animal experimental procedures was approved by the Animal Care and Use Committee of Poultry Research Institute, Ankara (Permit Number: 2003–2).

A total of 396, 17-week-old ATAK-S hybrid brown pullets with similar body weights (1316 ± 24 g) were used in this study. The pullets, at the beginning of the trial, were moved from the rearing facility to the experimental laying house. The birds were kept in three-tier enriched colony cages (240 cm wide × 60 cm deep × 57 cm height; Kutlusan® Cage Equipment, İzmir-Turkey) in an environmentally controlled room at 22 ± 3°C and 59 ± 4% relative humidity. They were each randomly allocated to one of the 36 cages containing 11 birds each at a stocking density of 1309 cm^2/bird. Each cage was equipped with a curtained nest area (172 cm^2/bird; red curtains), perches running lengthwise in the middle of the cage (21 cm/bird), a scratch mat (88 cm^2/bird; rubber mat surface), a feeder mounted on the front of the cage outside the bird space (21.8 cm/bird) and nipple drinkers (1.4 birds/nipple). Circular metal perches (40 mm diameter) and mushroom-type plastic perches (38.7 mm wide and 31.64 mm height) were used in the experiment. The perches were positioned 10 cm above the ground and the distance between the top of the perch and the ceiling was 40 cm. New feed was added into the through feeders once a day (at 0800 h) to reduce feed spillage during both growth and laying periods. Diets and drinking water were offered *ad libitum*.

Pullets were reared in conventional growing cages and did not have access to perches or any other enrichment equipment. During the growth period, birds were vaccinated against main diseases (infectious bronchitis disease, infectious bursal disease, Newcastle disease, Coryza, Fowl Pox and Salmonellosis) according to commercial practices. The pullets were kept on a 23 h/d light programme for the first weeks of life and then light was decreased 2 h/weeks until reaching 12 h at 6 weeks of age. From 7 to 17 weeks of age, the light programme was kept constant. The lighting schedule was L12: D12 at 18 weeks (lights on 6:30–18:30) and increased weekly by 1 hour of light until it reached L16: D8 at 22 weeks of age (lights on 6:30–22:30). During the growing period, each pullet was allocated a through feeder with a length of 6.9 cm.

Each cage was randomly allocated to one of four treatments in a 2 × 2 factorial design. The factors included perch design (circular steel *vs.* mushroom-shaped plastic) and feed form (mash *vs.* crumble). Nine replicate groups of 11 hens each were allotted to each treatment in a randomised design.

Experimental diets

One identical basal experimental diet was produced. This diet was formulated mainly with maize and soybean meal, according to the recommendation of NRC (1994) for laying hens at peak production phase (*i.e.*, 20 to 45 weeks). Maize, the main cereal grain used in this diet, was ground using a hammer mill (Münch-Edelstahl GmbH Weststraße 2 640 721-Hilden, Germany) through a 5-mm screen. After grinding, all components of the mash diet were mixed in a horizontal mixer (KT 1000 K; Kocamaz Machine Industry 35 860-Izmir, Turkey) capable of mixing 3000 kg of feed/h. Birds were fed a pullet grower diet (based on ground maize, ground wheat and soybean meal) from weeks seven to 16, a pre-lay diet from weeks 17 to 19 and a layer diet from weeks 19 to the 40. The grower and pre-lay diets were formulated to meet the recommendations from NRC (1994) and prepared in mash form. The assay diets were offered 2 weeks prior to the data collection period (24–40 weeks) as an adaptation period.

The basal mash diet was not subjected to heat treatments or crumbled. To form crumble, the mash diet was processed in a steam conditioner at 85°C for 20–30 s, then steam pelleted in a mill (pelleter; Münch-Edelstahl GmbH Weststraße 2 640 721-Hilden, Germany) capable of manufacturing 2000 kg of feed/h with a die size of 4-mm and 35-mm thickness. The pellets were then crushed to form crumbles measuring 2–3-mm. Within 8–9 min after pelleting, the crumbles were cooled to ~ 24°C.

Laying performance and egg quality

All hens were weighed individually at 24 and 40 weeks of age to determine hen body weight (BW) and weight gain (BWG). During the experimental period, eggs were collected daily to calculate egg production rate. The average daily feed intake per hen (ADFI) and feed conversion ratio (FCR) were determined at 7 d intervals per cage replicate. The FCR was expressed as eggs produced (kg feed/kg egg). Egg mass was calculated by multiplying egg weight by production rate. Production variables such as ADFI and egg production were adjusted for hen mortality. Any mortality was recorded and the weight of each dead hen was determined. The cracked-broken egg percentage and shell-less egg percentage (defined as an egg without a shell but with an intact membrane) were calculated by dividing the total number of deformed eggs by the total number of eggs in each treatment. All production variables were determined cumulatively (24–40 weeks of age) by cage replicate.

A total of 18 eggs were randomly collected from each experimental group (two eggs per cage) every 28 d to assess eggshell quality variables. The shape index was measured using the FHK egg shape determinator (Fujihira Industry Co., Tokyo, Japan). The weight of albumen, yolk and eggshell were divided into whole egg weight and then multiplied by 100 to determine percentage weight. Eggshell strength was determined by applying increased pressure to the broad end of each egg, using a force gauge (Egg Force Reader, SANOVO Technology A/S, Odense, Denmark). Thickness was measured at the two ends and in the middle section of the eggshell with a digital micrometre (model IT-014UT-Mitutoyo, Kawasaki, Japan). Egg weight, albumen height and Haugh units (HU) were assessed with multi-tester equipment (Egg Analyzer®, ORKA Technology LLC, Ramat HaSharon 47 100, Israel) as per the method published by Bozkurt et al. (2012). Yolk height and diameter were evaluated using a micrometre (model IT-014UT-Mitutoyo, Kawasaki, Japan). The Haugh unit was based on the formula proposed by Haugh (1937) as follows:

$$\text{HU}\ (\%) = 100 \times \log\ (\text{H} + 7.57 - 1.7\text{W}^{\,0.37})$$

The intensity of the yolk colour was appreciated to match the colour numbers in the Roche yolk colour fan scale (Vuilleumier 1969).

Determination of weight and length of digestive organs and pH of proximal GIT

At the end of the 40 weeks of age, two birds per replicate were randomly selected, weighed individually and killed by cervical dislocation. The digestive tract (from the beginning of the crop to the cloaca, including digesta content) and the liver and the pancreas were removed aseptically. Then, the crop, proventriculus and gizzard were emptied from any digesta, cleaned, dried with desiccant paper and weighed. The weight of the crop, proventriculus, gizzard, liver and pancreas were expressed relative to live BW. In addition, the length of the duodenum (from gizzard to pancreo-biliary ducts), jejunum (from pancreobiliary ducts to Meckel's diverticulum), ileum (from Meckel's diverticulum to the ileo-caecal junction), and of the two caeca (from the ostium to the tip of the right and left caeca) was measured on a glass surface using a flexible tape with a precision of 1 mm and expressed relative to live BW. The pH of the crop, proventriculus and gizzard contents were recorded by immersing the electrode (Sensorex, S200C Epoxy, Garden Grove 92 841, CA) of a digital pH metre (Hanna Instruments, HI 2211, Woonsocket 02 895 RI) into the centre of the organs. The pancreas and small intestines of these birds were used for analysis of pancreatic enzyme activities and histomorphology of the villus, respectively.

Determination of pancreatic enzyme activities

Tissue samples were immediately weighed and washed in ice-cold PBS (pH 7.4) solution to remove excess blood, homogenised (2,000 rpm/min for 1 min, 1:10 w/v) using a stirrer (Stuart SHM 1, UK) in PBS in an ice bath. Then, homogenate was centrifuged at 5000×g for 5 min at 4°C to obtain the supernatant. The resultant supernatant was used for analyses. Trypsin (chicken trypsin ELISA kit, Bioassay Technology Laboratory: BT Lab, E0186Ch, China), pancreatic alpha-amylase (chicken pancreatic alpha-amylase ELISA kit, Bioassay Technology Laboratory: BT Lab, E0185Ch, China), lipase (chicken lipase ELISA kit, Bioassay Technology Laboratory: BT Lab, E0301Ch, China) levels were measured using a commercial kit as instructed by the manufacturer on an ELISA reader (Thermo Multiskan FC, U.S.A.).

Apparent total tract digestibility coefficients of nutrients

The apparent total tract digestibility (ATTD) coefficients of nutrients were determined *via* the addition of chromic oxide (Cr_2O_3) as an indigestible analytical marker. The chromic oxide was carefully added and mixed into each of the four experimental diets for the digestibility experiment at a final concentration of 2 g Cr_2O_3/kg diet, at the expense of maize.

From week 38 onwards, all birds were fed diets containing Cr_2O_3. The digestibility experiment used a 4 d pre-experimental adaptation period and a 3 d collection period. At 39 weeks of age, during the 3 d collection period, excreta from each cage was collected twice daily (*i.e.*, 1000 to 1200 h and 1800 to 2000 h) and stored in sealed bags at −20°C. The remaining feed and feathers in the excreta trays were carefully removed. Excreta collected per cage during the 3d collection period was pooled, resulting in nine samples for each of the four treatments. The following equation was used to calculate ATTD (M. L. Scott, Nesheim, and Young 1976):

$\text{ATTD}\ (\%) = 100 - [(\text{diet } Cr_2O_3/\text{excreta } Cr_2O_3) \times (\text{nutrient in excreta}/\text{nutrient in diet})] \times 100.$

Laboratory analyses

The nutrient content of the diets was determined by proximate analysis. Representative samples of feeds were ground in a laboratory mill fitted with a 1 mm screen (Retsch Model Z-I, Stuttgart, Germany) and analysed for dry matter, crude protein (N × 6.25, Kjeldahl Procedure; Vapodest, 30S, Gerhardt GmbH & Co. KG, Königswinter, Germany), ether extract, crude ash, crude fibre, starch, sugar, total Ca and P content. All methods used were those published by the Association of German Agricultural Analysis and Research Institutes (VDLUFA) for the chemical analysis of feedstuff (Naumann and Bassler 1993). Chromium in the diet and excrete were determined by atomic absorption spectrometry (Perkin Elmer AAnalyst 400, Waltham, MA, U.S.A.) according to the method published by Fenton and Fenton (1979). Metabolisable energy concentrations (ME) in the diet was estimated using the equation by Carpenter and Clegg (1956):

$\text{ME (Kcal/kg)} = 53 + 38 \times [\text{CP } (\%) + 2.25 \times \text{ether extract } (\%) + 1.1 \times \text{starch } (\%) + 1.05 \text{ sugar } (\%)]$

The chemical analyses of the study diets in the form of mash and crumbled pellets are shown in Table 1. Before chemical analysis, excrete samples were dried at 57°C for 72 h. Dried excrete were milled (0.75 mm mesh) and analysed for dry matter, crude ash, crude protein and ether extract as previously described.

Particle size distribution and mean in the mash and crumble diets, expressed as geometric mean diameter

Table 1. Ingredient composition and nutrient content of the basal layer diet fed hens from 24 to 40 weeks of age.

Ingredient (g/kg as fed basis, unless otherwise indicated)	Mash	Crumble
Maize	470.0	470.0
Soybean meal, 46.5% CP	139.4	139.4
Full fat soybean	140.0	140.0
Sunflower meal, 26.8% CP	128.0	128.0
Soybean oil	10.0	10.0
Ground limestone	88.0	88.0
Di calcium phosphate	8.0	8.0
Sodium chloride	2.0	2.0
Sodium bicarbonate	2.0	2.0
Vitamin Premix[1]	1.0	1.0
Mineral Premix[2]	1.0	1.0
DL-methionine, 98%	2.1	2.1
L-Lysine	2.0	2.0
Threonine	0.8	0.8
Choline chloride, 70%	0.7	0.7
Salmacid[3]	4.0	4.0
Rononzyme+Hypos[4]	1.0	1.0
Analysed nutrient content		
Dry matter	891.62	908.91
Crude protein (N x 6.25)	173.88	171.97
Ether extract	57.22	55.85
Crude fibre	44.73	44.08
Crude ash	127.47	126.49
Starch	352.98	350.66
Sucrose	28.05	26.97
Calcium	39.03	39.67
Total phosphorus	6.84	6.42
Calculated nutrient content[5]		
Available phosphorus	4.23	3.97
Lysine	8.95	8.85
Methionine	4.92	4.87
Methionine + Cysteine	7.84	7.75
Threonine	5.63	5.57
Tryptophan	2.01	1.99
Linoleic acid	26.24	25.61
AME (MJ/kg)[6]	11.67	11.53

[1] Supplied per kilogram diet: vitamin A (trans-retinylacetate), 4.12 mg; vitamin D_3 (cholecalciferol), 66 µg; vitamin E (all-*rac*-tocopherol-acetate), 20 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_6, 2.4 mg; vitamin B_{12} (cyanocobalamin), 0.020 mg; vitamin K_3 (bisulphatemenadione complex), 4.5 mg; nicotinicacid, 40 mg; pantothenicacid (D-calciumpantothenate), 12 mg; folicacid, 0.6 mg; D-biotin. [2] Supplied per kilogram diet: 60 mg; zinc (ZnO), 60 mg; manganese (MnO), 80 mg; iron (Fe SO_4), 40 mg; copper ($CuSO_4{\cdot}5\,H_2O$), 5 mg; cobalt ($CoCo_3$), 0.1 mg; iodine (CaI), 0.4 mg; selenium (Na_2SeO_3), 0.2 mg. [3] Supplied per kilogram diet: 3080 mg formic acid, 400 mg sodium formate, 280 mg glycerilpolietilen glicol and 2 mg aromatizer. [4] Supplied per kilogram diet: 36 IU of amylase, 20 IU of xylanase, 60 IU of cellulase, and 500 FTU phytase. [5] Based on the values tabulated in the publication of the NRC (1994). [6] Based on the equation by Carpenter and Clegg (1956).

Table 2. Particle size distribution (%), geometric mean diameter (GMD, μm) geometric standard deviation (GSD) and bulk density (g/l) of the basal feed mixture fed hens in the form as mash and crumble.

Sieve Size (μm)[1,2]	Mash	Crumble
2500	12.7	54.21
1250	28.65	23.92
630	27.06	14.34
315	23.02	5.25
160	8.41	1.54
80	1.06	0.74
GMD[3]	957	2058
GSD[4]	2.28	2.07
BD[5]	0.66	0.70

[1] The percentage of particles smaller than 80 μm or bigger than 2,500 μm was negligible for all diets. [2] Cereals passed through the 5-mm screen for grinding the basal mash feed. [3] GMD = Geometric mean diameter (dgw); dgw = log-1 │Σ (Wi log đi/Σ Wi)│Where: Wi = weight fraction on the ith sieve di = diameter of sieve openings of the first sieve di + 1 = diameter of openings in the next larger than previous sieve (just above in set) đ = geometric mean diameter of particles on the ith sieve (di x di + 1).

[4] GSD=Geometric standard deviation (Sgw); Sgw = log−1 │Σ Wi (log đi − log dgw)/Σ Wi│½ 5 BD = Bulk density (g/l).

(GMD), were determined by dried sieving, as outlined by ASAE (1995) using 100 g samples using a shaker (Retsch AS 200, Retsch GmbH 42 781 Haan, Germany) using eight sieves ranging in mesh from 5,000 to 40 μm (5000, 2500, 1250, 630, 315, 130, 80 and 40 μm screen). The results are shown in Table 2.

The dry-sieving analyses of the experimental diets were performed as described by Röhe et al. (2014). In brief, a representative 100 g sample of each diet was passed through for 10 min at an amplitude of eight. After the shaking process, the amount of particles retained on each screen was determined by subtracting the weight of the sieve and the retained feed from the blank weight of the sieve. All the analyses and determination of geometric mean diameter (GMD) of the diets were conducted in triplicate.

To determine bulk density of the diets, a filling hopper on top of a cylinder, with a known volume of 1 l, was filled with mash. Hopper and cylinder were separated by a slide with a fall weight on top of it. After removing the slide, the weight fell, thereby pulling down the mash. Access of feed was removed by placing the slide back in the cylinder. Bulk density was determined by dividing the net weight of the mash by the volume of the container (Van Krimpen et al. 2009).

Intestinal tract histomorphology

Samples from the small intestine (duodenum, jejunum, and ileum) were removed and immediately fixed in 10% neutral buffered formalin for 24–48 h. After initial processing, tissues were embedded in paraffin. Sections measuring 5 μm were taken from the formalin-fixed paraffin-embedded (FFPE) blocks were stained with haematoxylin and eosin (HE; Luna 1968). Villus height, width, and crypt depth were measured on eight villi from each intestinal segment using a microscope (Olympus BX51-DP71, Tokyo, Japan) with Olympus cellSens software (CS-ST-V1.8). The villus surface area was calculated using the formula as described by Sakamoto et al. (2000):

$$\text{Villus surface area} = (2\pi) \times (\text{villus width}/2) \times (\text{villus height})$$

Behaviour

Behavioural data were collected by analysing video recordings over 48 h from each colony cage (experimental unit of 11 hens each) at 24 and 40 weeks of ages. The scan sampling method was used to assess the number of hens on perches and at feeders (defined as beak being placed on feed) at the cage level. Perching behaviour was observed by counting hens on perches once every hour during the daytime between 07:00 and 22:00 h, giving a total of 32 scans per cage at each age (24 and 40 weeks of age). Only daytime perch utility rates of hens are presented, based on the earlier reports that hens had very high motivation to perch at night (Abrahamsson and Tauson 1994; K. Liu et al. 2018) and did not show any preference for perch type (Struelens et al. 2009) during the night-time. Feeding behaviour was observed by counting hens at the feeder once every minute during a 10-min period at three-time points (morning, 09:00 h; noon 13:00 h; evening, 18:00 h). Thus a total of 60 records per cage/age were used to assess feeding behaviour. The number of hens recorded on perches and at feeders was divided by the number of hens in each cage to calculate the percentage of hens using perches and feeding.

Keel bone and foot pad health

At the age of 40 weeks, six hens per cage were randomly selected for keel bone and foot pad health assessment. Keel bone fractures and deviations were recorded using a binomial one-zero scaling to indicate the presence or absence of the problem. All the birds were examined by the same technician. During palpation, callus formation, bumps or indentations were considered to distinguish fractures while abnormalities varying from a straight line between caudal and distal points were described as deviation (Casey-Trott et al. 2015).

Both fractures and deviations were considered to be one single trait *i.e.*, keel bone damage (KBD). This approach took into account the limitations of the palpation method on live animals to distinguish fractures and deviations based on the conclusions (Casey-Trott et al. 2015).

Footpad health was assessed using a three-scale scoring by checking both feet, which were scored as 0: normal, 1: necrosis or proliferation of epithelium or mild bumble foot, 2: severe dermatitis with swollen bumble foot (Welfare Quality© 2009). However, there were few hens with score 2; thus hens with scores 1 and 2 were pooled resulting in a binomial one-zero scaling for foot pad dermatitis.

Statistical analysis

The experimental unit was cage for BW, BWG, feed intake, laying performance traits, egg quality characteristics and ATTD. For digestive organ size, pancreatic enzyme activity and villus morphology, the experimental unit was denoted the two birds chosen at random from each cage replicate. Data were analysed using the GLM Procedure of SAS software (SAS Institute 2003). Arc sine transformation was applied to the percentage values before testing for differences. When the model was significant, Tukey's test was used to separate treatment means. Variability in the data was expressed as the standard error of the means and, differences between treatments were considered significant at $p < 0.05$.

Behaviour data were checked for normality using the Shapiro–Wilk test to ensure normality. Perch utility data were analysed using a linear model, with feed form and perch design as the main effects and their interaction. For feeding behaviour, the model included feed form, perch design and observation time effects and any possible interactions. Non-significant ($p > 0.05$) interactions were removed from the model. Cage within feed form and perch design was described as the random effect for both data. Keel bone damage and FPD data were analysed using the Chi-square test of independence separately for dependent variables. Significance was denoted when $p < 0.05$.

Results

No significant interactions between perch design and feed form were seen for any of the traits studied and so only the main effects are presented. Except for the behavioural attributes, none of the variables studied were influenced by perch design whilst the hens' responses to feed form were more pronounced.

Body weight, hen productivity and egg quality

The effects of feed form and perch design on BW, BWG and productive performance of laying hens are presented in Table 3. The final BW and BWG for hens fed crumbles (2026 g) was 46 g heavier ($p < 0.001$) than those fed the mash diet (1980 g). Mortality throughout the experiment was low (0.5%) and not related to any treatment (data not shown).

Feeding hens on pellets instead of mash did not affect any of the performance parameters studied except ADFI and FCR. Significant ($p < 0.001$) responses of ADFI and FCR to changes in feed form were observed after the 16 week feeding period. The average daily feed intake per hen (ADFI) fed crumbles was 4.6 g higher than that fed mash feed, which resulted in a more unfavourable FCR (2.40 *vs.* 2.30; $p < 0.01$). The negative effect on FCR was due to a marked increase in ADFI though egg mass was comparable between mash and crumble treatments.

The cracked-broken egg percentage was not influenced by feed form ($p = 0.448$) or perch design ($p = 0.881$). The same was true for the percentage of shell-less eggs ($p = 0.144$). As presented in Table 4, none of the egg quality parameters studied were influenced by either feed form or perch design ($p > 0.05$).

Weight and pH of digestive organs

The relative weight of the digestive organs, small intestines and caecum are summarised in Table 5. At the end of the experiment, the gizzard was heavier in hens fed mash than in hens fed crumble (21.0 *vs.* 16.8 g/1000 g BW; $p < 0.01$). However, the relative weight of crop, proventriculus, pancreas and liver was unaffected by feed form. The relative length of the duodenum, jejunum, ileum, caecum and entire small intestines were not significantly affected by feed form. The relative length of the small intestine segments were as follows: duodenum (12.6%), jejunum (27.5%), ileum (26.7%), caecum (6.72%) and entire small intestines (66.9%). Birds fed the mash diet showed decreased pH values

Table 3. Influence of feed form and perch design on body weight and productive performance of the laying hens from 24 to 40 weeks of age.

Item	Body weight (g) 24 weeks	Body weight (g) 40 weeks	Body weight gain (g)	Egg production (%)	Egg weight (g)	Egg mass (g)	Feed intake (g/hen/day)	FCR (kg feed /kg egg)
Feed form								
Mash	1684	1980^{b}	298^{b}	94.29	58.47	55.12	129^{b}	2.34^{b}
Crumble	1682	2026^{a}	344^{a}	94.08	58.74	55.26	133^{a}	2.40^{a}
Perch design								
Circular steel	1689	2003	314	94.31	58.69	55.34	131	2.36
Mushroom plastic	1677	2004	327	94.06	58.52	55.05	131	2.38
Pooled SEM[1]	9.29	8.96	3.74	0.13	0.16	0.32	0.57	0.02
Source of variation				Probability				
Feed Form	0.917	0.002	0.001	0.278	0.245	0.758	0.001	0.007
Perch design	0.361	0.972	0.088	0.175	0.478	0.517	0.498	0.314

[1] Data are means of 9 replicate pens of 11 hens each per treatment. ^{a,b}Means within columns with different superscripts are different at ($p < 0.05$).

Table 4. Influence of feed form and perch design on table egg quality characteristics of the laying hens from 24 to 40 weeks of age.

Item	Egg weight (g)	Egg shape index	Shell weight (%)	Shell thickness (μm)	Shell break. strength (kg/cm^{2})	Albumen weight (%)	Yolk weight (%)	Albumen height (mm)	Yolk height (mm)	Haugh unit	Yolk colour Score
Feed form											
Mash	58.32	76.72	9.36	379	43.51	65.37	25.28	7.33	18.92	85.50	12.15
Crumble	58.84	76.96	9.32	383	43.73	65.71	25.14	7.42	19.08	85.79	12.15
Perch design											
Circular steel	58.39	77.00	9.33	382	43.69	65.69	25.13	7.46	18.87	85.92	12.15
Mushroom plastic	58.77	76.69	9.35	381	43.55	65.39	25.30	7.30	19.13	85.37	12.14
Pooled SEM[1]	0.37	0.20	0.07	1.0	0.43	0.25	0.21	0.08	0.10	0.44	0.09
Source of variation					Probability						
Feed Form	0.327	0.384	0.648	0.089	0.720	0.330	0.647	0.469	0.252	0.639	0.979
Perch design	0.474	0.256	0.765	0.741	0.816	0.384	0.570	0.172	0.059	0.377	0.933

[1] Data are means of randomly sampled 18 eggs per treatment (2 eggs per replicate) with 4 weeks intervals from 24 to 40 weeks of age.

Table 5. Influence of feed form and perch design on the relative weight and pH of digestive organs in the hens at 40 weeks of age.

	Relative weight (g/1000 g BW)						pH	
Item	Crop	Proventriculus	Gizzard	Pancreas	Liver	Crop	Proventriculus	Gizzard
Feed form								
Mash	3.39	5.78	21.05^{a}	2.04	18.14	4.55	3.47^{b}	3.02^{b}
Crumble	3.19	5.49	16.82^{b}	2.00	18.29	4.47	3.89^{a}	3.46^{a}
Perch design								
Circular steel	3.26	5.65	19.01	2.02	18.18	4.57	3.72	3.29
Mushroom plastic	3.33	5.62	18.86	2.02	18.24	4.48	3.64	3.19
Pooled SEM[2]	0.13	0.18	0.47	0.07	0.43	0.05	0.13	0.12
Source of variation			Probability				Probability	
Feed Form	0.292	0.265	0.001	0.657	0.806	0.179	0.029	0.013
Perch design	0.685	0.892	0.817	0.965	0.926	0.109	0.672	0.544

[1] Total length of the small intestines including the length of duodenum, jejunum and ileum. [2] Data are means of 18 hens per treatment (2 birds per each replicate pen). ^{a,b}Means within columns with different superscripts are different at ($p < 0.05$).

Table 6. Influence of feed form and perch design on pancreatic enzyme activities and total apparent tract digestibility coefficients of nutrients (%) in the hens at 40 weeks of age.

	Pancreatic enzymes				Digestibility coefficients		
Item	Chymo-trypsin	Amylase	Lipase	Dry matter	Crude ash	Crude protein	Ether extract
Feed form							
Mash	163^{a}	148^{a}	619^{a}	75.3	53.5	64.8^{a}	79.2^{b}
Crumble	142^{b}	127^{b}	551^{b}	74.8	53.5	62.1^{b}	83.3^{a}
Perch design							
Circular steel	159	142	597	74.7	53.9	63.2	81.7
Mushroom plastic	146	133	573	75.3	53.2	63.7	80.7
Pooled SEM	5.45	4.51	16.89	0.432	0.595	0.349	1.206
Source of variation				Probability			
Feed Form	0.039	0.041	0.013	0.471	0.960	0.0001	0.0243
Perch design	0.096	0.129	0.324	0.292	0.385	0.396	0.553

[1] Data are means of 18 hens per treatment (2 birds per replicate pen). ^{a,b}Means within columns with different superscripts are different at ($p < 0.05$).

in the proventriculus and the gizzard ($p < 0.01$) compared with those fed the crumbled feed. However, no significant differences due to feed form for the pH in the crop.

Pancreatic enzyme activities and nutrient utilisation coefficients

Table 6 shows the effects on pancreatic enzyme activities and the percentage of apparent total tract digestibility (ATTD). Activities of pancreatic chymotrypsin, amylase and lipase were higher ($p < 0.05$) in mash-fed hens than those fed crumble. The ATTD for dry matter and crude ash did not vary significantly with feed form, whereas the digestibility coefficient of protein and ether extract were different. The ATTD of protein in hens fed crumbles was lower ($p < 0.05$) than in those fed mash, however, crumbling the feed increased ($p < 0.05$) the ATTD of ether extract compared to mash.

Intestinal tract histomorphology

Villus height (VH), crypt depth (CD), villus width and surface area and VH/CD ratio of the duodenum, jejunum and ileum in hens are shown in Table 7. Villus height and villus surface area of the duodenum were greater ($p < 0.01$) in hens fed mash compared to crumbles, whereas the opposite was observed for crypt depth ($p < 0.05$). None of the jejunal and ileal villus measurements were affected by feed form.

Feeding behaviour and perch utility

Feed form did not affect the frequency of hens at the feeder on weeks 24 or weeks 40 (Table 8). However, feeding frequency significantly differed due to perch design, being higher in the circular steel group than mushroom-type plastic perches at 40 weeks of age ($p < 0.05$). No interactions were seen between feed form and perch design. The time of observation showed different feeding behaviour of hens, being lowest in the morning (9:00 h) and the highest in the evening (18:00 h) and intermediate at noon (13:00 h). The frequency of perching did not differ with feed form, perch design and there was no interaction (Table 8).

Keel bone damage and foot pad health

The percentage of hens with normal or fractured/deviated keel bone was not associated with feed form and perch design (Table 9). In total, 55.35% of hens examined at 40 weeks of age had keel bone problems. The overall incidence of FPD was 48.84% in the experiment. Chi-square test revealed that the incidence of foot pad dermatitis significantly depended on perch design while feed form had no effect (Table 9). The percentage of hens with FPD (62.86%) was significantly higher for the circular steel compared with the mushroom plastic perches (37.14%).

Discussion

It was hypothesised that perch material and design can affect perch utility and maintenance energy demand by laying hens, consequently influencing feed form preferences and daily feed

Table 7. Histomorphology of small intestines in laying hens as influenced by alterations of feed form and perch design.

	Histomorphologic criteria of villus				
Item	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	VH/CD[1]	Surface area (mm$^{2)}$)
			Duedonum		
Feed form					
Mash	1193^{a}	155	175^{b}	6.98	0.58^{a}
Crumble	1104^{b}	152	190^{a}	6.75	0.53^{b}
Perch design					
Circular steel	1154	151	182	6.78	0.55
Mushroom plastic	1144	156	183	6.95	0.56
Pooled SEM[2]	17.31	2.75	4.88	0.19	0.01
Source of variation			Probability		
Feed Form	0.001	0.420	0.035	0.378	0.008
Perch design	0.678	0.156	0.823	0.524	0.626
Feed Form x Perch design	0.094	0.735	0.652	0.907	0.511
			Jejunum		
Feed form					
Mash	751	142	151	5.75	0.33
Crumble	734	139	145	5.39	0.32
Perch design					
Circular steel	757	138	148	5.46	0.33
Mushroom plastic	728	142	148	5.67	0.32
Pooled SEM	13.48	2.83	4.77	0.18	0.01
Source of variation			Probability		
Feed Form	0.380	0.400	0.388	0.156	0.357
Perch design	0.137	0.279	0.921	0.409	0.319
Feed Form x Perch design	0.699	0.486	0.713	0.270	0.866
			Ileum		
Feed form					
Mash	576	132^{a}	112	5.27	0.24
Crumble	564	121^{b}	109	5.38	0.22
Perch design					
Circular steel	578	127	111	5.39	0.23
Mushroom plastic	561	126	110	5.26	0.22
Pooled SEM	14.85	2.47	2.72	0.13	0.01
Source of variation			Probability		
Feed Form	0.580	0.002	0.386	0.567	0.055
Perch design	0.429	0.699	0.766	0.471	0.303

[1] VH/CD=Villous height-to-crypt depth ratio. [2] Data are means of 18 hens per treatment (2 birds per each replicate pen). [a,b] Means within columns with different superscripts are different at ($p < 0.05$).

Table 8. Influence of feed form, perch design and observation time on feeding behaviour (%) and perch utility (%) of laying hens at 24 and 40 weeks of age.

	Feeding Behaviour %		Perching Behaviour %	
	Week 24	Week 40	Week 24	Week 40
Feed form				
Mash	42.12	38.54	32.81	44.90
Crumble	40.39	39.22	32.09	44.93
Pooled SEM	1.19	1.16	1.57	2.16
Perch design				
Circular steel	40.47	41.19^{a}	33.20	44.15
Mushroom plastic	42.03	36.57^{b}	31.71	45.69
Pooled SEM[1]	1.19	1.16	1.56	2.16
Observation time				
Morning	35.04^{c}	31.90^{c}	–	–
Noon	40.32^{b}	38.11^{b}	–	–
Evening	48.40^{a}	46.64^{a}	–	–
Pooled SEM	1.28	1.38	–	–
Source of variation		Probability		
Feed form	0.313	0.680	0.746	0.991
Perch design	0.362	0.008	0.504	0.619
Feed form × Perch design	0.617	0.174	0.859	0.132
Observation time	<0.000	<0.000	–	–

[a,b] Means within columns with different superscripts are different at ($p < 0.05$).

Table 9. Influence of feed form and perch design on keel bone fractures and deviations and food pad dermatitis (%).

	Keel bone damage				Foot pad dermatitis			
	Normal		Fractured/deviated		Normal		Affected	
	n	%	n	%	n	%	n	%
Feed form								
Mash	44	45.83	63	47.06	56	50.91	51	48.57
Crumble	52	54.17	56	52.94	54	49.09	54	51.43
Total	96	44.65	119	54.80	110	51.16	105	48.84
χ2			1.075				0.117	
Probability			0.299				0.732	
Perch Design								
Circular steel	49	48.96	60	51.26	42	38.18	66	62.86
Mushroom plastic	47	51.04	58	48.74	68	61.82	39	37.14
Total	96	44.65	119	55.35	110	51.16	105	48.84
χ2			0.113				13.084	
Probability			0.737				0.0003	

consumption. It was likely that hens with access to circular steel perches would have difficulties maintaining stability to achieve successive perching due to its slippery surface (Pickel, Scholz, and Schrader 2010). Subsequently, this could increase maintenance energy requirements and ultimately change feed form preference in chickens perching on steel pipes. However, current findings did not support this, as the results showed that circular steel perches did not create conditions that affected additional energy requirements and feed form preference in layers. As there was no statistically significant interaction between feed form and perch design for any of the traits examined, only main effects are discussed.

Productive performance

Even though pelleting and crumbling broiler diets has been common practise in the broiler industry over the half-century, available scientific information on egg layer chickens reported quite divergent and often unsatisfactory results with manipulations in the macro structure of feed (Ege et al. 2019; Herrera et al. 2017; Kandasami et al. 2023; Koçer et al. 2016; Safaa et al. 2009; Wan et al. 2022). Of note, all of these studies were carried out in conventional cages where less space and no environmental enrichment, such as nests and perches, were provided to birds. However, published data for alternative housing conditions for laying hens are scarce. Therefore, in order to examine the implications of feed form under non-cage rearing conditions, the results of the current experiment were compared with those from two earlier studies in which hens were kept in enriched cages (Ege et al. 2019) and aviary (Wahlström, Tauson, and Elwinger 1999) systems with perch availability.

In the present experiment, the ADFI of crumble-fed hens was 4.6 g (3.6%) higher ($p < 0.001$) than those hens fed mash resulting in increased (worsened) FCR ($p < 0.001$) due to unchanged ($p = 0.292$) egg mass production between mash and crumble treatments. However, the opposite pattern was found in another experiment (Wahlström, Tauson, and Elwinger 1999) where hens fed the crumbled feed yielded higher egg weight and mass than those fed mash, without any significant changes in ADFI and FCR. Different methodologies between the two studies in terms of ingredient composition of feed, experimental period, strain of the hen, space allowed to birds, arrangement of perches and access to litter area complicates comparisons between the two studies.

Nevertheless, the results of the present experiment are in line with the study by Ege et al. (2019), which showed 10 g (7.7%) higher daily feed intake per Lohmann LSL hens with crumbles compared to mash feeds. Higher dietary intakes for crumbled feed compared to mash were likely due to the allocation of less energy for maintenance and enhanced gastrointestinal emptying, as suggested by Svihus et al. (2004). In addition, there has been speculation that the gelatinisation of starch during pelleting may have contributed to higher ADFI in broilers (Abdollahi, Ravindran, and Svihus 2013), which corroborates the findings of the current study. However, the poorer FCR with crumble feeding regimen in the current experiment and by Ege et al. (2019) contradicted this hypothesis (Pepper et al. 1968; Savory 1974).

Higher final BW seen in hens fed crumbles compared with mash was most likely due to greater ADFI. This is not in agreement with Ege et al. (2019), who showed no effect when feeding crumbles on BW of hens after 32 weeks feeding period. In the present study, part of the energy consumed from almost 5 g more crumbled feed per day increased body fat deposition rather than an increase in egg mass. In accordance with the results of the present experiment, numerically higher BW were observed for hens from two laying hen strains fed the crumble diet instead of mash under the aviary rearing system have been reported (Wahlström, Tauson, and Elwinger 1999). However, limited evidence from a study conducted 25 years ago may not be comprehensive enough to accurately represent the actual response of hens to changes in feed form. When rearing in enriched cages and aviary systems, the ingredient composition of diets and physical quality of basal mash, pellets and crumbles should be well defined.

Egg quality

Data obtained from the current experiment clearly indicated that egg quality was barely affected by feed form. This was in line with a limited number of studies conducted over the past seven decades, as reviewed by Bozkurt et al. (2020), which showed that hens could consume adequate amounts of nutrients fundamental for egg formation, irrespective of feed form. However, it is evident from several earlier experiments that the xanthophyll content of eggs from hens fed pellets was lower than for mash diets (Ege et al. 2019; Hafeez et al.

2015; Koçer et al. 2016). This was likely due to the detrimental effects of higher temperatures during processing, resulting in the destruction of oxycarotenoid pigments present in feed ingredients (Karunajeewa et al. 1984; Koçer et al. 2016; Zheng et al. 2020). However, in the present experiment, the intensity of yolk colour was comparable between mash and pellet treatments presumably due to relatively higher feed and, ultimately, carotenoid intake for units of egg yolk mass in crumble-fed hens compared to those which were fed mash.

Gastrointestinal tract characteristics

With regard to digestive organ size, the major difference between the mash- and crumble-fed hens was gizzard weight. Results from earlier studies with pullets and laying hens (Bozkurt et al. 2019; Ege et al. 2019; Frikha et al. 2009; Herrera et al. 2017) indicated that mash diets increased proventriculus and gizzard weight over pelleted or crumbled diets, similar to that found in the present study. This can be associated with rapid disintegration of crumbles when they are moistened in the upper digestive tract, which may result in further reduction in number of particles, subsequently decreasing mechanical stimulation by the feed (Svihus 2006; Amerah et al. 2007a,2007b). In support of this, Engberg et al. (2002) and Abdollahi et al. (2011) demonstrated the harmful effect of concomitant degradation of larger particles during the pelleting process if the mash feed was finely ground. This might have been the case in the present experiment, where cereals were quite finely ground in a hammer mill passing through a 5 mm sieve size (to improve the physical quality of pellets) and crumbled after pelleting, which gave further particle reduction.

The relative weight of other digestive organs and the length of small intestines and caecum were not influenced by feed form. The transit rate increase by almost 4% for crumbles through intestines without change on nutrient utilisation or productive performance may have been related to negative physiological consequences of crumbles, such as higher feed passage rate and GIT motility, in turn, increased luminal viscosity (Abdollahi, Ravindran, and Svihus 2013). Results from other studies indicated that feed form did not significantly affect intestinal length in pullets (Bozkurt et al. 2019; Frikha et al. 2009; Saldaña et al. 2015) or laying hens (Ege et al. 2019; Herrera et al. 2017; Koçer et al. 2016; Röhe et al. 2014), corroborating the data from the current study.

Providing feed as crumbed pellets to laying hens heightened proventriculus and gizzard pH by almost 13% in relation to those hens given mash feed. The logical explanation for this was the poor grinding activity and resultant under-development of gizzard, the main site of hydrochloric acid production, presumably as a result of concomitant degradation of larger particles during processing (Amerah et al. 2007a; Engberg, Hedemann, and Jensen 2002). Consistent with the findings of the present experiment, Ege et al. (2019) reported that the pH of the proventriculus and gizzard content in crumble-fed hens was significantly higher than that for mash-fed counterparts (4.48 *vs.* 4.04 and 4.18 *vs.* 3.46) following a 32-week feeding period. Data from the present study were in agreement with the observations from pullets at 17 weeks of age whereby pH of the proventriculus and gizzard content decreased by a magnitude between 0.3 and 0.8 units when mash feed was replaced with crumbles (Bozkurt et al. 2019; Saldaña et al. 2015).

In the present study, pancreatic activities of chymotrypsin, amylase and lipase in crumble-fed pullets were lower than those of their mash-fed counterparts. Greater pancreatic enzyme activity with mash feed would be the logical consequence of a higher acidic environment in the gizzard, which is the prominent physiological limit to optimise endogenous and exogenous enzyme activation in the duodenum. Altogether, the results supported a correlation among particle size, gizzard function and pH, GIT motility and pancreatic enzyme secretion (Amerah et al. 2007a, 2007b; González-Alvarado et al. 2008; Svihus 2011).

Intestinal microstructure and nutrient utilisation

Intestinal microarchitecture plays a crucial role in the function of intestinal digestion and absorption in the intestines (Paiva, Walk, and McElroy 2014; Xu et al. 2003). Higher villus height and VH/CD ratio are indications of greater luminal absorptive surface areas for nutrients (Montagne, Pluske, and Hampson 2003). In the present study, hens fed a mash diet exhibited higher villus height, VH/CD ratio and ultimately villus area, compared to those fed crumbles, resulting in a significant improvement in FCR. Conversely, hens fed crumbles, which had lower nutrient absorption capacity, gained more weight than those fed mash. The relatively higher ADFI capacity in ATAK-S hybrid than in other brown layer strains might have given rise to such a pattern.

Optimisation of nutrient utilisation is important for performance in poultry and involves a number of physiological and physical functions that work together to maintain efficient nutrient utilisation (S. Y. Liu, Truong, and Selle 2015; Svihus and Hetland 2001). In the present study, ATTD of dry matter and crude ash did not vary significantly with alterations in feed form, and effects on the digestibility coefficient of ether extract and crude protein were evident. A significant decrease in the digestibility of protein for crumbles may have been associated with the denaturation of protein due to the thermo-mechanical treatments during processing (Voragen et al. 1995). This concurs with Abdollahi et al. (2018), who found that pelleted diets may increase the passage rate of feed and, when paired with an increase in ADFI, nutrient digestibility may be compromised. The ATTD of ether extract was higher in hens fed crumbles compared to those fed mash, in contrast to the ATTD of protein. The beneficial effects of steam-cooking of maize on fat digestibility in broilers shown by Jiménez-Moreno et al. (2016) prompted speculation that steam-cooking may accomplish this by disrupting the cell wall matrix and releasing encapsulated lipids. The report by Wahlström et al. (1999b) partly corroborated these statements, as they demonstrated unchanged total tract digestibility for organic matter, sucrose and crude protein but improved crude fat digestibility in laying hens fed crumbles in relation to those fed mash. In the present experiment, several significant changes in absorptive capacity and pancreatic enzyme production with mash feeding positively mirrored improvements in FCR, which agreed with data from Ege et al. (2019) in laying hens kept in enriched cages.

Behaviour

Environmental enrichment material supply and additional space offered to laying hens in enriched colony cages leads to the expectation that energy requirements and daily feed intake would increase due to the accompanying increase in locomotor activity. However, the results from various studies are conflicting. The literature includes reports of studies in which lower (Elson and Croxall 2006; Valkonen et al. 2006) or higher (Hetland et al. 2004) feed intake was observed in hens housed in enriched cages equipped with round steel perches than in hens kept in conventional cages without a perch. Some of the studies reported no significant effect of cage design on the feed intake of laying hens (Dikmen et al. 2016; Hetland et al. 2004; K. Liu et al. 2018; Onbaşılar et al. 2015; Valkonen et al. 2008). However, the impact of feeding management, which includes nutrient density and structural characteristics of the feed, on these differences in studies has not been adequately measured (Hetland et al. 2004; Valkonen et al. 2008). In the present experiment, significant alteration in both material and design of the perch (circular-shaped steel versus mushroom-shaped plastic) resulted in a lack of birds' response to all of the traits measured, though hens given round steel perches displayed more feeding activity at 40 weeks of age than those roosting on plastic perches during the light period of the day. The perching rates were not different between types or feed forms. This might have resulted from the limitation of the current sampling method for monitoring feeding behaviour. However, the possibility that a reduced rate of feeding activity of hens in the mushroom plastic perches group at 40 weeks of age would indicate that these differences cannot be excluded and might become more apparent in later ages. Because perching is one of the most important behavioural needs of hens (EFSA 2015) hens might have chosen to perch regardless of type (circular metal and mushroom-shaped plastic) in the current experiment. This demonstrated that perch design in enriched cages did not affect perching in laying hens and perch-motivated locomotor activity was not so great to generate additional maintenance energy demand by 40 weeks of age. Therefore, a longer experimental period to the end of the lay and different perch designs should be considered in future research.

Insignificant interactions found between feed form and perch design for all traits examined nullified the hypothesis that hens may have reduced perch utility due to difficulty in balancing on the round steel perches in furnished cages and thus would expend additive energy than those perching on mushroom-shaped ones with wider and slightly sloping perching surface. The results did not indicate a significant effect of perch type on energy expenditure and energy requirements for perching. Although it was not possible to conclude entirely, due to limitations of sampling methods for behaviour which did not allow us to count hens getting on and off the perches, similar perching rates confirmed that hens did not show preference to the types used in this study. The present study was conducted using brown egg layers. However, significant differences in perch utility rates between the white and brown commercial laying hens with higher use with white hens reported (Wall and Tauson 2007). Therefore, different egg-laying genotypes should be considered in future studies.

Foot pad and keel bone damage

The results showed that perch design and material could impact foot health by lowering the percentages of hens with FPD in mushroom-type plastic perches in enriched cages. This was in accordance with an earlier study by Pickel et al. (2011) who reported that foot pad problems are associated with perch design and circular metal perches led to a higher peak force on foot pad area than mushroom-type plastic perches. They concluded that circular metal perches are not suitable for both keel bone and foot pad health. Furthermore, Scholz et al. (2014) revealed that mushroom-type perches had better grip and slipperiness than those metal perches.

Although a large accumulation of information has indicated that perch design, material, location of perches and genotype may be factors affecting KBD, there are still uncertain issues regarding the causes of such damage (Hester et al. 2013; Kappeli et al. 2011; Tauson and Abrahamsson 1994, 1996).

It was expected that mushroom plastic perches would improve keel bone status and foot pad health compared to circular steel perches. However, keel bone data failed to support this hypothesis. Because Pickel et al. (2011) reported five-times higher peak force on the keel bone in circular metal perches as compared with the mushroom-type plastic perches; it was expected that perch design affected keel bone problems, especially deviations. The lack of response may partly be related to the young age of the hens (40 weeks) at which KBD was assessed. Kappeli et al. (2011) reported a higher KBD incidence in rubber-coated metal in comparison to plastic perches. Although there were limitations of the palpation method (Casey-Trott et al. 2015) used in this study, overall KBD incidence was 55.35% at 40 weeks of age and within the range of 36 to 86% from earlier reports in different housing systems (Wilkins et al. 2011) and 62% in enriched cages (Rodenburg et al. 2008).

Conclusions

Neither the perch utility rate nor feed consumption in hens was affected by perch type. This indicated that there was no additional maintenance energy demand for hens provided with circular steel compared to mushroom-shaped plastic perches. Feeding hens on crumbled pellets promoted their daily feed intake while worsening FCR. Perch type did not affect energy demands (due to perch type) or consequent feed form preference of brown laying hens at peak production period.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This project was supported by the Republic of Turkey Ministry of Food, Agriculture and Livestock, Project No: [TAGEM/HAYSUD/Ü/20/A4/

P4]. The authors wish to thank to director and technicians of Adnan Menderes University Agricultural Biotechnology and Food Safety Application and Research Center (ADU- AgBioCenter, TARBIYOMER) for their support and contribution in performing the proximate analysis in feed and excrete.

ORCID

H. Ö. Bayır http://orcid.org/0000-0003-3972-6987

References

Abdollahi, M. R., V. Ravindran, and B. Svihus. 2013. “Pelleting of Broiler Diets: An Overview with Emphasis on Pellet Quality and Nutritional Value.” *Animal Feed Science and Technology* 179 (1–4): 1–23. https://doi.org/10.1016/j.anifeedsci.2012.10.011.

Abdollahi, M. R., V. Ravindran, T. J. Wester, G. Ravindran, and D. V. Thomas. 2011. “Influence of Feed Form and Conditioning Temperature on Performance, Apparent Metabolisable Energy and Ileal Digestibility of Starch and Nitrogen in Broiler Starters Fed Wheat-Based Diet.” *Animal Feed Science and Technology* 168 (1–2): 88–99. https://doi.org/10.1016/j.anifeedsci.2011.03.014.

Abdollahi, M. R., F. Zaefarian, V. Ravindran, and P. H. Selle. 2018. “The Interactive Influence of Dietary Nutrient Density and Feed Form on the Performance of Broiler Chickens.” *Animal Feed Science and Technology* 239:33–43. https://doi.org/10.1016/j.anifeedsci.2018.03.005.

Abrahamsson, P., and R. Tauson. 1994. “Effect of Perches at Different Positions in Conventional Cages for Laying Hens of Two Different Strains.” *Acta Agriculturae Scandinavica Section A - Animal Science* 43 (4): 228–235. https://doi.org/10.1080/09064709309410171.

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007a. “Feed Particle Size: Implications on the Digestion and Performance of Poultry.” *World’s Poultry Science Journal* 63:439–451. https://doi.org/10.1017/S0043933907001560.

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007b. “Influence of Feed Particle Size and Feed form on the Performance, Energy Utilisation, Digestive Tract Development and Digesta Parameters of Broiler Starters.” *Poultry Science* 86 (12): 2615–2623. https://doi.org/10.3382/ps.2007-00212.

Appleby, M. C., S. F. Smith, and B. O. Hughes. 1992. “Individual Perching Behaviour of Laying Hens and Its Effects in Cages.” *British Poultry Science* 33 (2): 227–238. https://doi.org/10.1080/00071669208417462.

ASAE. 1995. “Method of Determining and Expressing Fineness of Feed Materials by Sieving. ASAE Standard S319.2.” In *Agriculture Engineers Yearbook of Standards*, edited by Electric Power and Processing Division Technical Committee, 461–462. St. Joseph, MO: American Society of Agriculture Engineers.

Bist, R. B., S. Subedi, L. Chai, P. Regmi, C. W. Ritz, W. K. Kim, and X. Yang. 2023. “Effects of Perching on Poultry Welfare and Production: A Review.” *Poultry* 2 (2): 134–157. https://doi.org/10.3390/poultry2020013.

Bozkurt, M., I. Giannenas, M. Çabuk, and A. E. Tüzün. 2020. “The Effect of Feed Structure on Gastrointestinal Tract Traits and Performance in Laying Hens: An Overview of 70 years’ Experience.” *World’s Poultry Science Journal* 76 (3): 493–516. https://doi.org/10.1080/00439339.2020.1790330.

Bozkurt, M., B. Koçer, G. Ege, A. E. Tüzün, H. H. Biyik, and E. Poyrazoğlu. 2019. “Influence of the Particle Size and Form of Feed on Growth Performance, Digestive Tract Traits and Nutrient Digestibility of White Egg-Laying Pullets from 1 to 112 D of Age.” *Poultry Science* 98 (9): 4016–4029. https://doi.org/10.3382/ps/pez144.

Bozkurt, M., K. Küçükyılmaz, A. U. Çatli, M. Çinar, E. Bintaş, and F. Çöven. 2012. “Performance, Egg Quality and Immune Response of Laying Hens Fed Diets Supplemented with Mannan Oligosaccharide or an Essential Oil Mixture Under Moderate and Hot Environmental Conditions.” *Poultry Science* 88 (11): 2368–2374. https://doi.org/10.3382/ps.2011-02023.

Carey, J. B., F. L. Kuo, and K. E. Anderson. 1995. “Effects of Cage Population on the Productive Performance of Layers.” *Poultry Science* 74 (4): 633–637. https://doi.org/10.3382/ps.0740633.

Carpenter, K. J., and K. M. Clegg. 1956. “The Metabolizable Energy of Poultry Feeding Stuffs in Relation to Their Chemical Composition.” *Journal Science Food and Agriculture* 7 (1): 45–51. https://doi.org/10.1002/jsfa.2740070109.

Casey-trott, T., J. L. Heerkens, M. Petrik, P. Regmi, L. Schrader, M. J. Toscano, and T. Widowski. 2015. “Methods for Assessment of Keel Bone Damage in Poultry.” *Poultry Science* 94 (10): 2339–2350. https://doi.org/10.3382/ps/pev223.

Chargo, N. J., C. I. Robison, S. L. Baker, M. J. Toscano, M. M. Makagon, and D. M. Karcher. 2019. “Keel Bone Damage Assessment: Consistency in Enriched Colony Laying Hens.” *Poultry Science* 98 (2): 1017–1022. https://doi.org/10.3382/ps/pey373.

Dikmen, B. Y., A. Ipek, U. Şahan, M. Petek, and A. Sözcü. 2016. “Egg Production and Welfare of Laying Hens Kept in Different Housing Systems (Conventional, Enriched Cage, and Free Range).” *Poultry Science* 95 (7): 1564–1572. https://doi.org/10.3382/ps/pew082.

Duncan, E. T., M. C. Appleby, and B. O. Hughes. 1992. “Effect of Perches in Laying Cages on Welfare and Production of Hens.” *British Poultry Science* 33 (1): 25–35. https://doi.org/10.1080/00071669208417441.

EFSA Panel on Animal Health and Animal Welfare. 2015. “Scientific Opinion on Welfare Aspects of the Use of Perches for Laying Hens.” *The EFSA Journal* 13 (6): 1–70. https://doi.org/10.2903/j.efsa.2015.4131.

Ege, G., M. Bozkurt, B. Koçer, A. E. Tüzün, M. Uygun, and G. Alkan. 2019. “Influence of Feed Particle Size and Feed Form on Productive Performance, Egg Quality, Gastrointestinal Tract Traits, Digestive Enzymes, Intestinal Morphology and Nutrient Digestibility of Laying Hens Reared in Enriched Cages.” *Poultry Science* 98 (9): 3787–3801. https://doi.org/10.3382/ps/pez082.

Elson, H. A., and R. Croxall. 2006. “European Study on the Comparative Welfare of Laying Hens in Cage and Non-Cage Systems.” *Archiv für Geflügelkunde* 70 (5): 194–198.

Engberg, R. M., M. S. Hedemann, and B. B. Jensen. 2002. “The Influence of Grinding and Pelleting of Feed on the Microbial Composition and Activity in the Digestive Tract of Broiler Chickens.” *British Poultry Science* 43 (4): 569–579. https://doi.org/10.1080/0007166022000004480.

Fenton, T. W., and M. Fenton. 1979. “An Improved Method for Chromic Oxide Determination in Feed and Feces.” *Canadian Journal of Animal Science* 59 (3): 631–634. https://doi.org/10.4141/cjas79-081.

Frikha, M., H. M. Safaa, E. Jiménez-moreno, R. P. Lázaro, and G. G. Mateos. 2009. “Influence of Energy Concentration and Feed Form of the Diet on Growth Performance and Digestive Traits of Brown Egg-Laying Pullets from 1 to 120 Days of Age.” *Animal Feed Science and Technology* 153 (3–4): 292–302. https://doi.org/10.1016/j.anifeedsci.2009.06.012.

González-alvarado, J. M., E. Jiménez-moreno, D. G. Valencia, R. P. Lázaro, and G. G. Mateos. 2008. “Effect of Fiber Source and Heat Processing of the Cereal on the Development and pH of the Gastrointestinal Tract of Broilers Fed Diets Based on Corn and Rice.” *Poultry Science* 87 (9): 1779–1795. https://doi.org/10.3382/ps.2008-00070.

Hafeez, A., A. Mader, I. Ruhnke, I. Röhe, F. Goodarzi Boroojeni, M. S. Yousaf, K. Männer, and J. Zentek. 2015. “Implication of Milling Methods, Thermal Treatment, and Particle Size of Feed in Layers on Mineral Digestibility and Retention of Minerals in Egg Contents.” *Poultry Science* 94 (2): 240–248. https://doi.org/10.3382/ps/peu070.

Haugh, R. R. 1937. “The Haugh Unit for Measuring Egg Quality.” *US Egg & Poultry Magazine* 43 (12): 552–573.

Herrera, J., B. Saldaña, P. Guzmán, L. Cámara, and G. G. Mateos. 2017. “Influence of Particle Size of the Main Cereal of the Diet on Egg Production, Gastrointestinal Tract Traits, and Body Measurements of Brown Laying Hens.” *Poultry Science* 96 (2): 440–448. https://doi.org/10.3382/ps/pew256.

Hester, P. Y., S. A. Enneking, B. K. Haley, H. W. Cheng, M. E. Einstein, and D. A. Rubin. 2013. “The Effect of Perch Availability During Pullet Rearing and Egg Laying on Musculoskeletal Health of Caged White Leghorn Hens.” *Poultry Science* 92 (8): 1972–1980. https://doi.org/10.3382/ps.2013-03008.

Hetland, H., R. O. Moe, R. Tauson, S. Lervik, and B. Svihus. 2004. “Effect of Including Whole Oats into Pellets on Performance and Plumage Condition in Laying Hens Housed in Conventional and Furnished Cages.” *Acta Agriculturae Scandinavica, Section A —*

Animal Science 54 (4): 206–212. https://doi.org/10.1080/09064700410010026.

Hetland, H., B. Svihus, S. Lervik, and R. Moe. 2003. "Effect of Feed Structure on Performance and Welfare in Laying Hens Housed in Conventional and Furnished Cages." *Acta Agriculturae Scandinavica Section A - Animal Science* 53 (2): 92–100. https://doi.org/10.1080/09064700310002387.

Jiménez-moreno, E., A. de Coca-sinova, J. M. González-alvarado, and G. G. Mateos. 2016. "Inclusion of Insoluble Fiber Sources in Mash or Pellet Diets for Young Broilers. 1. Effects on Growth Performance and Water Intake." *Poultry Science* 95 (1): 41–52. https://doi.org/10.1016/j.anifeedsci.2013.03.011.

Kandasami, R., R. Sangameswaran, C. Sudharsan, T. Sathyabama, P. Selvaraj, M. Moorthy, and P. Vasanthakumar. 2023. "Effect of the Cold-Press Pellet and Mash Feed Forms on the Productive Performance of Laying Hens." *Agricultural Mechanisation in Asia, Africa and Latin America* 54 (7): 14767–14773.

Kappeli, S., S. G. Gebhardt-henrich, E. Fröhlich, A. Pfulg, H. Schäublin, and M. H. Stoffel. 2011. "Effects of Housing, Perches, Genetics, and 25-Hydroxycholecalciferol on Keel Bone Deformities in Laying Hens." *Poultry Science* 90 (8): 1637–1644. https://doi.org/10.3382/ps.2011-01379.

Karunajeewa, H., R. J. Hughes, M. W. McDonald, and F. S. Shenstone. 1984. "A Review of Factors Influencing Pigmentation of Egg Yolks." *World's Poultry Science Journal* 40:52–65. https://doi.org/10.1079/WPS19840006.

Koçer, B., M. Bozkurt, K. Küçükyilmaz, G. Ege, H. Akşit, A. Orojpour, S. Topbaş, A. E. Tüzün, E. Bintaş, and K. Seyrek. 2016. "Effects of Particle Sizes and Physical Form of the Diet on Performance, Egg Quality and Size of the Digestive Organs in Laying Hens." *European Poultry Science* 80. https://doi.org/10.1399/eps.2016.159.

Konkol, D., E. Popiela, and M. Korczynski. 2020. "The Effect of an Enriched Laying Environment on Welfare, Performance, and Egg Quality Parameters of Laying Hens Kept in a Cage System." *Poultry Science* 99 (8): 3771–3776. https://doi.org/10.1016/j.psj.2020.04.01.

Liu, K., H. Xina, T. Shepherd, and Y. Zhao. 2018. "Perch-Shape Preference and Perching Behaviors of Young Laying Hens." *Applied Animal Behaviour Science* 203:34–41. https://doi.org/10.1016/j.applanim.2018.02.009.

Liu, S. Y., H. H. Truong, and P. H. Selle. 2015. "Whole-Grain Feeding for Chicken-Meat Production: Possible Mechanisms Driving Enhanced Energy Utilisation and Feed Conversion." *Animal Production Science* 55 (5): 559–572. https://doi.org/10.1071/AN13417.

Luna, L. G. 1968. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. New York: Blakiston Division, McGraw-Hill.

Mench, J. A., D. A. Summer, and J. Rosen-molin. 2011. "Sustainability of Egg Production in the United States—The Policy and Market Context." *Poultry Science* 90 (1): 229–240. https://doi.org/10.3382/ps.2010-00844.

Montagne, L., J. R. Pluske, and D. J. Hampson. 2003. "A Review of Interactions Between Dietary Fibre and the Intestinal Mucosa, and Their Consequences on Digestive Health in Young Non-Ruminant Animals." *Animal Feed Science and Technology* 108 (1–4): 95–117. https://doi.org/10.1016/S0377-8401(03)00163-9.

Naumann, C., and R. Bassler. 1993. "Chemical Analyses of Feedstuff." In *Method Book III*. 3rd ed., p. 119. Darmstadt, Germany: VDLUFA-Press.

NRC. 1994. *Nutrient Requirements of Poultry: 1994*. Washington, DC: National Academic Press.

Oester, H. 1994. "Different Types of Perches and Their Influence of the Development of Bumble Feet in Laying Hens." *Archiv für Geflugelkunde* 58 (5): 231–238.

Onbaşilar, E. E., N. Unal, E. Erdem, A. Kocakaya, and B. Yaranoglu. 2015. "Production Performance, Use of Nest Box, and External Appearance of Two Strains of Laying Hens Kept in Conventional and Enriched Cages." *Poultry Science* 94 (4): 559–564. https://doi.org/10.3382/ps/pev009.

Paiva, D., C. Walk, and A. McElroy. 2014. "Dietary Calcium, Phosphorus, and Phytase Effects on Bird Performance, Intestinal Morphology, Mineral Digestibility, and Bone Ash During a Natural Necrotic Enteritis Episode." *Poultry Science* 93 (11): 2752–2762. https://doi.org/10.3382/ps.2014-04148.

Pepper, W. F., J. D. Summers, S. J. Slinger, and G. C. Ashton. 1968. "Effect of Steam Pelleting on the Performance of Hens Fed Various Laying Diets." *Canadian Journal of Animal Science* 48 (2): 229–234. https://doi.org/10.4141/cjas68-030.

Pickel, T., B. Scholz, and L. Schrader. 2010. "Perch Material and Diameter Affects Particular Perching Behaviours in Laying Hens." *Applied Animal Behaviour Science* 127 (1–2): 37–42. https://doi.org/10.1016/j.applanim.2010.08.005.

Pickel, T., L. Schrader, and B. Scholz. 2011. "Pressure Load on Keel Bone and Foot Pads in Perching Laying Hens in Relation to Perch Design." *Poultry Science* 90 (4): 715–724. https://doi.org/10.3382/ps.2010-01025.

Pohle, K., and H. W. Cheng. 2009. "Furnished Cage System and Hen Well-Being: Comparative Effects of Furnished Cages and Battery Cages on Behavioral Exhibitions in White Leghorn Chickens." *Poultry Science* 88 (8): 1559–1564. https://doi.org/10.3382/ps.2009-00045.

Preisingir, R. 2000. "Lohmann Tradition, Praxiserfahrung und Entwicklungsperspektiven." *Lohmann Information* 3 (1): 13–16.

Rodenburg, T. B., F. A. M. Tuyttens, K. de Reu, L. Herman, J. Zoons, and B. Sonck. 2008. "Welfare Assessment of Laying Hens in Furnished Cages and Non-Cage Systems: An On-Farm Comparison." *Animal Welfare* 17 (4): 363–373. https://doi.org/10.1017/S096272860002786X.

Röhe, I., I. Ruhnke, F. Knorr, A. Mader, F. Goodarzi Boroojeni, R. Löwe, and J. Zentek. 2014. "Effects of Grinding Method, Particle Size, and Physical Form of the Diet on Gastrointestinal Morphology and Jejunal Glucose Transport in Laying Hens." *Poultry Science* 93 (8): 2060–2068. https://doi.org/10.3382/ps.2013-03783.

Safaa, H. M., E. Jimènez-moreno, D. G. Valencia, M. Frikha, M. P. Serrano, and G. G. Mateos. 2009. "Effect of Main Cereal of the Diet and Particle Size of the Cereal on Productive Performance and Egg Quality of Brown Egg-Laying Hens in Early Phase of Production." *Poultry Science* 88 (3): 608–614. https://doi.org/10.3382/ps.2008-00328.

Sakamoto, K., H. Hirose, A. Onizuka, M. Hayashi, N. Futamura, Y. Kawamura, and T. Ezaki. 2000. "Quantitative Study of Changes in Intestinal Morphology and Mucus Gel on Total Parenteral Nutrition in Rats." *Journal of Surgery Research* 94 (2): 99–106. https://doi.org/10.1006/jsre.2000.5937.

Saldaña, B., P. Guzmán, L. Camara, J. García, and G. G. Mateos. 2015. "Feed Form and Energy Concentration of the Diet Affect Growth Performance and Digestive Tract Traits of Brown-Egg Laying Pullets from Hatching to 17 Weeks of Age." *Poultry Science* 94 (8): 1879–1893. https://doi.org/10.3382/ps/pev145.

SAS Institute. 2003. *SAS User's Guide. Version 8.1*. Cary, NC: SAS Institute Inc.

Savory, C. J. 1974. "Growth and Behaviour of Chicks Fed on Pellets or Mash." *British Poultry Science* 15 (3): 281–286. https://doi.org/10.1080/00071667408416108.

Scholz, B., J. B. Kjaerand, and L. Schrad. 2014. "Analysis of Landing Behaviour of Three Layer Lines on Different Perch Designs." *British Poultry Science* 55 (4): 419–426. https://doi.org/10.1080/00071668.2014.933175.

Scott, G. B., and G. MacAngus. 2004. "The Ability of Laying Hens to Negotiate Perches of Different Materials with Clean or Dirty Surfaces." *Animal Welfare* 13 (3): 361–365. https://doi.org/10.1017/S0962728600028487.

Scott, M. L., M. C. Nesheim, and R. J. Young. 1976. *Nutrition of the Chicken*. Ithaca, N.Y: Scott & Associates.

Skanberg, L., C. B. K. Nielsen, and L. J. Keeling. 2021. "Litter and Perch Type Matter Already from the Start: Exploring Preferences and Perch Balance in Laying Hen Chicks." *Poultry Science* 100 (2): 431–440. https://doi.org/10.1016/j.psj.2020.11.041.

Struelens, E., F. A. M. Tuyttens, B. Ampe, F. Ödberg, B. Sonck, and L. Duchateau. 2009. "Perch Width Preferences of Laying Hens." *British Poultry Science* 50 (4): 418–423. https://doi.org/10.1080/00071660903110885.

Svihus, B. 2006. "The Role of Feed Processing on Gastrointestinal Function and Health in Poultry." In *Avian Gut Function in Health and Disease*, edited by G. C. Perry, 183–194. Wallingford, U.K: CAB International, Cromwell Press. https://doi.org/10.1079/9781845931803.0000.

Svihus, B. 2011. “The gizzard: function, influence of diet structure and effects on nutrient availability.” *World’s Poultry Science Journal* 67 (2): 207–224. https://doi.org/10.1017/S0043933911000249.

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.” *BritishPoultry Science* 42 (5): 633–637. https://doi.org/10.1080/00071660120088461.

Svihus, B., K. H. Kløvstad, V. Perez, O. Zimonja, S. Sahlström, R. B. Schüller, W. K. Jeksrud, and E. Prestløkken. 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 (3–4): 281–293. https://doi.org/10.1016/j.anifeedsci.2004.08.009.

Tactacan, G. B., W. Guenter, N. J. Lewis, J. C. Rodriguez-lecompte, and J. D. House. 2009. “Performance and Welfare of Laying Hens in Conventional and Enriched Cages.” *Poultry Science* 88 (4): 698–707. https://doi.org/10.3382/ps.2008-00369.

Tauson, R. 2002. “Furnished Cages and Aviaries: Production and Health.” *World’s Poultry Science Journal* 58:49–63. https://doi.org/10.1079/WPS20020007.

Tauson, R., and P. Abrahamsson. 1994. “Foot and Skeletal Disorders in Laying Hens-Effects of Perch Design, Hybrid, Housing System and Stocking Density.” *Acta Agriculturae Scandinavica Section A - Animal Science* 44 (2): 110–119. https://doi.org/10.1080/09064709409410189.

Tauson, R., and P. Abrahamsson. 1996. “Foot and Keel Bone Disorders in Laying Hens: Effects of Artificial Perch Material and Hybrid.” *Acta Agriculturae Scandinavica Section A - Animal Science* 46 (4): 239–246. https://doi.org/10.1080/09064709609415876.

Valkonen, E., E. Venäläinen, L. Rossow, and J. Valaja. 2006. “Effects of Dietary Protein on Egg Production of Laying Hens Housed in Furnished or Conventional Cages.” *Acta Agriculturae Scandinavica Section A - Animal Science* 56 (1): 33–41. https://doi.org/10.1080/09064700600608631.

Valkonen, E., E. Venäläinen, L. Rossow, and J. Valaja. 2008. “Laying Hens in Furnished and Conventional Cages.” *Poultry Science* 87 (5): 844–852. https://doi.org/10.3382/ps.2007-00237.

van Krimpen, M. M., R. P. Kwakkel, C. M. C. van der Peet-schwering, L. A. den Hartog, and M. W. A. Verstegen. 2009. “Effects of Nutrient Dilution and Nonstarch Polysaccharide Concentration in Rearing and Laying Diets on Eating Behavior and Feather Damage of Rearing and Laying Hens.” *Poultry Science* 88 (4): 759–773. https://doi.org/10.3382/ps.2008-00194.

Voragen, A. G. J., H. Gruppen, G. J. P. Marsman, and A. J. Mul. 1995. “Effect of Some Manufacturing Technologies on Chemical, Physical and Nutritional Properties of Feed.” In *Recent Advances in Animal Nutrition, University of Nottingham Feed Manufacturers Conference*, edited by P. C. Garnsworthy and D. J. A. Cole, 93–126. Nottingham, U. K.: Nottingham University Press.

Vuilleumier, J. P. 1969. “The ‘Roche Yolk Colour Fan‘—An Instrument for Measuring Yolk Colour.” *Poultry Science* 48 (3): 767–779. https://doi.org/10.3382/ps.0480767.

Wahlström, A., K. Elwinger, and S. Thomke. 1999. “Total Tract and Ileal Nutrient Digestibility of a Diet Fed as Mash or Crumbled Pellets to Two Laying Hybrids.” *Animal Feed Science and Technology* 77 (3–4): 229–239. https://doi.org/10.1016/S0377-8401(98)00255-7.

Wahlström, A., R. Tauson, and K. Elwinger. 1999. “Production and Egg Quality as Influenced by Mash or Crumbled Diets Fed to Laying Hens in an Aviary System.” *Poultry Science* 78 (12): 1675–1680. https://doi.org/10.1093/ps/78.12.1675.

Wall, H., and R. Tauson. 2007. ““Perch Arrangements in Small-Group Furnished Cages for Laying Hens.” *Journal of Applid Poultry Research* 16 (3): 322–330. https://doi.org/10.1093/japr/16.3.322.

Wan, Y., R. Ma, A. Khalid, L. Chai, R. Qi, W. Liu, J. Li, Y. Li, and K. Zhan. 2022. “Effect of the Pellet and Mash Feed Forms on the Productive Performance, Egg Quality, Nutrient Metabolism, and Intestinal Morphology of Two Laying Hen Breeds.” *Animals* 11 (3): 701. https://doi.org/10.3390/ani11030701.

Welfare Quality©, 2009. *The Welfare Quality® Assessment Protocol for Poultry (Broilers, Laying Hens)*. The Netherlands: The Welfare Quality® Consortium, Lelystad.

Wilkins, L. J., J. L. McKinstry, N. C. Avery, T. G. Knowles, S. N. Brown, J. Tarlton, and C. J. Nicol. 2011. “Influence of Housing System and Design on Bone Strength and Keel Bone Fractures in Laying Hens.” *The Veterinary Record* 169 (16): 414. https://doi.org/10.1136/vr.d4831.

Xu, Z. R., C. H. Hu, M. S. Xia, X. A. Zhan, and M. Q. Wang. 2003. “Effects of Dietary Fructooligosaccharide on Digestive Enzyme Activities, Intestinal Microflora and Morphology of Male Broilers.” *Poultry Science* 82 (6): 648–654. https://doi.org/10.1093/ps/82.6.1030.

Zheng, Y. W., L. H. Zhao, Y. M. Wei, Q. G. Ma, C. Ji, and J. Y. Zhang. 2020. “Effects of Main Cereal Type and Feed Form on Production Performance, Egg Quality and Egg Sanitary Indices of Laying Hens.” *British Poultry Science* 61 (2): 164–168. https://doi.org/10.1080/00071668.2019.1704685.