THESIS OF DOCTORAL (PhD) DISSERTATION

BOGLÁRKA HORVÁTH

GEORGIKON CAMPUS
KESZTHELY

2025

HUNGARIAN UNIVERSITY OF AGRICULTURAL
AND LIFE SCIENCES

GEORGIKON CAMPUS

INVESTIGATIONS OF THE EFFECTS OF SPLIT FEEDING SYSTEM ON EGG PRODUCTION, ENVIRONMENTAL AND DIGESTIVE PHYSIOLOGICAL PARAMETERS IN LAYING HENS

Boglárka Horváth

Keszthely
2025

Name of the doctoral school: Festetics Doctoral School

Study field: Animal Husbandry

Head of the doctoral school: Dr. Angéla Anda, DSc,
university professor,

Hungarian University of Agriculture and Life Sciences, Georgikon Campus,
Institute of Crop Sciences,
Department of Agronomy

Doctoral supervisor: Dr. László Pál, PhD,
university professor

Hungarian University of Agriculture and Life Sciences, Georgikon Campus,
Institute of Animal Physiology and Nutrition,
Department of Animal Nutrition and Nutritional Physiology

The candidate has fulfilled all the requirements of the Doctoral Regulations of the Hungarian University of Agricultural and Life Sciences and has taken into account the comments and suggestions made in the workshop discussion of the thesis when revising it, therefore the thesis may be submitted for the defence procedure.

…… ……

Approval by the Head of the Doctoral School

Approval of the Supervisor

1. Background and objectives

The traditional and widely used practice in the feeding of laying hens is to feed a complete diet of the same composition and nutrient content throughout the day. Alternative systems, such as free-choice, split and loose-mix feeding, can more precisely follow the nutrient and calcium requirements of the daily egg-laying cycle, which vary hourly and at different times of the day, and in some cases allow selection between energy, protein and calcium sources (Molnár et.al,2018). Some technologies can potentially use local whole grains, reducing the energy needed to transport, mill and blend the grain and the carbon footprint of feed production. In the sequential or split feeding system, morning and afternoon diet of different composition is distributed in the same feeding trough and may be the most suitable alternative system for large-scale laying hen farms (Molnár et.al., 2018). Two types of split feeding system have been developed. In the first type, which does not use whole grains, animals consume a more energy- and protein-rich and calcium-poor diet in the morning due to the formation of egg white, and a more calcium-rich diet in the afternoon due to the greater calcium need for shell formation, compared to the technological recommendation (Lee and Ohh, 2002). In the second technology, which uses whole grains, the grain is fed alone during the morning, which is rich in energy but low in protein and calcium. In the afternoon, an energy-poor, but protein- and calcium-rich diet is fed (Umar-Faruk et al., 2010). The split system has been shown in several experiments to achieve comparable egg production, lower feed intake and better feed conversion ratio compared to the conventional feeding (Leeson and Summers, 1978; Lee, K.H.; Ohh, Y.S. (2002); de Los Mozos et.al, 2015; Umar Faruk et al., 2010).

During my doctoral research, the first type of split system was investigated, and I tried to adapt the objectives of the experiments to the current feeding challenges of laying hens. Modern genotypes are already suitable for egg production cycles lasting up to 100 weeks of age and their feeding in the split system has not been adequately investigated even at the model level. In the system developed during my experiments, I placed great emphasis on examining the crude protein levels of diets, on implementing the so-called low protein feeding. The split system can also help in the more efficient calcium supply of intensively producing modern laying hens, which was also examined in my experiments. During my research, in addition to the egg production and egg quality parameters, several parameters related to the environmental load were investigated in a novel way to evaluate the split technology as comprehensively as possible.

Based on the above, the objectives of the thesis can be summarised as follows:

1. For my experiments, I aimed to develop a new split feeding technology with a morning diet that has a crude protein content that is identical to the value indicated in the technology recommendations, and a crude protein content in the afternoon diet that is lower than this value. In addition to adjusting the crude protein content, during the optimisation of the amino acid content of diets, the standardised ileal digestible (SID) concentrations of eight essential amino acids (lysine, methionine, arginine, valine, threonine, leucine, isoleucine, tryptophan) were considered for a more accurate amino acid supply.

2. My goal was to compare the developed split system and its reduced dietary crude protein (LP) version to investigate the effects of different crude protein and amino acid supplies. The LP diets had 2% lower crude protein content in both the morning and afternoon diets compared to the control split feeding

diets, but the concentrations of SID lysine, methionine, methionine+cystine, threonine, valine and arginine were the same.

3. A further aim using the developed split feeding system was to compare the control calcium supply (calcium content in the morning diet is about 20% lower than the requirement, and in the afternoon diet is about 20% higher), with the effect of the calcium supply providing a greater difference between the calcium contents in the morning and afternoon diets (calcium content in the morning diet is 38% lower than the requirement, and in the afternoon diet is 35% higher).

2. Materials and methods

During my doctoral research, three model trials and one large-scale farm experiment were conducted with the following topics:

1. Comparison of conventional and split feeding technology (model 1 and large-scale experiment)

2. Comparison of the effect of different protein and amino acid supply in split feeding technology (model 2 experiment)

3. Comparison of the effect of different calcium supply in split feeding technology (model experiment 3)

2.1. Materials and methods for model experiments

2.1.1. Experimental animals and their housing

The experiments were carried out at the experimental site of the Institute of Animal Physiology and Nutrition of the Hungarian University of Agricultural and Life Sciences, Georgikon Campus. Model experiment 1 was carried out with 48 Nick Brown layer hybrids, which were 29 weeks of age at the beginning of the experiment. In model experiments 2 and 3, 48 laying hens of the Hy-Line Brown genetics were used which were 47 and 62 weeks old at the start of experiments, respectively. The experimental animals arrived from the Fuchs Egg Ltd. farms one week before the experiments and were fed the feed consumed on the farm for seven days. The laying hens were housed in two-storey individual cages (1056 cm^2 floor area per bird), each equipped with a nipple drinker and a manually filled feeding trough. Six animals were placed within a floor so that the animals next to each other belonged to a separate treatment and there was a total of 8 replicates of this block of 6 animals in the experimental room. The temperature of the room was 20 ± 0.6°C and the light

programme was set to 16 h light and 8 h dark periods at 30 lux. The experiments were approved by the Institutional Ethics Committee (Animal Welfare Committee, Georgikon Campus, Hungarian University of Agricultural and Life Sciences) under licence number MÁB-2/2021.

2.1.2 Experimental dietary treatments

2.1.2.1 Comparison of conventional and split feeding technology (model experiment 1)

Of the 48 animals used in the experiment, 24 were fed conventional (control, C) diets and 24 were fed a split (SF) diet. The experimental diets were produced at the MATE ÉTI experimental plant in Keszthely. The experimental diets were fed for 12 weeks, when the laying hens were 29-40 weeks old. In both feeding systems the daily feed ration was 120 g feed/day. At 8 am at the beginning of the light period, the animals in both treatment groups received 40% of their daily feed ration (48 g) and had 7 hours to consume it until the second ration was distributed (6.85 g/hour). The remaining 60% of the daily feed ration (72 g) was received at 3 pm and consumed for 9 hours until the end of the light period (8.00 g/hour). At the time of feed distribution, any feed residue remaining in the feeder was always weighed and removed. The diets of the control group were formulated according to the breeder's recommendations (Brown Nick Management Guide, 2024; H&N International), with a daily feed intake of 120 g and egg production above 90%, and the same composition was used for both the morning and afternoon diets (laying phase II). During the preparation of all the feed recipes, the actual measured nutrient content of the raw materials was used and the AMEn values were calculated by the Bestmix software for each raw material and used for formulation of diets. The morning diet of the split-feeding group had higher energy (AMEn; 102.7%) and lower Ca content

(80.4%) compared to the C group, while the afternoon diet had lower energy (96.4%) and higher Ca concentration (113.4%) compared to the control group (100%). The crude protein content of the morning diet of the split technology was the same as the crude protein content of the diet of the conventional system, but the crude protein concentration of the afternoon diet of the split feeding was lower (92%) than the value calculated for the control (100%). The SID Lys, Met, Met+Cys concentrations in the morning and afternoon diets of the SF technology and the SID concentrations of other important essential amino acids (Arg, Val, Thr, Leu, Ile, Trp) in the morning diet of the SF technology were almost the same as in the experimental diet of the C system. The levels of SID Arg, Val, Thr, Leu, Ile, Trp in the afternoon diet of the split feeding were lower than those in the diet of the C group to a similar extent to the crude protein level. Both treatments had the same ratio of fine to coarse limestone sources (70:30%).

2.1.2.2. Comparison of the effect of different protein and amino acid supply in a split feeding technology (model experiment 2)

In this experiment, two split feeding treatments were compared: a control (C) and an experimental treatment containing reduced crude protein (LP) diets. The experiment was conducted for 6 weeks, with the experimental animals aged 47-53 weeks. The daily feed ration and its distribution were the same as described for model experiment 1. As in model experiment 1, the nutrient content of the raw materials was measured and the AMEn content of raw materials was updated before the recipes were prepared. The energy (AMEn) and Ca content of the morning and afternoon diets of the two split treatment groups were nearly identical and showed similar differences compared to the commercial layer diets as recommended by the breeder company (Hy-Line Performance Guide, 2024; Hy-Line International). The AMEn ratio of the morning and afternoon diets of treatment C was nearly identical to the ratio

used in model experiment 1 (treatment SF). The crude protein and essential amino acid SID contents of the morning diet of the C treatment were determined following the recommendations of the breeding company (Hy-Line Performance Guide, 2024; Hy-Line International). Also similar to experiment 1, the crude protein level of the afternoon diet was 92% of that of the morning diet, and the essential amino acid SID levels were 88-93% in this comparison. The diets of the morning and afternoon phases of the LP treatment contained 2% less crude protein compared to the corresponding diets of the C treatment. However, SID Lys, Met, Met+Cys, Thr, and Val concentrations did not differ in this way between the diets of the two treatments, being identical in the morning and afternoon mixtures of the C and LP treatments, respectively. Crystalline amino acids Arg, Leu, Ile, and Trp were not used in the diets, so their SID levels were reduced in the lower crude protein diets of the LP treatment compared to the diets of the C treatment. The 2% absolute crude protein reduction between the two treatments was 13-14% on a relative basis, while the reduction in SID levels of the aforementioned amino acids was about 20% between the C and LP diets.

2.1.2.3. Comparison of the effect of different calcium supply in split feeding technology (model experiment 3)

In the experiment, two split feeding treatments were compared: a control (C) and a treatment containing modified calcium experimental diets (CA). The experiment was carried out for 6 weeks between the 62nd and 68th week of age of the animals. The daily feed ration and its distribution were the same as described for model experiment 1. As in the model experiments 1 and 2, the nutrient content of the raw materials was measured and the AMEn content per raw material was updated before the recipes were prepared. The energy (AMEn) and crude protein contents and SID concentrations of essential amino acids in the morning and afternoon diets of the two split treatment groups were

almost identical. The ratios of AMEn, crude protein and essential amino acid SID levels in the morning and afternoon diets followed the ratio used in model 2 experiment (treatment C). Crude protein and essential amino acid SID levels in the morning diets were determined by following the recommendations of the breeding company (Hy-Line Performance Guide, 2024; Hy-Line International). Compared to the Ca level corresponding to the Ca requirement of the experimental animals (4.38%), the morning diet of the C treatment had a Ca level about 20% lower and the afternoon diet about 20% higher. The same principle was followed in model experiment 2, while in model experiment 1 the Ca level in the afternoon mixture was only about 13% higher than the requirement. For the CA treatment, a greater reduction than before in the morning diet and a greater increase in the afternoon diet was applied: the Ca content in the morning diet was 38% less than the requirement and the afternoon diet contained 35% higher Ca than the requirement.

2.1.3 Measurements and sampling procedures

The body weight of the experimental animals was measured individually on a weekly basis. The feed intake (g/day/hen) of the morning and afternoon phases, egg production (%) and egg weights (g) were recorded daily. From the measured data, an arithmetic mean was calculated for each hen for the entire experiment, and then the means were statistically evaluated (n=24/group). The following data were calculated for one laying hen and for the entire experiment: average daily, morning and afternoon feed intake (g/day/hen), average daily, morning and afternoon protein, calcium and phosphorus intake (g/day/hen), daily egg yield (g/day/hen) and feed conversion ratio (kg feed/kg egg). On the last day of the experiment, one egg was collected from each animal for egg quality measurement, which were stored at 4 °C. After 24 hours of storage, the eggs were left to stand at room temperature for 30 minutes, and then the egg quality parameters (eggshell strength, albumen height, Haugh

unit, yolk color, yolk diameter, yolk height, yolk index, shell thickness) were measured using a Digital Egg Tester 6500 (Nabel Co., Kyoto 601-8444 Japan).

In the last week, the experimental diets were supplemented with 0.5% titanium dioxide indicator. After a four-day adaptation period, representative excreta samples were collected daily for two consecutive days after homogenization of the entire daily excreta. The two-day samples from each bird were combined, thoroughly homogenized, and stored at -20°C until later measurements. Before examining the excreta samples, they were gently thawed and homogenized. The dry matter content, total-N, ammonium-N (NH_4^+-N) and uric acid-N content of the excreta were measured. On the last day of the experiment, at 8:00 a.m., the animals were exsanguinated under carbon dioxide anesthesia by cutting the jugular vein and an ileal content sample was collected from each animal. During the sampling, the first section of the small intestine from Meckel's diverticulum to 2 cm before the ileocecal opening was removed. The intestinal content of the samples were obtained by rinsing the terminal two-thirds of the removed small intestine section with distilled water. After sampling, due to the small amounts, intestinal content samples from three animals were pooled, so a total of 8 pooled samples per treatment were stored at -20°C until analysis. Both tibiae of the slaughtered animals were collected and cleaned them of muscle and cartilage residues by boiling. The cleaned bones were stored frozen at -20°C until chemical analyses and fracture toughness measurements were performed.

2.1.4. Analytical methods and calculations

The analytical measurements, except for the determination of the fracture toughness of the tibia samples, were carried out in the laboratory of the Department of Animal Nutrition and Nutritional Physiology, MATE ÉTI, at

the Festetics Imre Bioinnovation Centre, Georgikon Campus. The collected excreta and intestinal samples were dried in an oven at 60 °C for 72 h before further processing and samples of all three sample types (feed, excreta, intestinal) were ground in a grinding mill (Retsch ZM 100, Retsch GmbH and Co., K.G., Haan, Germany). For each sample two analytical measurements were performed and the results were averaged. The dry matter content of the ground samples was determined after drying in a drying oven (105 °C for 24 hours to constant weight) (ISO 6496:2001). The total N (crude protein) content of the samples was determined according to the Kjeldahl method (ISO 5983-1:2005) using a Foss-Kjeltec 8400 analyser (Nils Foss Allé 1, DK-3400 Hilleroed, Denmark). NH_4^+-N was determined from excreta samples according to the method of Peters (Peters et al., 2003), while uric acid-N was determined according to the method of Marquardt (Marquardt et al., 1983). All N parameters were calculated on a dry matter basis. Urinary N content was determined as the sum of ammonium-N and uric acid-N (O'dell, et al., 1960).

The crude fat (ISO 6492) and crude fibre (ISO 6865:2001) contents of the experimental diets were determined. Calcium and phosphorus contents were also measured from the feed and excreta samples. After preparation resulting in dry ash, the calcium content of the supernatant fraction of the samples was measured by atomic absorption spectrophotometry (ISO 6869:2001). The phosphorus content of the samples was determined by photometry (ISO 6491:2001) using a Biochrom Libra S12 UV-VIS spectrophotometer (Biochrom Ltd. Cambridge, UK). The concentration of amino acids in the feed and in the excreta contents was also determined. Samples were first oxidised in a mixture of hydrogen peroxide and formic acid and then hydrolysed in 6 M HCl solution (MSZ EN ISO 13903:2005). An automatic amino acid analyser (Amino Acid Analyser AAA 400; Ingos, Czech Republic) was used for the separation and detection of amino acids. The TiO_2 content of the

experimental diets, excreta and ileal samples was determined by UV spectroscopy (Ferguson et al., 1998). In model experiment 1, the left tibia of the two tibiae collected and prepared for testing per animal was transported to the MATE Agricultural Science Testing Laboratory in Kaposvár. The bone strength was tested with a Zwick Roell Z005 universal tensile testing machine, controlled by the associated testXpert V11.0 testing software. The right tibia was dried in an oven at 100 °C for 24 hours and defatted in a Soxhlet apparatus using a petroleum ether for 16 hours. Subsequently, the samples were dried overnight in a drying oven at 100 °C to determine the weight of the dry, defatted bone, and then ashed in an oven at 600 °C for 16 h (AOAC Method 972.15; AOAC, 1990). The ash content of the tibia was calculated using the residual ash weight of the dry, defatted bone mass. Ca and P from the bone ash were determined using the methods described for feed samples.

To determine the retention of nitrogen, calcium and phosphorus, the following equation was used (Bregendahl et al. 2002): Apparent N retention = 1-[([TiO_2] feed / [TiO_2] excreta) x ([Element] excreta / [Element] feed)]. In the equation, [TiO_2] is the TiO_2 concentration in the feed or excreta, while [Element] is the N, Ca and P concentration in the feed or excreta. The above equation was used to determine the apparent ileal digestibility of amino acids, but in addition to the TiO_2 concentration. The concentration of each amino acid in the diet and in the gut contents was measured. For the split-technology experimental diets, the concentrations of indicator, nitrogen, macronutrients and amino acids were calculated using the formula: average concentration = (mid-morning feed concentration x 0.4) + (mid-afternoon feed concentration x 0.6).

2.1.5. Statistical analysis

Data preparation for statistical analysis was carried out using Microsoft Office Excel 2016. After checking the data for normality of distribution (Kolmogorov-Szmirnov test) and homogeneity of variances (Levene's test), the effect of dietary treatments was evaluated using a two-sample (independent) t-test. If the prerequisite of normal distribution of data or homogeneity of variances was not met, the Mann-Whitney test was used. All statistical analyses were performed using the SPSS 22.0 software package (IBM Corp., Armonk, NY, USA). Statistical significance was defined at $P<0.05$

2.2 Materials and methods of the large-scale farm experiment

2.2.1 Experimental animals and their housing

The large-scale farm experiment was carried out at Fuchs Egg Ltd., a partner of UBM Feed Company, at the Ltd's plant No. II in Ajka-Bakonygyepes, where split feeding was used in practice. The experiment was carried out in two 700-700 m^2 deep-litter barns with the same housing technology, equipped with an automatic feeding and drinking system. In the two experimental barns, 6000-6000 Nick Brown hybrids were introduced at 16 weeks of age, of which 5975 (Barn B) and 5962 (Barn C) animals started to consume the experimental diets. The temperature of the barns was 20 ± 0.9 °C and the light programme was set at 16 h light and 8 h dark periods at 30 lux.

2.2.2. Experimental dietary treatments

Prior to the experiment, hens in both barns were fed laying starter I for 10 days as a preparatory transition diet to allow a more gradual transition from the previously fed laying starter to the experimental diet. In barn C, this transitional diet was also distributed with a split technology for technological

preparation. The feeding of the experimental diets started at 29 weeks of age, using conventional feeding in barn B and split feeding in barn C. The experiment ran in parallel with model experiment 1 for 10 weeks until the animals were 38 weeks old. The experimental diets were produced in the UBM Group's feed mixing plant in Szeleste. The calculated nutrient contents of the experimental diets were almost identical to those of the diets used in model 1, since the same genotype and age of the herd were fed with the same experimental treatments. However, the proportions of some feed ingredients differed from those of model 1, because the measured nutrient contents of the raw materials differed slightly and a mixture of herbs (thyme 0.10% + chamomile flower 0.05%) was included in the formulation of the large-scale experiment. The diet of the control group (C, n=6000) was the same for both the morning and afternoon ration, as recommended by the breeding company (Brown Nick Management Guide, 2024; H&N International). The split-feeding group (SF; n=6000) consumed a diet with different composition in the morning and afternoon. The energy/nutrient ratios of the morning and afternoon diets of the control and split treatment were the same as in the model 1 experiment. The daily ration to be distributed was planned on the basis of feed consumption on the previous three days. In both treatments, the animals were fed 40% of the daily ration in the morning (4:00 h) and a further 60% of the daily ration in the afternoon in two portions (40% at 12:00 h and 20% at 17:00 h), using a computer-controlled automatic feeder. In the case of treatment SF, the morning feed was distributed from the morning diet and the other two feed distributions from the afternoon diet. The following data were automatically recorded daily: number of animals, mortalities, number of eggs, egg production %, feed consumption. I measured animal body weight and egg weight independently of the program. Animal body weights were measured weekly at the beginning of the experiment and thereafter, by individually

measuring the body weights of 25 animals per barn, randomly selected from five points (n=5/sample point) in the barns.

2.2.3 Measurements and sampling procedures

Data collection of egg production parameters was partly carried out by the on-farm data collection program Livestocker, distributed by Animalsoft Ltd. in both experimental barns. Over the whole experiment, 250 animals per treatment (per barn) were measured. The data were arithmetically averaged (n=25/treatment) at each measurement time and then the average weight for the whole experiment (n=10/treatment) was calculated for both treatments. The number of deaths was recorded daily and then the data were evaluated for the whole experiment. The quantity of intact eggs produced and deposited in the nest (number; excluding broken and litter eggs) and the resulting egg production intensity (%) were also automatically recorded daily and averages per treatment for the whole experiment were evaluated. At the start of the experiment and weekly thereafter, 30 fresh eggs were randomly selected from both barns during the morning and their individual weights were measured and averaged in situ (n=30/treatment) and treatment averages calculated for the whole experiment (n=10/treatment). At the beginning and at the end of the experiment, after the on-site egg weight measurement, the eggs collected per barn were transported to the MATE ÉTI laboratory in Keszthely and the egg quality parameters (eggshell firmness, egg white height, Haugh unit, yolk colour, yolk diameter, yolk height, yolk index, shell thickness) described in the model experiments were measured on the same day using the Digital Egg Tester 6500 (Nabel Co., Kyoto 601-8444 Japan). In the case of feed consumption, the amount of feed consumed from the morning and afternoon mixtures could not be recorded separately, and the daily feed consumption per treatment was measured and the individual daily feed intake calculated using the on-farm software. Using these data, I evaluated the average individual

daily feed intake (g/day/chick) for the whole experiment. Using the measured crude protein content of the experimental mixtures and the daily feed intake data, I calculated the individual daily crude protein intake, and then calculated and evaluated the average individual daily crude protein intake for the whole experiment for the two treatment groups.

2.2.4. Analytical and statistical methods

During the experiment, analytical measurements were carried out on the feed mixture samples in the laboratory of the Department of Animal Nutrition and Animal Nutrition Physiology of MATE ÉTI, at the Georgikon Campus Festetics Imre Bioinnovation Center, according to the methods described in the model experiments. The data preparation and statistical analysis were carried out as described in the model experiments.

3. Results and discussion

3.1 Comparison of conventional and split feeding technology (model 1 and large-scale farm trial)

The feed intake of the experimental animals is shown in Table 1. In the model experiment, split feeding significantly reduced the feed and crude protein intake of hens in both the morning and afternoon periods and for the whole day (P<0.001). Total daily and afternoon energy (AMEn) intake was lower in the split feeding group, but energy intake of the two groups of hens from the morning diet was not significantly different. In the deep-litter farm experiment, there was no demonstrable difference in daily feed and energy intake between the two feeding technologies, but again, the split feeding technology resulted in significantly lower crude protein intake (P<0.001).

Table 1. Daily feed intake in the model and in the farm experiment (g/day/animal; mean ± SEM)

Experiment[1]	Model experiment (week 29-40)			Farm experiment (week 29-38)
	Morning	Afternoon	Summa	
C	45.1 ± 0.4	69.0 ± 0.5	114.0 ± 0.9	129.7 ± 1.0
SF	42.9 ± 0.4	65.0 ± 0.4	107.8 ± 0.8	131.3 ± 0.6
P value	<0.001	<0.001	<0.001	NS[2]

[1]C - conventional feeding (control), SF - split feeding; [2] NS - not significant (P>0.05)
Model experiment: n=23/treatment, Farm experiment: n=74/treatment

The results of the egg production parameters of the experimental animals are shown in Table 2 (model experiment) and Table 3 (farm experiment). The egg production intensity, egg weight, daily egg weight for the whole period of the experiments did not differ significantly in any of the experiments (P>0.05) when comparing the two feeding regimes. In the model experiment, split feeding resulted in significantly better feed conversion ratio compared to

conventional feeding (P<0.001). In the large-scale farm experiment, feeding technology did not affect feed conversion ratio of laying hens (P>0.05).

Table.2 Egg production characteristics in the model experiment (29-40 weeks of age; mean ± SEM)

Experiment[1]	Egg production intensity (%)	Egg weight (g)	Daily egg weight (g/day)	Feed consumption (kg/kg)
C	98.5 ± 0.4	60.1 ± 0.6	59.1 ± 0.6	1.93 ± 0.01
SF	98.0 ± 0.2	60.3 ± 0.7	59.0 ± 0.7	1.83 ± 0.02
P value	NS[2]	NS	NS	<0.001

[1]C – conventional feeding (control), SF – split feeding; n=23/treatment; [2] NS – not significant (P>0.05)

Table 3. Egg production characteristics in the farm experiment (29-40 weeks of life; mean ± SEM)

Experiment[1]	Egg production intensity (%)	Egg weight (g)	Feed conversion ratio (kg/kg)
C	92.4 ± 0.2	61.3 ± 0.3	2.15 ± 0.04
SF	93.0 ± 0.2	61.0 ± 0.7	2.16 ± 0.02
P value	NS[2]	NS	NS

[1]C – conventional feeding (control), SF – split feeding; [2] NS– not significant (P>0.05), egg production intensity: n=59, egg weight and feed conversion ratio: n=10

The daily N intake of laying hens during split feeding was significantly lower than that of conventional feeding (P<0.001; Table 4). The average N retention was similar in the two groups, thus we demonstrated significantly lower N emission due to the split treatment (P<0.05).

Table 4. Nitrogen intake, retention and emission (29-40 weeks of age) (mean ± SEM)

Experiment[1]	N intake (g/day/animal)	N retention (%)	N emission (g/day/animal)
C	2.80 ± 0.02	42.93 ± 1.00	1.60 ± 0.26
SF	2.51 ± 0.02	40.52 ± 0.80	1.49 ± 0.28
P value	<0.001	NS [2]	<0.05

[1]C - conventional feeding (control), SF - split feeding; [2] NS - not significant (P>0.05)

Significant differences were not found in the dry matter content of the excreta, but in the different N forms of the excreta and in the total N content (Table 5). Split feeding significantly reduced the concentrations of fecal-N, NH_4^+-N, uric acid-N, urine-N and total N in the excreta compared to the control feeding.

Table 5. Dry matter content and concentration of N forms of the excreta (29-40 weeks of age; mean ± SEM)

	Treatment [1]		P value
Parameter	**C**	**SF**	
Dry matter (%)	20.18 ± 0.62	21.60 ± 0.41	NS [3]
Feces-N (mg/g dry.m.)	21.73 ± 0.43	20.53 ± 0.29	<0.05
NH_4^+-N (mg/g dry.m.)	7.12 ± 0.10	6.63 ± 0.10	<0.001
Uric acid-N (mg/dry.m.)	21.39 ± 0.20	20.11 ± 0.19	<0.001
Urine-N [2] (mg/g dry.m.)	28.51 ± 0.28	26.75 ± 0.25	<0.001
Total N (mg/dry m.)	50.24 ± 0.54	47.28 ± 0.47	<0.001

[1] C – conventional (control) feeding, SF – split feeding; [2] sum of NH4+-N and uric acid-N; [3] NS – not significant (P>0.05)

3.2. Comparison of the effects of different protein and amino acid supply in split feeding technology (model experiment 2)

The morning, afternoon and daily feed intake of the experimental animals was similar in the two treatment groups (P>0.05, Table 6). The use of diets with reduced crude protein content did not affect the AMEn intake of the experimental animals, but significantly lower morning, afternoon and daily crude protein intake was measured compared to the control treatment (P<0.001, Table 6)

Table 6. Feed intake and crude protein intake of the experimental animals (47-53 weeks of age; mean ± SEM)

Experiment[1]	Feed intake (g/day/animal)			Crude protein intake (g/day/animal)		
	Morning	**Afternoon**	**Total**	**Morning**	**Afternoon**	**Total**
C	44.5 ± 0.3	67.9 ± 0.4	112.5 ± 0.7	7.2 ± 0.1	10.2 ± 0.1	17.4 ± 0.1
LP	44.6 ± 0.3	67.7 ± 0.5	112.3 ± 0.8	6.5 ± 0.1	8.9 ± 0.1	15.4 ± 0.1
P value	NS[2]	NS	NS	<0.001	<0.001	<0.001

[1]C – Control treatment, LP – Reduced crude protein treatment; n=24/treatment; [2]NS – not significant (P>0.05)

The effects of the experimental treatments on egg production parameters are shown in Table 7. Consumption of reduced crude protein diets increased the egg production intensity of the experimental animals, but at the same time reduced egg weight (P<0.001), and thus did not significantly affect the daily egg weight produced. Feed conversion ratio did not differ significantly between the two experimental groups (P>0.05).

Table 7 Effect of experimental treatments on egg production parameters (47-53 weeks of age; mean ± SEM)

Experiment[1]	Egg production (%)	Egg weight (g)	Daily egg weight (g/day)	Feed conversion ratio (kg/kg)
C	90.78 ± 1.4	63.9 ± 0.7	57.3 ± 0.8	1.97 ± 0.02
LP	94.75 ± 0.8	59.9 ± 0.7	56.7 ± 0.8	1.99 ± 0.03
P value	<0.05	<0.001	NS[3]	NS

[1]C – Control treatment, LP – Reduced crude protein treatment; n=24/treatment; [2]NS – not significant (P>0.05)

In our experiment, feeding the reduced protein content mixtures resulted in significantly lower N intake of laying hens (P<0.001), but N retention and N emission did not differ significantly compared to the control treatment (Table 8).

Table 8 Nitrogen intake, retention and emission (47-53 weeks of age; mean ± SEM)

Experiment[1]	N intake (g/day/animal)	N retention (%)	N emission (g/day/animal)
C	2.67 ± 0.02	38.23 ± 1.80	1.65 ± 0.05
LP	2.42 ± 0.02	35.60 ± 2.16	1.57 ± 0.06
P value	<0.001	NS[2]	NS

[1]C – Control treatment, LP – Reduced crude protein treatment; n=24/treatment; [2]NS – not significant (P>0.05)

3.3. Comparison of the effect of different calcium supply in split feeding technology (3rd model experiment)

We could not detect significant differences in the feed intake of laying hens between the two treatment groups (P>0.05). The different Ca supply did not significantly affect the egg production intensity, egg weight, daily egg weight and feed sales value. In the case of egg production intensity, I showed a strong

trend in favor of the CA treatment, in which group the egg production % was approximately 2% higher compared to the C group (P=0.05).

Table 9. Effect of experimental treatments on feed intake and egg production parameters (62-68 weeks of age; mean ± SEM)

Experi-ment[1]	Feed intake (g/day)	Egg production (%)	Egg weight (g)	Daily egg weight (g/day)	Feed conversion ratio (kg/kg)
C	113.7 ± 0.7	92.56 ± 0.9	62.3 ± 0.9	57.2 ± 0.8	1.99 ± 0.03
CA	113.0 ± 0.7	94.87 ± 1.0	61.1 ± 0.6	57.5 ± 0.9	1.97 ± 0.03
P value	NS	NS[2] (=0.05)	NS	NS	NS

[1]C – Control treatment, CA – Calcium treatment; n=24/treatment; [2]NS – not significant (P>0.05)

In terms of egg quality characteristics, the yolk index of eggs produced under CA treatment was significantly higher than that of eggs produced in the C group (P<0.05; Table 10).

Table 10 Calcium intake, eggshell quality parameters and tibia composition (62-68 weeks of age; mean ± SEM)

Parameter	Treatment[1]		*P* value
	C	CA	
Calcium intake morning (g/day)	1.76 ± 0.01	1.38 ± 0.01	<0.001
Calcium intake afternoon (g/nap)	3.81 ± 0.02	3.98 ± 0.03	<0.001
Calcium intake summa (g/nap)	5.56 ± 0.03	5.36 ± 0.04	<0.001
Eggshell strenght (kgf) [2]	3.72 ± 0.31	3.78 ± 0.24	NS[3]
Eggshell thickness(mm)	0.45 ± 0.01	0.44 ± 0.01	NS
Tibia bone ash content (%)	52.27 ± 0.26	52.98 ± 0.22	NS
Tibia bone calcium content (%)	18.68 ± 0.17	18.72 ± 0.20	NS
Tibia bone phosphrus content (%)	8.60 ± 0.15	8.72 ± 0.17	NS

[1]C – Control treatment, CA: calcium treatment; n=24/treatment; [2]NS – not significant (P>0.05)

The effects of the experimental treatments on nitrogen uptake, retention and emission of laying hens are shown in Table 11. CA treatment did not affect the N intake of the experimental animals, but resulted in higher N retention and lower N emission compared to the results of the C treatment (P<0.001; Table 11). The dry matter content of the excreta did not differ between the two feeding treatments, but significantly lower values of all tested N forms were measured under the CA treatment compared to the C treatment (P<0.05; Table 12).

Table 11. Nitrogen intake, retention and emission
(62-68 weeks of age; mean ± SEM)

Experiment[1]	N intake (g/day/animal)	N retention (%)	N emission (g/day/animal)
C	2.93 ± 0.02	44.18 ± 1.46	1.64 ± 0.04
CA	2.95 ± 0.02	51.79 ± 1.44	1.42 ± 0.04
P value	NS[2]	<0.001	<0.001

[1]C – Control treatment, CA – Calcium treatment; n=24/treatment; [2]NS – not significant (P>0.05)

Table 12. Dry matter content and concentration of N-forms in the excreta
(62-68 weeks of age; mean ± SEM)

Parameter	Treatment [1]		P value
	C	CA	
Dry matter (%)	26.65 ± 0.50	25.25 ± 0.53	NS[3]
Feces-N (mg/g dry m.)	24.17 ± 0.60	22.30 ± 0.69	<0.05
NH_4^+-N (mg/g dry m..)	4.51 ± 0.13	3.86 ± 0.19	<0.05
Uric acid-N (mg/g dry m.)	12.18 ± 0.24	11.44 ± 0.25	<0.05
Urine-N [2] (mg/g dry m.)	16.69 ± 0.31	15.30 ± 0.32	<0.05
Summa N (mg/g dry m..)	40.86 ± 0.81	37.60 ± 0.91	<0.05

[1] C – Control treatment, CA – Calcium treatment; n=24/treatment; [2] NS – not significant (P>0.05); [2] is the sum of NH4+-N and uric acid-N; [3] NS – not significant (P>0.05)

4. Conclusions and recommendations

In my experiments, when examining the split feeding system, I applied several new modifications to the previously used technology in terms of protein and amino acid supply. According to previous practice, the crude protein content of the morning diet of the split system without whole grains exceeded the recommended requirement value in traditional technology. During my studies, morning diets were composed whose crude protein content was in a novel way equal to the value indicated in the technological recommendations, while the crude protein content of the afternoon diet was lower than this value. As a condition for this modification, when optimizing the amino acid content of the mixtures, I took into account the SID concentration of eight essential amino acids (lysine, methionine, arginine, valine, threonine, leucine, isoleucine, tryptophan) in order to provide a more accurate amino acid supply. Due to the lower crude protein content of the morning diet, the new system is not only more economically advantageous, but also environmental-friendly due to lower nitrogen emissions. During my experiments, I tested the new split system with two layer hybrids (Nick Brown and Hy-Line Brown); in the future, it would be recommended to test the technology with other genotypes producing table eggs, as well as with broiler breeder flocks. The scientific results formulated during the research relate to the specific experimental periods of the laying cycle that were examined, which did not cover the entire egg production period in either topic. Therefore, it would be advisable to extend the studies to the entire production period.

I compared the experimental split diets based on the described principles with the traditional technology in the 1st model and large-scale farm experiment during the peak production period. During the comparison, I did not experience any negative effects of the split system on the body weight of the

experimental animals, egg production parameters, or egg quality. In the 1st model experiment, despite the lower feed intake, energy (AMEn) and crude protein intake measured in the split system, the egg production intensity and egg weight were the same as in the conventional feeding group, thus a more favourable feed conversion ratio was measured than in the control. Regarding egg quality, the higher eggshell thickness obtained in the split system compared to the conventional system should be highlighted. The composition and fracture strength of the tibia of the laying hens in peak production were similar in the two systems examined, and the health of the bone was not endangered by the split technology. Based on my favourable results, our split system under study can also be recommended for testing under large-scale conditions with furnished cage technology. Before the full-cycle large-scale application, I recommend performing a comparative model experiment with old experimental animals at the end of the production cycle. If the split diets can be produced at the same or a lower price than the traditional diets, then the split system can enable more economical production during operation. In the split system currently operated by Fuchs Egg Ltd., I was able to conduct a farm experiment under deep litter conditions, where similar production results were achieved compared to the traditional technology, despite the lower crude protein intake of the layers. My relatively short study under farm conditions would be worth extending to the entire laying cycle, as well as to aviary and free-range keeping technologies.

During my experiments, I showed for the first time that laying hens producing in a split feeding system not only emit less nitrogen into their environment, primarily due to their lower N intake than those receiving traditional feeding, but also that the concentration of N forms present in the droppings is more favourable. Based on my results, the excreta produced in the split technology is more advantageous in terms of ammonia emission, which contains a lower

concentration of urine-N calculated as the sum of NH_4^+- and uric acid-N. In the case of experimental animals producing in the split system, calcium taken up in a smaller daily amount than in the control, but in a daily rhythm more appropriate to physiological needs, ensured the same production level and at the same time the daily amount of calcium released into the environment per animal also decreased. Split feeding, on the other hand, is not suitable for reducing the phosphorus emission of laying hens, because in my model experiment, the P emission of traditional and split system laying hens did not differ significantly.

In the 2nd model experiment, I compared the split system used in the 1st model experiment and its reduced crude protein level version (LP), which has not been studied before. Compared to the control split diets, both the morning and afternoon LP mixtures had a 2% lower crude protein level, but the SID lysine, methionine, methionine+cystine, threonine, valine and arginine concentrations were the same. As a result of feeding the LP diets, the laying hens produced more eggs, but of lower weight, during the six weeks of the experiment. In my opinion, the solution to prevent the decrease in egg weight could be to optimize the SID leucine, isoleucine and tryptophan levels of the LP diets. In the droppings of laying hens fed diets with reduced crude protein contents, although their nitrogen intake was lower, there was no significant decrease in the total N content or in the concentrations of the different N forms compared to the control treatment.

My 3rd model experiment allowed the comparison of two different calcium supplies in a split system in a much more precise way than previous studies, ignoring the influencing effects of different energy, protein and phosphorus supplies. Compared to the control calcium supply, the CA treatment had a lower Ca content in the morning diet and a higher Ca content in the afternoon diet. The CA diet combination, which provided a greater difference between

morning and afternoon dietary Ca contents, did not significantly affect egg production characteristics, eggshell thickness and shell fracture strength, tibia composition ($P>0.05$), but resulted in better N retention, thus lower N emissions and a more favourable N composition in terms of ammonia emissions. The CA treatment probably provides egg production with lower and more favourable N emissions in large-scale farms, and I definitely recommend testing it.

5. New scientific results

1. In my studies with laying hens (Nick Brown and Hy-Line Brown hybrids), a new split feeding system was developed and used for the first time where the crude protein content of the morning diet was the same as the value indicated in the technological recommendations (100%), and the crude protein content of the afternoon diet was lower (92%). As a condition for this modification, the SID concentration of eight essential amino acids (lysine, methionine, arginine, valine, threonine, leucine, isoleucine, tryptophan) was considered during optimization in order to provide more accurate amino acid supply.

2. The investigated new split feeding system during the peak production period (29-40 weeks of age) in furnished cages provides lower feed intake and the same egg production intensity and egg weight as the traditional feeding technology, thus providing a more favourable feed conversion ratio. Furthermore, it is suitable for achieving egg production results (egg production intensity, egg weight, feed conversion ratio) similar to the traditional feeding technology under deep litter operating conditions (29-38 weeks of age).

3. The laying hens in the new split feeding system emit less nitrogen into their environment due to their lower nitrogen intake than those in the conventional feeding system, and the concentration of nitrogen forms present in the excreta is more favourable in terms of ammonia emission due to the lower concentration of urine nitrogen (NH_4^+ and uric acid content).

4. The investigated new split feeding system in furnished cage technology (47-53 weeks of age) composed of diets with a 2% reduced crude protein content but the same SID lysine, methionine, methionine+cysteine, threonine, valine and arginine concentrations, results in similar feed intake and feed conversion

ratio but lower crude protein intake, higher egg production intensity and lower egg weight compared to the control split feeding diets.

5. In the new split feeding system tested, adjusting the calcium content in the morning diet to 38% lower than the requirement and in the afternoon diet to 35% higher, does not significantly affect egg production parameters, eggshell thickness and breaking strength, and tibia composition. However, it results in higher nitrogen retention and lower nitrogen emission, and a more favourable nitrogen composition of the excreta in terms of ammonia emission in model conditions between 62-68 weeks of age, in furnished cage housing.

6. Publications related to the topic of the dissertation

I. Publications in peer-reviewed journals in foreign language

Horváth, Boglárka; Strifler, Patrik; Such, Nikoletta; Wágner, László; Dublecz, Károly; Baranyay, Henrik; Bustyaházai, László; Pál, László (2024): Developing a more sustainable protein and amino acid supply of laying hens in a split feeding system. Animals, 14 (20), 3006. https://doi.org/10.3390/ani14203006

II. Publications in peer-reviewed journals in Hungarian

Horváth Boglárka, Baranyay Henrik, Such Nikoletta, Wágner László, Dublecz Károly, Pál László (2025): Alternatív tojótyúk takarmányozási rendszerek lehetőségei és korlátai. Állattenyésztés és Takarmányozás. 2025.74.2. (accepted for publication).

Horváth Boglárka, Baranyay Henrik, Such Nikoletta, Wágner László, Dublecz Károly, Pál László: Tojótyúkok eltérő kalcium ellátásának vizsgálata osztott etetési technológiában. Állattenyésztés és Takarmányozás. 2025.74.2. (accepted for publication).

III. Publications in full length in peer-reviewed conference proceedings

Horváth Boglárka, Strifler Patrik, Such Nikoletta Amanda, Janecskó Szilvia, Baranyay Henrik, Pál László (2021): Osztott takarmányozási rendszer hatásának vizsgálata tojótyúkok termelési eredményeire üzemi körülmények között In: Bene, Szabolcs (szerk.) XXVII. Ifjúsági Tudományos Fórum, Keszthely, (2021.05.20), Konferenciakötet, Magyar Agrár- és Élettudományi Egyetem, Georgikon Campus, ISBN 978-615-6338-04-4, pp. 15-20., 6 p.

IV. Publications in abstract form in conference proceedings

Horváth Boglárka, Strifler Patrik, Such Nikoletta, Pál László, Janecskó Szilvia, Baranyai Henrik, Pál László (2021): Osztott takarmányozási rendszer hatásának vizsgálata tojótyúkok nitrogén és kalcium retenciójára LXIII. Georgikon Napok Keszthely, 2021.10.7-8., Kivonatkötet, p. 24.

Horváth, Boglárka; Strifler, Patrik; Such, Nikoletta; Janecskó, Szilvia; Baranyai, Henrik; Pál; László (2022): Effect of split feeding system on nitrogen retention of laying hens. In: Proceedings of the 26th World1's Poultry Congress, Paris, France (7-11. August 2022), p. 246.

7. Bibliography

Bregendahl, K., Sell, J.L., Zimmerman, D.R. (2002) Effect of Low-Protein Diets on Growth Performance and Body Composition of Broiler Chicks. In: Poultry Sciene 81: 1156–1167. p. doi: 10.1093/ps/81.8.1156

de Los Mozos, J.; Navarro-Villa, A.; García-Ruíz, A.I. (2015) Splitfeeding System Reduces Second Grade Eggs in the Late Production Phase of Laying Hens. In Proceedings of the 20th European Symposium on Poultry Nutrition, Prague, Czech Republic, 24–27 August 2015.

Ferguson, N.S., Gates, R.S., Taraba, J.L., Cantor, A.H., Pescatore, A.J., Ford, M.J., Burnham, D.J. (1998) The Effect of Dietary Crude Protein on Growth, Ammonia Concentration, and Litter Composition in Broilers. In: *Poultry Science,* 77: 1481–1487.p

Lee, K.H.; Ohh, Y.S. (2002) Effects of Nutrient Levels and Feeding Regimen of a.m. and p.m. Diets on Laying Hen Performances and Feed Cost. In: *Korean Journal of Poultry Science*, 29: 195–204 p.

Marquardt, R.R.; Ward, A.T.; Campbell, L.D. (1983) A Rapid High-Performance Liquid Chromatographic Method for the Quantitationor Uric Acid in Excreta and Tissue Samples. In: *Poultry Science*, 62 (10): 2099-2105 p, doi: 10.3382/ps.0622099

Molnár, A.; Hamelin, C.; Delezie, E.; Nys, Y. (2018) Sequential and Choice Feeding in Laying Hens: Adapting Nutrient Supply to Requirements during the Egg Formation Cycle. In: *World's Poultry Science Journal,* 74 (2): 199–210 p. doi: 10.1017/S0043933918000247

O'dell, B.L., Woods, W.D., Laerdal, O.A., Jeffay, A.M., Savage, J.E. (1960) Distribution of the major nitrogenous compounds and amino acids in chicken Urine1. *Poultry Science* 39: 426–432.p. doi: 10.3382/ps.0390426

Umar Faruk, M., Bouvarel, I., Même, N., Rideau, N., Roffidal, L., Tukur, H.M., Bastianelli, D., Nys, Y. and Lescoat, P. (2010) Sequential feeding using whole wheat and a separate protein-mineral concentrate improved feed efficiency in laying hens. In: *Poultry Science* 89: 785-96. p. doi:10.3382/ps.2009-00360