THESIS OF DOCTORAL (PhD) DISSERTATION

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THE IMPORTANCE OF VALINE SUPPLY IN BROILER CHICKEN NUTRITION

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1. RESEARCH BACKGROUND

Due to the continuously increasing cost of protein sources used in broiler feeds it is of crucial importance to study the potential for reduced dietary protein levels which can only be achieved by optimizing the amino acid supply (Sterling et al. 2003; Rezaei et al. 2004; Harn et al. 2019; Cho et al. 2023; Selle et al. 2023). Reducing the dietary protein content of poultry feeds would not only yield physiological and production advantages, but is also important from economical and environmental aspects (Alleman and Leclercq, 2007; Chrystal et al. 2020). Lower N excretion levels mean a lower N load on the environment, and this is a major consideration for countries involved in intensive livestock production (Williams 1995; Belloir et al. 2017; Ramos and Girish 2018). The use of industrial amino acids in broiler nutrition has long been in the focus of research as not only can they help to improve production and other parameters (e.g. dressing percentage, carcass composition, immunity, etc.) but can also contribute to reducing the ratio of one of the costliest feed ingredients, i.e. the protein sources (e.g. extracted soybean meal) in feed formulas. The latest research results highlight the possibility of valine being the fourth limiting amino acid in corn-soybean meal based broiler diets, which implies that if there is a sufficient number of positive trial results the continued and rational use of valine can be predicted (Etienne Corrent, 2009; Kidd et al, 2015; Kaplan and Yildiz 2017; Allameh et al. 2019). The relevant objectives of the European Green Deal (2020) demand that greenhouse gas emissions be 55% lower by 2030, and full climate neutrality be achieved by 2050. Compared to other gases responsible for global warming (N_2O, CH_4) carbon dioxide is of utmost significance. Nielsen et al. (2011) were among the first to publish data pertaining to broiler production, finding its average carbon footprint to be 2.88 kg CO_2 eq (equivalent) per a unit (kg) of weight gain, of which 91% originated from the feed. In addition to the CO_2 footprint of broiler production the attention will be increasingly focused on nitrogen load and release - present in the production chain in the form of ammonia. Since there is a close correlation between the rate of ammonia release and the crude protein content of feed (Kriseldi et al. 2018; Such et al. 2021), reducing the dietary crude protein levels appears to be the most obvious method for reducing the nitrogen load from broiler production (Caufal et al. 2006; Ramos and Girish, 2018). Consequently, the daily protein intake and protein utilization performance of different genotypes should also be a primary consideration (Harn et al, 2019). As yet there are only limited data available in the literature pertaining to the valine requirements of intensive broiler breeds and the results of the studies are often contradictory. For this reason, it is necessary to improve the accuracy of the requirements based on the feed formulas adapted to the feed ingredient supply available in the various regions.

Based on the foregoing, it appears expedient to study how the different crude protein and valine contents of the diets affect the live weight, weight gain, feed conversion rate and protein utilization of roosters, and the dietary CO_2 footprint of broiler production.

2. RESEARCH OBJECTIVES

Our objective was that using the literature data presented we study how the the live weight, weight gain, feed conversion rate and protein utilization of roosters, and the dietary CO_2 footprint of broiler production change when feeding corn-soybean meal based diets with different crude protein and valine contents.

Another objective was to determine in the course of the digestibility studies the ileal digestibility and absorption of the crude protein and amino acid contents of all diets fed in the different phases of growth (7 per phase; 21 diets in total).

3. MATERIALS AND METHODS

In my PhD work I studied the effect of different crystalline valine supplementation levels added to reduced crude protein diets on the performance parameters and the dietary CO_2 footprint, beside the ileal digestibility of dietary crude protein and amino acid in high genetic potential Ross308 broiler hybrid roosters.

3.1. Performance studies

3.1.1. Trial birds and housing

The experimental protocol complied with the European Union regulations concerning the protection of animals used for experimental and other scientific purposes (EU Directive 2010/63/EU). The trials were conducted at the Department of Animal Nutrition of the former University of Kaposvár Faculty of Animal and Environmental Science based on the permission issued by the Food Safety and Animal Health Directorate of the Somogy County Department of Agriculture (document number: SOI/31/446-7/2014), which was valid for the duration between 2014.04.02 to 2019.04.02. The trial used a total of 1680 Ross308 broiler hybrid roosters (30 birds/cage; 8 repetitions x 30 birds = 240 birds/treatment). The trial birds equipped with individual wing tags at day one of age were placed in deep litter pens, at a 12.5 bird/m^2 density. The temperature of the room, and the length and intensity of lighting were set according to requirements of the birds following the recommendations of the breeding company (Aviagen, 2014).

3.1.2. Treatments, trial diets

The trial series used a three-phase feeding system, consisting of a starter diet fed during days 1-14, a grower diet between days 15-21 and a finisher diet from day 22 to day 35 of age. All diets were formulated on a corn-soybean meal basis. The trial series consisted of 7 treatments. In the positive control treatment (PC) the diet was formulated with crude protein and valine contents following the recommendations of Aviagen (2014) and the commercial feed products used in the region (starter: 210 g/kg crude protein, 1.20 g L-valine supplementation/kg of diet; grower: 190 g/kg crude protein, 0.70 g L-valine supplementation/kg of diet, finisher: 180g/kg crude protein, 0.40 g L-valine supplementation/kg of diet). This corresponded to 10.5 g/kg total valine (TV) in the starter phase; while in the grower phase these parameters were 9.1 g/kg (TV); and in the finisher phase they were 8.4 g/kg (TV), respectively.

In the next 6 treatments the diets were formulated with reduced protein levels (LP) (crude protein contents: 190 g/kg starter, 170 g/kg grower, 160 g/kg finisher) and increasing L-valine (V) levels (LPV0, LPV1, LPV2, LPV3, LPV4 and LPV5) starter (0-0.50-1.00-1.50-2.00-2.50 g L-valine supplementation/kg of diet), grower (0-0.50-1.00-1.60-2.10-2.70 g L-valine supplementation/kg of diet) and finisher (0-0.50-1.10-1.60-2.10-2.70 g L-valine supplementation/kg of diet). The valine contents of the broiler diets were gradually increased across all phases of the trial series (starter, grower, finisher), and as a result the TV and SIDV grew by +0.5 g/kg increments in each treatment. Consequently, the changes of the TV and SIDV levels in the starter, grower and finisher phases were TV 8.7-11.3 g/kg diet (SIDV: 7.9-10.4 g/kg diet), TV 7.6-10.3 g/kg diet (SIDV: 7.0-9.5 g/kg diet) and TV 7.3-10.0 g/kg diet (SIDV: 6.7-9.2g/kg diet), respectively. Diets were fed in the starter phase in mash form, while in the grower and finisher phases the birds received their feed in pellet form (3 mm).

3.1.3. Recording the trial data

In the course of the trial the individual live weight of broilers were determined on days 1, 14, 21 and 35 corresponding with the trial diets fed, using a Sartorius CP16001S balance (Germany, Göttingen) equipped with an adapter for weighing live animals. Wing tags, live weights, day and cause of mortality of lost birds were continuously recorded. Feed intake of the birds was determined by groups (by cage) in the intervals between live weight measurements.

3.2. Digestibility studies

3.2.1. Trial birds and housing

The ileal digestibility studies were conducted *post mortem* on days 14, 21 and 35 of the trial with 6 birds per treatment and trial phase, ie with a total of 126 birds. Chyme was collected at the end of the three-day preparatory stage following the two-stage carbon dioxide gas stunning and subsequent bleed-out of the birds. The housing of the trial animals was as described in *section 3.1.1.*

3.2.2. Treatments, trial diets

The treatments and the composition, nutrient and amino acid contents of the trial diets were the same as those in *section 3.1.2* with the exception that on the last 3 days of the studies the diets were supplemented with titanium dioxide (TiO_2). Diets were available to the birds *ad libitum*. Drinking water was also available *ad libitum* from open water bowls.

3.2.3. Methodology of chyme collection

At the end of the 3-day preparatory stage – during which the birds were fed the diets supplemented with titanium dioxide (TiO_2) – the chyme was collected following the two-stage carbon dioxide gas stunning and subsequent bleed-out of the birds, in adherence to the relevant animal protection and animal welfare rules.

After the birds were bled out the abdominal cavity was opened and the section of the ileum between the Meckel's diverticulum (*diverticulum ductus vitellointestinalis*) and the caudal part of the *valvula ileorectalis* was dissected Next the chyme in this section was carefully collected without delay. The collected sample was weighed with gram precision and stored at a temperature below –18 ^{o}C until further processing. The live weight of the birds was measured directly before the start of the chyme collection procedure. Prior to the start of the analyses the ileum chyme samples were prepared for the laboratory studies by careful drying at 65 ^{o}C.

3.3. Laboratory studies

3.3.1 Chemical studies

The dry matter, crude protein, crude fat, crude fiber, crude ash, calcium and phosphorus contents of the feed ingredients and trial diets were determined in accordance with the Hungarian Standards (MSz) (Hungarian Feedstuffs Codex, 2004) provisions. The provisions of MSz 6830/377 were applied to determine the moisture content; MSz 6830-4:1981 for crude protein content; MSz 6830/6-78 for crude fat content; MSz 6830/7-81 for crude fiber content; MSz 6830/8-78 for crude ash content; MSz 6830/20-80 for calcium content and MSz-ISO 6491 for phosphorus content. The titanium dioxide contents of the diet were determined according to AOAC (1996). The amino acid analysis of the diets was conducted according to Bech-Andersen et al. (1990).

3.3.2 Calculating the CO_2 footprint of the diets

The carbon footprint of the trial diets was determined using the data base of GFLI 2.0 (Global Feed LCA Institute) and AFP 6.3 (Agri-footprint).

3.4 Statistical analysis of the trial data

The statistical analyses were performed in the SPSS Statistics for Windows v.20 software (IBM Corp., Armonk, N.Y., USA). Normal distribution was tested by Kolmogorov–Smirnov tests. The means were compared by one-way analysis of variance (ANOVA with Bonferroni correction) or Mann–Whitney nonparametric tests. Differences were considered statistically significant at $P<0.05$. The general model for the variance analysis was:

$$Y_{ijk} = \mu + A_i + B_j + (A \times B)_{ij} + e_{ijk}$$

where:

Y_{ijk}	=	dependent variable;
μ	=	grand mean;
A_i	=	treatment
B_j	=	number of replicates
$(A \times B)$	=	interaction between treatment and replicate
e_{ijk}	=	residual error.

The relationships between the dietary valine content and the digestibility and absorption of amino acids were studied using non-linear regression analysis.

4. RESULTS

4.1 Results of the performance studies

Changes of the live weight and weight gain of the birds

The initial weight of the broilers (day 1 of age) was the same across all treatments in our studies, which is a prerequisite of measuring the treatment effects objectively. The study of live weights on days 14, 21 and 35 found them to be the highest in birds fed the diet with the recommended crude protein and valine levels (PC) (430 g, 1027 g, 2569 g). In comparison, the live weight of birds fed the diet with reduced protein content and without valine supplementation (LPV0) was significantly lower ($P<0.001$) on each of the studied days (388 g, 917 g, 2385 g). Supplementing the reduced protein base diet (LPV0) with valine (LPV1, LPV2, LPV3, LPV4, and LPV5) had no significant effect on the live weight of the broilers ($P>0.05$). At the end of the starter phase the average live weight of birds fed the reduced protein diets (LPV0, LPV1, LPV2, LPV3, LPV4, and LPV5) was 41 gram lower than the live weight of PC birds (430 g/bird vs 389 g/bird). By the end of the grower phase this difference became 98 g/bird (1027 g vs 929 g), and at the end of the trial it stabilized at 141 g/bird (2569 g vs 2428 g). Compared to the PC treatment (2569 g/bird) the lowest live weight on day 35 of age was recorded for the birds in the treatment with reduced protein and without valine supplementation (LPV0), where the live weight of the birds was only 2385 gram. This indicates that a lower protein intake coupled with valine deficiency leads to a pronounced decrease of live weight. It is remarkable, however, that while both on day 14 and on day 21 of age the live weight of PC birds was 9.5% higher than the average live weight of birds fed the reduced protein diets, at the end of the trial (day 35) this difference fell to 7.2%.

The average daily weight gain of birds in the PC treatment exceeded that of the LPV0 birds by 2.3 g/d in the starter phase (days 1-14), and by 8.5 g/d in the grower phase (days 15-21) ($p<0.001$), which corresponds to a relative difference of 9.3% and 11.4%, respectively. During the last two weeks of rearing (days 22-35) this difference dropped to 4.3% and was no more statistically verifiable ($p>0.05$). In this phase of rearing the daily weight gain was found to be the same across all treatments, irrespective of the crude protein content and valine supplementation level of the diets ($P=0.085$). During the entire trial period (days 1-35) the average daily weight gain of the PC birds was only 4.8 g/d higher than that of their LPV0 peers ($P<0.001$). It is remarkable, however, that the average daily weight gain of birds fed the diet with reduced protein content but supplemented with 1.0/1.0/1.05 g/kg valine (LPV2) was the same as the weight gain of PC birds ($P>0.05$), which suggests that in this phase the protein and valine requirements of the birds were actually met.

4.1.2. Feed intake and feed conversion rate

According to our data the feed intake of the birds was the same across all treatments ($P>0.05$) during the phase of feeding the starter diets (days 1-14). The grower phase showed similar trends with the difference that in this phase the LPV1 birds consumed 6.2% less feed than their PC peers

(P<0.05). The feed intake of birds fed the reduced protein diets was the same across all treatments (P>0.05). The feed intake of birds fed the LP diets was the same in the finisher phase (days 22-35) and also during the full trial period (days 1-35) (P>0.05). It should be noted, however, that during the full trial period the LPV0 birds consumed 4.6% less diet than their PC peers (P>0.001).

When looking at the feed conversion rate of birds, statistically verifiable differences were essentially found in the starter (days 1-4) and in the grower (days 15-21) phases. In these phases the PC birds needed 4.5% and 5.5% less diet to achieve a unit of weight gain compared to their LPV0 peers (P<0.05). During the phase of feeding the finisher diets the feed conversion rates (FCR) were the same across all treatments (P>0.05), irrespective of the protein content and valine supplementation levels of the diets. In consequence, also when looking at the entire trial duration there was no statistically verifiable difference found in the feed conversions rates (FCR) of the birds in the individual treatments (P>0.05).

4.1.3 Protein intake and protein utilization

According to our data the broilers fed the diet formulated with the recommended protein content (PC) consumed 7.3 g/d crude protein in the starter phase, 20.2 g/d in the grower phase and 31.9 g/d in the finisher phase, which means a 19.7 g/d crude protein intake when taking into account the full trial period. In contrast, the crude protein intake of LPV0 birds was 6.3 g/d (starter), 17.2 g/d (grower), 26.8 g/d (finisher) and 16.7 g/d between days 1-35 of age, meaning a 15.2% lower protein intake over the full 35-day trial period (LPV0 vs PC). All of the differences were statistically verifiable (P<0.05). The average daily crude protein intake of birds fed the reduced protein diets with valine supplementation was the same as the crude protein intake of LPV0 birds in all of the trial phases (P>0.05).

The roosters in PC treatment used 268 g/kg (starter), 242 g/kg (grower) and 293 g/kg (finisher) crude protein to produce a unit of weight gain. In contrast, the average protein conversion rate of the LPV0 birds was 255 g/kg (starter), 230 g/kg (grower) and 257 g/kg (finisher). During the full trial period (days 1-35) these birds needed 249 g crude protein to produce 1 kg weight gain, which - similarly to the intake - was also lower by 9.5% compared to the value determined for the PC birds. The differences were significant in all instances ($P<0.05$). Although the average crude protein utilization of the birds fed the diets with reduced protein content and supplemented with valine was 1.9% better (numerically) than the value determined for the LPV0 treatment, this difference was not significant ($P>0.05$). When the average protein conversion rate of treatments using the diets with reduced protein content but supplemented with valine is compared to that of the PC birds, it is apparent that the former produced a unit of weight gain using 11.2% less protein ($P<0.05$).

4.1.4 The effect of protein reduction on the dietary CO_2 footprint of broilers

The CO_2 footprint of trial diets was calculated based on the GFLI 2.0 and AFP 6.3 data bases. According to our results the positive control (PC) diet had a 1.818 kg CO_2 eq/kg value in the starter phase, while the reduced protein diets (LPV0) were characterized by a value of 1.552 kg CO_2 eq/kg, which is 14.6% lower than the PC base value. This difference was 19.3% for the grower diets (1.629 kg CO_2 eq/kg vs 1.366 kg CO_2 eq/kg), and 20.9% for the finisher diets (1.538 kg CO_2 eq/kg vs 1.272 kg CO_2 eq/kg).
Our results show that the broilers fed the diets with the recommended protein content (PC) produced a unit of weight gain beside generating a dietary CO_2 footprint of 2.32 CO_2 eq/kg in the starter phase, 2.09 CO_2 eq/kg in the grower phase and 2.48 CO_2 eq/kg in the finisher phase, while over the full trial duration this value was 2.35 CO_2 eq/kg. In contrast, the dietary CO_2 footprint of the weight gain of birds fed the diet with reduced

protein contents (LPV0 and LPV1-5) was on average 2.07 CO_2 eq/kg (starter), 1.84 CO_2 eq/kg (grower) and 2.02 CO_2 eq/kg (finisher), which overall resulted a 1.99 CO_2 eq/kg carbon footprint during the 35-day trial period. This value is 15.3% better than the value calculated for the PC treatment ($P<0.001$). The dietary CO_2 footprint of the weight gain of birds fed the reduced protein but valine supplemented diets is similar to this value, being an average 1.99 CO_2 eq/kg for the 35-day trial period ($P>0.05$).

4.2 Results of the digestibility studies

The analysis of data recorded in the course of the digestibility studies due to space limitations only covers from the studied parameters crude protein, and the most important amino acids in poultry nutrition: lysine, methionine, methionine+cystine, threonine and valine, and total amino acids (collectively). However, the digestibility and absorption values for the other amino acids are also presented in the relevant tables of the dissertation. The comparison of the results of the digestibility studies with data published in the literature is relatively limited, as such studies typically focus on the ileal digestibility of individual ingredients, and less frequently on their absorption.

4.2.1 Apparent ileal digestibility of crude protein and amino acids during the starter diet phase (days 1-14)

According to our studies the ileal digestibility of **crude protein** was 81.8% in the case of the reduced protein diet without valine supplementation (LPV0), which is 2.6% better than the value of the standard diet (PC) (79.2%, $P<0.05$). This improvement of digestibility can be explained with the better absorption seen beside a reduced protein supply. In consequence of valine supplementation the digestibility improved further and reached its peak (83.9%) at 10.4 g/kg valine content. This relationship can be described with a polynomial equation ($R^2 = 0.8445$). The digestibility of

lysine was 88.1% in the LPV0 diet, exceeding the PC value by 4% (84.1%, $P<0.05$). We reached the peak of lysine digestibility (89.1%) at 10.7 g/kg valine content showing a moderate correlation ($R^2 = 0.6383$). The digestibility of **methionine** was 95.0% in the LPV0 diet, being 2.3% higher than the PC value (92.7%, $P<0.05$). The peak (95.9%) was found by 11.5 g/kg valine, with a moderate correlation ($R^2 = 0.5674$). In case of **methionine+cystine** the peak of digestibility was 89.8%, achieved at 11.4 g/kg valine content. The calculated digestibility peak was 5.2% higher than the PC value.

The digestibility threonine was 77.1% in the LPV0 diet, and this is 5.1% better than the PC value (72.0%, $P<0.05$). The peak (81.2%) was reached at 10.8 g/kg valine content, beside a strong correlation ($R^2 = 0.9627$). The digestibility of **valine** achieved the peak (87.2%) outside the studied range at 12.0 g/kg valien content. This was a strong correlation ($R^2 = 0.9938$).

4.2.2 Apparent ileal absorption of crude protein amd amino acids during the starter diet phase (days 1-14)

On day 14 of age birds in the PC treatment absorbed 9.4 g **crude protein** daily, while the birds fed the diet with reduced protein without valine supplementation (LPV0) absorbed only 7.5 g, which represents a 20.2% decrease ($P<0.05$). The relationship between valine content and crude protein absorption can be described with a polynomial equation, which reaches its peak outside the studied range ($R^2 = 0.5918$). As for lysine, methionine, methionine+cystine and threonine the birds fed the reduced protein diet absorbed less amino acid compared to their PC peers numerically only ($P>0.05$), which can be attributed to the fact that the reduced crude protein diet (LPV0) contained of these amino acids the same amount of lysine, methionine, methionine+cystine and threonine as the diet of the PC birds. This indicates that in case we reduce the crude protein content of the diets but supplement the amino acids in crystalline form up to the amino acid level of the diet with the recommended crude protein and amino acid content (PC) the quantity of absorbed amino acid for these amino acids will not decrease ($P< 0.05$). For **valine** however the LPV0 diet resulted in a 28.3% lower absorption ($P<0.05$). The peak of absorption was outside the studied range, because almost 100% of crystalline valine is absorbed. The absorption of valine shows a polynomial relationship with the increase of the valine content in the diets ($R^2 = 0.9896$).

The absorption of **total amino acids** was 9.2 g/day for the PC birds, while in the case of the LPV0 diet it was 7.2 g/day, representing a 21.7% decline ($P<0.05$). The peak of absorption was attained at 10.9 g/kg valine, meaning an absorption of 7.6 g/day ($R^2 = 0.932$), but even so it remained 17.4% below the value for the PC birds. This difference was also visible in the weight gain: an 8.5% reduction was seen in the birds of the LPV0 treatment, although this is less than the absorption difference. This can probably be attributed to the fact that the birds fed the diet with the reduced protein content excreted less amino acid in the urine, and consequently the difference in the quantity of truly available amino acids was less.

4.2.3 Apparent ileal digestibility of crude protein and amino acids during the grower diet phase (days 15-21)

In the grower phase, between days 15 to 21, the digestibility of **crude protein** was 77.9% in the PC treatment, which is very similar to the 79.9% found by Barua et al. (2020). In the LPV0 treatment with reduced protein and no valine supplementation the digestibility reached 79.5%, but this improvement was not significant ($P>0.05$). Valine supplementation (LPV1-LPV5) did not improve the protein digestibility ($P>0.05$). The peak of protein digestibility (78.9%) was found at 9.0 g/kg valine content, and this was only 1% higher than the PC value, but the correlation was weak ($R^2=0.2009$). The digestibility of **lysine** was 83.8% in the PC treatment, and 86.0% on the LPV0 group, this 2.2% improvement, however, was not significant ($P>0.05$). In the LPV1 and LPV3 treatments the valine supplementation significantly improved the digestibility of lysine

(P<0.05), and thus exceeded the PC value by 3.8-3.3%. The peak of lysine digestibility (86.5%) was found at 8.4 g/kg valine. The correlation between the digestibility of valine and lysine was weak (R^2=0.1675). The digestibility of **methionine** was 92.2% in the PC treatment, and 93.3% in the LPV0 group, but this improvement was not found to be significant (P>0.05). The peak of methionine digestibility was 93.5% beside 8.1 g/kg valine. The correlation was weak (R^2 = 0.3892). The combined digestibility of **methionine+cystine** also showed a similar tendency, reaching its peak (86.7%) at 5.3 g/kg valine, which is outside our studied range and the correlation was moderate (R^2=0.5271). There was a numerical improvement of **threonine** digestibility in the LPV0 group (69.0% vs 73.1%) but this difference was not significant (P>0.05). The peak of digestibility was 73.3% at 8.8 g/kg valine, which is 1.3% higher than the PC value. The correlation is very weak (R^2 = 0.0567). The digestibility of **valine** showed a strong correlation with the valine content (R^2=0.7929), reaching its peak (81.9%) at 10.1 g/kg valine, exceeding the PC value by 12.9%. The digestibility of **total amino acids** showed no significant difference across the treatments (P>0.05). The peak digestibility (80.9%) was found at 8.5 g/kg valine, and this was 2.0% higher than the PC value. The correlation was very weak (R^2=0.0026).

4.2.4 Apparent ileal absorption of crude protein and amino acids during the grower diet phase (days 15-21)

According to the measurements at day 21 of age birds in the PC group absorbed 21.6 g **crude protein** per day, while in the case of the LPV0 treatment, which contained reduced protein and no valine supplementation, the absorption fell to 16.1 g, remaining 25.5% below the PC value (P<0.05). In case of the reduced protein diets there is a strong polynomial relationship (R^2 = 0.767) between the valine level and the crude protein absorption, the peak of 17.7 g/day can be achieved at 10.2 g/kg valine content, and this is 18% lower than the absorption of the PC birds. As for **lysine**, the LPV0 birds absorbed 3.7% more lysine (1110 mg/day) than the PC group birds (1070 mg/day), showing a significant difference ($P<0.05$). The valine supplementation did not increase further the absorption of lysine, indicating that the lysine supply of the reduced protein diets was adequate. The absorption of **methionine** in the LPV0 birds was 35.4% higher than the PC value ($P<0.05$), while the absorption of **methionine+cystine** showed no difference across the treatments, meaning these amino acids were present in sufficient quantities. The absorption of **valine** in the LPV0 group was 23.3% lower than in the PC group ($P<0.05$). The peak of valine absorption was 1088 mg/day at 12 g/kg valine content, and this exceeded the PC value by 20.9% but it was outside the studied range. The increase of the valine level showed a strong correlation with absorption ($R^2 = 0.996$). The absorption of **total amino acids** in the PC birds was 19.9 g/day, and 15.8 g/day in the LPV0 group, representing a 20.6% decrease ($P<0,05$). The peak of 17.0 g/day was achieved with 9.5 g/kg valine, but even this remained below the PC value by 14.6%. The correlation was very strong ($R^2 = 0.9422$). Due to the reduced protein diets the decrease of live weight gain and weight gain was modest: the difference in the LPV0 group was -4.1% and in the LPV2 treatment only -0.6%. This can probably be attributed to the explanation in the starter phase section, ie. in case of restrictive crude protein supply the urinary amino acid excretion will be less as well (Babinszky et al., 2003; Whitacre and Tanner, 2018). In consequence the difference between the amino acids available for maintenance and tissue accretion was less, which was reflected in the diminished reduction of the weight gain.

4.2.5 Apparent ileal digestibility of crude protein and amino acids during the finisher diet phase (days 22-35)

According to the trial data the **crude protein** digestibility during the grower phase was measured to be the lowest in the PC treatment, being 72.4%. The difference of the crude protein digestibility of the LPV0 treatment which had reduced protein content and no valine supplementation (72.7%) compared to the former was statistically not verifiable (P>0.05). The LPV1-5 treatments which were supplemented with valine improved the crude protein digestibility significantly (P<0.05) both compared to the PC and to the LPV0 treatments, while no further statistically verifiable differences were found between the LPV1-5 treatments (P>0,05). The crude protein digestibility was found to be the best in the LPV3 treatment (78.8%) which was 6.4% higher than the value measured in the PC treatment (72.4%). The improvement can probably be explained with the findings of the previous phases, that allowed the more efficient absorption of nutrients. In the finisher phase the crystalline valine supplementation significantly improved (P<0.05) the digestibility of crude protein compared to the PC and to the LPV0 treatments. The peak of the crude protein digestibility (78.9%) can be achieved by 9.0 g/kg valine content, and this is the same as the value of the LPV3 treatment. The correlation is very strong ($R^2 = 0.8766$). The digestibility of **lysine** tended to be similar to the crude protein digestibility. In the PC birds we found a 78.9% lysine digestibility, and in the LPV0 treatment 81.6%, which was 2.7% higher than the PC value but this difference was statistically not significant (P>0.05). Increasing the valine dosage resulted in a significant increase of the lysine digestibility (P<0.05). Lysine digestibility was found to be the highest in the LPV2 and LPV3 treatments (86.2% and 86%), and these exceeded the PC values by 7.3% and 7.1%, respectively. The peak of lysine digestibility (86.4%) can be reached at 9.0 g/kg valine content, which is the same as the values in the LPV2 and LPV3 treatments. The correlation is very strong ($R^2 = 0.9661$). The digestibility of **methionine** yielded similar results as well. For PC birds we measured 89.3% methionine digestibility, while in the case of the LPV0 treatment 88.9% ($P>0.05$). In the LPV2, LPV3, LPV4 and LPV5 treatments we observed significantly higher methionine digestibility compared to the PC, LPV0 and LPV1 treatments ($P<0.05$); the value was found to be the highest in the LPV3 treatment (92.4%) beside an 8.9 g/kg valine content. The peak of methionine digestibility (92,4%) can be achieved at 9.1 g/kg valine content. The correlation I strong ($R^2 = 0,8596$). The digestibility of **methionine+cystine** showed no significant difference between the PC and LPV0 treatments (82.1% vs 82.0%, $P>0.05$), but between the LPV1-5 treatments significant improvement was found ($P<0.05$). The methinone+cystine digestibility was found to be the highest in the LPV3 and LPV5 treatments (85.0%). The peak (85.2%) can be achieved at a valine content of 9.2 g/kg, which is very similar to the values measured in the LPV3 and LPV5 treatments. The correlation is strong ($R^2 = 0.7353$). **Threonine** digestibility in the LPV0 treatment was 7.5% higher than in the PC treatment (64.5% vs 57.0%) ($P>0.05$). The threonine digestibility was also higher in the LPV1-2-3 treatments compared to the PC and LPV0 treatments, but the differences were not found to be significant. The peak (70.7%) can be reached at 8.6 g/kg valine content, which is 0.2 g/kg higher than the valine content of the finisher diet of the PC group. The relationship can be described with a polynomial equation ($R^2 = 0.5167$). The digestibility of **valine** showed a similar tendency, and was essentially the same between the PC and LPV0 treatments (71.6% vs 72.0%, $P>0.05$). In case of the LPV5 treatment however the difference was significant (81.9%, $P<0.05$). The peak (81.7%) is reached at 9.6 g/kg valine content, which shows a very strong correlation ($R^2 = 0.9294$), and this is 10.1% higher than the value measured for the PC birds. The valine digestibility in treatment LPV5 and its peak are practically the same (81.7% vs 81.9%). The digestibility of **total amino acids** (without tryptophane) showed a similar tendenchy. The digestibility measured in the PC and LPV0 treatments was the same (73.3% vs 73.4%, P>0.05). The valine supplementation in the LPV1-5 treatments significantly increased the digestibility of total amino acids (P<0.05); the peak (79.6%) can be reached at 9.1 g/kg valine content, and this is 6.3% higher than the result of the PC treatment. The relationship is polynomial (R^2 = 0.8266), similarly to the findings for most amino acids, and the peak is reached at an 0.7 g/kg higher valine content.

4.2.6 Apparent ileal absorption of crude protein and amino acids during the finisher diet phase (days 22-35)

According to our studies on day 25 of age of the finisher phase (days 22-35) the birds in the PC treatment absorbed 32.9 g **crude protein** per day. In contrast the crude protein absorption of birds fed the reduced protein diet without valine supplementation (LPV0) was measured to be 20.7% lower (26.1 g), and this difference was statistically verifiable (P<0.05). With valine supplementation a significant increase of crude protein absorption was observed in the LPV1, LPV3 and LPV4 treatments compared to LPV0 (P<0.05), but these values did not reach the cp absorption level measured in the PC treatment (P>0.05). Peak absorption can be reached by 8.9 g/kg valine content, which meant the daily absorption of 29.3 g total amino acids, and this is 10.9% less than in the PC group. The relationship can be described with a polynomial equation of very high strength (R^2 = 0.9905). The absorption of **lysine**, **methionine**, **methionine+cystine** and **valine** in case of the reduced protein diet without valine supplementation (LPV0) was significantly lower when compared to the PC treatment (P<0.05). Valine supplementation improved the absorption, but none of the treatments reached the values measured for the PC treatment. The absorption of methionine and methionine+cystine was significantly higher than the LPV0 treatment value in the case of LPV2, LPV4 and LPV5 only (P<0.05), but they did not reach the PC group level. In respect of **threonine** the absorption of PC and LPV0 birds was the same

(P>0.05), but as a result of valine supplementation the level of absorbed threonine – with the exception of LPV5 treatment – exceeded even the absorption measured in the PC birds (P<0.05). **Valine** supplementation also improved the valine absorption which in the LPV3, LPV4 and LPV5 treatments exceeded even the valine absorption of the PC treatment (P<0.05). According to our calculations the peak of valine absorption is outside the range we studied, but can be reached at the level of 1846 mg/day at 11 g/kg valine content, and this is 23.9% higher than the absorption in the PC treatment. The relationship can be described with a polynomial equation and the reliability is extremely strong ($R^2 = 0.994$). According to our trial data the birds in the PC treatment absorbed 32.0 g **total amino acids** per day on their day 35 of age. In contrast the daily total amino acids absorption of birds fed the diet with reduced protein and no valine supplementation (LPV0) was 25.2 g, and this is 21.3% less than the ileal amino acid absorption of the PC birds (P<0.05). The peak of absorption was found at 9.0 g/kg valine content, being 28.6 g/day absorbed total amino acids, which is 10.6% less than the absorption measured for the PC birds. The correlation is very strong ($R^2 = 0.9576$). The difference however did not affect the performance of the birds, since the weight gains and feed conversion rates were the same across all treatments. This is probably due to the physiological response whereby the excess amino acids are excreted in the urine from the birds (Babinszky et al., 2003), and the excretion of the amino acids changes subject to the actual physiological requirements. Consequently, the very similar performance necessitates that the level of amino acids available for the birds should also be similar.

5. CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions that can be drawn from the results of the performance studies

Compared to the Aviagen (2014) hybrid standard, the birds in the positive control (PC) and LPV0-5 treatments achieved numerically higher live weight and better feed conversion ratios. However, compared to the equivalent baseline values of Aviagen (2022), the live weight of the birds in all experimental groups also exceeded the standard, while the feed conversion ratio was numerically less favorable.Reducing the protein content of the diets (PC) by 20 g/kg per phase (starter, grower, finisher) – beside maintaining the lysine, methionine+cystine, threonine and tryptophane levels (LPV0) – led to a decline of weight gain and feed conversion values up to days 21 of age of the birds which was not improved significantly even when adding valine supplementation. In the finishing phase (days 22 – 35 of age) the weight gain and feed conversion of the birds was the same across all treatments, meaning the reduced protein content did not affect their growth adversely anymore. In terms of live weight, birds fed the recommended (PC) diet with standard protein and valine content achieved better results compared to those on reduced-protein diets without valine supplementation or with valine supplementation. Over the total duration of the rearing period (days 1 – 35) feed conversion was not affected by the different protein and valine levels. Birds fed the diet with reduced protein content and supplemented with valine could produce a unit of weight gain from 11.2 % less protein than their peers fed the dieta with the recommended protein and valine contents (PC). Due to the improved protein utilization rate the birds fed the reduced protein diet and the reduced protein diet with valine supplementation achieved during the 35-day trial period a 15.3 % improvement in the dietary CO_2 footprint of their weight gain compared to that of their PC peers. The concept applied in our trial series could be a feasible option to reduce the dietary CO_2 footprint and it would be worthwhile to confirm this assumption in further targeted studies. Our results have provided important information for the further development of sustainable, environmentally conscious broiler production, which is expected to become increasingly significant, and a prerequisite of which is to reduce the dietary CO_2 footprint of production.

5.2 Digestibility and absorption conclusions drawn from the digestibility studies

Based on the results of the digestibility studies it can be concluded that **in the starter diet phase (measured on day 14)** the apparent ileal digestibility of the amino acids in the diet with reduced protein content and without crystalline valine supplementation for most of the amino acids is significantly higher than the value found for the diet formulated with the recommended crude protein and amino acid contents (Aviagen, 2014) (PC). In case of the diets with reduced protein content and supplemented with valine the ileal digestibility of most amino acids only tended to be higher ($P>0.05$). When feeding the diets with reduced protein content and supplemented with valine the peak digestibility of total amino acid exceeds the digestibility measured for the diet containing the recommended protein and amino acid levels. At the time of the study the crude protein absorption of the birds fed the diet with reduced protein content and without valine supplementation (LPV0) remained below the crude protein absorption of the birds fed the diet with the recommended protein and amino acid contents (PC) is significantly lower. In respect of lysine, methionine, methionine+cystine and threonine the birds fed the diets with reduced protein content and with or without valine supplementation absorbed less amino acid numerically only compared to their PC peers ($P>0.05$). In respect of valine the birds fed the diet with reduced protein content and without valine supplementation absorbed 28.3% less valine than their PC peers.

With the increase of the valine levels in the reduced crude protein diets also the quantity of absorbed valine increases and the reliability of the correlation is very strong (R^2 0.9896). Compared to the total amino acids absorption of the birds fed the diet with the recommended crude protein and valine contents the birds fed the diet with reduced protein content and without valine supplementation (LPV0) absorbed 21.7% less total amino acids. The absorption peak of total amino acids is 17.4% below the value measured for the PC birds. The difference in the absorption of total amino acids is also reflected in the live weight and weight gain of the birds. This negative difference in weight gain however is less than the difference found for absorption.

It can be concluded from the results of our digestibility studies that **measured at the end of the grower diet phase (day 21)** the apparent ileal digestibility of most amino acids in the diet with reduced protein and without crystalline valine supplementation is significantly higher than the value measured for the diet formulated with the recommended crude protein and amino acid contents (PC) (Aviagen, 2014). When supplementing the reduced crude protein basal diet (LPV0) with crystalline valine the ileal digestibility of lysine, threonine and valine increases significantly ($P<0.05$); in the case of crude protein, methionine, methionine+cystine and total amino acids, however, only a numerical improvement of digestibility can be expected ($P>0.05$). The peak digestibility of valine exceeds the digestibility measured for the diet with the recommended protein and amino acid contents (PC) by 12.9%. In respect of the diets with reduced amino acid content but supplemented with valine the peak digestibility of total amino acids exceeds the digestibility measured for the diet with the recommended protein and amino acid levels (PC) by 2.0% but the reliability of this relationship is very weak (R^2 0.0026). Based on these findings it can be concluded, that the crystalline valine supplementation at this phase does not affect the digestibility of total amino acids. According to our data at the time of the studies compared to the crude protein absorption of the birds in the PC treatment the crude protein absorption of birds fed the diet with reduced protein and without valine supplementation (LPV0) is 14.5% lower. In respect of valine, the birds fed the diet with reduced protein content and without valine supplementation absorbed 23.3% less valine than their PC peers. Compared to the total amino acids absorption of the birds in the PC treatment the birds fed the diet with the reduced protein content and without valine supplementation (LPV0) are able to absorb 20.6% less total amino acids. The difference in the absorption of total amino acids is also reflected in the live weight and weight gain of the birds. This negative difference in weight gain however is less than the difference found for absorption.

Based on the data measured **at the finisher diet phase (measured on day 35 of age)** it can be concluded that the apparent ileal digestibility of the amino acid content of the diet with reduced protein content and without crystalline valine supplementation (LPV0) in respect of most amino acids does not differ significantly (P>0.05) from the values measured for the diet formulated with the recommended crude protein and amino acid levels (Aviagen, 2014). Supplementing the reduced crude protein basal diet (LPV0) with crystalline valine significantly improved the digestibility of several amino acids, including methionine, valine, glycine, proline, aspartic acid, glutamic acid, and alanine, compared to the LPV0 treatment (mostly independent of dose). Meanwhile, the ileal digestibility of other amino acids showed only a tendency to increase. When supplementing the same basal diet with valine the peak digestibility of threonine exceeds the digestibility measured for the diet containing the recommended protein and amino acid levels by 13.7%. The peak digestibility of total amino acids is 6.3% higher than the PC value. At the time of the studies the birds in the PC treatment absorbed 20.7% more crude protein daily then their peers fed the diet with reduced protein content and without valine supplementation (LPV0). In respect of lysine, methionine, and methionine+cystine the birds fed the diets with reduced protein content and without valine supplementation absorbed significantly less amino acid compared to their PC peers ($P<0.05$) and this did not change when the diet was supplemented with valine. In respect of valine the birds fed the diet with reduced protein content and without valine supplementation absorbed 22.8% less valine than the PC broilers. At the same time, birds in the LPV3-5 treatments, which consumed reduced-protein diets supplemented with valine, absorbed significantly more valine than their counterparts on the PC and LPV0 diets. On day 35 of age birds in the PC treatment are able to absorb 21.3% more total amino acids than the birds fed the diet with reduced protein content and without valine supplementation (LPV0). The difference measured in the absorption of total amino acids is not reflected in the weight gain of the birds anymore, which can probably be attributed to the almost identical quantity of available amino acid.

The performance and crude protein and amino acid digestibility data obtained through the concept applied in our trial series can provide a practical option to reduce the dietary CO_2 footprint which would be worthwhile to confirm in further targeted studies. Our results have provided important information for the further development of sustainable, environmentally conscious broiler production, which is expected to become increasingly significant, and a prerequisite of which is to reduce the dietary CO_2 footprint.

5.3 Recommendations

The results of the trial series indicate that in the first 3 weeks of rearing, ie at the time the starter and grower diet are fed the rate of protein decrease (20 g/kg) impacts the growth of broilers negatively, but this effect is eliminated in the last two weeks of rearing, and then the birds are capable to achieve similar daily weight gains irrespective of the crude protein content of the diets. Bearing this interrelation in mind it could be useful to reduce to some extent the protein supply during the starter and grower phase beside maintaining the protein and amino acid levels applied in the finisher phase.

Based on our trial results it appears justifiable to conduct further performance and digestibility studies that use other high genetic potential broiler hybrids. Such could be for instance the Cobb500 - while the market share of this hybrid is limited in Hungary and the European Union, it is quite significant in the USA and South America.

Both from the scientific and practical aspect it is recommendable to also set up parallel studies that would serve to characterize the differences between the two genotypes and to further improve the accuracy of genetic profile based feeding technologies but also extend to examine differences between sexes.

Due to the changes of new consumption trends it would be worthwhile to include in these studies the slow-growing (heritage) broilers as well.

The results of further supplementary studies could contribute to the optimization of the different crude protein and valine contents of the diets, and to reducing the dietary CO_2 footprint of broiler production.

It would be useful to augment the digestibility studies with N-retention studies as well. The results of these studies could provide informative data about the possibility of reducing the rate of N-emissions, and help to interpret the improved protein conversion.

In the interest of cost efficient and environmentally conscious broiler production it is also recommendable to set up studies in which the effect of new, commercially available crystalline amino acids (e.g. L-Arginine) was detected in the interest ofn reducing N-emissions and the dietary CO_2 footprint.

6. NEW SCIENTIFIC RESULTS

1) When the crude protein content of Ross308 broiler diets formulated in accordance with the Aviagen (2014) crude protein and amino acid recommendations is reduced by 20 g/kg diet (starter: 210 → 190 g/kg diet; grower: 190 → 170 g/kg diet; finisher: 180 → 160 g/kg diet) the weight gain of broiler roosters up to day 21 of age is significantly lowered ($P<0.05$), and this does not improve even when valine is supplemented. In the final phase of rearing (21–35 days of age), feeding the reduced crude protein diets used in the study, either alone or with valine supplementation, no longer had a depressive effect on the birds' feed conversion ratio or average daily weight gain ($P>0.05$).

2) During the finisher diet phase (between days 22-35 of age) the ileal digestible valine/lysine ratio of the Ross308 broiler rooster diet is 0.72, which is 6 percentage point lower than the 0.78 value set forth in the Aviagen (2022) recommendations. With this ileal digestible amino acid ratio the birds fed the reduced protein diets (160 g/kg diet) achieved the same weight gain ($P>0.05$) in the last phase of rearing as their peers fed the diets with the standard crude protein contents (180 g/kg diet).

3) The Ross308 broiler roosters fed the reduced protein (starter: 210 → 190 g/kg diet; grower: 190 → 170 g/kg diet; finisher: 180 → 160 g/kg diet) diets without valine supplementation or with valine supplementation are able to produce a unit of weight gain from 11.2% less crude protein - as per the average of all treatments - than their peers fed the diet formulated with the Aviagen (2014) recommendations. As a result, the dietary CO_2 footprint of their weight gain is 15.53% better during the 35-day rearing period.

4) In the reduced crude protein diets without crystalline L-valine supplementation the ileal digestibility of crude protein in the starter phase (days 1-14) significantly exceeds ($P<0.05$) the value measured for the diets formulated according to the Aviagen (2014) recommendations (81.8% vs 79.2%), and this is not improved any further by a further crystalline L-valine supplementation ($P>0.05$). When feeding finisher diets (between days 22-35 of age) the ileal digestibility of crude protein in the reduced protein diet supplemented with crystalline L-valine is significantly higher ($P<0.05$) than the value measured for the reduced protein diet without L-valine supplementation and for the diet formulated with the crude protein and amino acid levels recommended by Aviagen (2014); and this finding is also true for the total amino acids.

5) In case of the diets with reduced crude protein content, with or without L-valine supplementation, the peak digestibility of total amino acids exceeds the value measured for the diets formulated in accordance with the Aviagen (2014) recommendations, both in the starter and in the finisher phases. In case of a restrictive protein supply there is a strong or very strong correlation between the peak digestibility of total amino acids and the valine content of the diets in the starter and finisher phases (starter: $R^2=0.950$, finisher: $R^2 = 0.827$).

6) In the finisher phase (between days 22-35 of age, Ross308 genotype) the amino acid digestibility in the diet with reduced protein and without crystalline L-valine supplementation showed no significant differences for most amino acids ($P>0.05$) when compared to the values measured for the diets formulated with the Aviagen (2014) recommendations.

7) The Ross308 roosters fed the diets formulated with the Aviagen (2014) recommendations absorbed 21.3% more total amino acids than their peers fed the diet with reduced protein and without valine supplementation. The difference found in the total amino acids absorption is not reflected anymore in the weight gain of the birds, which can probably be attributed to the almost identical quantity of available amino acids.

7. PUBLICATIONS IN THE SUBJECT OF THE DISSERTATION

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