PHD THESIS BOOKLET

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EVALUATION THE NUTRITIVE VALUE AND FEEDABILITY OF EXTRACTED SUNFLOWER MEAL IN PULLETS AND LAYING HENS

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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 when revising the thesis, therefore the thesis may be submitted for the thesis defence procedure.

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Approval by the Head of School Approval of the Thesis supervisor

1. Background and objectives of the work

Poultry meat and eggs are the most abundant animal products and the most important source of protein for mankind today. As human population and food demand grows, the role of the poultry industry will become even more important, as it is able to produce a high quality and high nutritional value product in a highly efficient way, with a low environmental footprint and without religious prohibitions (Zoltán, 2023). The price of the main raw materials used in poultry feed is a key element in the profitability of poultry production. However, weather conditions and increasing global demand are causing continuous changes in raw material prices, challenging feed professionals. In practice, one of the methods used is to convert feed rations using cost-efficient alternative feedstocks such as extracted sunflower meal as a soybean alternative (Nardone et al., 2010). Extracted sunflower meal (SFM), a low-cost by-product of agro-industrial origin, is one of the promising alternative feed ingredients that can partially replace extracted soybean meal in poultry diets (Bilal et al., 2017). However, the use of SFM in poultry feeds may be limited by its chemical composition, which has two main components that limit its use: namely high fibre and low lysine content (Nolte et al., 2021; Saleh et al., 2021a). Several studies have investigated the incorporation of SFM in egg feeds at different levels. In most of these studies, SFM was able to replace 50-100% of soy protein without adversely affecting the production performance of laying hens.

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

In my PhD thesis I was trying to answer the question of the variability of the nutrients of extracted sunflower (SFM) meal, which is available in our country at relatively low prices, and how accurately the NIR instrument,

commonly used in feed qualification, can estimate the composition of SFM nutrients. We also wanted to know if there is a correlation between the nutrients in sunflower meal. As SFM is primarily a protein feed, we also planned to investigate to what extent the amino acid composition of sunflower protein can be considered constant and how its amino acid composition is related to the amino acid requirements of the shearers and hens.

By conducting animal experiments, we wanted to find out whether there is a limit to the incorporation of sunflower seed meal in laying hens and pullets that reduces amino acid digestibility. Since, to date, the ileal digestibility studies for SFM have been predominantly performed in broiler chickens, we also aimed to determine the amino acid digestibility coefficients for pullets and hens.

The fibre composition of SFM is very heterogeneous, we do not have enzyme supplements specifically developed for sunflower-based diets. Therefore, we also sought to answer whether the exogenous enzymes currently used, and their combinations affect the ileal digestibility of amino acids, the viscosity of the gut contents and, in hens, egg production and egg quality in SFM diets.

2. Materials and methods

2.1. Nutrient content of extracted sunflower meal

In my first study, I examined a total of 20 extracted sunflower (SFM) samples from different sources in Hungary, using classical laboratory methods and near-infrared spectroscopy (NIR) to investigate their content parameters. From the measurement results, the variance of the nutrients, the estimation accuracy of the NIR instrument and the interaction of the different nutrients were determined.

2.1.1. Feed analysis methods

In the course of our work, we collected a representative number of SFM samples from the domestic market. In total 20 samples were analysed, for which the NIR measurements were performed by Agrofeed Ltd. We analysed 20 samples at the plant of Agrofeeders Ltd. near Szalkszentmárton, Hungary, using a Foss NIR BS 2500 with calibration for sunflower (Evonik Ltd., AminoNIR AA Calibration, 26.06.2015, ID: 9414), followed by laboratory measurements according to the nutrient categories used for NIR estimation, using standard methods at the Laboratory of Food and Feed Analysis, Institute of Food and Feed Science, Georgikon Campus, Hungarian University of Agricultural and Life Sciences, Institute of Life Sciences and Nutrition, Georgikon Campus, Hungary. In addition to crude fibre, crude fat, crude protein, crude ash, ADF, NDF (ISO 6865:2001), total sugars (Luff Schoorl method, EG 152. 2009), total phosphorus (ISO 6491:2001), phosphorus phytin (Megazyme, K-Phyt 5/17), amino acids (Ingos Amino Acid Analyzer AAA 400; ISO 13903:2005) and gross energy (GE; IKA C6000, IKA-Werke GmbH & Co. KG Janke-Kunkel Str. 10. 79219 Staufen, Germany) were also determined

for SFM samples. The names of the tested amino acids were abbreviated as follows in the paper: cystine - CYS, aspartic acid - ASP, methionine - MET, threonine - THR, serine - SER, glutamic acid - GLU, proline - PRO, glycine - GLY, alanine - ALA, valine - VAL, isoleucine - ILE, leucine - LEU, tyrosine - TYR, phenylalanine - PHE, lysine - LYS, tyrosine - TYR, histidine - HIS, arginine - ARG.

2.1.2. Statistical analysis and calculations

We calculated the mean, minimum-maximum level, and variance of nutrients. The variance of nutrients was evaluated by the coefficient of variation (CV). The relationship between the measured and NIR-predicted nutrient categories was tested by bivariate correlation analysis. Interactions between different nutrients and between SFM crude protein and individual amino acids were evaluated using Pearson's correlation. The protein quality of SFM, extracted soybean meal, maize and wheat was evaluated by calculating the chemical score (CS) and the essential amino acid index (EAAI). For this comparison, the amino acid composition of wheat, maize and soybean meal proteins was determined from the European Feedstock Production Report database of Evonik Ltd (Evonik Nutrition and Care Ltd 2017). In the case of CS, the essential amino acid content of the feed proteins was divided by the essential amino acid content corresponding to the needs of laying hens. The needs of laying hens were expressed in the amino acid composition of the protein of the compound feed. EAAI was calculated as the geometric mean of the amino acid ratios of the CS calculation. All statistical analyses were performed using the statistical software package SPSS 23.0.

2.2. First animal experiment

In my first animal experiment, I performed digestion experiments with pullets and laying hens, using SFM at 10-20 and 30% in the diets and investigating the effect of different doses on ileal amino acid digestibility. The values obtained were compared with the tabulated values used in practice.

2.2.1. Animals and treatments

The animal experiment has been approved by the Institutional Ethics Committee (Animal Welfare Committee, Georgikon Faculty, University of Pannonia) under the licence number MÁB-11/2019.

In the first part of the experiment, a total of 32 Tetra SL pullets were housed in metabolic cages. The special feeders allowed for an accurate measurement of the daily feed intake. Water was available ad libitum through valve drinkers. At the start of the study, the boars were 10 weeks old and had an average body weight of 638 g. In addition to the control diets of maize, wheat and maize starch (K), three diets containing SFM in gradual doses were used. For this purpose, commercial SFM available on the market was used in the proportions of 10, 20 and 30% (SFM10, SFM20, SFM30). Each diet was fed in 8 replicates.

Sunflower meal was fed at the expense of wheat starch, and consequently the increase in protein and amino acid content of the experimental diets came exclusively from SFM. Titanium dioxide (TiO2) 0.5% was used as an indigestible marker. All diets were fed in mash form and the daily feed intake was adjusted according to the technological recommendations (Tetra

Ltd. 2019). The length of the light and dark periods was 10 and 14 h, respectively. Computer controlled climatic conditions were maintained throughout the experiment in accordance with the technological recommendations (Tetra Ltd. 2019).

In the second part of the experiment, a total of 32 Tetra SL laying hens were used, housed in individual cages as described in the first part of the experiment. At the start of the experiment, the hens were 50 weeks old and had an average body weight of 1941 grams. The light period in this case was 16 hours with 8 hours of darkness. All housing and technological conditions were the same as in the first experiment.

2.2.2. Feed analysis methods

The dry matter (ISO 6496), crude protein (ISO 5983-1: 2005), crude fat (ISO 6492), crude fibre (ISO 6865: 2001), total P (ISO 6491: 2001) and Ca (ISO 6869: 2001) contents of the experimental diets were determined. The starch content was measured by polarimetric method according to European Directive 152/2009. The AMEn content of ND and the diets was calculated using the Fisher and McNab equation (Fisher C. and McNab 1987). As can be seen, the increased inclusion of SFM in the formulation increased both the crude protein and crude fibre content of the diets.

2.2.3. Intestinal content sampling

During the five-day acclimatisation period, both the gilts and the laying hens adapted to their individual cages and fully consumed their daily ration. On day 7, the birds were slaughtered after stunning with carbon dioxide and faecal contents were collected. Samples were taken 1 cm from the Meckel's diverticulum, before the ileo-caecal insertion. The ileum was cut

into short pieces and the contents were carefully extracted, homogenised, and stored in Eppendorf tubes at -20 °C until further analysis.

2.2.4. Analytical methods, calculations, and statistics

SFM and feeds were analysed using official methods. The amino acid content of feed and faecal samples was determined using an automatic amino acid analyser (Ingos Amino Acid Analyzer AAA 400) after 24 h acid hydrolysis with 6 M aqueous HCl at 110 °C. To avoid loss of methionine (MET) and cystine (CYS), samples were oxidized with formic acid before hydrolysis. The tryptophan content was not determined. The apparent amino acid digestibility of the diets was calculated from the ileal digestible amino acid and TiO2 content of the diets. TiO2 content was determined by spectrophotometer (Jenway 6100) at 410 nm according to the method of Short, Wiseman and Boorman (1996).

The coefficient of digestibility (DC) of amino acids for each feed was calculated according to the following equation:

$$(\mathbf{DC_{AA\ Feed}}: (\mathbf{AA_{\ Feed}} - (\mathbf{AA_{Intestinal}}\ \mathbf{x\ TiO_{2\ Feed}}\ \mathbf{/TiO_{2\ Intestinal}}))\ \mathbf{/AA_{Feed}}$$

The ileal amino acid digestibility of sunflower diets was calculated by linear regression between daily amino acid intake and the amount of preabsorbed amino acids, based on the work of Rodehutscord et al. (2004). The daily intake of amino acids (mg/day) was calculated by multiplying the feed intake (g/day) by the amino acid content of the feed (mg/g). The amount of pre-caecally absorbed amino acids was calculated by multiplying the amino acid intake (mg/day) by the ileal digestibility of the feed (DCAA Feed). The amino acid digestibility of SFM was the slope of the linear regression equation in this case. Measured amino acid

digestibility of SFM was compared with data in tables (NRC, 1994; Redshaw et al., 2010; Blok and Dekker, 2017).

The amino acid digestibility of the diets was compared by one-way analysis of variance (ANOVA), while the measured and tabulated values were compared by paired sample t-test using the SPSS 24.0 for Windows (SPSS Inc., Chicago, IL, USA) software package. Differences were considered significant at $P<0.05$.

2.3. Second animal experiment

I carried out my 3rd experiment, also with pullets and laying hens, with the 20% mixing rate that was considered the best in the first animal experiment. In addition to the inclusion of 16 % SFM in the diets of the pullets and 20 % SFM in the diets of the laying hens, the effects of different enzyme supplements on ileal digestibility, body weight gain of pullets, egg production of laying hens and viscosity of ileal and jejunal contents were investigated.

2.3.1. Animals and treatments

The licence number of the experiment is MÁB-3/2020. The experiment was also divided into 2 parts, using 48 Tetra SL pullets and 48 laying hens. In the first half of the experiment, the pullets were housed in individual metabolic cages at 10 weeks of age. Afterwards, their diet was gradually changed from the commercial colony diet they had previously been fed to the experimental diets over a 5-day acclimatisation period. For the pullets, the experiment lasted 7 weeks, during which the animals' body weights were measured weekly. The daily light and dark periods were 16 and 8

hours respectively. The housing and experimental conditions of the animals were the same as in the second experiment.

In the second half of the experiment, laying hens were placed in individual metabolic cages at 50 weeks of age and then acclimatised as described for the pullets. The hens were kept for 4 weeks, during which the weight of the eggs was measured daily, and the amount of feed consumed every two days. The hens had an average initial weight of 2.09 kg and a final weight of 2.19 kg.

The experiment was set up with 1 control and 5 treatment groups: Control, maize and soybean based diet (K); diets containing 16 and 20% sunflower (ND); 16 and 20% sunflower + NSP breakdown enzyme supplement (NSP); 16 and 20% sunflower + protease supplement (P); 16 and 20% sunflower + NSP breakdown enzyme + protease enzyme (NSP+P); 16 and 20% sunflower with extra phytase supplement (F) for the pullets and egg diets. The pullet diets contained only 16% ND because we used diets without oil supplementation similar to practical conditions. A diet with more than 16% sunflower meal would have required energy supplementation, which would have affected the growth of the pullets compared to the control diet. The NSP-degrading enzyme supplement contained endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase (Axtra® XB 201 TPT, Danisco Animal Nutrition, Marlborough, UK). The extra phytase enzyme supplement was an enzyme produced by the bacterial species Buttiauxella (Axtra PHY 20000 TPT2, Danisco Animal Nutrition, Marlborough, UK), which increased the content of the basal diet by 300 FTU. The protease enzyme preparation (dehydrated yeast culture, dried Bacillus licheniformis fermentation solution, wheat bran; Eazypro®, JEFO

Nutrition Inc. The enzymes mixed with the diets reported the following enzyme activities: Axtra® XB 201 TPT: 2440 U endo-1,4-beta-xylanase; 304 U endo-1,3(4)-beta-glucanase; Axtra PHY 20000 TPT2: 1000 FTU; Eazypro®: 15000 U.

Birds have ad libitum access to water. Enzymes were included in the feed formulation according to the manufacturer's recommendations. It can be seen that the most significant difference in the composition of the diets was that the sunflower-based diets contained less wheat and more oil supplements. As a result, the sunflower-based diets had higher crude fat and fibre content and lower starch content than the control diets. There was no significant difference in protein and amino acid content of the diets. Among the amino acids, the control diets contained more lysine and the SFM diets more methionine. In all cases, the amino acids met the needs of the pullets and laying hens.

2.3.2. Feed analysis methods

The feed analysis methods were the same as described in the previous experiment. For viscosity measurements, frozen samples were centrifuged (12,000 G for 10 min) after draining. The viscosity of the supernatant (0.5 mL) was measured using a Brookfield DV II+ viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA) at 25 C° CP with 40 cone heads and a shear rate of 60-600s-1.

2.3.3. Egg quality testing

For egg quality, 1 egg per bird was sampled every 2 weeks at 3 time points: at the beginning of the experiment, after 2 weeks and at the end of the experiment (4 weeks in total). The samples taken at the beginning of the

experiment were taken at the beginning of the sunflower feeding period, this sampling is the control measurement where the effect of sunflower feeding is not yet visible. Egg analyses were carried out using a DET6000 egg analyser (Figure 19) and the following parameters were measured: egg weight (Wt), eggshell firmness (Str), protein height (ht), Haugh unit (HU), yolk colour (YF), yolk height (YH), yolk diameter (YD), yolk index (YI) and eggshell thickness (Thk). The yolk index is the ratio of yolk height to yolk diameter, which is also an indicator of egg freshness, and varies similarly with the Haugh unit.

2.3.4. Intestinal content sampling

In both cases, samples were taken at the end of the 7th week in pullets and at the end of the 4th week in laying hens from the entire iliac intestine, from Meckel's diverticulum to 1 cm before the cecum was aspirated. During sampling, the ilium was cut into short pieces of 5-6 cm and the intestinal contents were carefully removed, homogenised, and stored in Eppendorf tubes at -20 °C until further use. The jejunum content was taken from the proximal part of the intestine. The amino acid digestibility assay method and analyses were the same as described in the previous experiment.

2.3.4. Statistical analysis

Statistical evaluation of results for ileal amino acid digestibility values was performed by two-factor analysis of variance using Tuckey's test. Statistically significant difference was defined at the $p \leq 0.05$ level. The two factors tested were treatment and age of the animals. For the viscosity study, statistical analysis of variance was also performed to determine differences between treatments and age groups using Tukey's test ($p<0.05$). Here, the main effects were sampling location, age of animals and

treatments. For egg quality testing, we also used a two-factor analysis of variance with Tukey's test, here the two variables were treatments and time elapsed. The SPSS 24.0 software package was used for statistical calculations.

3. Results and disscussion

Samples measured under laboratory conditions contained on average 38.5% crude protein, 1.1% crude fat and 16.6% crude fibre, ranging from 34.3-46.5%; 0.61-1.78% and 6.96-23.02%, respectively. In both the measured and NIR estimated results, the largest variance (CV%) was observed for crude fat 20.3%, crude fibre 21.9%, ADF 21.3% and NDF 18.7%. Smaller variations were found for crude protein, sugar, and phosphorus. Gross energy was the parameter with the lowest variability. All CV values were higher for the measured parameters compared to the NIR estimates. Except for GE, the relationship between predicted and measured nutrient content was significant. The "r" values showed high precision for crude protein, different fibre fractions and phosphorus. For crude fat, dry matter and phytin phosphorus, lower correlation coefficients indicate lower accuracy of NIR.

The measured amino acid content of the SFM samples showed a higher variance and the degree of variance was amino acid dependent. Among the essential amino acids, MET, LYS, THR and HIS had the highest variance, while CYS, TYR, ARG, LEU, ILE and VAL had lower variance. The variance of the NIR results was lower and more balanced for all amino acids (CV% = 7.74-9.53). Despite the differences in the coefficients of variation, the accuracy of the NIR prediction was significant in all cases with high R-values.

We found several significant interactions between different nutrients. As expected, there is a negative correlation between crude protein content and the different fibre fractions. On the other hand, sugar, phosphorus and phytin phosphorus were positively correlated with the crude protein content of sunflower hulls.

Since SFM is an important source of protein in animal feed, the relationship between essential amino acids of sunflower protein and the stability of amino acid composition was also evaluated. As shown in the data in Table 19, MET and HIS showed the most significant interaction with the other essential amino acids. MET showed negative correlation with TYR, ARG, LEU, VAL and PHE and positive correlation with HIS. Variation of HIS in sunflower protein showed positive correlation with THR and MET and negative correlation with CYS, TYR, ARG, LEU, VAL, and ILE.

Changes in crude protein content of SFM did not affect the relative LYS and THR content. However, the relative MET and HIS content increased when protein was higher. A significant negative correlation was found between the relative proportion of other essential amino acids and crude protein.

Comparing the essential amino acid composition of SFM protein with that of other forages, it contains less LYS and more sulphur amino acids and ARG than soybean meal (Figure 1). The other essential amino acid content of SFM protein is close to that of maize and wheat, except for ARG, whose proportion is almost twice as high in SFM and LEU which is the dominant essential amino acid in maize protein.

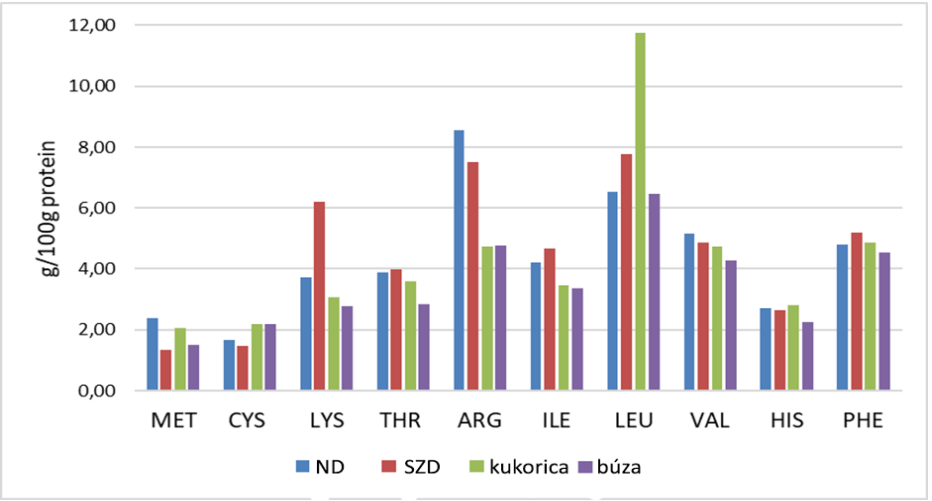

Figure 1: Amino acid composition of sunflower (ND), soybean (SZD), maize (kukorica) and wheat (búza).

The relative amino acid content of different protein sources was also compared with the amino acid requirements of laying hens (Figure 2). The closer the ratios of essential amino acids are to the requirement (100%, red line), the more balanced the protein, i.e. less deficit and surplus. The graph shows that the arginine content of both extracted meals is about 60-80% higher than the chicken requirement. The same is true for the leucine content of maize. All other amino acids are around 100% or below. The graph also shows that the lysine fraction of soybean meal and the MET fraction of sunflower meal both meet the needs of chickens.

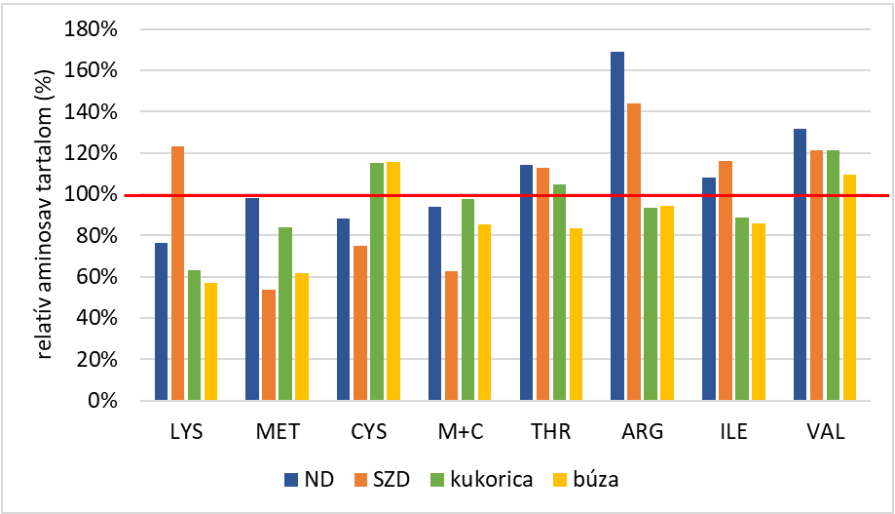

Figure 2: Proportion of amino acids in sunflower meal (ND), soybean meal (SZD), maize (kukorica) and wheat (búza) protein in comparison with the amino acid requirements of laying hens.

In the first animal experiment, the average daily feed intake of the pullets in groups K, SFM10, SFM20 and SFM30 was 53g, 59g, 58g and 58g respectively. The birds therefore consumed slightly more of the diets containing SFM, but this difference was not significant. The amino acid digestibility of the diets of the pullet ranged from 58.6% to 88.9%, with the lowest and highest values for threonine and glutamic acid. Despite the higher fibre content of SFM-containing diets, the absorption of some amino acids was significantly improved. Among essential amino acids, SFM significantly increased the digestibility of THR, VAL, LYS ARG. In the case of THR, digestibility increased in proportion to the SFM incorporation rate, with an overall increase of 12.6% at the 30% incorporation rate ($p=0.000$). In the case of VAL, this improvement in digestibility was only trend-like at the 10 and 30% incorporation rates but improved by 5.8% at the 20% incorporation rate ($p<0.030$). For LYS, a similar trend was seen,

with a statistically verifiable 6% improvement only at 30% mixing ($p<0.027$). For ARG, a significant result was also detected in two cases, at 20 and 30% mixing ($p=0.001$). LEU was the only essential amino acid whose digestibility was negatively affected. The digestibility of the three non-essential amino acids GLY (by 5.9%, $p=0.031$) and ASP (by 6.7%, $p=0.007$) increased in pullet diets only after SFM20 treatment. In the laying hen experiment, the average daily feed intake decreased with increasing SFM ratio (control: 117 g, SFM10: 101 g, SFM20: 86 g and SFM30: 77 g). The birds consumed 31% less feed in the N20 treatment and 34% less feed in the N30 treatment ($p=0.000$). The digestibility interval of amino acids ranged from 73.6% to 93.6%. In the laying hen trial, feeding SFM did not change the digestibility of amino acids. The only significant difference was a decrease in the digestibility of ILE in the 20% treatment compared to the 10% treatment (by 7.33%, $p=0.025$), but it was not different from the C treatment.

Details of the regression analyses are presented in Table 1. The linear regression between daily amino acid intake and the amount of amino acids absorbed before the appendix was significant in all cases. The table shows the slopes, constants, and squares of the correlation coefficients. In this methodology, slopes represent the digestibility of SFM amino acids. As can be seen, the slopes of the regression lines in the pullet experiment ranged from 0.70 (THR) to 0.86 (ARG, GLU). For laying hens, the lowest slope was also associated with THR (0.74), while the highest slopes were associated with MET and ARG (0.89). For all amino acids, higher slopes were obtained for laying hens than for pullets. The differences between the two groups of animals were low for TYR (1.4%), GLU (2.0%), PRO

(2.2%) and VAL (2.9) and high for CYS (9.1%) and LEU (8.8%). Two examples of linear regression are shown in Figures 3. and 4.

Table 1. Parameters of the linear regression equations describing the relationship between daily amino acid intake (x) and daily ileal amino acid absorption (y).

	Pullets			Laying hens		
	coefficient	constant	r2	coefficient	constant	r2
Cisztin	0.7371	0.1628	0.9873	0.8278	0.1086	0.9958
Aszparagin	0.7664	-5.8059	0.9943	0.8208	0.4473	0.9952
Metionin	0.8516	-0.0779	0.9951	0.8902	0.0972	0.9970
Treonin	0.7007	-9.9535	0.9895	0.7482	0.2153	0.9883
Szerin	0.7502	-3.5986	0.9888	0.8038	0.2252	0.9898
Glutamin	0.8646	13.639	0.9938	0.8846	3.6644	0.9974
Prolin	0.8293	-5.1777	0.9890	0.8516	0.8581	0.9972
Glicin	0.7441	-3.3842	0.9938	0.7948	0.3901	0.9897
Alanin	0.7664	3.5366	0.9905	0.8195	0.8044	0.9938
Valin	0.8056	-7.8719	0.9923	0.8350	0.3577	0.9932
Izoleucin	0.8095	-1.6082	0.9936	0.8469	0.2460	0.9951
Leucin	0.7758	21.214	0.9905	0.8639	0.3352	0.9938
Tirozin	0.8300	-9.2035	0.9913	0.8436	0.0835	0.9942
Fenilalanin	0.8225	1.0595	0.9934	0.8619	0.5313	0.9973
Hisztidin	0.7730	-0.1798	0.9906	0.8289	0.2909	0.9933
Lizin	0.7508	-4.0101	0.9931	0.7985	0.3294	0.9909
Arginin	0.8610	-8.3682	0.9971	0.8918	-0.0051	0.9976

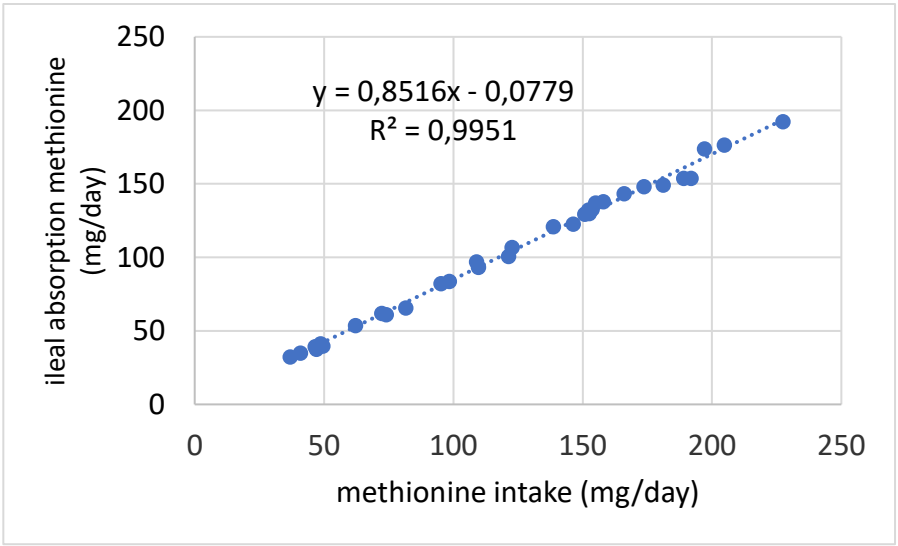

Figure 3: Relationship between methionine intake and ileal absorption in pullets.

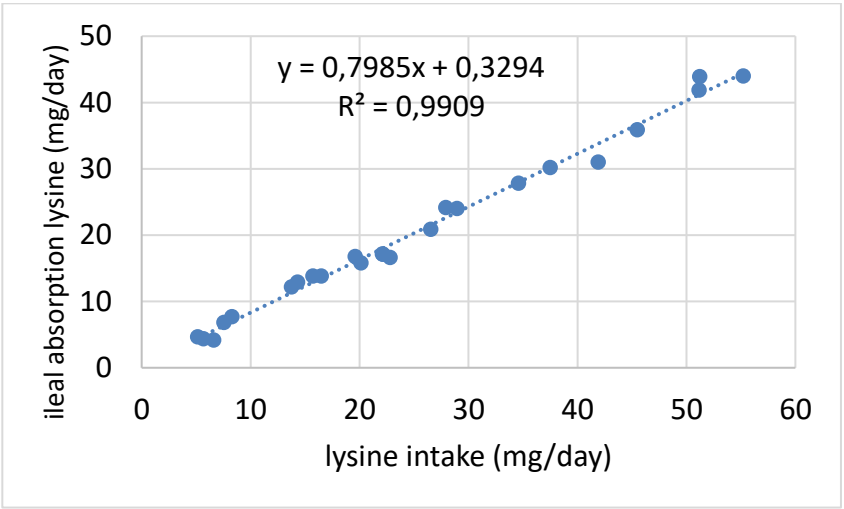

Figure 4: Relationship between lysine intake and ileal absorption in laying hens.

By comparing our results with some commonly used tabulated values (Redshaw et al. 2010, National Research Council 1994, Blok and Dekker 2017), we can see that the digestibility coefficients measured with pullets were lower than the tabulated values in all cases except for histidine (CVB)

and cystine (CVB). For laying hens, LEU, VAL, PHE and HIS showed the highest deviation (p=0.000) compared to international recommendations (Table 2). When comparing the measured and table values using one-sample t-test, the highest similarity between coefficients measured in hens and CVB values was obtained (Table 3).

Table 2. Comparison of the measured amino acid digestibility values of sunflower samples with the internationally used literature values by one-sample t-test for pullets.

	Internationally literature values			**Measured**
	Evonik (2017)	**CVB (2017)**	**NRC (1994)**	**Pullets**
lysine	0.87	0.82	0.84	0.75
p-value	*0.000*	*0.000*	*0.000*	
methionine	0.92	0.92	0.93	0.85
p-value	*0.000*	*0.000*	*0.000*	
cystine	0.80	0.73	0.78	0.74
p-value	*0.000*	0.091	*0.000*	
threonine	0.82	0.76	0.85	0.70
p-value	*0.000*	*0.000*	*0.000*	
arginine	0.93	0.91	0.93	0.86
p-value	*0.000*	*0.000*	*0.000*	
isoleucine	0.89	0.85	0.90	0.81
p-value	*0.000*	*0.000*	*0.000*	
leucine	0.88	0.84	0.91	0.78
p-value	*0.000*	*0.006*	*0.000*	
valin	0.87	0.83	0.86	0.81
p-value	*0.000*	*0.000*	*0.000*	
histidine	0.88	0.77	0.87	0.77
p-value	*0.000*	0.700	*0.000*	
phenylalanine	0.90	0.87	0.93	0.82
p-value	*0.000*	*0.000*	*0.000*	

Table 3. Comparison of the measured amino acid digestibility values of sunflower samples with the internationally used literature values by one-sample t-test for laying hens.

	Internationally literature values			**Measured**
	Evonik (2017)	**CVB (2017)**	**NRC (1994)**	**Laying hens**
lysine	0.87	0.82	0.84	0.80
p-value	*0.000*	0.644	0.054	
methionine	0.92	0.92	0.93	0.89
p-value	*0.001*	*0.001*	*0.000*	
cystine	0.80	0.73	0.78	0.83
p-value	*0.011*	*0.000*	*0.000*	
threonine	0.82	0.76	0.85	0.75
p-value	*0.001*	0.606	*0.000*	
arginine	0.93	0.91	0.93	0.89
p-value	*0.000*	*0.036*	*0.000*	
isoleucine	0.89	0.85	0.90	0.85
p-value	*0.005*	0.183	*0.000*	
leucine	0.88	0.84	0.91	0.86
p-value	*0.000*	*0.000*	*0.000*	
valin	0.87	0.83	0.86	0.84
p-value	*0.000*	*0.000*	*0.000*	
histidine	0.88	0.77	0.87	0.83
p-value	*0.000*	*0.000*	*0.000*	
phenylalanine	0.90	0.87	0.93	0.86
p-value	*0.000*	*0.000*	*0.000*	

In our second animal experiment, the pullets were fed the same amount of feed as recommended by the breeder. The 10-week-old animals started with 53 g of feed per day, increasing to 68 g by week 14. The pullets consumed all the feed rationed. There was no significant difference in the average weight of the animals at the start of the experiment (K: 682.1 g; ND: 674.1 g; NSP: 702.5 g; P: 749.5 g; NP: 717.8 g; F: 724.6 g) and different treatments did not result in a difference (p=0.906) in mean weights at the

end of the experiment (K: 1294.8 g; ND: 1286.0 g; NSP: 1324.8 g; P: 1354.2 g; NP: 1337.3 g; F: 1317.2 g). Nevertheless, it can be noted that, surprisingly, we found the lowest values for the F treatment and the highest values for the NSP treatment, with an average difference of 29.7 g between the two treatments (Figure 5). However, this difference was not significant (p=0.906).

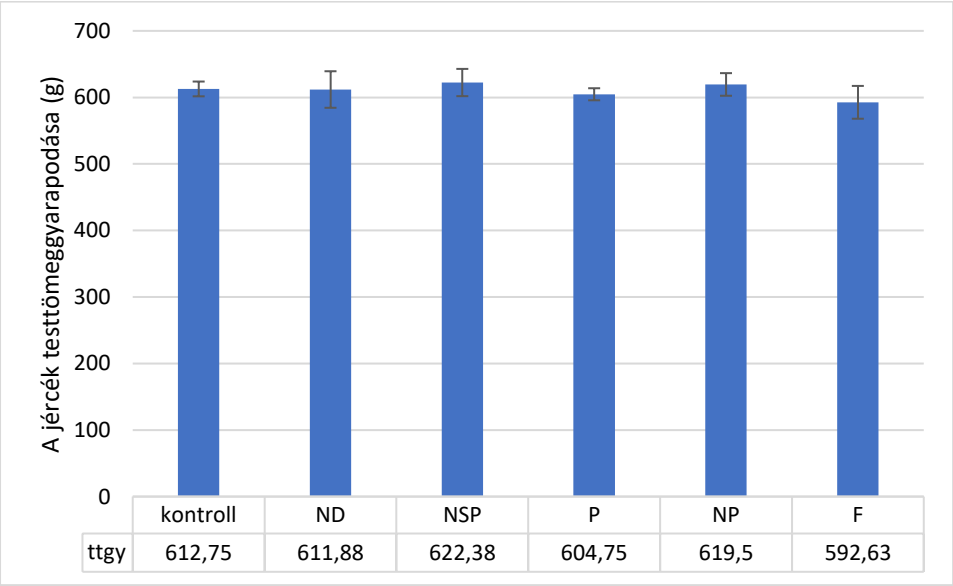

Figure 5. Effect of treatments on the average body weight gain of pullets.

Looking at the main effects, it can be seen that different enzyme treatments and age also had a strong influence on the digestibility of amino acids.

Among the enzyme treatment main averages, the best digestibility values for all amino acids were obtained for the phytase treatment. Treatment C caused the statistically weakest digestibility results for a total of 7 amino acids. This improvement compared to C treatment F was 6.3% for ASP, 11.0% for THR, 6.3% for GLY, 5.5% for VAL, 11.0% for TYR, 5.5% for LYS, and 6.3% for ARG (p=0.000). LEU was the only amino acid where

the result obtained in the C treatment was not different from the result obtained in the F treatment, with significantly poorer digestibility measured in the ND, NSP, P and NP treatments (p=0.000), representing a difference of 2.2% for ND, 2.1% for NSP, 2.1% for P and also 2.2% for NP. For MET, the P treatment did not differ from F, but we obtained worse scores for C, ND, NSP and NP (p=0.000), similar to PHE (p=0.000).

For SER and GLU, we obtained the poorest values for the NP treatment compared to the F treatment (4.6% for SER, 2.8% for GLU, p=0.000). PRO was statistically significantly better digested than the F treatment for all treatments (2.5-3.6%, p=0.000). For ALA, all treatments were significantly different from F (2-3.9%, p=0.000). For ILE, only treatment ND was not different from F, and for HIS, C and ND, all other treatments resulted in significantly worse digestibility (1.3-2.6% for ILE, p=0.000, 2-3.4% for HIS, p=0.002).

Regarding the effect of age, it can be stated that the amino acid digestion of laying hens was statistically better than that of the pullets except for two amino acids (MET, GLU).

The treatment and age interactions were significant for all amino acid digestibility. The reason being that the NSP treatment resulted lowest digestibility values in pullets, while in laying hens at 50 weeks of age the lowest coefficients were found at the control treatment.

In terms of feed intake, average egg weight, total egg weight and specific feed consumption, our results showed that feeding extracted sunflower and enzyme supplementation had no statistically significant effect on these parameters. There was a tendency to observe that hens consumed more sunflower meal-based diets and their specific feed conversion increased,

but the differences were not significant. Our results suggest that a 20% SFM incorporation is safe if the energy, protein, and amino acid levels of the diets meet the needs of the hens.

The results of the egg quality study showed that only two of the nine quantitative and qualitative parameters examined, egg weight (Wt) and yolk colour (YF), showed statistically significant differences between treatments. For egg weight, the ND treatment resulted in the largest number of eggs (p=0.034), differing by 6.5% from group C. The mean of NSP, P, NP and F treatments did not differ. For yolk colour, all treatments resulted in statistically higher values compared to treatment C (p=0.000). In contrast, sampling time resulted in a significant difference between sampling times for seven of the parameters tested (shell firmness (Str), protein height (Ht), Haugh unit (HU), yolk colour (YF), yolk diameter (YD), yolk index (YI) and shell thickness (Thk). The highest mean values for both shell strength and shell thickness were obtained at the first time point (p=0.015 and p=0.000, respectively). A similar trend was observed for protein height and Haugh unit, but for these the difference was only statistically verifiable compared to the 2nd time point measured (p=0.016 and p=0.009), not compared to the 3rd time point. For yolk colour and yolk index, the first time point resulted in the higher values and was significantly different from both time points 2 and 3 (p=0.000 and p=0.000). The only exception where the lower value resulted from the first time point was for yolk diameter, with higher values for both later measurements (p=0.000).

In the interaction between the two main effects (treatment x sampling time), it was found that in a total of two of the nine different quantitative and

qualitative parameters studied, the interaction was significant for yolk index (YI) and skin thickness (Thk).

For viscosity test data from ileum and jejunum sampling of pullets and laying hens, the independent site effect, age group effect and treatment effect tests and their interactions showed that there were no differences between sampling sites (Table 4). For the age group effect and the treatment effect, there are clear statistically significant differences. The viscosity of the small intestine content of laying hens was higher compared to pullets (p=0.000). The viscosity of the small intestine was reduced by 29.8% when the soy content of the compound feed was reduced and by 23.2% when supplemented with 20% SFM and by 23.2% when supplemented with NSP above this level (p=0.000). The results of the P, NP and F treatments did not differ from the C treatment. When examining the treatment effect, we found that the control group was significantly different from the ND and NSP treatments. Furthermore, statistically verifiably higher viscosity values were recorded for the F treatment than for the ND treatment (p=0.000). In our experiment, the lowest viscosity values were obtained with the ND treatment, which contained 20% extracted sunflower. The viscosity values of the NSP, P and NP treatments did not differ from the ND treatment, only the K and F treatments differed significantly. It can be assumed that the difference in feed composition may have at least partly accounted for this difference, as the K treatment contained 10% more wheat in laying hens, which due to the soluble arabinoxylans it contains may increase the viscosity of the gut contents without xylanase enzyme supplementation (Smits and Annison 1996, Choct and Annison 1992, Parsaie et al. The higher viscosity values of the F treatment could be due to the fact that the phytase enzyme breaks down not only phytic acids but also

proteins, starch and fibres bound to phytates. This may increase the proportion of soluble fibres released in the glandular and friable stomach under acidic pH conditions. NSP, P and NP treatments did not affect viscosity values measured after feeding diets containing sunflower. This is because sunflower has a very diverse fibre composition and contains relatively less of the soluble beta-glucan, arabinoxylans, which the exogenous enzymes in our experiments were developed to break down (Choct 2006).

Table 4. The results of the jejunal and ileal viscosity

Jejunum				Ileum			
(mPas)							
pullet		laying hen		pullet		laying hen	
K	3.800	K	5.043	K	3,991	K	5.127
ND	3.100	ND	4.228	ND	3,144	ND	3.484
NSP	3.587	NSP	3.633	NSP	3,462	NSP	3.849
P	3.805	P	3.608	P	4,250	P	3.511
NP	3.458	NP	4.308	NP	3,805	NP	4.213
F	4.070	F	4.493	F	3,934	F	5.005

Sampling site effect	
Jejunum	3.949
Ileum	3.965
Age group effect	
Pullet	3.721[b]
Laying hen	4.181[a]
Treatment effect	
K	4.492[a]
ND	3.461[c]
NSP	3.647[bc]
P	3.803[abc]
NP	3.991[abc]
F	4.307[ab]
Average standard deviation	*0.987*

p-value	
Sampling site	0.702
Age group	**0.000**
Treatment	**0.000**
Sampling site * Age group	0.598
Sampling site * Treatment	0.881
Age group * Treatment	**0.012**
Sampling site * Age group * Treatment	0.688

[a, b] *Values with different letters indicate a significant difference. Statistically significant values are in bold.*

4. Conclusions

Extracted sunflower meal is a locally readily available alternative to extracted soy meal in many countries. In our research, we found that the estimation of the nutrient content of extracted sunflower meal by NIR instruments was of reasonable accuracy, with high correlation coefficients for all nutrient categories except crude fat and gross energy, and for amino acids. However, the amino acid composition of sunflower meal protein is not constant. Among the essential amino acids, the proportions of lysine and threonine are not varied. The proportion of protein methionine and histidine increases, while the proportion of other essential amino acids decreases as the protein content of sunflower meal increases. The practical significance of this is that the amino acid content of feed materials is usually calculated from the crude protein content using regression equations.

The results of the digestion experiment show that the higher fibre content of sunflower meal is tolerated by the pullets and laying hens. Even a 30% inclusion does not negatively affect the amino acid digestibility of the feed mixtures. In young pullets, the digestibility of several amino acids was also increased by feeding sunflower, probably due to the crushing stimulating effect of the structural fibres of sunflower. This effect was not observed in laying hens. Our result highlights the importance of age and species-specific amino acid digestion coefficients for more fibrous diets. Differences were observed between the measured and published digestion coefficients of amino acids in sunflower grain. This is mainly due to differences between animal models for digestibility determination. A remarkable and significant difference in amino acid digestibility was

observed between pullets and hens. Taking this into account would also be important from a practical point of view. Phytase improved the digestibility of amino acids to the greatest extent in both the pullets and the hens when fed diets containing sunflower meal.

From the results of our second animal experiment, we can conclude that, with adequate energy and amino acid supplementation, 20% of extracted sunflower meal can be safely used in the diets of both pullets and hens. This incorporation rate does not modify the growth of the pullets nor the egg production of the hens. Feeding sunflower increased egg weight and also increased yolk colour. Supplementing sunflower diets with extra phytase, NSP-degrading enzymes or protease had no effect on production results. Contrary to our expectations, the feeding of sunflower diets did not increase but decreased the viscosity of the small intestine contents. Among the exogenous enzymes, viscosity was increased by phytase, but not affected by the other exogenous enzymes. The efficacy of the different enzyme preparations is limited when feeding sunflower.

5. New scientific results

1. Changes in the protein content of extracted sunflower meal do not affect the lysine and threonine content of the protein. However, the methionine and histidine levels increase, and the other essential amino acids decrease as the protein content of sunflower meal increases.
2. Supplementation of 10-20-30% sunflower meal in pullet diets improves the digestibility of threonine, valine, lysine and arginine, but impairs the absorption of leucine. In laying hens, the effect of sunflower meal was small, only the digestibility of isoleucine was affected by feeding sunflower meal.
3. By comparing the measured amino acid digestibility values with those can be found in the tables, we can conclude that our measured values are generally lower. The digestibility coefficients determined with pullets were significantly lower in all comparisons.
4. The extra phytase supplementation in pullets and laying hens diets containing extracted sunflower seed meal resulted the greatest improvement in amino acid digestibility.
5. Feeding the laying hens with a 20% sunflower meal containing diet increases the egg mass and yolk colour.
6. The viscosity of the small intestine content is reduced in both pullets and hens when 20% sunflower meal was fed. The viscosity values of the jejunum and ileum contents of hens are significantly higher than those of the pullets. The addition of extra phytase to sunflower diets increases the viscosity of the intestinal contents.

6. Publications related to the topic of the thesis

I. Article in a foreign language journal with impact factor:

Mezőlaki Á., Such, N., Wágner, L., Rawash, M. A., Tewelde, K. G., Pál, L., Poór, J., Dublecz, K. (2023): Evaluation the nutrient composition of extracted sunflower meal samples, determined with wet chemistry and near infrared spectroscopy. Journal of Central European Agriculture, 2023, 24(3), p.613-623 (Q4; IF: 0.7)

Such N, **Mezőlaki** A, Tewelde KG, Pál L, Horváth B, Poór J and Dublecz K (2024): Feeding sunflower meal with pullets and laying hens even at a 30% inclusion rate does not impair the ileal digestibility of most amino acids. Front. Vet. Sci. 11:1347374. doi: 10.3389/fvets.2024.1347374. (Q1, IF: 3.2)

II. Published in full in a conference publication:

Mezőlaki Á., Such N., Pál L., M. A. Rawash, Márton A., Horváth B., Strifler P., Dublecz K. (2022): Comparative evaluation of the nutrient content of extracted sunflower meal measured by NIRS and laboratory methods. Simon-Gáspár, Brigitta; Simon, Szabina (eds.) Youth for science: A volume of studies. MATE, Georgikon Campus, Keszthely, Hungary, pp. 159-163.

Such, N., **Mezőlaki, A.,** Kiss, B., Pál, L. Rawash, M. A., Tewelde, K. G., Dublecz, K. (2024): Effect of feeding extracted sunflower meal-based diets, with and without NSP degrading enzyme, on the viscosity of the jejunal and ileal intestinal content of pullets and laying hens. 22. Boku-Symposium Tierernährung, 29. Februar, Wien, proceedingbook, pp.184-187.