THESIS OF DOCTORAL (PhD) DISSERTATION

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THE EFFECT OF THE LYSINE/ENERGY RATIO OF THE FEED ON THE ILEAL DIGESTIBILITY OF AMINO ACIDS AND THE N-RETENTION UNDER THE FATTENING PERIOD OF THE PIGS WITH DIFFERENT GENOTYPE

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1. RESEARCH BACKGROUND

The current size of the total pigstock in Western European countries with developed economies has gradually decreased in recent years, but according to the available data, the reproductive efficiency of the pig sector has improved significantly in the last decade (Eurostat, 2023). In our country, the annual amount of pork purchased by households in 2019 was 33.1 kg/person, which is the highest officially registered value since 1993 (KSH, 2023). This amount represents 43.2% of the total meat consumption, therefore it can be concluded that the demand for high-quality pork products still represent a significant volume.

Due to the above, the aim of the pork industry is to put into production livestock with high lean meat production capacity, favorable feed conversion rate and high reproductive performance, to be able to produce high-quality pork, which, in addition to the important volume, meets the consumer needs that have changed. The animal product can be considered as high-quality meat if it can fully satisfy both the consumer and processor needs, and, by adapting to the needs of healthy eating, it has a relatively low fat, and high protein content.

One of the basic conditions for the production of pork with a favorable protein / fat ratio is the optimum lysine / energy ratio provided for the animals, which is considered to be common to take into consideration in nowadays practical pig feeding. However, according to Schinckel and De Lange (1996), there may be up to 30% difference in the performance of genotypes, used in pork production, suitable for industrial production, in the same farm conditions. The efficient providing of the protein and amino acid supply of pigs may be nuanced more by the research results according to which there may be differences also in the potential for protein and amino acid digestibility of populations used for intensive production.

Due to all of these, it seems necessary to investigate the effect of different lysine-energy ratios of feed on ileal digestibility and N-retention in the case of growing and fattening pigs with different genetic potential, and with different performance.

2. OBJECTIVES

In my doctoral dissertation I aimed to clarify the following questions in the first (30-60 kg) and second (60-110 kg) fattening phases in pigs with different genetic potential:

I. How the different lysine/energy ratios of the feed affect the apparent ileal digestibility of crude protein, amino acids, total amino acid content, and the protein bound amino acids.

II. Depending on the genotype and on the lysine/energy ratio of the feed, how the N-retention of experimental animals changes.

3. MATERIAL AND METHODS

3.1 ILEAL DIGESTIBILITY TRIALS

The digestibility trials were conducted in the 30-60 kg and 60-110 kg liveweight interval with a total number of ninety crossbred growing pigs with 3 different genotype (30 animals/genotype) and genetic potential (3 genotypes x 6 treatments x 5 animals/treatment x 2 fattening phases x 2 replicates; n=360). The experiment series were conducted in the experimental animal house of Kaposvár University at the Department of Animal Nutrition, in Hungary, between 2012 and 2015. The ethics approval for this study was issued by the National Scientific Ethical Committee on Animal Experimentation, Hungary, prior to the initiation of the experiment (approval numbers: 23.1/01027/005/2007.; SOI/31/446-7/2014. In the studies, we involved animals *(Table 1)* of the genotypes that are used in the industrial pork production in Hungary and whose genetic potential can be well differentiated according to their fattening performance (weight gain). In all cases, the animals were free from brucellosis, leptospirosis, Aujeszky's disease and Porcine Reproductive and Respiratory Syndrome (PRRS). The structure of the digestibility trials is shown in *Table 2.* At the start of the experiments, the live

weight of growing pigs was 40.9 ± 8.5 kg, while that of fattening pigs was 80.8 ± 9.3 kg. Prior to starting the tests, the experimental animals were cannulated with PVTC cannula (Post Valve T-Cannula) as described by Van Leeuwen et al. (1991).

Table 1: The genetic potential of the experimental animals based on the classification of Close (1994)

ILEAL DIGESTIBILITY AND N–RETENTION TRIALS		
Genetic potential	**Average daily weight gain (g/nap)**	**Genotype**
High	1000-1200	Danbred (danish largewhite x danish landrace) x danish duroc
Medium	800-1000	Hungahib 39 (largewhite x landrace) x (hampshire x pietrain)
Normal	<800	(hungarian largewhite x hungarian landrace) x duroc

Experimental diets were formulated on corn soy and barley base in the first (30-60 kg) and second phase (60-110 kg) of fattening period based on the recommendation of NRC (2012), taking into consideration the ideal protein concept. In our experiments performed with growing and fattening pigs, we studied the effects of the same DE-, crude protein- and crude fiber content of the feed, and the effect of 6 different lysine levels on ileal digestibility.

Table 2: The structure of the ileal digestibility trials

PREPARATION AND COLLECTION PERIOD							
Surgery period (5 days)	**Regeneration period (10 days)**	**Adaptation period (5 days)**	**Collecting period (5 days)**				
			Collection day	Rest day	Collection day	Rest day	Collection day

Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). Titanium dioxide (TiO_2/Tioxide® A-HR) was added on top (5 g/kg) to the diets as an indigestible marker. Pigs were fed

two times daily (8:30 am and 4:30 pm) in two equal portions at a level of 2.8 times the maintenance energy requirement (450 kJ ME/kg BW $^{0.75}$/d). Animals had free access to water throughout the experiment.

The digestibility studies consisted of a 5 days surgery period, a minimum 10 day regeneration period, 5 day adaptation and 3 day collection period. At the beginning and at the end of each period, the body weight of each pig was individually recorded, and the feed allowance for each animal was adjusted. Between the collection days (Monday, Wednesday and Friday) we provided rest days (Tuesday and Thursday) for the animals. Chymus was continuously collected for 8 hours in polyethylene bags fixed on PVTC cannula, it was measured and immediately frozen. Laboratory testing of feed components, experimental feed and collected samples was determined in accordance with requirements of the Hungarian standard and AOAC (1996).

The effect of treatments on ileal digestibility for each nutrient (dry matter, crude protein and amino acids) was analyzed by two-way analysis of variance (ANOVA). In case of a significant effect, the statistical reliability of the differences between treatments was verified by a Tukey-test. For statistical procedures, SAS statistics program was used (SAS/STAT® 9.4, 2013).

The relationship between the lysine level of the experimental feeds and the ileal digestibility of the crude protein and the amino acids was tested by nonlinear regression analysis.

3.2 N-RETENTION TRIALS

The N-retention tests were also established in the live weight range of 30-60 kg and 60-110 kg with a total of 90 barrows (3 genotypes x 6 treatments x 4 animals/treatment x 2 fattening phases x 4 animals/treatment x 2 repetitions; n=288). At the start of the experiments, the average live weight of intact growing pigs was 42.9 ± 4.9 kg, while that of fattening pigs was 75.0 ± 6.5 kg. The genotype and animal health status of the experimental animals involved in the

tests were the same as that of the animals in the digestibility studies. Before starting the tests and in the rest periods, the animals were housed in individual pens, while during the main phase of the tests, the use of individual metabolic cages allowed the separate collection of urine and faeces. At the start of the experiment, the live weight of intact growing pigs was 42.9 ± 4.9 kg, while that of fattening pigs was 75.0 ± 6.5 kg.

Experimental diets were formulated on corn soy and barley base in the first (30-60 kg) and second phase (60-110 kg) of fattening period based on the recommendation of NRC (2012), taking into consideration the ideal protein concept. In our experiments performed with growing and fattening pigs, we studied the effects of the same DE-, crude protein- and crude fiber content of the feed, and the effect of 6 different lysine levels on ileal digestibility. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). Titanium dioxide (TiO_2/Tioxide® A-HR) was added on top (5 g/kg) to the diets as an indigestible marker. Pigs were fed two times daily (8:30 am and 4:30 pm) in two equal portions at a level of 2.8 times the maintenance energy requirement (450 kJ ME/kg BW $^{0.75}$/d). Animals had free access to water throughout the experiment.

N-retention trials consisted of 8 day pre-feeding and 6 day collection phases *(Table 3).* The amount of faeces produced was measured twice a day (at 8:00 am and 3:30 pm) to the nearest gram and the whole quantity was frozen. At the end of the experiment, the collected faeces were homogenized, 20% of the total amount was dried by freeze drying, up to a constant weight and then it was prepared for laboratory testing by milling (1 mm screen diameter). Laboratory testing of feed components, experimental feed and collected samples was determined in accordance with requirements of the Hungarian standard and AOAC (1996). The effects of the treatments on N-retention, dry matter- and N-digestibility, N-intake, all N-, faecal N- and urinary N-excretion were analyzed by two-way analysis of variance (ANOVA).

Table 3: The structure of the N-retention trials

Pre-feeding phase								Collecting phase					
Su	Mo	Tu	We	Th	Fr	Sa	Su	Mo	Tu	We	Th	Fr	Sa
Individual floor pens								Metabolic cages					

In the case of a significant effect, the statistical reliability of the differences between treatments was verified by a Tukey-test. For statistical procedures SAS statistics program was used (SAS/STAT® 9.4, 2013). The relationship between the lysine level of the experimental feeds and the crude protein, and N-retention was tested by nonlinear regression analysis.

4. RESULTS

4.1 RESULTS OF DIGESTIBILITY TRIALS

Based on the data obtained during the trials, we can conclude that in growing pigs and fattening pigs with different genetic potential, there are differences in the ileal digestibility of crude protein, total lysine and total amino acid content of the feed. Ileal digestibility of crude protein, total lysine and total amino acid content reaches its maximum with the different Lys/DE ratio ($GSID_{max}$) of the feed. The most effective absorption site was determined by calculating the absorption maximum points per genotype and nutrient. By comparing the absorption maximum points calculated in case of the genotype specific evaluation method (GSID), we can state that the Lys/DE ratio of the feed influence the digestibility of the listed nutrients in a different way in each genotype, therefore the genotype-independent evaluation method (GIID) over-evaluates, and in some cases, under-evaluates the crude protein and the amino acid digestibility.

Available literature data only show changes in total digestibility in regard of the amino acid supplementation, however changes in the digestibility of protein bound amino acids have not been studied during the experiments. Therefore, in our tests, we also aimed to study the digestibility of protein bound amino acids

too. Based on the results of our experiment series, we can conclude that the higher Lys/DE ratio achieved by increasing the ratio of crystalline amino acids has significantly effects ($P < 0.05$) on the digestibility of protein bound amino acids (lysine, methionine, threnine). There is a close, negative correlation between the increase of Lys/DE ratio of feed and the absorption of protein bound amino acids, regardless of the genotype.

The decrease in digestibility of protein bound amino acids results from the different absorption phases of crystalline and "native" forms.

4.2 RESULTS OF N-RETENTION TRIALS

Based on our results it can be established that the N-retention of growing and fattening pigs with different genetic potential is the most effective with the different Lys/DE ratio of the feed, and the amount of retained nitrogen also shows significant differences depending on the genetic potential and the Lys/DE ratio of the feed, therefore the data obtained from the analysis of the genotype specific nitrogen retention (GSNR) also contribute to the development of feeding concepts based on genetics for growing and fattening pigs.

Based on our data, it is proven that nitrogen metabolism - including N-retention of animals-, in addition to the crude protein content of feed, is largely influenced by the amino acid content and the the crystalline amino acid/protein bound amino acid ratio of feed.

By comparing the results of digestibility and N-retention trials, it can be seen that irrespective of the genotype, based on the highest ileal digestibility ($GIID_{max}$) of crude protein content and all AS-contents of the feed, the Lys/DE ratio of feed can be estimated with high precision, with which the N-retention of fattening pigs is maximized. In our opinion, based on the ileal digestibility of crude protein and amino acids, as well as differences in N-retention of animals, it can be stated that the use of genetic profile-based feeding technologies can be considered as unavoidable already at the absorption level of amino acids.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS FROM THE ILEAL DIGESTIBILITY TRIALS (30-60 KG)

Ileal digestibility of crude protein, total lysine and total amino acid content of the feed reaches its maximum ($GSID_{max}$) with the different Lys/DE ratio of the feed in growing pigs with different genetic potential.

Close polynomial relationship (R^2=0,72-0,97) can be described between the genotype specific ileal digestibility (GSID) of the crude protein, the total lysine and the total amino acid content of the feed and the lysine-energy ratio of the feed in the first phase of fattening (30-60 kg) despite the different development of the efficiency of absorption per genotype.

The protein digestion of growing pigs with high genetic potential is the most effective when fed with feed in which the proportion of crystalline amino acids used is lower than protein bound amino acids. In this range, in respect of crude protein digestion, these have a significant advantage over the two other genotypes studied (hungarian largewhite x hungarian landrace x duroc and Hungahib 39).

The ileal digestibility of protein-bound amino acids (lysine, methionine, threonine), studied independently (GIID) of the genotype is statistically demonstrated to decrease ($P < 0.05$) by the increase in the lysine-energy ratio.

5.2 CONCLUSIONS FROM THE ILEAL DIGESTIBILITY TRIALS (60-110 KG)

Compared to the first phase of fattening (30-60 kg), the lysine-energy ratio of feed has a lower effect on ileal digestibility of crude protein in the second phase of fattening (60-110 kg). The relationship between the genotype specific (GSID) and the genotype-independent ileal digestibility (GIID) of crude protein, and the lysine / energy ratio of feed in the second phase of fattening is only moderately strong (R^2 = 0.56-0.68).

Ileal digestibility of crude protein, total lysine and total amino acid content reaches its maximum with the different Lys/DE ratio ($GSID_{max}$) of the feed in the pigs with different genetic potential.

The ileal digestibility of protein-bound amino acids (lysine, methionine, threonine), studied independently (GIID) of the genotype is statistically demonstrated to decrease ($P < 0.05$) by the increase in the lysine-energy ratio.

5.3 CONCLUSIONS FROM THE N-RETENTION TRIALS (30-60 KG)

With the same crude protein content, the more extended Lys/DE ratio obtained with the AS supplementation of experimental feeds does not have any effect on faecal, urinal, or all nitrogen excretion in the hungarian largewhite x hungarian landrace x duroc and Hungahib 39 experimental animals ($P > 0.05$), resulting in the same N-retention.

It is statistically verifiable that the same crude protein content of the feed, but its different Lys/DE ratio influences the N-retention of the Danbred hybrids with high genetic potential ($P < 0.05$). With the N-retention, the urinary- and total N-excretion of these animals change in a statistically verifiable way ($P < 0.05$). The N-retention of growing pigs with high genetic potential is significantly higher than the two other genotypes involved in our tests.

Irrespective of the genotype, based on the highest ileal digestibility ($GIID_{max}$) of crude protein content and all AA-contents of the feed, the Lys/DE ratio of feed can be estimated with high precision, with which the N-retention of growing pigs is maximized.

5.4 CONCLUSIONS FROM THE N-RETENTION TRIALS (60-110 KG)

With the same crude protein content, the more extended Lys/DE ratio obtained with the AA supplementation of experimental feeds does not have any effect on

faecal, urinary-, or total nitrogen-excretion in the hungarian largewhite x hungarian landrace x duroc experimental animals (P>0.05), resulting in the unchanged N-retention.

In the Hungahib 39 animals with medium genetic potential, the Lys/DE ratio of the feed results in a statistically verifiable increase in urinary N-excretion (P <0.05), due to which the N-retention decreases (P <0.05). This can be explained by the shifting between the absorption phase of the amino acids from different sources, and by the energy supply of the animals below their needs.

The N-retention of Danbred hybrids with high genetic potential is influenced by the Lys / DE ratio in a statistically verifiable way (P <0.05). With the N-retention, the urinary-, and total N-excretion of these animals change significantly (P <0.05). The N-retention of Danbred fattening pigs is significantly higher than the two other genotypes involved in our tests.

By comparing the results of digestibility and N-retention tests, it can be seen that irrespective of the genotype, based on the highest ileal digestibility ($GIID_{max}$) of crude protein content and all AA-contents of the feed, the Lys/DE ratio of feed can be estimated with high precision, with which the N-retention of fattening pigs is maximized.

5.5 RECOMMENDATIONS

In the future, it is justified to carry out additional digestibility and N-retention tests by including several breeds with decisive importance in the industrial production of pork, used in large-scale production (largewhite, landrace, duroc, pietrain), and its cross-breeds, and different breeding lines, which tests will provide data about the crude protein and amino acid digestibility and N-retention of growing and fattening pigs as a function of the Lys/DE ratio.

Based on our research findings, these studies should be directed to the comparative study of hybrids with high genetic potential that can respond to the question if there are detectable differences in the digestibility and N-retention of crude protein and amino acids in pigs with the same genetic potential.

In order to produce economically viable, environmentally friendly pork that is best suited to consumer needs, it is recommended to take into account the results of the genotype specific (GSID) digestibility and N-retention tests when developing feeding techniques and recommendations based on genetic profiling.

In the future, it would be useful to combine the digestion-physiological researches with molecular genetic studies that can be traced back to the genetic basis (genes, QTLs) to detect the differences between the crude protein and amino acid digestibility and N-retention of animals.

6. NEW SCIENTIFIC RESULTS

I. The ileal digestibility of the crude protein is higher in the Danbred hybrids with high genetic potential compared to pigs with a normal (HLW x HL x Du) and medium (LW x L x H x Pi) genetic potential in the first phase of fattening (30-60 kg). Their crude protein digestion is most effective when the ratio of crystalline amino acids is lower than the protein bound amino acids. In the second part of fattening (60-110 kg) this advantage is eliminated.

II. There is a close, negative correlation between the increase of Lys/DE ratio –with more crystalline amino acids– and the absorption of protein bound amino acids within the examined lysine levels, regardless of the genotype.

III. The N-rtention of Danbred growing and fattening pigs with high genetic potential is higher than the genotypes with normal and medium genetic potential when increasing the lysine content and the Lys/DE ratio of the feed.

IV. The ileal digestibility of the crude protein-, total lysine- and total amino acid content of the feed reaches its maximum with different Lys/DE content of the diets in growing and fattening pigs with different genetic potential. It justifies the development of the genotype specific feeding system.

V. The maximum of the genotype-independent ileal digestibility ($GIID_{max}$) of crude protein and total amino acid content and the maximum of the genotype-independent N-retention ($GINR_{max}$) are approximately by the same lysine content lizintartalomnál (9.68 g/kg vs. 9.97 g/kg és 9.16 g/kg vs. 8.76 g/kg) (Lys/DE ratio). This allows a relatively accurate estimation of the Lys/DE-ratio in the feed at which the highest N-retention in growing and fattening pigs is achieved, independently from genotype.

7. PUBLICATIONS IN THE SUBJECT OF THE DISSERTATION

Scientific publications in Hungarian:

Tenke, J., Horák, A., Tischler, A., Tolnai, L., Tossenberger, J. (2011).: A lizin ellátás hatása a fehérje és az aminosavak ileális emészthetőségére növendék sertésekben. *Acta Agraria Kaposváriensis.* **15 (2):** 47-54. (ISSN 1418-1789).

Tenke, J., Babinszky, L. (2012). A molekuláris genetika eredményeinek alkalmazása a hízósertések takarmányozásában. *Magyar Állatorvosok Lapja.* **134 (3):** 179-188.

Papers published fully in conference proceedings:

Tenke J., Tolnai, L., Tossenberger, J. (2011). A takarmányok lizin-kiegészítésének hatása a fehérje és az aminosavak ileális emészthetőségére hízó sertésekben. *Fenntarthatóság és versenyképesség? LIII. Georgikon napok. Nemzetközi Tudományos Konferencia.* Keszthely, 2011. szeptember 29-30. Az LIII. Georgikon Napok Elektronikus Kiadványa. (ISBN 978-963-9639-44-7).

Tenke J., Obrácz, V., Tossenberger, J. (2012). A takarmányok elrtérő lizin-tartalmának hatása az aminosavak ileális emészthetőségére növendék sertésekben. *A magyar mezőgazdaság-lehetőségek, források, új gondolatok. XXXIV. Óvári tudományos nap.* Mosonmagyaróvár, 2012. október 5. 557-562. (ISBN 978-963-9883-93-2).

Abstracts published in Hungarian conference publications:

Tenke, J., Horák, A., Tischler, A., Tolnai, L., Tossenberger, J. (2011). A lizin ellátás hatása a fehérjék és az aminosavak ileális emészthetőségére növendék sertésekben. *A takarmányozás aktuális kérdései. 15. Nemzetközi Takarmányozási Szimpózium.* Kaposvár, 2011. jún. 3. 61-62. (ISBN 978-963-9821-32-3).

Tenke J., Tolnai L., Tossenberger, J. (2011). A takarmányok lizin-kiegészítésének hatása a fehérje és az aminosavak ileális emészthetőségére hízó sertésekben. *Fenntarthatóság és versenyképesség? LIII. Georgikon napok. Nemzetközi Tudományos Konferencia.* Keszthely, 2011. szeptember 29-30. 125. (ISBN 978-963-9639-43-0).

8. PUBLICATIONS OUTSIDE THE SUBJECT OF THE THE DISSERTATION

Scientific publications in Hungarian:

Tossenberger, J., Halas, V., Tóthi, R., Horák, A., Tenke, J., Tischler, A. (2011). Tartalékok és lehetőségek a tojótyúkok költséghatékony takarmányozásában. *Acta Agraria Kaposváriensis.* **15 (2):** 1-13. (ISSN 1418-1789).

Szabó-Fodor, J., Bóta, B., Mihucz, G., Sulyok, M., Tenke, J., Kovács, M. Hazai sertéstakarmányok multi-mikotoxin szennyezettségének felmérése. (2018). *Magyar Állatorvosok Lapja***. 140 (3):** 349-360.

Vida, O., Egri, B., Tenke, J., Horák, A., Tóth, T. (2018). Szilárd és folyékony glicerinkiegészítés hatása a szoptató kocák teljesítménymutatóira, tejtermelésére és néhány vérparaméterére. *Állatteny. Tak.* **67 (2):** 78-91.

Vida, O., Tenke, J., Czompó, K., Tanai, A., Fábián, J. (2019). A terápiás dózisban alkalmazott cink-oxid kiegészítés és a különböző rostforrások hatása a választott malacok teljesítményére. *Agronapló.* **23 (9):** 68-69.

Materials published fully in conference proceedings:

Tossenberger, J., Halas, V., Tóthi, R., Horák, A., Tenke, J., Tischler, A. (2011). Tartalékok és lehetőségek a tojótyúkok költséghatékony takarmányozásában. *A takarmányozás aktuális kérdései. 15. Nemzetközi Takarmányozási Szimpózium.* Kaposvár, 2011. jún. 3. 3-16. (ISBN 978-963-9821-32-3).

Abstracts published in Hungarian conference publications:

Tenke, J., Seregi, J., Varga, L., Tossenberger, J. (2013). Nagy teljesítményű hibrid sertések háztáji tartásban – Egy hízlalási vizsgálat eredményei. *A jövő farmja. LV. Georgikon napok. Nemzetközi Tudományos Konferencia.* Keszthely, 2013. szeptember 26-27.

Abstracts published in international conference publications:

Tossenberger, J., Tenke, J., Donkó, T., Repa, I., Horák, A., Tischler, A., Kühn, I. (2011). The effect of phytase and phosphorus supply on different bone parameters estimated by computer-tomography in broilers. *18th European Symposium on Poultry Nutrition.* Çeşme-İzmir, Turkey, 2011. okt. 31 - nov. 4. 671-673.

Tossenberger, J., Tenke, J., Horák, A. P., Tischler, A., Feuerstein, D. (2011). Effect of xylanase and beta glucanase supplementation of the diet on the ileal and postileal digestibility of nutrients in growing pigs. *10. BOKU-Symposium Tierernährung.* Bécs, Ausztria, 2011. ápr. 28. 166-170. (ISBN 978-3-900962-94-4).