

Effect of Reduced Crude Protein Level on Fecal Nitrogen Excretion and Blood Profile in Finishing Pigs

Lukáš Bujňák^{1*}, Pavel Nad¹, Tomáš Mihok¹, Alena Hreško Šamudovská¹, František Zigo¹

¹ Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

*Corresponding author's email: lukas.bujnak@uvlf.sk

¹ Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

Email: pavel.nad@uvlf.sk

¹ Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

Email: tomas.mihok@uvlf.sk

¹ Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

Email: alena.hreskosamudovska@uvlf.sk

¹ Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

Email: frantisek.zigo@uvlf.sk

ABSTRACT— *The purpose of this experiment was to evaluate the effects of a low-protein diet on the selected variables of protein and energy metabolism in the blood serum as well as on the indicators of fermentation in the faeces (fecal crude protein, ammonia, pH, volatile fatty acids, and dry matter). Twelve finishing pigs (initial body weight: 64.6 ± 3.35 kg) were randomly divided into two groups and fed basal diets with crude protein (CP) levels of 13.6% and 15.4%, respectively. The Lysine, Methionine, Threonine, and Tryptophan levels in the 13.6% CP group were the same as those in the 15.4% CP group after the addition of four crystalline amino acids—Lysine, Methionine, Threonine, and Tryptophan to the diet. The blood and faeces samples were taken from the cranial vena cava and from the rectum at the end of the study period (44 days). The results showed that with the decrease in dietary CP level from 15.4% to 13.6% (a decrease of 1.8%), the fecal crude protein, the fecal ammonia, as well as the blood urea level decreased in the experimental group compared to the control group ($P < 0.05$ and 0.01 , respectively).*

Keywords—amino acids, crude protein, pigs, urea, ammonia

1. INTRODUCTION

In recent years, environmental pollution caused by nitrogen emissions has become an increasingly serious problem. Due to the requirement for more efficient use of proteins in the nutrition of pigs, the total concentration of dietary crude protein in feed mixtures is often reduced while ensuring their adequate biological value. The correct determination of the optimal need for amino acids is important for the full use of the genetic potential of pigs, for the maximum conversion of ingested proteins, and for the reduction of nitrogen excretion into the environment [1; 2]. An excessive amount of total protein in the feed can increase the amount of nitrogen entering the large intestine [3].

Reducing nitrogen emissions has been researched for a long time, and the technology of using low-protein diets appears to be one of the most successful. According to the theory of the balance of protein-amino acid nutrition, a low-protein feed compound is a diet that makes it possible to reduce the protein levels in the feed ration and nitrogen emissions by adding suitable types and amounts of synthetic amino acids, without negative effects on production and product quality. It has been shown that reducing the amount of dietary crude protein in feed mixtures intended for growing pigs can effectively reduce nitrogen excretion from pigs [4].

Diets with a lower content of crude protein lead to a decrease in blood urea nitrogen content [5; 6], which indicates an improvement in the efficiency of amino acid utilization [7]. It should be emphasized, however, that if the amounts of crude protein in the mixture are not professionally reduced, even though the needs of the main limiting amino acids are balanced, the lack of some other amino acids, which may become limiting, can have a negative effect on the use of protein in the feed ration. Reducing nitrogen excretion in manure can be effectively achieved by reducing protein intake in feed. Reducing the protein concentration in the feed without the addition of crystalline amino acids, however, reduces growth performance and the quality of meat or carcasses [4] and leads to the limitation of protein synthesis [8].

The goal of this work was to evaluate the effect of a reduced concentration of dietary crude protein in the total feed mixture on the levels of selected serum parameters and indicators of the fermentation process in the large intestine in a 44-day experiment with a balanced intake of limiting amino acids by analyzing selected indicators in pig faeces.

2. MATERIAL AND METHOD

The evaluation of the influence of the reduced concentration of crude protein in the feed mixture on selected indicators of protein and energy metabolism in the blood serum as well as on the level of the fermentation process in the large intestine by the analysis of faeces was carried out in an experiment (44 days) in two groups of pigs in the final fattening phase. Twelve fattening pigs were included in the experiment, which was divided into two sex- and weight-balanced groups with six animals in each group. The pigs' average live weight at the start of the experiment was 64.6 ± 3.35 kg.

The pigs were fed a complete feed mixture with a lower content of crude protein (13.6% in the experimental group compared to 15.4% in the control group). Both mixtures contained equal amounts of the main limiting amino acids: lysine, methionine, threonine, and tryptophan, balanced for the needs of meat-type pigs. Supplementing the missing proportion of amino acids in the experimental feed mixture with a lower content of crude protein was realized by using crystalline forms of these synthetic amino acids. The compositions or recipes of both feed mixtures are shown in Table 1.

The animals were fed twice a day and had unlimited access to drinking water. The content of dry matter, crude protein, crude fat, crude fibre was analyzed in the feed mixtures, and the value of metabolizable energy was calculated. The amounts of amino acids in the feed mixtures were calculated using the pig ration formulation program. The study was carried out in the animal quarters of the Department of Animal Nutrition and Husbandry at the University of Veterinary Medicine and Pharmacy in Košice in compliance with the EU regulations concerning the welfare of animals.

Table 1: Composition of complete feed mixtures for fattening pigs (meat type)

Ingredients (%)	Control diet	Experimental diet
Corn	24	25
Wheat	26.35	30
Barley	30	31.5
Soybean meal	11.5	6.5
Rapeseed meal 00	5	3.5
Premix Vitamin-Minerals	3	3
Lysine	0.15	0.36
Methionine		0.04
Threonine		0.08
Tryptophan		0.02

On the last day of the experimental period (day 44), blood was collected from all animals in both monitored groups from the anterior vena cava. An analytical examination of blood serum (total proteins, urea, albumin, glucose, and cholesterol) was performed on an automatic analyzer. On the same day, samples of faeces from animals in both groups were also taken for examination. Faeces samples were obtained from animals individually after morning feeding. Analytical examination of the faeces determined the content of dry matter and total protein. Ammonia content was determined in fresh faeces samples using a Kjeltex analyser. Quantitative determination of volatile fatty acids (VFAs) was performed using the isotachopheresis method on a double capillary analyzer (EA100, VILLA LABECO, Slovakia). After a 4-hour infusion (2 g fresh dung + 20 ml distilled water), the pH of the faeces samples was measured.

The mean standard deviation was used to report all data. The differences between means were determined according to the unpaired t-test using the Graph-Pad Prism statistical program (Graph Prism software, USA). Differences ($P < 0.05$) were considered statistically significant using conventional criteria.

3. RESULTS AND DISCUSSION

The results of the analyses of the nutritional value of the complete feed mixtures used in the experimental and control groups are presented in Table 2. Both feed mixtures (experimental and control) had the same concentration of metabolizable energy. Compared to the control, the content of dietary crude protein was 1.8% lower in the experimental feed mixture.

The feed mixture intended for the experimental group was supplemented with an extra addition of limiting amino acids: lysine, methionine, threonine, and tryptophan to match the level of their content in the control group and be balanced to meet their needs for the meat type of pigs in the final fattening phase.

Table 2: Results of analyses of complete feed mixtures for fattening pigs

Parameters	Control diet	Experimental diet
Dry matter (g/kg)	876.3	876
Crude protein (g/kg)	153.7	135.7
Crude fat (g/kg)	22.9	22.8
Crude fibre (g/kg)	37.8	36.5
Lysine (g/kg)	8.20	8.20
Threonine (g/kg)	5.56	5.40
Methionine+Cysteine (g/kg)	2.49	2.50
Tryptophan (g/kg)	1.65	1.60
Metabolizable energy (MJ/kg)	12.76	12.77

Table 3 shows the evaluation of metabolism by blood serum analysis in fattening pigs in the experimental and control groups. When evaluating the levels of total proteins, no significant differences were detected between the groups. When evaluating the level of protein nutrition by monitoring the level of urea in the blood, it is necessary to take into account the quantitative amount of total protein in the ration. The average urea concentration in serum, an important indicator of protein nutrition quality, was significantly lower in the experimental group compared to the control group ($P<0.05$). No statistically significant differences in serum concentrations of albumin, glucose, and cholesterol were observed between the groups. All values of monitored serum parameters in both groups were within the reference range for these indicators, according to authors Kraft and Durr [9]; Doubek et al. [10].

Table 3: Evaluation of metabolism by blood serum analysis in fattening pigs

Parameters	Reference values	Control group	Experimental group
Total proteins (g/l)	< 86	63.85 ± 3.12	61.85 ± 2.43
Albumin (g/l)	18 - 31	32.80 ± 1.68	31.35 ± 2.02
Urea (mmol/l)	3.3 - 8.3	4.71 ± 0.35 ^a	3.61 ± 0.35 ^b
Glucose (mmol/l)	3.9 - 6.4	5.22 ± 1.86	5.02 ± 1.32
Cholesterol (mmol/l)	2 - 3.3	2.07 ± 0.26	2.00 ± 0.16

a, b – significant differences ($P<0.05$)

Table 4: Evaluation of the fermentation process in the large intestine by the analysis of fresh faeces in fattening pigs

Parameters	Control group	Experimental group
Dry matter (%)	27.49 ± 1.68	29.47 ± 2.02
Crude protein (%)	6.72 ± 0.44 ^a	5.98 ± 0.44 ^b
Ammonia (mg/kg)	912.52 ± 147.56 ^a	682.51 ± 100.49 ^c
pH	6.32 ± 0.25	6.64 ± 0.32
tVFAs (g/kg)	12.69 ± 1.59	11.69 ± 1.57

tVFAs – total Volatile Fatty Acids; a, b – significant differences ($P<0.05$); a, c – significant differences ($P<0.01$)

In Table 4, the level of the fermentation process and excretion is evaluated by analyzing the content of dry matter, crude protein, ammonia, pH, and volatile fatty acids (VFAs) in the faeces of pigs in the experimental and control groups. The experimental group had a significantly lower content of crude protein ($P<0.05$). Also, the analysis of the amount of ammonia in the faeces samples in the experimental group confirmed lower average ammonia values in the experimental group compared to the control ($P<0.01$). The differences in the dry matter content of faeces, their pH, and the content of total VFAs

were not statistically significant between the groups. Large intestine pH and fermentation products such as ammonia and VFAs are considered indicators of intestinal health [11].

Synthetic amino acids in the diet are known to increase amino acid (nitrogen) digestibility to promote growth intensity [12]. The addition of synthetic amino acids can lead to a decrease in the nitrogen content of the manure due to better utilization of proteins [13]. It is true that most of the nitrogen in pigs is excreted in urine, and to a lesser extent, in faeces. The total amount of nitrogen excreted by these two routes changes little. Ammonia is the final product of organic nitrogen compounds from the urine and faeces of farm animals.

Pig excretory products are a significant source of environmental nitrogen pollution [14]. In feeding management, controlled reduction of protein feed to reduce total protein levels in the ration and addition of synthetic forms of amino acids to meet their needs has become an effective way to reduce nitrogen emissions [15]. This manipulation to reduce ammonia emissions appears to be a highly effective and efficient method in pig farming [16; 17].

5. CONCLUSION

By reducing the content of dietary crude protein in the feed mixture of fattening pigs (-1.8%) in the experimental group compared to the control and adding synthetic amino acids to increase the needs of the four main limiting amino acids for meat-type pigs in the category of fattening pigs in the final phase of fattening, we noted by evaluating the metabolism, the analysis of selected blood serum parameters, and the evaluation of the fermentation process in the experimental group that there was a significant decrease in the values of ammonia and total protein in faeces as well as serum urea concentrations.

6. CONFLICTS OF INTERESTS

Authors declare that there are no conflicts of interest.

7. ACKNOWLEDGEMENT

This work was supported by the Slovak project VEGA No. 1/0402/20.

8. REFERENCES

- [1] Heo, J. M., Kim, J. C., Hansen, C. F., Mullan, B. P., Hampson, D. J., Pluske, J. R. (2008). "Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning". *Archives of Animal Nutrition*, 62(5), 343-358.
- [2] He, L., Wu, L., Xu, Z., Li, T., Yao, K., Cui, Z., ... & Wu, G. (2016). "Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs". *Amino acids*, 48(1), 21-30.
- [3] Stein, H. H., Kil, D. Y. (2006). "Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 2". *Animal biotechnology*, 17(2), 217-231.
- [4] Kumar, A.; Bhar, R.; Mandal, A.B.; Mendiratta, S.K. (2012). "Effect of low protein diets and lysine supplementation on growth performance and carcass characteristics of growing pigs". *Afr. J. Biotechnol.*, 11, 12128–12134.
- [5] Nyachoti, C. M., Omogbenigun, F. O., Rademacher, M., Blank, G. (2006). "Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets". *Journal of Animal Science*, 84(1), 125-134.
- [6] Fang, L. H., Jin, Y. H., Do, S. H., Hong, J. S., Kim, B. O., Han, T. H., Kim, Y. Y. (2019). "Effects of dietary energy and crude protein levels on growth performance, blood profiles, and nutrient digestibility in weaning pigs". *Asian-Australasian journal of animal sciences*, 32(4): 556.
- [7] Kohn, R. A., Dinneen, M. M., Russek-Cohen, E. (2005). "Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats". *Journal of animal science*, 83(4), 879-889.
- [8] Wang, D., Wan, X., Peng, J., Xiong, Q., Niu, H., Li, H., ... & Jiang, S. (2017). "The effects of reduced dietary protein level on amino acid transporters and mTOR signaling pathway in pigs". *Biochem. Bioph. Res. Co.*, 485, 319–327.
- [9] Kraft, W., Dürer, M. U. (2001). 30. Reference values. In Hajko & Hajková: *Clinical Laboratory Diagnosis in Veterinary Medicine* (Slovak/Czech edition). Bratislava: VEDA. 365 pp.
- [10] Doubek, J., Šlosárková, S., Řeháková, K., Bouda, J., Scheer, P., Piperisová, I., Tomenendálová, J., Matalová, E. (2007). "Interpretation of Basic Biochemistry and Haematology Findings in Animals" (*In Czech*). Noviko a.s., Brno, 102 p.

- [11] Htoo, J. K., Araiza, B. A., Sauer, W. C., Rademacher, M., Zhang, Y., Cervantes, M., Zijlstra, R. T. (2007). “Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs”. *Journal of animal science*, 85(12), 3303-3312.
- [12] Han, I.K., Lee, J.H. (2000). “The role of synthetic amino acids in monogastric animal production”. *Asian-Australasian Journal of Animal Sciences*, 13, 543–560.
- [13] Nahm, K.H. (2002). “Efficient feed nutrient utilization to reduce pollutants in poultry and swine manure”. *Critical Rev Environ Sci Technol.*, 32 (1):1–16.
- [14] Yang, Z., He, T., Bumbie, G. Z., Hu, H., Chen, Q., Lu, C., & Tang, Z. (2020). “Effects of dietary crude protein levels on fecal crude protein, amino acids flow amount, fecal and ileal microbial amino acids composition and amino acid digestibility in growing pigs”. *Animals*, 10(11), 2092.
- [15] Zhao, L.; Guo, H.L.; Sun, H. (2020). “Effects of low-protein diet supplementation with alpha etoglutarate on growth performance, nitrogen metabolism and mTOR signalling pathway of skeletal muscle in piglets”. *J. Anim. Physiol. An. N.*, 104, 300–309.
- [16] Portejoie, S., Dourmad, J. Y., Martinez, J., Lebreton, Y. (2004). “Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs”. *Livest. Prod. Sci.*, 91, 45–55.
- [17] Hansen, M.J.; Rgaard, J.V.N.; Adamsen, A.P.S.; Poulsen, H.D. (2014). “Effect of reduced crude protein on ammonia, methane, and chemical odorants emitted from pig houses”. *Livest. Sci.*, 169, 118–124.