International Journal of Veterinary Science and Agriculture Research
Volume 6 Issue 4, November-December 2024
ISSN: 2582-4112, Available at www.ijvsar.com

Effect of reduced crude protein level on faecal nitrogen excretion and fermentation activity in the large intestine of growing pigs

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Keywords: *crude protein, fermentation, limiting amino acid, nitrogen, pigs*

Abstract: *In this study, 12 crossbred weaned pigs were used to assess the effects of a lower crude protein content in the feed mixture supplemented with crystalline amino acids on the large intestine fermentation markers and nitrogen excretion in the faeces. Each of the two sets of weaned piglets had six piglets. Crude protein content in the experimental diet was 16.7%, while the control diet was 19.5%. At the conclusion of the 28-day study period, the faeces were collected directly from the rectum. The experimental group's dietary crude protein content had reduced, as evidenced by significantly greater levels of ammonia (P <0.001) in fresh faeces and crude protein content in dry matter faeces (P <0.01) as compared to the control group. When compared to the control group, the experimental group's dietary crude protein content decreased, as evidenced by a non-significant rise in the pH of their faeces. There were no discernible statistical differences between the experimental and control groups in terms of the proportion of acetic, propionic, and butyric acids or the quantity of total volatile fatty acids.*

I. INTRODUCTION

Excretion of nutrients into the external environment poses significant challenges related to environmental issues and feed costs in pig farming. Furthermore, high levels of total nitrogen in young pig feed mixtures are associated with a higher incidence of post-weaning diarrhoea as well as excessive protein fermentation in the colon compared to diets with lower protein content. The findings of Han et al. [5] suggest that low-protein diets, when supplemented with appropriate amino acids, do not negatively affect growth performance or nutrient digestibility from weaning to the final fattening phase of pigs.

There has long been a known effort in pig farming to reduce the level of nitrogen excretion in an attempt to minimize its impact on the environment, and therefore it is assumed that reducing the total nitrogen content of mixtures reduces the negative impact of high-protein diets. The impact of high- or low-protein diets may vary depending on the farm environment, pig growth development, and genetics. Therefore, recommendations for

reducing total nitrogen content with simultaneous amino acid supplementation to improve feed efficiency take into account various environmental factors.

Undigested proteins in pig diets pass into the caecum and colon, where they are fermented by enzymes of the gut microbiota [16]. However, this process is associated with the production of toxic metabolites (sulphur compounds, indole, phenolic compounds, amines, and ammonia), which can inhibit normal microbial activity and prevent epithelial cell proliferation in the colon [18].

The use of low-protein diets is associated with several advantages, such as reduced nitrogen emissions in the faeces, lower energy costs, reduced incidence of post-weaning diarrhoea, or mitigation of odour emissions at the farm level [16].

Determining the optimal amino acid requirement is important for full utilisation of genetic potential, increased conversion of ingested protein, and reduced nitrogen excretion into the environment [6, 7].
Results of several studies have shown that reducing the nitrogen content of weaning diets reduces the prevalence of diarrhoeal diseases [7, 17]. Diets with lower nitrogen content lead to a decrease in blood urea nitrogen [13], suggesting improved amino acid utilisation efficiency [10]. However, it is true that too extensive a reduction in total nitrogen in the feed can affect growth rate due to limited synthesis of non-essential amino acids and limited availability of bioactive compounds from protein supplements, even if the requirements for limiting amino acids are met [2].

The aim of this work was to evaluate, in a 4-week experiment with a balanced intake of limiting essential amino acids, the impact of a reduced concentration of crude protein in the feed mixture of young growing pigs on the level of the fermentation process in the colon by analysing pH, ammonia, and concentrations of volatile fatty acids, as well as the content of dry matter and crude protein in faeces samples.

II. Materials and Methods

The evaluation of the impact of reduced crude protein concentration in the feed mixture of weaned pigs on the fermentation process in the large intestine and on the level of excretion was carried out in a 4-week experiment in two groups of young pigs. The experiment included 12 weaned pigs, which were divided into two groups, with 6 pigs in each group, balanced in gender and weight. Weaning of the pigs was carried out at the age of 28 days. The initial average live weight of the weaned pigs in the control group was 8.70 ± 0.53 kg. This group was fed a complete feed mixture with a higher content of crude protein of 19.5%. The experimental group of weaned pigs, with an average live weight of 8.65 ± 0.60 kg, was fed a complete feed mixture with a lower crude protein content of 16.7% and with the addition of limiting essential amino acids—lysine, methionine, and threonine—slightly above the level of these amino acids in the control group. The animals were fed three times a day and had unlimited access to drinking water. The content of dry matter, crude protein, crude fat, crude fibre, ash, and the content of metabolisable energy were analysed in the feed mixtures. The amounts of amino acids in the feed mixtures were calculated from the program for compiling feed rations for pigs. The feed mixture in the control group was balanced in terms of the content of limiting amino acids to meet the needs of supermeat-type pigs according to the Czech standard [14].

On the last day of the experimental period, faecal samples from animals in both groups were taken for examination. Faecal samples were obtained from animals individually after morning feeding. Analytical examination of the faeces determined the content of dry matter and crude protein. In fresh faeces samples, the ammonia content was determined using a Kjeltec analyser (TECATOR, Sweden). Quantitative determination of volatile fatty acids (VFA) was performed by the isotachophoresis method on a double-capillary EA100 analyser (Villa Labeco, Slovak republic). The pH of the faeces samples was measured with a pH meter with an injection-combined glass electrode with a polymer electrolyte, suitable for measuring solid samples.

The obtained results were evaluated statistically using an unpaired t-test with significance levels $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively.

III. Results and Discussion

The results of the analyses of the nutritional value of the complete feed mixtures used are presented in Table 1. When evaluating the analysed parameters of the complete feed mixtures used in the experiment, it can be said that the diets mentioned had the same content of metabolisable energy. In the experimental feed mixture, the content of crude protein was 2.8% lower than in the control. The feed mixture intended for the experimental group was supplemented with an extra addition of limiting amino acids (lysine, methionine, and threonine) above the level of these amino acids in the control group and balanced to meet their needs for supermeat-type pigs.

Table 1: Results of analyses of complete feed mixtures.

Indicators	Test	Control
Dry matter (g/kg)	887.5	888.4
Crude protein (g/kg)	167.0	195.0
Crude fat (g/kg)	20.4	21.5
Crude fibre (g/kg)	32.9	33.1
Ash (g/kg)	54.6	57.0
Lysine (g/kg)	13.8	12.8
Threonine (g/kg)	8.7	7.6
Methionine + cysteine (g/kg)	7.9	6.6
Metabolisable energy (MJ/kg)	13.15	13.2

Table 2: Evaluation of the fermentation process and the level of excretion in feces.

Parameter	Test	Control
	(average ± SD)	(average ± SD)
Ammonia (mg/kg)	383.0 ± 15.1***	438.0 ± 13.2
pH	6.74 ± 0.31	6.46 ± 0.32
Acetic acid (g/kg)	4.98 ± 0.59	5.47 ± 0.56
Propionic acid (g/kg)	3.78 ± 0.47	3.80 ± 0.54
Butyric acid (g/kg)	1.67 ± 0.20	1.80 ± 0.12
The sum of the VFA (g/kg)	10.30 ± 1.10	11.13 ± 1.22
C2 (%)	48.3 ± 2.25	49.1 ± 2.23
C3 (%)	36.7 ± 2.12	34.1 ± 2.17
C4 (%)	16.2 ± 1.23	16.2 ± 0.89
Dry matter (%)	26.30 ± 1.60	25.20 ± 1.34
Crude protein (as DM)	19.90 ± 1.86**	23.80 ± 1.29

Abbreviations: VFA – total volatile fatty acids; StD – the standard deviation; C2 – acetic acid; C3 – propionic acid; C4 – butyric acid; DM – dry matter, ***P* <0,01; ****P* <0,001

Table 2 evaluates the level of fermentation process and excretion by analysing the content of dry matter, crude protein, ammonia, pH, and volatile fatty acids (VFA) in the faeces of animals from the experimental and control groups. In the experimental group, a significantly lower content of crude protein in the dry matter of faeces was analysed (P <0.01). Analysis of ammonia levels in fresh faeces samples in the experimental group confirmed lower average ammonia values (P <0.001). Differences in the content of faeces dry matter, faeces pH, concentrations of acetic, propionic, butyric acids, and total VFA were not statistically significant between groups. pH in the colon and fermentation products, such as ammonia and VFA, are indicators of intestinal health

[8]. The microflora in the large intestine of weanlings is unstable in the immediate post-weaning period and takes 2 to 3 weeks to develop its fermentative capacity [9].

High-protein starter diets may increase microbial colonisation and fermentation due to the higher content of undigested nutrients that pass through the stomach into the lower gastrointestinal tract [1]. These undigested nutrients may provide substrates for microbial growth in the large intestine. This results in an increase in the end products of microbial fermentation, such as volatile fatty acids (VFA) and ammonia. The majority of the VFA in faeces consists of short chain VFA, such as acetic, propionic, and butyric acids, which constitute 91% of the total VFA content [11]. The amount of VFA is correlated with the intensity of fermentation in the digestive tract, which depends on the availability of nutrients and/or representation of microorganisms in the intestine.

In line with our results, Mun et al. [11]. also reported no significant changes in the concentrations of acetic, propionic, and butyric acids in pig faeces in experiments using low levels of crude protein in diets supplemented with essential amino acids to replace the composition of volatile fatty acids in growing pigs.

Reducing the levels of total protein is effective in reducing the negative impact of pollution in excrements with lower excretion of nitrogen in faeces and urine. The concentration of nitrogen in manure is relatively high, and its excess is converted into substances such as ammonia, which are harmful to the environment [15].

Nutritional advances in determining the amino acid requirements of pigs, coupled with the increased availability of synthetic amino acids, allow for the reduction of total protein levels in diets through the application of the ideal protein concept, which aims to supply pigs with amino acids in amounts that meet their requirements without excess or deficiency, provide higher protein storage efficiency, and lower nitrogen excretion [3, 15]. Synthetic forms of amino acids in diets increase the digestibility of amino acids to support growth rate [4], and their addition may lead to a reduction in the nitrogen content of manure, suggesting better nitrogen utilisation [12].

IV. Conclusion

The reduction of total crude protein content in the feed mixture of young, growing pigs in the experimental group (- 2.8% compared to the control) and the addition of synthetic forms of amino acids to increase the needs of limiting amino acids for meat-type pigs resulted in a significant decrease in ammonia and crude protein values in faecal samples.

V. Acknowledgements

This work was supported by the Slovak grant VEGA no. 1/0698/24.

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