Original Paper

Effects of low protein diets with amino acids supplementation on biochemical and faeces parameters in weaned piglets

Lukáš Bujňák*, Ľubor Bindas, Iveta Maskaľová, Tomáš Mihok, Petra Timkovičová Lacková, Pavel Naď
University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic

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The goal of this study was to determine the effects of a low-protein diet supplemented with crystalline amino acids on the biochemical parameters in the blood serum, and the indicators of fermentation in the faeces in 12 crossbred piglets. The weaned piglets (at 28 days of age) were divided into two groups with 6 piglets each. The control diet contained 195 g/kg crude protein and the experimental diet contained 167 g/kg. The experimental diet was supplemented with lysine, methionine and threonine to achieve a more ideal amino acid pattern. The blood collections from the *sinus ophthalmicus* for the determination of the biochemical parameters were performed 2 times at 2 weekly intervals in both groups. The faeces were taken from the rectum at the end of the study period. The decrease in the dietary crude protein content of the experimental group was manifested by a significant decrease of the blood urea level (3.77 mmol/l average concentration) compared to the control group (4.97 mmol/l average concentration) (P <0.001). The serum concentrations of other components showed no significant statistical changes between the control and experimental groups. The results of the fermentation process analysis indicated that the acetate and the butyrate concentration decreased in the experimental group compared to the control group (P <0.05; 0.01, respectively). The decrease crude protein intake in the experimental group revealed significant lover levels of ammonia (P <0.001) and crude protein (P <0.01) compared to the control group.

Keywords: pigs, amino acids, proteins, metabolism; fermentation

1 Introduction

Dietary protein is the fundamental source of amino acids for pigs. The high inclusion of dietary protein and the imbalance of amino acid (AA) composition in animal husbandry result in inefficient utilization of protein resources and increased nitrogen excretion. Therefore, an efficient approach to alleviate the nitrogen excretion and increase the utilization of protein resources is to formulate the AA-balance protein-restricted diet with crystalline AA supplementation (Kim, Chen and Parnsen, 2019). Because of the increased availability of crystalline AA (lysine, methionine, and threonine, including the 'new' amino acids isoleucine and valine), and the continual need to improve the utilization of nutrients to reduce the impact of livestock production on the environment, there is always a need to more fully understand amino acid nutrition of non-ruminants (Kerr, 2006). Lysine, the first-limiting AA in typical swine diets, plays very important roles in exerting many metabolic and physiological functions in pigs (Liao, Wang, and Regmi, 2015). Threonine is considered an essential amino acid and is commonly the second or third limiting AA in pig diets based on corn and soybean meal; however, it may be the first limiting AA when diets are supplemented with synthetic lysine (Saldana et al., 1994).Threonine is critical for maintenance because it is used for the synthesis of muscle protein, mucin in the gastrointestinal system, and immunoglobulins (Nichols and Bertolo, 2008). A reduction of dietary crude protein (CP) could limit the growth performance of growing pigs, but a low-protein diet, supplemented with deficient amino acids, could reduce the excretion of nitrogen into the environment without affecting weight gain (Ball et al., 2013; He et al., 2016; Wang et al., 2018). It was also concluded that the supplementation of limited amounts of synthetic amino acids to diets for swine could spare 2 to 3 percentage units of dietary protein and substantially

***Corresponding Author:** Lukáš Bujňák, Department of Nutrition, Dietetics and Animal Breeding, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovak Republic. e-mail: lukas.bujnak@uvlf.sk

reduce the nutrient excretion, especially of nitrogen (Han and Lee, 2000). More studies indicated that the reduction of dietary CP and the supplementation of limiting AA could effectively decrease the nitrogen emission (Heo et al., 2008; Toledo et al., 2014). This fact motivated us to determine the effects of reducing the dietary CP content from 19.5 to 16.7% on serum biochemical parameters and indicators of fermentation processes in category of weaned piglets.

2 Material and methods

2.1 Animals and diets

Twelve crossbred piglets (Slovakian White × Landrace) were divided in two groups (6 animals in the control group and 6 animals in the experimental group; 50% male: 50% female in both of groups); with an initial average body weight (BW) of 8.70 ±0.53 in the control and 8.65 ±0.60 kg in the experimental group with weaning at 28 days of age in both groups. The experimental period lasted 4 weeks.

The same basic ingredients for the control and experimental groups were used in the study. The diets were formulated based on corn, wheat, barley, soybean meal, vitamin + mineral premix, and salt. The animals were divided into two groups according to the two different CP levels of diet (19.5% and 16.7%) with different soybean meal concentration in diets (25 vs. 18.5%). The addition of limiting amino acids (AA) – lysine Lys, methionine Met, and threonine Thr was used in the experimental diet according to the National Research Council amino acids recommendations (NRC, 2012) for limiting amino acids in category of growing pigs with body weight up to 25 kg. The concentrations of dietary metabolizable energy were calculated according to Šimeček, Zeman and Heger (1994). Feed and water were allowed on an *ad libitum* basis. The feed composition of the diets used in the study and their nutrient content are shown in Table 1.

Table 1 Composition and chemical composition (g/kg; as fed basis) of diets containing different levels of crude protein for piglets

Ingredients (%)	Control diet	Experimental diet
Corn	28.5	29.0
Wheat	33.4	38.4
Barley	10.0	10.0
Soybean meal	25.0	18.5
Premix Vitamin Mineral	3.0	3.0
Salt	0.1	0.1
L-Lysine HCl 78%	–	0.49
DL-Methionine	–	0.25
L-Threonine 98%	–	0.26
Parameters (g/kg)		
Dry mater	888.4	887.5
Crude protein	195.0	167.0
Etheric extract	21.5	20.4
Crude fiber	33.1	32.9
Neutral detergent fiber	198.7	162.8
Acid detergent fiber	54.1	54.6
Ash	57	54.6
Nitrogen free extract	581.8	612.6
Lysine	12.8	14
Threonine	7.6	8.7
Methionine+Cysteine	6.6	7.9
Metabolizable energy	13.2	13.1

The investigation was carried out in the animal quarters of the Institute of Animal Nutrition and Dietetics at the University of Veterinary Medicine in Košice in compliance with the EU regulations concerning the welfare of experimental animals

2.2 Analysis

The diets were analysed for their dry mater, crude protein (CP), crude fiber, acid detergent fiber, neutral detergent fiber, ether extract and ash by the AOAC (2001). The nitrogen free extract was mathematically calculated from previous parameters. The amino acids content in both diets were calculated according to the program for formulation of diets for pigs from AA composition of feeds and the addition of crystalline limiting amino acids. The blood collection from the *sinus ophthalmicus* for the determination of the biochemical parameters was performed 2 times at 2 weekly intervals in the control and experimental group, 4–5 hours after feeding. The biochemical parameters of the blood serum (total proteins, albumin, urea, glucose, triglycerides, cholesterol, AST aspartate aminotransferase, and AP alkaline phosphatase) were determined using a fully automatic random access benchtop analyser Ellipse (Italy).

The faeces were taken directly from the rectum at the end of the investigation. The quantitative determination of the short chain fatty acids (SCFAs) was done by the method of isotachophoresis employing a two-capillary analyser EA100 (Villa Labeco, Slovakia). The content CP and ammonia in the faeces was determined according to the AOAC (2001).

2.3 Statistical methods

All data were reported as the mean ± S.D. (standard deviation). The differences between means were determined according to the unpaired *t*-test using GraphPad Prism statistical program (Graph Prism software, USA). By conventional criteria, differences ($P < 0.05$; $P < 0.01$; $P < 0.001$) were considered to be statistically significant.

3 Results and discussion

The first goal of our study was performed to investigate the effects on the biochemical parameters in the blood serum following the feeding of a low crude protein diet to piglets. The metabolic variables in the blood serum are shown in Table 2.

No significant differences were seen between the control and experimental groups in the serum total protein and albumin. The average concentrations of total protein from two collections of the study period were slightly higher in the experimental group compared to the control group. Opposite tendency was observed in the albumin concentration. The average total protein concentrations determined in both groups were lower than the reference values reported by Doubek et al. (2010) (65–90 g/l).These differences could be due to the very young category of the animals used in our investigations. The reference concentration of albumin in serum ranges between 19 and 39 g/l according to Doubek et al. (2010). The average albumin concentrations determined in both groups were consistent with those reference values. The blood urea level as an important indicator of protein nutrition showed marked changes. Throughout the study, the serum urea concentration was significantly lower (P <0.001) in pigs fed the experimental diet, with the lower CP content supplemented with essential limiting amino acids (Lys, Met, Thr), compared to the control diet which contained higher CP. Significantly higher levels of blood urea in our work were recorded in the control group compared to the experimental group at both intervals (week 2. and 4.). The mean values in the control group corresponded to the reference values of Doubek et al. (2010) (3.6–10.7 mmol/l). In the experimental group, the mean values of the urea parameter in the monitored intervals were just above the lower reference range. No significant differences between groups in other serum parameters were found. The concentrations of glucose, cholesterol, triglycerides, AST, and AP in the blood serum in weaning pigs were within the physiological values for pigs (Kraft and Dürr, 2001; Doubek et al., 2010).

Protein synthesis requires a complete set of AAs presented at the synthesis site simultaneously, and when the first-limiting AA is used up, protein synthesis stops and the remaining free or unbound AAs will be catabolized via deamination (NRC, 2012; Liao et al., 2015). The increasing availability of synthetic amino acids allows for the reduction of the crude protein level in piglet diets in association with adequate AA supplementation, which maintains sufficient essential AA supply (Figueroa et al., 2002). Lysine is the first-limiting amino acid in typical swine diets and plays very important roles in promoting growth performance of pigs. Limiting dietary lysine supply to late-stage finishing pigs can increase the blood plasma concentrations of urea nitrogen and total cholesterol (Regmi et al., 2018).

In our study the reduction of CP (19.5% vs.16.7%) only slightly influenced the concentrations of the serum parameters in comparison with the control group, except for the blood urea level. The urea excreted in urine is the main nitrogenous end-product from amino acids catabolism in pigs and plasma or serum urea concentrations may be indicative of excreted nitrogen in urine (Roth and Raczek, 2003). A lower blood urea nitrogen indicated higher availability of dietary nitrogen and a better use for amino acids with the CP reduction (Toledo et al., 2014). Fang et al. (2019) observed significant decrease of the blood urea nitrogen concentration as CP dietary level decreased in the weaning period.

The supplementary part of our study was to investigate the effects of feeding a low CP diet to piglets on short chain fatty acids profile and nitrogen excretion in faeces are shown in Table 3.

Table 2 Effects of different dietary CP content on biochemical parameters of piglets

Parameters	Control diet (19.5% CP)			Experimental diet (16.7% CP)		
Week	2.	4.	2.–4.	2.	4.	2.–4.
Total protein (g/l)	51.04±2.91	53.53±2.06	52.29±2.48	52.70±2.78	53.95±2.44	53.33±2.61
Urea (mmol/l)	4.48±0.31[a]	5.45±0.41[a]	4.97±0.36 [a]	3.44±0.26[b]	4.10±0.30[b]	3.77±0.28[b]
Albumin (g/l)	34.90±1.86	33.35±1.52	34.13±1.69	33.85±2.76	31.50±2.38	32.68±2.57
Glucose (mmol/l)	5.79±0.29	5.54±0.48	5.66±0.38	5.66±0.20	5.29±0.30	5.47±0.25
Triglycerides (mmol/l)	0.37±0.05	0.43±0.04	0.40±0.04	0.33±0.03	0.43±0.22	0.38±0.12
Cholesterol (mmol/l)	2.14±0.13	1.93±0.13	2.03±0.13	2.01±0.11	2.02±0.15	2.01±0.13
AST (μkat/l)	0.38±0.03	0.36±0.01	0.37±0.02	0.35±0.03	0.34±0.03	0.34±0.03
AP (μkat/l)	7.66±0.61	7.83±0.49	7.75±0.55	7.91±0.28	7.77±0.23	7.84±0.26

[a, b] – significant differences (P <0.001); AST – aspartate aminotransferase; AP – alkaline phosphatase

Table 3 Effects of different dietary CP content on faeces indicators of piglets (g/kg dry matter)

Parameters	Control group (19.5% CP)	Experimental group (16.7% CP)
Crude protein	244.50 ±10.80a	203.00 ±11.60c
NH3	1,464.00 ±52.30a	1,176.00 ±55.50b
Acetate	21.70 ±2.21a	18.17 ±2.27d
Propionate	15.12 ±2.24	14.53 ±1.83
Butyrate	7.36 ±0.49a	5.83 ±0.77c
The total SCFAs	44.18 ±4.96	38.53 ±4.89

a, b – significant differences (*P* <0.001); a, c – significant differences (*P* <0.01); a, d – significant differences (*P* <0.05); NH_3 – ammonia; SCFAs – short chain fatty acids

The evaluation of the fermentation processes through the determination of the SCFAs in the faeces showed decreasing tendency in individual acids (acetic, propionic, butyric) as well as in the total short chain fatty acids concentration in the group that received the lower level of dietary CP (experimental group). Short-chain fatty acids (SCFAs), also referred to as volatile fatty acids (VFAs), the end products of fermentation by the anaerobic intestinal microbiota, have been shown to exert multiple beneficial effects on mammalian energy metabolism. In our work, we recorded the decrease of short chain fatty acids contents (significantly for acetic acid, *P* <0.05 and butyric acid, *P* <0.01) in the experimental group than in the control group. Decreased concentration of propionic acid and the total SCFAs in the faeces of the experimental group was also observed in our study, but was not significantly affected. The ammonia concentration and the CP content in the faeces revealed an increasing tendency with the higher dietary crude protein concentration. Concentration of crude protein in dry matter of faeces of piglets in the experimental group was significantly lower than in the control group (*P* <0.01). According to our results the reduction of crude protein in faeces may lead to decreased production of volatile ammonia through microbial fermentation in faeces. The ammonia level in dry matter of faeces of piglets in the experimental group was significantly lower compared to the control group (*P* <0.001). Our results support the results of Wang et al. (2018) who reported that every 1% reduction of dietary CP can decrease ammonia emission from faeces and urine by 8% to 10%. The high dietary CP concentration, as is common in diets for early-weaned pigs, may increase microbial fermentation of undigested protein. In agreement with our findings also Htoo et al. (2007) detected that the reduction in CP content from 24 to 20% in weaned pigs leads to decreased faecal ammonia nitrogen (*P* <0.05), acetic acid and volatile fatty acids (VFAs) concentrations. In the study by Heo et al. (2008) feeding low-protein treatments had no effect on the total VFAs level for 14 days after weaning.

Diets with high crude protein (CP) content are commonly used for early-weaned pigs. With the development of industrial synthetic AA technology, supplementary feed grade AA, such as L-valine and L-isoleucine have become available for use in livestock diets, resulting in the potential for further reduction in dietary CP (Wang et al., 2018). According to the results of the Jiao et al. (2016), crystalline amino acids supplementation allows the reduction of dietary CP levels by 3 to 4 percentage units with no effects on carcass traits for finishing gilts. The results of Peng et al. (2016) indicate that reducing dietary CP level from 20% to 15.30%, supplemented with indispensable AA, had no significant effect on growth performance and had a limited effect on immunological parameters. However, a further reduction of dietary CP level up to 13.9% would lead to poor growth performance and organ development, associated with the modifications of intestinal morphology and immune function. Indeed, feeding weaned pigs a lower level of crude protein caused lower ammonia concentrations in the small intestine (Bikker et al., 2006) and decreased plasma urea nitrogen, ammonia nitrogen and volatile fatty acids in the ileal digesta (Nyachoti et al., 2006).

4 Conclusions

Our study demonstrated that feeding lower CP content in the diet with the addition of limiting amino acids (lysine, methionine, and threonine) for recommendation of ideal amino acids pattern for piglets after weaning, significantly reduces the blood urea concentration (average concentrations from two weekly collection 3.77 vs. 4.97 mmol/l). The statistically significant differences among the groups were found in the acetate and butyric acid concentration in the faeces. Also, lower concentrations of ammonia and CP in the faeces of the experimental group were observed (-288 mg/kg DM and -41.5 g/kg DM, respectively) compared to the control group. The use of crystalline limiting amino acids improved the use of nitrogen from the diet in metabolism, with lower nitrogen excretion into the environment.

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