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The effects of a low protein intake on the growth and blood parameters of weaning pigs

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The effects of dietary protein content on health and productivity were studied in 41 weaned Danube white breed pigs growing from 9 to 25 kg live weight. The duration of the experiment was 41 days. The compound feeds for the two groups had the same component composition and were calculated to match the needs of the animals, but differed in the amount of crude protein. Animals were offered two diets providing 190.2 and 160.2 g crude protein per kg and 9.3 g lysine per kg, respectively. When reducing dietary protein, it is important to pay attention to lysine, as it is key to growth and general animal health. The equalisation of the lysine content in the compound feeds was done by synthetic lysine. Pigs were fed ad libitum equal rations. In conclusion: Reduced protein levels did not significantly affect the growth of weaned pigs (386 g/d and 380 g/d) when the compound feeds were equalised for lysine content. Pigs fed low protein levels had better utilisation of crude protein (p<0.001) and poor utilisation of crude fat (p=0.045). Reduced protein levels have a statistically significant positive effect on haematological and biochemical blood parameters associated with health status in weaned pigs. The results can be used to practically solve some health problems when weaning pigs.

KEY WORDS: blood biochemical parameter / blood haematological parameter / feed utilisation / protein level / weaned pig

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The digestive system of small piglets is morphologically and functionally not sufficiently developed to digest solid food. With age, the enzyme activity of the digestive glands changes significantly [Kanev *et al*. 1989]. The weaning period of pigs is often associated with intestinal diseases such as inflammation of the mucosa, intestinal dysfunction, and diarrhoea. Decades ago, antibiotics were used as a preventive measure against post-weaning digestive problems in pigs.

Because of the risk of resistance to antibiotics and the potential damage to human health, an EC ban for their use in animal husbandry was introduced in 2006 [Council Directive 70/524/EU-ECR, 1998]. Pig nutrition science is therefore facing an open problem in the search for alternatives to deal with digestive problems in post-weaning pigs.

Pluske *et al.* [2002] noted that both ration levels and protein sources were major causes of post-weaning diarrhoea in pigs. Applying lower levels of protein (<18%) reduces digestive problems in pigs [Weellock *et al*. 2008], but a lower level may impair performance [Nyachoti *et al*. 2006, Weellock *et al*. 2008, Yue and Qiao 2008].

Lu *et al*. [2018] tested probiotics as an alternative to post-weaning antibiotics to reduce stress and ensure piglet's gut health. Post-weaning diarrhoea (PWD) according to Nadeau *et al*. [2017] and Jorgensen *et al*. [2016] is mainly caused by conditioned pathogens and enterotoxins. The authors point out that improving the gut microbiota is a potential method to control PWD.

One of the methods to deal with nutritional problems is by limiting the ration level immediately after weaning or by applying lower levels of protein and amino acids as an alternative technology for post-weaning feeding [Nyachoti 2017]. According to some authors, limited feeding during the first days after weaning can be a preventive measure against digestive disorders and help the enzyme system of the digestive tract to better adapt to the new feed. According to Nyachoti [2017] and Wang *et al.* [2018] the undegraded protein undergoes bacterial fermentation in the colon, which produces metabolites with toxic effects and favours the proliferation of pathogenic bacteria. This can damage gut health. On the other hand, Wang *et al*. [2018] indicated that pig rations with high protein content resulted in excess amino acids and excretion of excess nitrogen in faeces and urine. The crude protein (CP) reduction level should be up to 4% units combined with added amino acids for a compensatory effect to occur at a later period [Nyachoti 2017].

Understanding the mechanisms by which low protein diets reduce growth performance may lead to the development of dietary strategies and feed products that are not only environmentally friendly but also have no negative impact on pig growth.

When reducing dietary protein, it is important to pay attention to lysine, as it is key to growth and general animal health.

Finding the optimal protein levels in compound feeds and the ratio size immediately after weaning are the subject of research. Studies in this direction in our country are too episodic, which provokes our interest in this direction.

The experiment aimed to investigate the effect of two protein levels in weaned pig feeding schemes ensuring good health and performance.

Material and methods

Experiment design

The scientific and economic experiment was held at the Agricultural Institute - Shumen with 2 groups of 20 and 21 animals in each (11 females and 10-9 males per group) or a total of 41 weaned pigs of the Danube white breed. One pig was excluded from the beginning of the experiment due to severe illness. The experiment was conducted according to the methodology described by Oslage and Angelova-Gocheva [1994], to exclude randomness as much as possible, resulting from individual differences of the experimental animals. Pigs were matched between groups for live weight, number, sex, and age.

The experiment started at 9,110-9,063 kg live weight (SD=1,13) and ended with 41 pigs, 21 and 20 per group reaching 24,952-24,650 kg live weight (LW) for the 1st and 2nd groups respectively. The duration of the experiment was 41 days.

The animals were bred, fed, and watered in groups, being distributed in 6 raised boxes of 7 animals per box.

Pigs were fed ad libitum the same rations. Each day, two assistants recorded the daily ration and the health status of the pigs. Forage left over from the previous day was also weighed and recorded. These data served to accurately calculate the feed consumed.

The compound feeds for both groups had the same component composition and were calculated to correspond to the needs of the animals, but differed in the amount of CP (Tab. 1). The compound feeds for the pigs of the 1st group (HPD – high protein diet) contained 19.02% protein, and those for the 2nd (LPD – low protein diet) – 16.02%. The amount of crude protein in the compound feeds was regulated by the amount of soybean meal, and the lysine content by synthetic lysine. The remaining amino acids were of a variable amount, which varied according to the amount of soybean meal in the respective compound feed (Tab. 2).

To establish the influence of the protein level on the productivity of the growing pigs, the following indicators were controlled and established: live weight - at the beginning and at the end of the experiment; quantification of feed consumption – daily; health status – daily.

Table 1. Scheme of the experiment

Indicators (g/kg)	Group	
	1	2
Crude protein	190.2	160.2
Lysine	9.3	9.3

Table 2. Component composition and nutrient content of compound feed

Indicators (%)	Groups	
	1	2
Corn	36.00	45.00
Wheat	32.07	31.00
Soy meal	25.00	16.77
Wheat bran	5.00	5.00
Lysine (98-%)	0.00	0.22
Vitamin-mineral premix	0.20	0.20
Ca	0.98	0.98
Dicalcium phosphate	0.55	0.63
NaCl	0.20	0.20
All	100.00	100.00
Energy and nutrient content in 1 kg of compound feed:		
Metabolizable energy (ME), MJ	12.66	12.61
Crude protein (g)	190.2	160.2
Lysine (g)	9.3	9.3
Methionine+cystine (g)	6.8	5.8
Threonine (g)	7.2	5.8
Tryptophan (g)	2.5	2.0
Crude fats (g)	20.9	21.1
Crude fibres (g)	34.8	32.1
Ca (g)	7.0	7.0
P (g)	6.0	6.0

The Scientific Council of the Scientific Department of Animal Sciences at the Agricultural Institute – Shumen, after discussing the methodology of the experiment, gives permission to conduct the experiment.

Haematological and biochemical tests

At the end of the experiment, blood samples were taken from 9 pigs from each group (5 females and 4 males) from the orbital venous sinus using a closed system method. All samples were collected in plastic blood collection tubes (Vacusera, Izmir, Turkey) and immediately inverted 10 times. Serum biochemistry samples were collected in serum tubes and allowed to clot at room temperature for 2-3 h before centrifugation (2000 × g for 15 min). The serum was collected and stored at -20°C for subsequent biochemical analysis. Whole blood samples were collected in EDTA (ethylenediaminetetraacetic acid) tubes and stored at room temperature for haematological analysis within 6 h of sampling. Analytical blood count procedures were performed with a SYSMEX XS 500i 5-type differential count automated haematology analyzer (Sysmex Europe GmbH, Norderstedt, Germany) and a Selectra Pro XL automatic biochemical analyzer (ELITech Group, Puteaux, France) by the instructions of the manufacturer. These include the count of leukocytes (WBC) by conductometric and visual optical method, erythrocytes (RBC) by conductometric method, haemoglobin (HGB) by cyan-methemoglobin method, hematocrit (HCT) by indirect based on the method of conductometric analysis, the mean volume of erythrocytes (MCV) by conductometric method, mean content of haemoglobin in erythrocytes (MCH), mean concentration of haemoglobin in erythrocytes (MCHC).

Statistical analysis

Data were processed using the methods of variance statistics and are shown as arithmetic mean, standard deviation, and standard error. To determine differences between groups and multiple comparisons, data were analysed by analysis of variance (ANOVA). Analysis of Variance (ANOVA) is a statistical method used to test differences between two or more means. ANOVA is based on comparing the variance (or variation) between the data samples to the variation within each particular sample. If the between-group variance is high and the within-group variance is low, this provides evidence that the means of the groups are significantly different. When using ANOVA, it is important to consider the assumptions of the method, such as normal distribution of data and homogeneity of variance. Samples were tested for homogeneity with the Homogeneity of Variances Test (Levene's) and for normality with the Normality Test (Shapiro-Wilk).

To establish and describe the degree and direction of the relationships, correlation coefficients were calculated among the traits – protein intake, average daily gain, and amount of protein per 1 kg of gain. Significance was defined as $p<0.05$.

Results and discussion

Protein in LPD compound feed was reduced by limiting the amount of soybean meal from 25.00 to 16.77%, which, according to Siera [2019], leads to a decrease in potassium content and thus to lower water consumption and smaller volume of faeces.

Pigs from both groups intake practically the same amount of feed, metabolic energy, lysine, and crude fats (CF). Group 2 consumed 16.19% (p=0.019) less CP, as shown in the scheme of the experiment. The rest of the accepted amino acids, except lysine, were in a proportional ratio with CP (Tab. 3).

The LPD did not significantly affect the average daily gain, as its difference with the HPD was -1.55%, which is insignificant and it can be assumed that the pigs of the two groups practically had the same growth rate – 386 g/d and 380 g/d. Our results are similar to those obtained by Spring et al. [2020] who reported that diets with reduced CP up to 25% supplemented with essential amino acids improved body weight and growth performance or had no negative effect on performance and feed efficiency of growing pigs while significantly reducing nitrogen excretion. A similar conclusion was reached by Hlatini *et al*. [2021] that protein levels can be reduced from the standard recommendation level of 193 g/kg to 135.1 g/kg without compromising the growth rate of growing pigs.

There was a tendency for better utilisation of compound feed and metabolic energy per 1 kg of growth by about 3.50% in HPD, and the utilisation of CF was statistically significantly better by 4.81% (p=0.045). When pigs are fed a diet with lower protein

Table 3. Performance results of weaned pigs

Indicators	Group	N	Mean	SD	SE	*p*
Feed intake (kg)	1 2	3 3	0.923 0.919	0.0532 0.0496	0.03073 0.02862	0.923
ME intake (MJ)	1 2	3 3	11.69 11.58	0.6780 0.6243	0.39142 0.36043	0.855
CP intake (g)	1 2	3 3	175.4 147.0	10.1297 7.9286	5.84836 4.57760	0.019
Lysine intake (g)	1 2	3 3	8.6 8.5	0.4933 0.4726	0.28480 0.27285	0.937
CF intake (g)	1 2	3 3	19.3 19.4	1.1358 1.0214	0.65574 0.58973	0.943
EDG (kg)	1 2	21 20	0.386 0.380	0.0781 0.0775	0.01704 0.01732	0.797
Feed conversion (kg/kg EDG)	1 2	3 3	2.389 2.478	0.0119 0.0682	0.00689 0.03940	0.089
ME conversion (MJ/kg EDG)	1 2	3 3	30.24 31.24	0.1514 0.8602	0.08743 0.49662	0.119
CP conversion (g/kg EDG)	1 2	3 3	453.9 396.4	2.3288 10.9258	1.34454 6.30802	<0.001
Lysine conversion (g/kg EDG)	1 2	3 3	22.2 23.0	0.1155 0.6429	0.06667 0.37118	0.101
CF conversion (g/kg EDG)	1 2	3 3	49.9 52.3	0.2646 1.4177	0.15275 0.81854	0.045
Live weight at start (kg)	1 2	21 20	9.110 9.063	1.0945 1.1947	0.23884 0.26715	0.896
Live weight at the end (kg)	1 2	21 20	24.952 24.650	3.6397 3.6023	0.79425 0.80549	0.791

ME – metabolizable energy; CP – crude protein; CF – crude fats; EDG – average daily gain.

content, carbohydrate metabolism is enhanced in the animals to compensate for the protein deficiency. Glucagon, which is directly related to carbohydrate metabolism, increases as an adaptive response of the body and thus reduces body fat stores and stimulates the breakdown of proteins to amino acids, which leads to the synthesis of new globin complexes [Spring *et al.* 2020].

We established that LPD had the strongest effect on CP utilisation, which was better by 12.67% (p<0.001) compared to HPD. In their study, Liu *et al*. [2021] found that pigs on a 15% CP diet showed significantly improved protein, amino acid, and energy utilisation. In addition, this diet optimises the composition of the intestinal microflora to a certain extent. Yoosuk *et al*. [2012] also reported that feed conversion decreased with increasing levels of protein and energy intake. The average daily gain increases with the increasing energy intake and dietary ideal protein content. The feed conversion ratio, which is the feed used and the scaled feed intake, decreases with increasing protein and energy intake levels. Increasing the diet protein content improves daily protein retention and total protein mass. Increasing the diet energy content also improves daily lipid retention and total lipid mass.

Table 4 shows the correlation coefficients between protein intake and feed conversion, metabolizable energy conversion, protein conversion, lysine conversion, fats conversion. From it, we can see that the strongest and very high positive relationship is between the protein intake and the amount of protein for 1kg of growth R=0.914 (p=0.011). The more protein the pigs intake, the more protein is used for 1 kg of weight gain. This correlation indicates that LPD pigs have better protein utilisation. In this way, the amount of undegraded protein reaching the colon is minimised, avoiding the formation of biogenic amines that negatively affect the intestinal microbiota and the protective function of the mucosa. Fermentation of this excess is also avoided, which reduces the risk of proliferation of pathogenic bacteria, such as E. coli, and therefore reduces the occurrence of diarrhoea [Siera, 2019]. We also established by 10.04% (p=0.116) fewer treated pigs for disease in the LPD group. Other authors reported that a high level of CP leads to a high microbial fermentation of undigested proteins, which can also lead to the proliferation of pathogenic bacteria in the gastrointestinal tract [Nyachoti *et al*. 2006, Bikker *et al*. 2007, Heo *et al*. 2008], create ammonia and toxic nitrogen compounds that can damage the gut [Piva *et al.* 1996, Pluske *et al.* 2002].

Table 4. Correlation coefficients between protein intake and feed conversion ratio, metabolizable energy conversion ratio, protein conversion ratio, lysine conversion ratio

Indicators	Protein taken (g)	p-value
Protein taken (g)	Pearson's r p-value	– –
Feed conversion ratio (kg)	Pearson's r p-value	-0.505 0.307
ME conversion ratio (MJ)	Pearson's r p-value	-0.458 0.361
Protein conversion ratio (g)	Pearson's r p-value	0.914 0.011
Lysine conversion ratio (g)	Pearson's r p-value	-0.478 0.338
Fats conversion ratio (g)	Pearson's r p-value	-0.599 0.209

Between CP intake and feed conversion ratio, energy conversion ratio, lysine conversion ratio and CF conversion ratio per 1 kg of growth, we found negative moderate correlations that were not statistically proven.

A moderate reduction in dietary protein can improve growth and metabolism by improving the gut microbiota in pigs [Liu et al., 2021]. When feeding a feed with a lower protein content, the available proteins are mainly used to build globin complexes. A greater amount of HGB is synthesised, which correlates with RBC (R=0.829; p<0.001), so the body is activated to build these vital protein complexes (Tab. 5). The products of the Krebs cycle, which is accelerated, are used to synthesise haemoglobin,

Table 5. Arithmetic mean values of haematological and biochemical indicators of the blood of weaned pigs

Indicators	Group	N	Mean	SD	SE	*p*
WBC (x109/l)	1	9	22.76111	6.8990	2.29966	0.225
	2	9	19.48111	3.6156	1.20521	
RBC (x1012/l)	1	9	6.40444	0.5979	0.19931	0.601
	2	9	6.26667	0.4926	0.16419	
HGB (g/l)	1	9	107.66667	8.7034	2.90115	0.352
	2	9	111.55556	8.5016	2.83388	
HCT (L/L)	1	9	0.35322	0.0291	0.00970	0.534
	2	9	0.36100	0.0224	0.00746	
MCV (fL)	1	9	55.27778	2.5912	0.86374	0.057
	2	9	57.71111	2.4287	0.80957	
MCH (pg)	1	9	16.84444	0.7452	0.24839	0.007
	2	9	17.81111	0.5840	0.19468	
MCHC (g/L)	1	9	305.00000	6.5574	2.18581	0.302
	2	9	308.77778	8.3633	2.78776	
PLT (x109/l)	1	9	444.11111	94.6975	31.56582	0.009
	2	9	337.33333	51.3907	17.13022	
RDW-SD (fL)	1	9	34.91111	1.7751	0.59171	0.341
	2	9	35.98889	2.7796	0.92653	
RDW-CV (%)	1	9	19.38889	1.2504	0.41681	0.491
	2	9	18.97778	1.2235	0.40783	
RDW (fL)	1	6	14.56667	1.2707	0.51876	0.253
	2	7	13.92857	0.5589	0.21125	
MPV (fL)	1	6	11.06667	0.6653	0.27162	0.249
	2	7	10.72857	0.2984	0.11279	
P-LCR (%)	1	6	35.43333	5.1251	2.09231	0.171
	2	7	32.24286	2.5072	0.94764	
PCT (L/L)	1	6	0.00497	8.85e-4	3.61e-4	0.006
	2	7	0.00363	5.31e-4	2.01e-4	
Urea (mmol/l)	1	9	6.37444	1.3971	0.46570	0.009
	2	9	4.81889	0.7400	0.24668	
Cholesterol (mmol/l)	1	9	2.05333	0.2322	0.07741	0.240
	2	9	2.20444	0.2897	0.09658	
HDL (mmol/l)	1	9	0.93444	0.1271	0.04236	0.058
	2	9	1.05889	0.1314	0.04379	
LDL (mmol/l)	1	9	0.41444	0.1242	0.04140	0.080
	2	9	0.53444	0.1467	0.04891	
Triglycerides (mmol/l)	1	9	1.03667	0.2557	0.08524	0.881
	2	9	1.02111	0.1679	0.05596	

WBC – count of leukocytes; RBC – erythrocytes; HGB – haemoglobin; HCT hematocrit; MCV – mean volume of erythrocytes; MCH – mean content of haemoglobin in erythrocytes; MCHC – mean concentration of haemoglobin in erythrocytes; PLT – platelets; RDW-SD – red cell distribution width standard deviation; RDW-CV – red cell distribution width coefficient of variation; RDW - red cell distribution width; MPV – mean platelet volume; P-LCR - platelet large cell ratio; PCT – procalcitonin; HDL – high-density lipoprotein; LDL – low-density lipoprotein.

which is also expressed in the results of blood tests, through the increased values of HGB by 3.61% and the changes of MCH by 5.39% (p=0.007).

The results of the haematological tests also show a decrease in platelet values by 24.04% (p=0.009) in the low-protein diet group, which can be explained by the shift of metabolism to carbohydrates. Under suitable conditions, the microorganisms in the large intestines cause fermentation, during which short-chain fatty acids are formed, among which is butyrate – a metabolite with a high concentration in the lumen of the large intestine. Due to the accelerated carbohydrate metabolism, with which the body compensates for the low intake of proteins, an amount of butyrate, an ester of butyric acid, accumulates. Silva [2013] reported that sorbitol butyrate (SB) may offer a therapeutic option for diseases involving platelet dysfunction, as it demonstrated a reduction in blood platelet counts from breeding pigs fed a 0.3% SB diet.

High-protein diets are associated with both increased costs and an increased incidence of enteritis in weaned pigs. This was also observed in the haematological indicators of pigs from HPD – increased values of WBC by 16.84% (p>0.05) and PCT by 36.91% (p=0.006). PCT is a precursor of calcitonin, which is among the markers by which sepsis or bacterial infection is diagnosed, and its level reflects the extent of the systemic inflammatory response.

Metabolic pathway analysis of serum metabolites revealed that nitrogen metabolism as well as BCAA (branched chain amino acids) metabolism and biosynthesis were significantly affected by the amount of dietary protein [Spring *et al.* 2020]. Our blood test results also showed decreased levels of urea concentration by 24.40% (p=0.009) in group 2. LPD feeding stimulated an increase in serum NAG (n-acetyltransferase), a major cofactor activator of carbamoyl-phosphate synthetase 1 (CPS1), which catalyses the synthesis of carbamoyl-phosphate, a key rate-limiting enzyme in urea synthesis. In addition, diets with LPD provide less ammonia, which is disposed of by glutamate binding. The preference for this type of reaction has several advantages over the ornithine cycle and urea synthesis – 1. Glutamine formation occurs in all tissues, not only in the liver; 2. The ammonia thus bound is mobile metabolically and topographically, it is used for various biosynthetic processes; 3. Glutamine synthesis is much more energetically beneficial than urea synthesis [Spring *et al*. 2020].

The level of total cholesterol was higher by 7.36% in LPD pigs, which is similar to the results reported by Spring *et al*. [2020] in that pigs fed moderately low protein and carbohydrate diets had higher serum cholesterol concentrations. This has also been shown in low-protein, low-carbohydrate diets in rodents and is associated with better cardiometabolic health and lifespan [Solon-Biet *et al.* 2015].

Blood tests showed an increased level of LDL-cholesterol by 28.95% (p=0.080) and of HDL-cholesterol by 13.32% (p=0.058), which is dependent on cholesterol values. Li *et al*. [2018] found that low protein diets can positively affect meat quality in pigs by regulating intramuscular fat content. This effect of diet is predictable because providing insufficient protein in the diet of LPD pigs results in inhibition of protein synthesis and muscle growth to some extent, and excess energy is diverted to fat accumulation. Lipid metabolism is concentrated in adipose tissue, which is the main depot of fatty acids [Wood *et al*. 2004].

The results of this research can be used to practically solve some health problems in weaning pigs. But more research can be conducted in this direction and under different technologies and growing conditions.

Conclusions

Reduced protein level did not significantly affect the growth of weaned pigs when compound feeds were equalised for lysine content.

There was a tendency for better utilisation of compound feed and metabolic energy per 1 kg of growth by about 3.50% in high protein diets pigs, and the utilisation of crude fats was statistically significantly better by 4.81% (p=0.045). Pigs fed low protein level had better utilisation of crude protein by 12.67% (p<0.001) and worse utilisation of crude fats (p=0.045).

Reduced protein level has a statistically significant positive effect on haematological and biochemical blood parameters associated with health status in weaned pigs.

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