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ORIGINAL PAPER

Effect of feeding guar (*Cyamopsis tetragonoloba*) meal on selected biochemical indices in blood and morphology of the liver of pigs*

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Abstract

Every year, the consumer demand for the quality of food products grows, and the question of the safety of feed components used to make animal food gains importance. The aim of this study was to determine the effects of guar meal, with different protein shares supplied in a diet, on the values of selected biochemical indices of dehydrated blood and liver of pigs. The pigs were divided into four groups. The animals in control group (1) were fed a diet containing soybean protein (SBM) as the main protein source. In the diets for experimental groups 2, 3 and 4, SBM protein was replaced with guar meal protein at 25%, 50% and 75% respectively. The material for the study came from 24 crossbred pigs that were fed the selected diets for 92 days. One week before expected slaughter, blood was drawn for biochemical analyses from the pigs in all groups. The use of a different protein source had no significant influence on changes in the investigated biochemical parameters in the blood of fattening pigs from the different experimental groups. The pigs were slaughtered according to the usual procedure in meat plants. Immediately after slaughter, sections of the liver were taken during carcass cutting. For the prepared specimens, a morphological assessment of the examined liver samples was made. Based on the investigations carried out and the results obtained, it can be concluded that the use of 50% and 75% guar meal protein as a substitute for SBM protein has negative effects on the pig organism. Most of the morphological changes were found in the livers of animals fed diets with a higher content of guar meal protein. The results of the study thus show that an evaluation of the biochemical indices in serum alone does not provide a complete picture of all changes in the organism of pigs under the influence of guar protein; only in combination with the results of the morphological examinations of the internal organs do they provide complete information about the health status of the animals.

Keywords: biochemical indicators, guar meal, health, pigs, protein

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INTRODUCTION

Ongoing breeding efforts in the pig population aimed at high productivity, i.e. im-proving fattening and slaughter traits, have led to a decline in the quality of pork (Żak et al. 2014) while forcing producers to intensify nutrition in order to exploit the growth potential of modern pigs. Changing legal, organizational and economic conditions make it necessary to look for ever newer solutions in animal nutrition. Feed accounts for about 70% of the costs in pig production, with protein components being among the most expensive. Feedstuffs that can improve taste, nutrition and meat quality are used in pig feeding (Lebret et al. 2015, Karpiesiuk et al. 2019). Modern pig production is mainly based on cereals and the protein component, which is mostly genetically modified soybean meal (GM SBM). Research related to the use of different protein sources (lupins, field beans, pellets, DDGS, rapeseed meal after extraction, insect protein) has been and is being conducted in individual research centers (Bugnacka and Falkowski 2001, Świątkiewicz et al. 2021). However, the use of more legumes in pig diets is limited by the presence of antinutritional factors. In addition to determining the quantities in which they can be used in pig generations with appropriate fattening and slaughter parameters, it is also important to study their effects on animal health. The evaluation of the components in terms of their health safety (Okorski et al. 2017) and the economic evaluation of the diet used (Karpiesiuk et al. 2018, Sońta et al. 2015) is also an important element. An additional argument for the search for new solutions in the field of nutrition is the issue of the impending ban on the use of GMO feeds and, in the case of organic farming, the current ban on the use of GMO feeds and the growing number of people interested in eating organic food from GMO-free crops. Other sources of feed protein should also be considered when looking for alternatives to GM soybean extraction meal for animal feed. The Covid 19 epidemic has shown the importance of diversifying the sources of raw materials for production. In the case of livestock production, we must also seek to diversify sources of feed components so that no single unpredictable factor upsets the production sector. In the search for alternatives to genetically modified soybean meal in animal feed, other sources of feed proteins should also be considered. One of these could be guar meal, which is a by-product of guar gum production. A by-product of gum extraction is guar meal (Sabahelkheir et al. 2012), which contains 33-47.5% (Salehpour et al. 2012) and as much as 55-60% crude protein (Mesgaran et al. 2010). In Poland, it is a little-known plant, and only some products derived from it are used on a larger scale, including guar gum. Most research on the use of guar meal in animal nutrition to date has focused on poultry and ruminants (Kamran et al. 2002, Milczarek et al. 2022, Chhikar et al. 2020), reflecting the fact that guar meal is grown in India and Pakistan and diets are based on poultry and lamb. Studies on the benefits of guar in pig diets have been sporadic (Karpiesiuk et al.

2018, 2023, Heo et al. 2009, Rainbrird, Low 1986). Guar seeds also contain antinutritional compounds that can have negative effects on animals by causing changes in their nervous, digestive, and reproductive systems (Bañuelos et al. 2005) and altering the biochemical profile of blood. The use of guar meal in poultry diets has been restricted because of reports of adverse effects such as diarrhoea, decreased growth rate, and increased mortality when relatively high amounts of guar protein are fed (Patel, McGinnis 1995). Other antinutritional substances present in guar meal, such as saponins, polyphenols, and tannins, may also adversely affect the liver, kidneys, and intestines, as has been found in mice and rats (Diwan et al. 2000).

The aim of the conducted study was to evaluate selected biochemical indices of blood and liver of pigs fed diets containing different levels of guar meal protein.

MATERIALS AND METHODS

The material for the study came from 24 hybrid pigs derived from a straight four cross [♀(♀Polish Landracez x ♂ Polish Large White) x ♂ (♀ Pietrain x ♂ Duroc) with an initial body weight of 30.1 kg and divided into four groups. Table 1 shows the composition of the experimental mixtures for the first and second fattening period. Pigs were fed complete diets containing 17% and 15% of total protein, respectively Pig Nutrient Requirements (1993) – Table 1.

Feed and water were available ad libitum. The experiment was approved by the Local Ethics Committee for Animal Experimentation in Olsztyn (decision No. 55/2018).

The chemical composition and metabolic energy of the experimental and control diets and guar meal presented in Table 2.

Samples of experimental diets were analyzed for nutrient content, including crude protein, crude fat, crude fiber, dry matter and crude ash, by standard methods, at the Analytical Laboratory of the Department of Animal Nutrition and Feed Science, University of Warmia and Mazury in Olsztyn. One week before the expected slaughter, blood was drawn from the pigs for biochemical analyses. Blood was collected intravenously in heparin tubes from the external jugular vein (vena jugularis externa). The sealed tubes, intended for laboratory analysis, were placed upright in racks and sealed in a thermal bag for blood transport. They were then immediately transported to the analytical laboratory, where biochemical analyses were performed using methods commonly used in laboratory analysis. All analyses were performed with the Cobas Integra 800 analyzer (protein was determined by the biuret assay; urea by the kinetic test with urease and glutamate dehydroge-

Table 1

Feed ingredients and the chemical composition of diets

Specification	Groups[#]			
	1	2	3	4
1st stage of fattening (30-70 kg body weight)				
Soybean meal	21.50	16.20	10.90	5.500
Guar meal	-	4.900	9.900	14.60
Wheat	30.00	30.00	30.00	30.00
Barley	45.50	45.90	46.20	46.90
Premix*	3.000	3.000	3.000	3.000
2nd stage of fattening (70-110 kg body weight)				
Soybean meal	15.00	11.25	7.500	3.750
Guar meal	-	3.400	6.800	10.300
Wheat	25.00	25.00	25.00	25.00
Barley	57.50	57.85	58.20	58.45
Premix*	2.500	2.500	2.500	2.500

[#] group 1 (C – control) where SBM was the main protein source; group 2 – where 25% of SBM protein was replaced with guar meal protein; 3 – group 3 – where 50% of SBM protein was replaced with guar meal protein; group 4 – where 75% of SBM protein was replaced with guar meal protein

* Premix: lysine – 8.4%, methionine – 2%, methionine and cystine – 2%, threonine – 2.5%, calcium – 17%, phosphorus – 2%, available phosphorus – 4%, total sodium – 4.4%, iron – 2000 mg, manganese – 1000 mg, zinc – 3500 mg, copper – 4000 mg, iodine – 26.6 mg, selenium – 6.6 mg, vitamins: A – 350 000 IU, D3 – 50 000 IU, E – 1 400 mg, K3 – 30 mg, B1 – 30 mg, B2 – 100 mg, B6 – 60 mg, B12 – 500 mg, folic acid – 40 mg, pantothenic acid – 350 mg, niacin – 400 mg, choline chloride – 7 500 mg, amino acids: L-lysine, L-threonine, DL-methionine, phytase, antioxidants

Table 2

Chemical composition and metabolic energy of the experimental and control diets

Specification	Groups								
	guar meal[#]	1		2		3		4	
		1st stage	2nd stage	1st stage	2nd stage	1st stage	2nd stage	1st stage	2nd stage
Dry matter (%)	91.54	90.86	90.82	90.89	90.78	90.96	90.92	91.02	91.01
Crude protein (%)	48.86	17.09	15.35	17.40	14.69	17.70	15.57	17.03	15.39
Crude fat (%)	4.890	0.960	0.840	1.220	1.030	1.340	1.330	1.470	1.750
Crude fiber (%)	7.150	2.750	3.210	3.540	3.260	3.550	3.800	3.820	4.100
Crude ash (%)	4.820	4.280	3.620	4.370	3.590	4.190	3.650	4.420	3.190
Metabolizable energy* MJ	13.00	13.00	12.93	13.16	13.04	13.33	13.15	13.48	13.27

* calculated value – WinPasze, [#] chemical composition and metabolic energy guar meal from Launtop Polska – antinutritional factors: trypsin inhibitor 3.96 mg g^{-1}, saponin 0.38%, guar gum ≤5%, tannin 1.71%

nase; albumin. LDH, creatinine, cortisol, CDP, AST ALT, ALP, total cholesterol and triglycerides by enzymatic colorimetric methods, HDL cholesterol by an enzymatic colorimetric test. LDL cholesterol levels were calculated based on Friedewald formula (Friedewald et al. 1972). The pigs were slaughtered according to the procedure commonly used in meat plants. Immediately after slaughter, sections of the liver were taken during carcass dissection. The sections were fixed in neutralised 10% formalin, pH 7.4, and then embedded in kerosene blocks, as is customary in histopathological studies. The microtome sections obtained from 5m were stained with hematoxylin and eosin (HE) and evaluated under a light microscope (BX52, Olympus, Tokyo, Japan) using Cell^B software (Olympus, Tokyo, Japan). The significance of the differences in the studied parameters of between the means in the groups was analysed using a one-way analysis of variance with Duncan's test. Calculations were performed using Statistica software PL ver. 13.3.

RESULTS AND DISCUSSION

Biochemical indicators of blood morphology of the digestive tract of pigs

Results of biochemical blood tests in experimental fattening pigs are presented in Table 3. The level of each indicator and their correlations provide information about the health status of the pigs. These correlations were

Table 3

Biochemical parameters in the serum of the experimental pigs

Specification	Groups			
	1	2	3	4
Alkaline phosphatase ALP (u L^{-1})	128,5	135,0	162,8	151,8
Alanine-aminotransferase ALAT (u L^{-1})	42,00	37,83	40,00	36,50
Aspartate aminotransferase ASPAT (u L^{-1})	21,00	20,80	23,50	23,70
Albumin ALB (g L^{-1})	43,00	41,70	43,10	38,20
Total protein TP (g L^{-1})	5,820	5,630	5,870	5,600
Creatinine (CREA)	1,160	1,170	1,120	1,060
Urea (UREA)	39,20	37,00	36,70	37,50
Total cholesterol (mml L^{-1})	2,150	2,045	2,140	2,179
HDL cholesterol (mml L^{-1})	1,120	0,970	1,040	1,130
LDL cholesterol (mml L^{-1})	0,930	0,890	0,880	0,900
Triacylglycerides (mml L^{-1})	0,260	0,260	0,550	0,400
Cortisol (µg dl^{-1})	6,790	4,470	6,370	4,910

not observed in any of the experimental groups, indicating the proper balance of the feed mixtures containing guar gum, among others.

Cholesterol content in the body is determined by genetic and environmental factors, with diet playing an important role. The dietary component that is often blamed for high blood cholesterol levels is fat, or more specifically, the high levels of saturated fatty acids in it. The highest total cholesterol concentration was found in group 4, ranging from 1.19% to 10.14% higher than in the animals of the other groups. The use of a different protein source in the diet did not affect the differences in HDL cholesterol content between the groups. According to Winnicka (2021), levels of HDL cholesterol in pig serum should account for at least 40% of total cholesterol concentrations, since a decrease in the HDL fraction below this value is undesirable. In our study, the proportions of HDL cholesterol in total cholesterol were very different, ranging from 48 to 52%. These differences were not confirmed statistically.

Serum triacylglycerol levels were elevated in the pigs studied. However, the differences between the mean values were not statistically confirmed; the increase in triacylglycerols was most likely influenced by the higher fat content in the mixtures of the groups.

In our study, the indices presented did not exceed the reference values. Only a tendency for an increase in serum alkaline phosphatase levels (ALP) in fattening pigs was observed with an increase in the proportion of guar meal in the diet. No such relationship was observed for the aminotransferases (ALAT and ASPAT). However, it is puzzling that ALAT levels were highest in the control group.

It has been shown that metabolic processes occurring in the body under homeostatic conditions have little effect on blood parameters. However, any disturbance in the normal functioning of tissues and organs shakes the existing balance of the organism and can change the level of tissue enzymes in the peripheral blood. The determination of biochemical serum indices such as macronutrients, enzyme activity, protein, urea, total cholesterol and its fractions, and triacylglycerols allows an evaluation of the organismic function of the animal (Elbers et al. 1992).

In study Hassan et al. 2020, they presented the effects of feeding guar meal on nutrient metabolism in pigs. There were no differences in plasma concentrations of urea nitrogen, total protein, albumin, glucose, total cholesterol or triglycerides among the pigs fed the five different diets (P>0.152). These results partially agreed with our findings.

Reduced protein and energy intake in the form of carbohydrates negatively affects serum protein and glucose levels and increases AST and ALAT levels (Gajęcki 1996). The total protein content did not exceed the reference values (Winnicka 2021), indicating a normal energy-protein ratio in the fed diets. Also, Sońta et al. 2020 determined no change in this parameter compared to the reference values in a trial on the use of blue lupins and peas

in the feeding of fattening pigs. In addition, an increased serum protein content of about 10% can occur simply due to intense exercise or excessive vascular pressure during blood sampling (Winnicka 2021). The total cholesterol content was slightly higher than the reference values in animals in groups 1, 3, and 4 (Winnicka 2021). Winnicka (2021) states that the serum cholesterol content of the HDL fraction should be at least 40% of the total cholesterol and it is unfavourable if the concentration of this fraction falls below such a level. Sońta et al. (2020) obtained higher serum total cholesterol and triglyceride levels in the study cited above than in their own study. Martins et al. (2005) and Sirtori et al. (2012) showed that legumes in compound feeds may have a hypocholesterolemic effect. In a study by Zhao et al. (2017), results showed that short-chain fatty acids (SCFAs) acetate, propionate, and butyrate significantly (P<0.05) reduced total plasma cholesterol levels in hamsters by 24%, 18%, and 17%, respectively.

Indicators of normal liver function are the enzymes ALAT, ASPAT, and ALP, while elevated levels indicate liver cell damage (Campbell 2012, Winnicka 2021). In all groups, the values of ALP were within the reference values given by Winnicka (2021).

According to Rotkiewicz et al. (1993), the increase in transaminase activity may indicate morphological damage to internal organs, possibly due to the guar gum antinutritive substances contained in mixtures 3 and 4 (Table 2). Therefore, it cannot be excluded that feeding diets containing guar meal-derived proteins for a longer period of time than in our study may have aggravated liver cell damage, as reflected by a significant increase in enzyme levels ALP, ALAT, and ASPAT.

In an Italian study on the use of field bean crops in pig feeding, no negative effects of these feed components on the pig body were found, since the ALP, ASPAT and ALAT concentrations in the blood of the experimental pigs were similar to those of the control group (Prandini et al. 2005). The values of biochemical indices and mineral concentrations in serum obtained in the present study were mostly within the reference standards (Winnicka 2021). The differences between the results discussed here and the literature data and reference values are due to the use of different legumes in the study (Martins et al. 2005, Prandini et al. 2005, Sońta et al. 2020) and the differences in their utilisation levels.

Research into protein sources that are safe for animal health, including swine, is urgently needed. Many hyopathologists point out that losses in pig production caused by disease syndromes with multifactorial aetiology are much higher than losses caused after importation, e.g., by CSF virus or ASF, due to their prevalence (Truszczyński, Pejsak 2012).

Production studies did not confirm that feed conversion should improve with intestinal villus growth and thus growth rate, and animals in groups 3 and 4 were characterised by significantly lower body weight gains and anecdotally significantly higher feed consumption per kg body weight gain

(Karpiesiuk et al. 2018). The antinutritive substances contained (tannins, saponins) can form complexes with proteins and thus reduce the digestibility of proteins and amino acids. This negative effect also occurs due to inhibition of endogenous enzymes (Jansman 1993).

Morphology liver in pigs

No studies on the effects of guar meal on liver morphology can be found in the available scientific literature. The morphological picture of the liver in each animal group is summarized in Table 4.

Table 4

Liver – morphological changes

Specification of morphological changes	Group 1 n=6	Group 2 n=6	Group 3 n=6	Group 4 n=6
Normal pouch and lobules	2	2	3	2
Sinusoidal vascular congestion in single lobules	4	3	3	4
Vascular congestion through ducts	1	2	3	1
Parenchymal degeneration in the central zone	0	2	3	4
Parenchymal degeneration of the entire lobe	0	2	2	1
Small foci of thrombotic necrosis	0	0	0	2
Proliferation of liver cells in the peripheral zone of the lobules	0	1	1	2
Presence of non-axial lobules	0	2	0	1
Connective tissue proliferation in the puncture sites	1	0	2	3
Infiltration of eosinophilic cells and lymphocytes in the stomata	0	1	2	2
Cysts in the parenchyma focally	1	0	0	0

Examination of the liver sections revealed obstruction of the central veins, portal vein, and sinusoidal vessels, which affected single or multiple lobules in individual animals but was most pronounced in the 4 group (Figure 1). Parenchymal degeneration of the central zone of lobules and parenchymal degeneration of all hepatic cells were found in the group 4, to a lesser extent in 3 and 2 animals and in individual animals of the groups 2, 3 and 4. In two pigs of the 4 group, small foci of thrombotic necrosis were found, each with several liver cells in small but numerous lobules. In some animals of groups 3 and 4, connective tissue proliferations in the ductal spaces, interlobular tissue, and cellular infiltrates were found, which were more numerous in groups 3 and 4. Hepatocyte proliferations and the presence of nonaxial lobules were noted in individual animals of the groups 2 and 4.

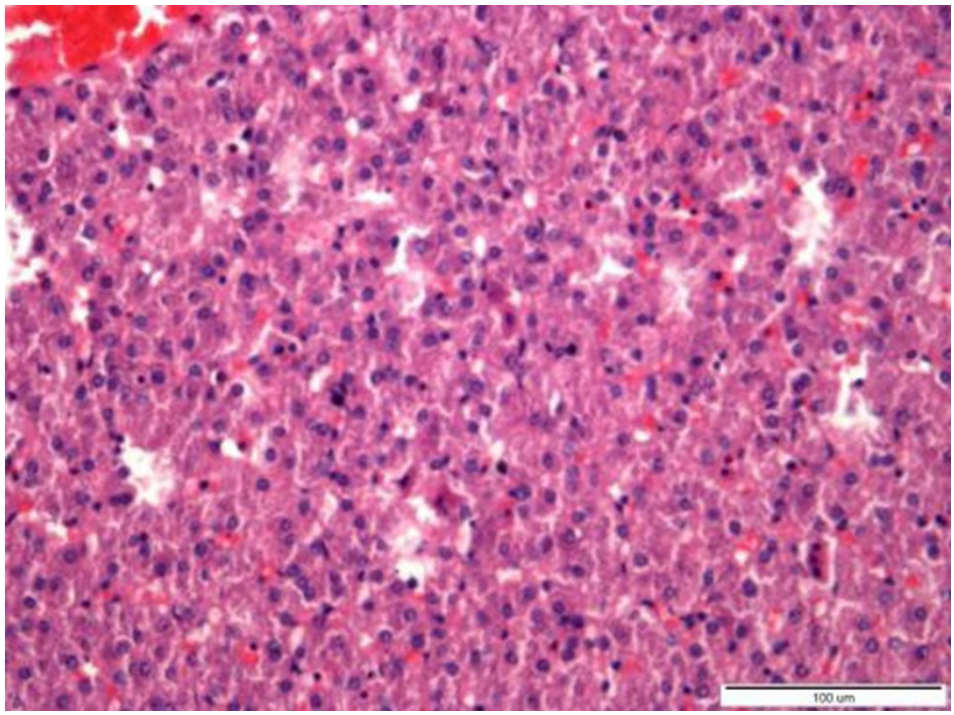

Fig. 1. Liver pattern with parenchymal degeneration of hepatocytes and congestion in group pigs 4

A study by Badr et al. (2015) on the effect of guar seed flour supplementation on the function and histology of kidney and liver in rats showed that the addition of 5% flour had a positive effect on the function of these organs. Histopathological examinations of the above organs also showed no morphological changes. Additions of 10% and 15% had a non-corrosive effect on the function and were the cause of the appearance of histological changes in the kidneys and liver of rats.

According to Rotkiewicz et al. (1993), the liver lesions observed in their study could be the reason for the impaired detoxification capacity of the liver. The studies conducted by showed a significant effect of field bean on changes in the digestive tract of pigs. Histopathological analysis showed cellular damage in liver, pancreas, stomach, duodenum and jejunum.

CONCLUSIONS

Consideration of the biochemical picture of serum biochemical indicators alone gives an incomplete picture of the changes that occur in the body of the pig under the influence of the applied diet. The combination of bio-

chemical and pathomorphological studies gives a more complete picture of the state of health of the animals. On the basis of the conducted studies and the obtained results, it can be concluded that under the described experimental conditions, the use of protein obtained from guar meal in the amount of 50% and 75% instead of protein obtained from soybean meal after extraction had negative effects on the organism of the experimental pigs. Most of the changes in the livers were also found in the animals fed higher guar meal protein content.

Author contributions

Conceptualization, K.K.; methodology, K.K., W.K., I.O-D., M.G.; validation, K.K. and W.K.; formal analysis, K.K.; investigation, K.K., W.K., A.O.-D., M.G., A.W.; resources, K.K.; data curation, K.K., A.O.-D., M.G.; writing – original draft preparation, K.K., W.K., I.O.-D., M.G.; writing – review and editing, K.K.; visualization, K.K, W.K., A.O.-D., M,G,; supervision, K.K.; project administration, K.K.; funding acquisition, K.K. and W.K. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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