



Sobotka W., Drazbo A. 2020.

Environmental impacts of diets containing different levels of crude protein and limiting amino acids fed to pigs in a phase feeding system.

J. Elem., 25(2): 645-655. DOI: 10.5601/jelem.2019.24.4.1926



RECEIVED: 31 October 2019

ACCEPTED: 24 January 2020

ORIGINAL PAPER

ENVIRONMENTAL IMPACTS OF DIETS CONTAINING DIFFERENT LEVELS OF CRUDE PROTEIN AND LIMITING AMINO ACIDS FED TO PIGS IN A PHASE FEEDING SYSTEM*

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ABSTRACT

The aim of this study was to determine the effect of complete diets containing different levels of crude protein and limiting amino acids fed to pigs in a two-phase feeding system on protein digestibility and N retention, utilization and excretion to the environment. A digestibility-balance trial was conducted using 24 growing-finishing pigs (hogs) divided into 3 groups. The animals were housed individually in metabolism crates and were fed diets: C – standard levels of protein and essential amino acids, R – protein and amino acid levels reduced by 15% relative to the standard levels, R+AA – reduced protein content and supplementation with crystalline lysine, methionine, threonine and tryptophan to the standard levels (as in diet C). Grower and finisher diets in which the levels of crude protein and total lysine were reduced by 15% fed to pigs in a two-phase feeding system decreased ($P \leq 0.01$) protein digestibility. However, a significant reduction in urinary N excretion and improvement in N utilization, relative to both N intake and N digestion ($P \leq 0.05$), were noted in this group. The supplementation of low-protein diets with crystalline essential amino acids improved protein digestibility, N retention and N utilization. According to estimates, a 15% reduction in crude protein (i.e. 2 to 2.5%) and amino acids concentrations in growing-finishing pig diets, relative to the standard levels, reduced N excretion to the environment by 18.7%. The supplementation of low-protein diets with essential amino acids contributed to a further reduction in N excretion to 22.5%. The results of this study indicate that the crude protein content of complete diets for growing-finishing pigs, in two-phase fattening, can be reduced by 2-2.5% relative to the standard level for this type of compound feed. However, enrichment of low-protein diets for pigs with lysine, methionine, threonine and tryptophan to the standard level remains a necessary condition.

Keywords: pigs, phase feeding, different levels of protein and limiting amino acids, nitrogen, excretion, environment pollution.

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* This study was financed by state funds allocated to the University of Warmia and Mazury in Olsztyn under the code no. 18.610.001-300 and project financially co-supported by the Minister of Science and Higher Education under the program titled "Regional Initiative of Excellence" for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.

INTRODUCTION

The conversion of plant products into foods of animal origin is accompanied by the emissions of unused metabolites to the environment. The growing demand for meat products leads to the intensification of animal production, and the associated environmental pollution. Livestock farming exerts adverse effects on the environment. Livestock are a major source of nitrogen (N) and phosphorus emissions. Animal excreta contain considerable amounts of pollutants that are released into the air, soil and surface waters, thus contributing to acid rain, the greenhouse effect and stratospheric ozone depletion (BOBRECKA-JAMRO, JANOWSKA-MIĄSIK 2014). Biogenic N and phosphorus compounds contained in animal excreta penetrate into the soil and inland waters, resulting in progressive degradation of aquatic ecosystems (SAPEK, SAPEK 2008, JANKOWIAK et al. 2010).

In recent years, much attention has been paid to environmental protection, including reduction of harmful gas emissions from both industrial and agricultural sources (AMANN 2012). One of the biggest environmental threats is posed by N from nitrites and ammonia salts present in synthetic fertilizers, and N contained in urea which is converted to ammonia in the presence of enzymes. According to many authors, N from natural fertilizers is more widely distributed in the environment than N from mineral fertilizers due to the volatilization of ammonia and nitric oxides as well as nitrate leaching to groundwater and incorporation into soil organic matter (BEKIER-JAWORSKA, SZOSTAK 2006). Nitrogen present in soil organic matter is converted to NH_4^+ during the process of mineralization that involves ammonization and ammonification. Amino acids are rapidly mineralized in soil. Ammonia released by mineralization is converted to N-NH_3 which is available to plants, and it is retained in the soil solution. Ammonia volatilization takes place under conducive conditions, contributing to soil acidification and eutrophication of groundwater and surface waters (HAYES et al. 2004, SZOSTAK et al. 2005, KALEEM ABBASI, ABDUL KHALIG 2016).

Monitoring and reduction of nitrogen emission from animal farms is one of strategic objectives of the European Union (AMANN 2012). It is estimated that nearly 50% of global production of the main source of nitrogen, that is ammonia, comes from pig farms (HAYES et al. 2004). The N content of pig excreta is not constant and may vary depending mostly on the quantity and quality of dietary protein. The quantitative and qualitative composition of N fractions in pig manure is determined by the dietary inclusion levels and quality of protein (PORTEJOIE et al. 2004). Feeding excess protein leads to the excretion of excess N. A similar effect is obtained due to diets deficient in limiting essential amino acids for pigs. In the latter case, deamination of amino acids contributes to N release, and readily soluble N compounds increase the share of N fractions in manure (NIEMI et al. 2010, HAUSCHILD et al. 2012).

In order to optimize protein utilization, the protein content of diets can be reduced and the quantity and quality of dietary protein can be adjusted to the nutrient requirements of pigs depending on their physiological status during the growing and finishing phases (GARRY et al. 2007, SEMENIUK, GRELA 2011, POWELL et al. 2011, ANDRETTA et al. 2016, WANG et al. 2018). However, complete diets have to be balanced accurately with respect to essential amino acids by selecting appropriate feed components or supplementing low-protein diets with essential crystalline amino acids.

The aim of this study was to determine the effect of complete diets containing different levels of crude protein and limiting amino acids fed to pigs in a two-phase feeding system on crude protein digestibility and N retention, utilization and excretion to the environment.

MATERIALS AND METHODS

The experiment was approved by Resolution no 5/2008 issued by the Animal Ethics Committee, the University of Warmia and Mazury in Olsztyn.

A digestibility-balance trial was performed to determine crude protein digestibility and N balance in pigs fed grower diets (experiment I) and finisher diets (experiment II) with different inclusion levels of crude protein and essential amino acid (lysine, methionine with cystine, threonine, tryptophan). The experimental materials comprised 24 growing-finishing hybrid (♀ Polish Landrace x Polish Large White) x ♂ Duroc pigs. The animals were divided into 3 groups, with 8 pigs per group, according to the following experimental design (Table 1).

The nutritional value of diets was determined for average daily gain of 850 g (Nutrient Requirements of Pigs 2014). The animals were housed individually in metabolism crates and were fed the diets whose composition is specified in Table 2. Feed in a friable form (feed/water ratio of 1:1) was administered twice daily at 7⁰⁰ a.m. and 2⁰⁰ p.m. Pigs had free access to water.

Protein content in experimental compound feed was determined according to standard methods (AOAC 2007). However, the digestible protein concentration was calculated based on the digestibility coefficients obtained from the authors' own research.

The amino acid composition of the protein in the feed materials was determined using a Biochrom 20 Plus amino acid analyzer. Hydrolysis of samples was carried out in 6 M HCl for 24 h and at 110°C. Sulfur amino acids were determined after oxidation of the samples with sulfuric acid, and tryptophan was determined according to the Polish Standard (PN-77/R-64820:1997). The results were used to calculate the content of lysine, methionine with cystine, threonine and tryptophan in the experimental compound feed.

Experimental design						
Experimental factor	2-phase feeding system					
	complete diets [#]					
	Grower from 30 to 70 kg body weight			Finisher from 70 to 110 kg body weight		
	C	R	R+AA	C	R	R+AA
Concentration of protein and essential amino acids						
Crude protein (%)	17.92	15.36	15.42	15.31	13.13	13.21
Total lysine (%)	0.99	0.85	0.99	0.83	0.71	0.83
Total methionine with cystine (%)	0.73	0.62	0.73	0.65	0.56	0.65
Total threonine (%)	0.64	0.55	0.64	0.54	0.45	0.54
Total tryptophan (%)	0.22	0.19	0.22	0.19	0.16	0.19
Number of pigs in the experimental group	8	8	8	8	8	8

[#] C – control diets with standard levels of crude protein and essential amino acid (total lysine, methionine with cystine, threonine, tryptophan);

R – low-protein diet with crude protein and amino acid levels reduced by 15% relative to the standard levels as in diets C;

R+AA – low-protein diet supplemented with crystalline lysine, methionine, threonine and tryptophan to the standard levels as in diets C.

During the digestibility-balance trial, feces and urine were collected, and quantitatively 5% samples of the total amount of feces excreted daily were saved for analysis and frozen. The N content of an average frozen feces sample was determined at the completion of the experiment. Urine was collected into plastic containers and was preserved with sulfuric acid to pH below 2. 5% samples were assayed for N content. The content of N in feces and urine was determined in 8 medium thawed samples for each experimental group, separated after the end of the digestibility-balance trial. The content of nitrogen in feces and urine was determined according to standard methods (AOAC 2007). Fecal and urinary N excretion was estimated mathematically in view of the experimental factors, based on N balance in pigs fed grower and finisher diets.

The results were analyzed statistically by one-way ANOVA with the use of Statistica ver. 13 PL software. The significance of differences between mean values of the analyzed experimental factors was estimated by the Duncan's test at the significance level $P \leq 0.05$ and $P \leq 0.01$.

RESULTS AND DISCUSSION

The component composition of the experimental diets and their nutritional value are shown in Table 2. The main sources of vegetable protein in the evaluated feed diets were post-extraction soybean and rapeseed meal

Table 2

Composition and nutritive value of diets for growing-finishing pigs

Specification	2-phase feeding system					
	complete diets [#]					
	Grower from 30 to 70 kg body weight			Finisher from 70 to 110 kg body weight		
	C	R	R+AA	C	R	R+AA
Ground wheat (%)	40.00	40.0	40.00	40.00	40.00	40.00
Ground barley (%)	36.78	41.95	41.52	43.57	49.17	48.82
Extraction soybean meal (%)	15.00	9.00	9.00	6.00	-	-
Extraction rapeseed meal „00” (%)	5.00	5.00	5.00	8.00	8.00	8.00
Rapeseed oil (%)	-	0.80	0.80	-	0.40	0.40
Mineral feed +premix ⁺ (%)	3.00	3.00	3.00	2.20	2.20	2.20
L-lysine HCL-78% (%)	0.22	0.25	0.41	0.23	0.23	0.42
DL-methionine 99% (%)	-	-	0.15	-	-	0.04
L-threonine 98% (%)	-	-	0.09	-	-	0.08
DL-tryptophan 98% (%)	-	-	0.03	-	-	0.04
Nutritional value of experimental mixtures						
Crude protein (%)	17.92	15.36	15.42	15.31	13.13	13.21
Digestible protein (g kg ⁻¹)	149	120	128	125	106	109
The proportion of digestible protein to total protein (%)	83.14	83.56	83.09	81.64	80.73	82.50
Total lysine (%)	0.99	0.85	0.99	0.83	0.71	0.83
Total methionine with cystine (%)	0.73	0.62	0.73	0.65	0.56	0.65
Total threonine (%)	0.64	0.55	0.64	0.54	0.45	0.54
Total tryptophan (%)	0.22	0.19	0.22	0.19	0.16	0.19
Crude fibre (%)	4.01	4.09	4.16	3.91	3.83	3.82
Metabolizable energy (MJ kg ⁻¹) ⁺⁺	13.15	13.06	13.11	13.18	13.02	13.11

[#] see Table 1, ⁺ limestone (1.0/0.7); dicalcium phosphate (0.7/0.5), salt (0.3/0.3), mineral-vitamin premix (1.0/0.7), ⁺⁺ values calculated on the basis of own research data (unpublished data SOBOTKA, DRAŽBO 2019) and data from the Nutrient Requirement of Pigs (2014).

“00”. The concentration of total protein in diet C grower/finisher was 17.92 and 15.31%, respectively. A 6% reduction in the share of soybean extraction meal contributed to a decrease in this nutrient in diet R grower/finisher from 2 to 2.5%, i.e. to levels of 15.36 and 13.13%, and a decrease in the concentrations of exogenous amino acids (lysine, methionine, threonine and tryptophan). Low-protein diets (R+AA grower/finisher) were supplemented with crystalline amino acids lysine, methionine, threonine and tryptophan in amounts ensuring that the levels of these amino acids were the same as in diet C.

The specific amount of digestible protein in the evaluated feed diets (Table 2) was different depending on the digestibility coefficient obtained (Tables 3 and 4) for this nutrient in the grower and finisher diets. It ranged from 120 to 149 g kg⁻¹ (diet grower) and from 106 to 125 g kg⁻¹ (diet finisher). In turn, the determination of the share of digestible protein in the total protein of diets was at a similar level. In grower diets, this share ranged from 83.09 to 83.56%, while in diets for the finisher pigs it made up to 80.73 to 82.50%. This was due to the fact that the R and R+AA diets (grower/finisher) had a lower concentration of total protein resulting from the experimental factor used.

Crude protein digestibility and N balance are presented in Tables 3 and 4. An analysis of the two-phase feeding system revealed that a reduction in the inclusion levels of crude protein and total lysine (the first limiting amino acid for pigs) in grower diets significantly decreased protein digestibility, from 82.9% in control group C to 78.4% in group R. The supplementation of a low-protein diet (group R vs. R+AA) with crystalline lysine, methionine, threonine and tryptophan increased the coefficient of crude protein digestibility from 78.4% to 83.3% ($P \leq 0.05$). The experimental factors (dietary

Table 3
Utilization of nitrogen (N) from diets in the growing period (experiment I)

Specification	Experimental diets Grower [#]			SEM	Level of significance $P \leq$		
	C	R	R+AA		C vs. R	R vs. R+AA	C vs. R+AA
Number of pigs in the experimental group (<i>n</i>)	8	8	8				
N x 6.25 digestion coefficient (%)	82.9	78.4	83.3	0.872	*	*	ns
Daily nitrogen balance							
N intake (g kg ⁻¹)	67.4	58.4	58.6	1.264	**	ns	**
N excreted in feces (g kg ⁻¹)	11.5	12.6	10.3	0.468	ns	ns	ns
N in feces/N intake (%)	17.1	21.6	17.6	0.387	*	*	ns
N digested (g kg ⁻¹)	55.9	45.6	48.3	0.956	**	ns	**
N excreted in urine (g kg ⁻¹)	26.8	18.2	19.3	1.268	**	ns	**
N in urine/N intake (%)	39.8	31.2	32.9	0.765	**	ns	**
N in urine/N digested (%)	47.9	39.9	32.9	0.618	*	**	**
N retention (g kg ⁻¹)	29.3	27.5	29.0	0.459	ns	ns	ns
N retention/N intake (%)	43.5	47.1	49.5	0.996	*	ns	*
N retention/N digested (%) ABV	52.4	60.1	60.6	1.408	*	ns	*

[#] see Table 1, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, ns – no statistical differences. The figures given in the table for the analyzed feature are arithmetic means of 8 individual samples.

Table 4

Utilization of nitrogen (N) from diets in the finishing period (experiment II)

Specification	Experimental diets Finisher [#]			SEM	Level of significance $P \leq$		
	C	R	R+AA		C vs. R	R vs. R+AA	C vs. R+AA
Number of animals in the experimental group (<i>n</i>)	8	8	8				
N x 6.25 digestion coefficient (%)	81.4	80.3	82.1	0.514	ns	ns	ns
Daily nitrogen balance							
N intake (g kg ⁻¹)	73.7	63.4	62.4	1.543	**	ns	**
N excreted in feces (g kg ⁻¹)	13.7	12.4	11.2	0.433	ns	ns	*
N in feces/N intake (g kg ⁻¹)	18.6	19.6	17.9	0.856	ns	ns	ns
N digested (g kg ⁻¹)	60.0	51.0	51.2	0.511	**	ns	**
N excreted in urine (g kg ⁻¹)	32.7	25.7	24.8	0.346	**	ns	**
N in urine/N intake (%)	44.4	40.5	39.7	0.945	**	ns	**
N in urine/N digested (%)	54.5	50.4	48.4	0.484	*	ns	**
N retention (g kg ⁻¹)	27.3	25.2	26.4	0.560	ns	ns	ns
N retention/N intake (%)	37.0	39.7	42.3	1.005	ns	ns	*
N retention/N digested (%) ABV	45.5	49.4	51.6	0.846	ns	ns	*

[#] see Table 1, * significant at $P \leq 0.05$; ** significant at $P \leq 0.01$, ns – no statistical differences. The figures given in the table for the analyzed feature are arithmetic means of 8 individual samples.

inclusion levels of crude protein and total lysine) had a positive effect on protein digestibility also in finisher diets, where the coefficient of crude protein digestibility was high at 81.4%, 80.3% and 82.1%, respectively.

In a study by WIĘCEK et al. (2006), crude protein digestibility was also affected by the levels of crude protein and amino acids in pig diets, and it exceeded 80%. However, KERR et al. (2003) demonstrated that feeding diets with the protein content reduced from 16% to 12% decreased protein digestibility by 5.3% in grower pigs without compromising the effect of fattening. According to some authors, e.g. LEEK et al. (2005), GRELA and KOWALCZUK-VASILEV (2010), protein digestibility increases with increasing dietary protein inclusion levels owing to higher digestibility of supplemental protein ingredients. This was demonstrated by the increase in N excretion in feces, especially when using the grower diets. In the present study, the supplementation of low-protein diets (R+AA) with crystalline lysine, methionine, threonine and tryptophan improved crude protein digestibility. It also reduced the excretion of nitrogen in the feces, both in the fattening phase grower and finisher, respectively from 12.6 to 10.3 g kg⁻¹/day and 12.4 to 11.2 g kg⁻¹/day (Tables 3 and 4).

The analyzed experimental factor had an effect on urinary nitrogen excretion. In both grower and finisher diets, lowering the total protein content reduced the excretion of this component. Group C feces excreted the most N in urine, 26.8 and 32.7 g kg⁻¹/day, respectively. On the other hand, pigs from group R had statistically significant smallest N excretion in urine in relation to pigs from group C. They were 18.2 g kg⁻¹/day in the fattening phase grower and 25.7 g kg⁻¹/day in the fattening phase finisher. The enrichment of the low-protein experimental diets R+AA with crystalline amino acids (lysine, methionine, threonine and tryptophan) did not have a statistically significant effect on a further reduction of urinary N excretion. The results of our own research indicate that the reduction of urinary nitrogen excretion may indicate a better balance of the amino acid composition of the experimental diets (R and R+AA) protein than the control diet (C) protein. In the research by SHRIVER et al. (2003) it was shown that supplementing the low-protein diet with lysine, methionine, threonine, tryptophan, and isoleucine and valine reduced the excretion of N in feces and urine. The KASPROWICZ-POTOCKA, FRANKIEWICZ (2011) studies showed that lowering the protein level in grower and finisher mixtures of pigs by 10 and 20%, respectively, significantly reduced urinary N excretion.

Both experimental factors, i.e. the dietary inclusion levels of crude protein and essential amino acid (total lysine, methionine with cystine), threonine, tryptophan) in grower and finisher diets, affected N retention in pigs (Tables 3 and 4). The lowest N retention was observed in pigs fed low-protein diets (group R) during both feeding phases, compared with those receiving control diets C. However, the values of the analyzed parameter were high (27.5/25.2 vs. 29.3/27.3 g kg⁻¹/day). The supplementation of low-protein diets with crystalline lysine, methionine, threonine and tryptophan (group R+AA) increased N retention in pigs from 27.5 to 29.0 g kg⁻¹/day and from 25.2 to 26.4 g kg⁻¹/day. The differences in mean values between experimental groups were not statistically significant.

A 15% decrease in the crude protein content of pig diets during the first and second phase significantly improved the apparent biological value (ABV) of protein (a ratio of N retention to N digestion). Pigs fed diets C with standard levels of crude protein and limiting amino acids were characterized by the lowest utilization of N digested (grower diets - 52.4%, finisher diets - 45.5%). Pigs fed diets R where crude protein levels were reduced by 15% relative to diets C were characterized by significantly higher utilization of N digested, which increased from 52.4% to 60.1% in the first phase of feeding and from 45.5% to 49.4% in the second phase. The supplementation of low-protein diets with essential amino acids (group R+AA) contributed to a further, significant increase in the utilization of N digested, particularly during the first phase. A similar relationship was also observed by GRELA and KOWALCZUK-VASILEV (2010). The cited authors found that crystalline lysine, methionine, threonine and tryptophan added to diets with reduced

Table 5

Nitrogen (N) excretion into the environment in two-phase fattening system depending on the level of protein and limiting amino acids (lysine, methionine, threonine, tryptophan)⁺

Specification	Experimental diets Grower/Finisher		
	C [#]	R [#]	R+AA [#]
N intake (g kg ⁻¹)	987.7	852.6	847.0
N excreted in feces (g kg ⁻¹)	176.4	175.0	150.5
N excreted in urine (g kg ⁻¹)	416.5	307.3	308.7
N excreted in feces and urine (g kg ⁻¹)	592.9	482.3	459.2
Reduction of nitrogen excretion into the environment depending on:			
from a 15% reduction in total protein and limiting amino acids			
(g kg ⁻¹)	0.0	-110.6	nd
(%)	100	81.3	nd
enrichment of low-protein diets with limiting amino acids:			
(g kg ⁻¹)	nd	0.0	-23.1
(%)	nd	100	95.2
enrichment of low-protein diets with limiting amino acids to the level as in standard feed:			
(g kg ⁻¹)	0.0	nd	-133.7
(%)	100	nd	77.5

⁺ data in the table are calculated arithmetic means, computed per fattening pig from the data obtained in digestibility-balance experiments I and II carried out for grower/finisher diets.

[#] see Table 1, nd – not applicable

(by 20%) protein content led to a 2% increase in the apparent biological value of protein.

The effect of protein and essential amino acid concentrations in grower and finisher diets on N excretion to the environment is shown in Table 5. It was estimated that N intake was highest (987.7 g kg⁻¹) in a pig fed control grower and finisher diets (C) with the highest crude protein content. The lowest N intake (847 g kg⁻¹) was noted in a pig fed diets with reduced protein content, supplemented with crystalline lysine, methionine, threonine and tryptophan (group R+AA). It was also found that feeding reduced protein diets (group R) contributed to a decrease in fecal and urinary N excretion. The improvement in protein quality in grower and finisher diets supplemented with crystalline amino acids (group R+AA) decreased fecal N excretion due to higher protein digestibility. However, the improvement in protein quality in low-protein diets supplemented with crystalline amino acids did not contribute to any further decrease in urinary N excretion.

The effect of the dietary inclusion levels of crude protein and essential amino acids (lysine, methionine, threonine and tryptophan) on fecal and urinary N excretion was evaluated based on the numerical data presented in Tables 3 and 4. Total fecal and urinary N excretion was highest (592.9 g kg⁻¹)

in the control group (C). A 15% reduction in the levels of crude protein and total lysine in group R decreased total fecal and urinary N excretion by 110.6 g kg⁻¹ (i.e. 18.7%). The supplementation of low-protein diets with crystalline lysine, methionine, threonine and tryptophan (group R+AA) contributed to a further decrease in fecal and urinary N excretion, by 23.1 g kg⁻¹ (i.e. 4.8%) relative to group R, and by 133.7 g (i.e. 22.5%) relative to group C. The results of studies by KASPROWICZ-POTOCKA, FRANKIEWICZ (2011), ANDRETTA et al. (2016) and WANG et al. (2018) show that feeding reduced protein and amino acids supplemented diets decreases N excretion in pigs to the environment.

CONCLUSIONS

The results of this study indicate that the crude protein content of complete diets for growing-finishing pigs, in two-phase fattening, can be reduced by 2-2.5% (approx. 15%) relative to the standard level for this type of compound feed. Enrichment of the reduced level of total protein with amino acids limiting the protein for pigs (lysine, methionine threonine and tryptophan) to a standard level remains a necessary condition. This allows one to achieve high protein digestibility of compound feed, high nitrogen retention in the pig's body and decreased N excretion to the environment.

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