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Nitrogen retention and growth performance of 25 to 50 kg pigs fed diets of two protein levels and different ratios of digestible threonine to lysine*

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ABSTRACT

The effect of supplementing threonine (Thr) to wheat-based diets containing about 18 (HP) and 16% (LP) protein was determined in pigs with high growth potential (barrows of synthetic line 990). Two basal, Thr deficient HP and LP diets of about 13 MJ ME/kg were used in digestibility experiment for determination of true ileal digestible amino acids content. Based on these results, four HP diets containing 0.86% true ileal digestible lysine and four Thr levels, and two LP diets with two Thr levels were formulated for N balance experiment (Trials 1 and 2, respectively) from the same ingredients and crystalline amino acids. In HP diets true digestible threonine:lysine ratios were 0.53, 0.60, 0.65 and 0.70, and in LP diets were of 0.53 and 0.65. Each trial was performed during 35 days on 6 pigs (per diet) in the range of body weight (BW) from about 25 to 50 kg with N balance at 35 and 45 kg of BW. In pigs fed on HP diets no response was found to increasing Thr, either in N retention or in pig performance. In contrast, in pigs fed the LP diets, Thr supplementation increased daily N retention from 19.2 to 20.7 g ($P \leq 0.03$) at 35 kg of BW. The effect of Thr supplementation on pig performance was not significant, however, it tended to increase average daily gain (774 vs 798 g) and lower feed/gain ratio (2.03 vs 1.97). The pigs fed LP diet excreted about 22% N less than the pigs fed on HP diet. In conclusion, growing pigs with high lean gain potential can optimize their nitrogen retention and growth performance on diets with protein level decreased to 16% and supplemented to contain about 0.86 and 0.56% of true ileal digestible lysine and threonine, respectively.

KEY WORDS: digestible threonine, nitrogen balance, pig performance

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INTRODUCTION

One of the most important factors affecting the protein utilization by pigs is the balance of essential amino acids (AA) in the dietary protein. Recently, a reduction in the quantity of protein fed to pigs has been highlighted to prevent increasing environmental pollution. When reducing the dietary protein, a correct proportion of individual AA in the diet has become more significant.

Threonine (Thr) is usually the second or third-limiting AA in practical pig diets with reduced protein level. Large variations exist among the reported requirements for pigs in a given weight range: for grower pigs (25 to 50 kg) the Thr requirement ranges from 0.39 to about 0.70% of the diet (Sowers and Meade, 1972; Taylor et al., 1982; Rademacher et al., 1999). These variations may result from a wide variability in ileal digestibility of Thr among common feedstuffs (Knabe et al., 1989; Conway et al., 1990; Schutte et al., 1997). Formulation of pig diets on a digestible AA basis would yield more uniform values and result in more precise diet formulation.

Also, recommendations expressed as dietary threonine:lysine (Thr:Lys) ratio differ and for grower pigs range from 0.60 (ARC, 1981) to 0.75 (Wang and Fuller, 1989). The optimum Thr:Lys ratio is not a constant value; it likely varies with lean growth rate, feeding level, and possibly diet composition (Sève et al., 1993; NRC, 1998; Moughan, 1999).

This study was conducted to evaluate the effectiveness of supplementing threonine to wheat-based diets, with normal and reduced protein content (18 or 16%), fed to growing pigs (25 to 50 kg) with high lean growth potential. The objective of the present study was also to determine the optimal Thr:Lys ratio that would maximize N retention and growth performance in pigs of about 35 and 45 kg of body weight (BW).

MATERIAL AND METHODS

Animals and feeding

Pigs with a high lean gain potential (390 g/d from 25 to 100 kg BW, synthetic line 990, Poland) were used in ileal digestibility and in nitrogen balance (two trials) experiments. The pigs were housed individually in metabolism cages with a slatted floor in an environmentally controlled room (22°C) and were given *ad libitum* access to water. Pigs were fed diets containing wheat and three protein feeds (soyabean meal, full fat soyabean and rapeseed meal). The energy was calculated based on the chemical composition of dietary ingredients (Table 1) using equations and digestibility coefficients as given in DLG (1991).

The experiments reported here were approved by the Local Animal Care Commission.

Table 1. Chemical composition of dietary ingredients (% DM) used to formulate diets containing about 18 (Trial 1) or 16% (Trial 2) crude protein

Item	Wheat		Soyabean meal	Soyax[1]		Rapeseed meal	
	Trial 1	Trial 2	Trials 1 and 2	Trial 1	Trial 2	Trial 1	Trial 2
Dry matter	86.5	87.3	87.9	89.4	88.3	88.1	89.3
Crude protein	15.3	12.8	49.3	37.5	38.1	36.3	37.4
Ether extract	1.64	1.87	2.04	22.2	22.9	5.81	2.33
Ash	1.84	1.67	8.34	5.32	5.78	6.96	7.01
Starch	53.3	54.9	3.04	2.81	1.19	4.53	1.38
Sugars	4.09	4.35	9.90	8.94	7.02	10.6	8.15
Crude fibre	2.86	3.52	6.83	5.85	6.89	14.3	12.5

[1] full fat soya

Digestibility experiment

Using ingredients presented in Table 1, two basal diets containing about 13.0 MJ ME and differing in protein level (about 18 and 16% of protein) were formulated for determination of true ileal digestibility of amino acids (Table 2) and then (after AA supplementation) for N balance experiment.

Based on AA analysis of feed ingredients and on digestibility coefficients given in Ami Pig (2000), the diets were designed to contain deficient level (about 0.47%) of true digestible Thr. For calculation the true digestibility of AA, protein-free diet was prepared according to Jondreville et al. (2001).

The experiment was carried out on barrows with initial BW of about 25 kg. After 7 days of adaptation, the pigs were surgically fitted with a post-valvular T-caecum cannula (PVTC) according to van Leeuwen et al. (1991). During the first ten days after cannulation, the animals were fed with increasing quantity of the basal diets. The recovery period was followed by 7-d periods of feeding the pigs with the basal diets (twice daily at 08.00 and 20.00) and at the end of the experiment with the protein-free diet. During the last 3-d of each experimental period, ileal digesta was collected using bags attached to the cannulas. The bags were changed approximately every hour and their content was immediately frozen at -20°C. After the collection, samples were thawed, pooled per animal within each experimental period, freeze dried and ground (0.5 mm) before chemical analysis. Each diet was fed to 6 animals.

Table 2. Composition of basal diets (for Trials 1 and 2) and protein free diet used in digestibility experiment, %[1]

Diet	Basal		Protein-free
	Trial 1	Trial 2	
Ingredient			
Wheat	77.00	77.00	-
soyabean meal	6.00	6.00	-
soyax [2]	7.00	7.00	-
rapeseed meal	6.00	5.80	-
maize starch	0.70	0.93	59.03
sucrose	-	-	30.00
soya oil	-	-	3.00
dicalcium phosphate	1.30	1.30	2.00
cellulose	-	-	3.50
limestone	0.90	0.87	0.44
NaCl	0.30	0.30	0.38
KCl	-	-	0.27
vit-min. premix, Optamix, PT-1-bis [3]	0.50	0.50	1.00
choline	-	-	0.08
Cr_2O_3	0.30	0.30	0.30
Nutrients analysed			
crude protein	17.25	15.80	0.33
total lysine	0.684	0.682	-
total methionine	0.267	0.235	-
total threonine	0.576	0.562	-
total tryptophan	0.190	0.179	-

[1] similarly composed diets supplemented in the place of starch with crystalline amino acids, were used in N balance experiment

[2] full fat soya

[3] premix Optamix contained in 1 kg: vit. A, 1,800,000 IU; vit. D_3, 400,000 IU; mg: vit. E, 6,000; vit. B_1, 300; vit. B_2, 700; biotin, 4; vit. B_6, 600; vit. B_{12}, 4; vit. K, 400; niacin, 4,008; folic acid, 50; Ca-pantothenate, 3,000; choline, 12,000; Mg, 30,000; Mn, 8,000; Zn, 17,000; Co, 100; Se, 60; Cu, 4,000; Fe, 20,000; J, 200; g: Ca, 267

Nitrogen balance experiment

Based on the results of amino acid digestibility estimation (Table 3), the diets containing 0.86 % true digestible lysine and different levels of threonine were formulated from the same ingredients and crystalline amino acids. The levels of the others indispensable AA were verified to be provided at ideal protein profile (Rademacher et al., 1999).

Four diets used in Trial 1 (18% protein) were designed to contain increasing true digestible Thr:Lys ratio of 0.53, 0.60, 0.65, and 0.70 and two diets used in

Trial 2 (16% protein) were of the ratios 0.53 and 0.65. Trial 2 followed Trial 1 and dietary treatments were replicated six times. Each trial was performed during 35 days on barrows in the range of BW from about 25 to 50 kg. For this purpose, barrows of about 20 kg initial BW were fed three times daily (08.00, 14.00 and 20.00) and allowed 10-d adjustment to the cages and to the experimental diets. The diet adjustment period was followed by two consecutive N balance periods at approximately 35 and 45 kg of BW when urine and faeces were collected during 6 days. The respective dietary allowances were 1.4 and 1.7 kg daily during the first and the second balance periods what means that the feeding level was about 0.90 *ad libitum*. Faeces were collected five times daily and were stored at -20°C. At the end of each collection period faeces was thawed, mixed and subsampled for subsequent analysis. Urine was collected continuously over the period in plastic container to which H_2SO_4 was added daily. Urine volume was measured and recorded daily and a 10% aliquot was stored (4°C) until it was analysed. During the 35-d N balance experiment, average daily gain (ADG) and feed:gain ratio (F/G) were measured.

Table 3. True ileal digestibility of amino acids of the basal diets (%) used in Trial 1 and Trial 2 and endogenous amino acid losses (g kg^{-1} DM intake) during feeding the protein-free diet (mean ± SD)

Amino acids	Basal diets Trial 1	Basal diets Trial 2	Protein-free diet amino acid losses
Lys	76.9 ± 1.2	75.4 ± 1.8	0.35 ± 0.08
Met	83.7 ± 2.0	86.2 ± 1.3	0.12 ± 0.03
Cys	83.1 ± 1.9	79.2 ± 1.4	0,21 ± 0.04
Thr	78.6 ± 1.7	75.6 ± 2.0	0.54 ± 0.07
Trp	80.4 ± 1.2	79.5 ± 1.5	0.13 ± 0.03
Ile	82.9 ± 1.1	81.4 ± 1.1	0.27 ± 0.06
Arg	88.0 ± 1.0	86.9 ± 1.3	0.38 ± 0.04
His	86.2 ± 1.2	84.4 ± 1.2	0.16 ± 0.02
Leu	84.4 ± 1.1	83.1 ± 1.3	0.46 ± 0.09
Phe	87.2 ± 0.9	83.0 ± 1.4	0.25 ± 0.05
Val	81.0 ± 1.3	79.1 ± 1.6	0.38 ± 0.07

Chemical analysis and calculations

Dry matter, N, ether extract, ash, total starch, sugar and crude fibre were analysed using standard methods (AOAC, 1990). The amino acid content of the feeds, diets and of digesta samples were determined according to procedures described by Buraczewska and Buraczewski (1984) using Beckman 6300 High Pressure Amino Acid Analyzer.

Calculation of AA ileal digestibility was based on chromium oxide (a marker) using equations given by Buraczewska et al. (1999) and Rademacher et al. (1999). Nitrogen retention (g) was calculated as: N intake (NI) - (faecal N output [NF] + urinary N output [NU]). Nitrogen retention (%) was calculated as: NI – [(NF + NU)/ NI × 100, and N digestibility was calculated as: [(NI – NF)/NI] × 100.

Data were subjected to statistical analysis using a completely randomized design according to the GLM procedure of Statistica 5.5 (Statsoft, Inc., Tulsa, OK). Individual pig was considered the experimental unit. One-way ANOVA was used to test the main effects of Thr:Lys ratio on daily (g and %) N retention, ADG and F/G, respectively in each experiment. Orthogonal polynomial contrasts were used to evaluate increasing Thr:Lys ratio in Trial 1.

RESULTS AND DISCUSSION

Digestibility experiment

The pigs remained healthy and consumed their meal allowances throughout the experiment. Postmortem examination, conducted at the conclusion of the experiment, revealed no intestinal abnormalities.

Amino acid digestibility of the basal diets is given in Table 3. Determination of the endogenous AA losses allowed to calculate true digestibility coefficients. The losses were found to be in the range estimated by many other authors using different methods, however, in comparision with average basal losses, recommended for calculation the standardized digestibility coefficients (Rademacher et al., 1999), our values were lower for majority of AA, i.e. for lysine (0.35 vs 0.40) for threonine (0.54 vs 0.60) and for tryptophan (0.13 vs 0.14 g/kg DM intake).

Amino acid digestibility of the basal diets were close to the values calculated on the basis of chemical analysis of dietary ingredients and their tabular digestibility of amino acids. However, true digestibility coefficients of the lower protein diet were 1-4 percentage units lower, than of the higher protein diet, in spite of similar composition. But, different batches of ingredients were used for the diets (Table 1). It is known that within each feedstuff, ileal digestibility of amino acids may differ considerably and discrepancies observed between studies to determine total Thr requirement may relate to differences in Thr digestibility in the basal diets among experiments (Conway et al., 1990; Schutte et al., 1997).

Nitrogen balance experiment

Generally, pigs in both trials appeared to be in good health and readily consumed their daily allowances.

In the nitrogen balance experiment, there was a need to supplement the diets not only with threonine according to experimental design, but also to enrich all diets with lysine to anticipated level of 0.86% true digestible lysine, and with some methionine too. The actual content of the main digestible amino acids in the diets prepared to feed pigs in the Trials 1 and 2 is given in Table 4.

Table 4. Actual content (%) of total and true ileal digestible amino acids in experimental diets containing different digestible threonine:lysine ratios used in Trials 1 and 2, as fed basis

Thr:lys ratio[1]	Trial 1				Trial 2	
	0.53[2]	0.60	0.65	0.70	0.53[3]	0.65
Crude protein	17.9				16.3	
Total lysine	1.04				1.05	
Total threonine	0.59	0.65	0.70	0.74	0.56	0.69
Total methionine	0.34				0.35	
Total tryptophan	0.19				0.18	
Digestible lysine	0.87				0.86	
Digestible threonine	0.47	0.53	0.57	0.61	0.43	0.56
Digestible methionine	0.30				0.32	
Digestible tryptophan	0.15				0.14	
Actual Thr:Lys ratio	0.54	0.60	0.65	0.70	0.50	0.65

[1] anticipated ratios

[2] this basal diet was supplemented per kg with 0.58, 1.01 or with 1.44 g crystalline Thr to receive the respective three diets with increasing Thr:Lys ratios of 0.60, 0.65 and 0.70

[3] this basal diet was supplemented per kg with 1.3 crystalline Thr to receive the diet with increased Thr:Lys ratio of 0.65

Trial 1. Nitrogen balance in pigs fed with higher protein diet (18%) determined at a body weight of about 35 and 45 kg, is presented in Table 5. There was no response in N digestibility and in N retention to increasing the Thr:Lys ratio from 0.53 to 0.70. At the lowest threonine concentration, average N retention was 19.8 and 23.3 g in pigs at body weight of about 35 and 45 kg, respectively. Daily N excretion during the successive balance periods was about 20 and 25 g.

No significant effect of increasing Thr:Lys ratio on ADG (743-774 g) and F/G were observed (Table 6). Feed conversion efficiency was high in all groups and ranged from 2.03 to 2.13 kg/kg.

Trial 2. Nitrogen balance in pigs fed diets containing 16.3% protein is shown in Table 7. At 35 kg of BW, increasing Thr:Lys ratio increased daily N retention from 19.2 to 20.7 g ($P \le 0.03$). Furthermore, increasing Thr:Lys ratio increased also daily N retention expressed as a percentage of daily N intake or daily N absorbed. Nitrogen digestibility was the same (83.7%) in both groups of pigs.

Table 5. Nitrogen balance in pigs fed four true ileal digestible threonine:lysine ratios (0.53, 0.60, 0.65 and 0.70) determined at a body weight of about 35 and 45 kg[1], Trial 1

Item	Threonine:lysine ratio				SEM	Effect (*P*-value)	
	0.53	0.60	0.65	0.70		main	linear
Pigs, 35 kg BW[1]							
N intake, g/d	40.1	40.2	40.3	40.3			
N digestibility, %	82.5	84.2	82.5	83.6	0.441	0.475	0.643
N excretion, g/d	20.3	19.6	19.9	20.1	0.180	0.674	0.778
N retention, g/d	19.8	20.6	20.4	20.3	0.180	0.490	0.441
N retention, %[2]	49.4	51.2	50.7	50.3	0.446	0.588	0.825
N retention, %[3]	59.9	60.8	60.7	60.9	0.512	0.906	0.565
Pigs, 45 kg BW[1]							
N intake, g/d	48.7	48.8	48.9	49.0			
N digestibility, %	85.1	85.2	85.7	85.7	0.413	0.933	0.551
N excretion, g/d	25.2	25.5	24.1	26.0	0.342	0.244	0.734
N retention, g/d	23.5	23.4	24.8	23.0	0.339	0.249	0.968
N retention, %[2]	48.3	47.9	50.7	47.0	0.696	0.250	0.857
N retention, % [3]	56.8	56.2	59.1	54.9	0.837	0.324	0.727

[1] each mean represents six barrows per treatment
[2] expressed in % of N intake
[3] expressed in % of digested N intake

Table 6. Pig performance during 35-d N balance experiment after feeding four true ileal digestible threonine:lysine ratios (0.53, 0.60, 0.65 and 0.70)[1], Trial 1

Item	Threonine:lysine ratio				SEM	Effect (*P*-value)	
	0.53	0.60	0.65	0.70		main	linear
Initial BW, kg	25.4	25.5	25.4	25.1			
Final BW, kg	51.9	51.5	52.0	52.2			
ADG, g	759	743	762	774	8.90	0.697	0.436
F/G, kg/kg	2.07	2.13	2.07	2.03	0.02	0.538	0.392

[1] each mean represents six barrows per treatment

At 45 kg of BW, N retention tended to increase when dietary Thr level increased. Similarly to Trial 1, N digestibility was not affected by Thr level, but increased with BW on average about 2 digestibility units. In both balance periods, total N excretion was on average about 22% lower than in Trial 1.

No significant effect of increasing Thr:Lys ratio on ADG and F/G were observed (Table 8), however, numerically higher ADG (774 vs 798 g) and lower F/G (2.03 vs 1.97) were found in pigs fed Thr supplemented diet, as compared to the basal diet.

Table 7. Nitrogen balance in pigs fed two true ileal digestible threonine:lysine ratios (0.53 and 0.65) determined at a body weight of about 35 and 45 kg[1], Trial 2

Item	Threonine:lysine ratio		SEM	Main effect
	0.53	0.65		*P*-value
Pigs, 35 kg BW				
N intake, g/d	36.5	36.7		
N digestibility, %	83.7	83.7	0.470	0.982
N excretion, g/d	17.3	16.0	0.340	0.039
N retention, g/d	19.2	20.7	0.354	0.026
N retention, %[2]	52.6	56.5	0.946	0.032
N retention, %[3]	62.8	67.5	1.108	0.027
Pigs, 45 kg BW				
N intake, g/d	44.4	44.5		
N digestibility, %	85.8	86.7	0.427	0.669
N excretion, g/d	20.8	19.0	0.585	0.141
N retention, g/d	23.6	25.5	0.599	0.111
N retention, %[2]	53.2	57.3	1.329	0.127
N retention, % [3]	62.0	66.5	1.380	0.108

[1] each mean represents six barrows per treatment
[2] expressed in % of N intake
[3] expressed in % of digested N intake

Table 8. Pig performance during 35-d N balance experiment after feeding two true ileal digestible threonine:lysine ratios (0.53 and 0.65)[1], Trial 2

Item	Threonine:lysine ratio		SEM	Main effect
	0.53	0.65		*P*-value
Initial BW, kg	24.9	24.9		
Final BW, kg	52.0	52.9		
ADG, g	774	798	9.49	0.219
F/G, kg/kg	2.03	1.97	0.02	0.221

[1] each mean represents six barrows per treatment

The results of Trial 1 are consistent with many other observations. In trials carried out on growing pigs by Schutte and de Long (1955, according to Le Bellego et al., 2002) and Chang et al. (2000), pig performance did not respond to changes in ileal digestible Thr:Lys ratio ranging from about 0.53 to above 0.65 when maize or maize and barley based diets (supplemented accordingly with protein bound and crystalline AA) were fed to the animals. Also, in experiment reported by Taylor et al. (1982) no positive effect in growth performance was found above the ratio of 0.53 when pigs were fed low protein diet (12.9%) based exclusively on barley supplemented with soyabean and fish meals and with AA. When expressing the total

Thr level as dietary concentration, the requirement was 0.56% and was similar to the requirement of boars (0.57%) found by Lenis et al. (1990).

On the other side, the positive response of pigs in Trial 2 supports considerable part of literature data. According to Prokop (1996) increasing Thr:Lys ratio from 0.50 to 0.60 increased protein deposition by about 4.5 percentage units in meat-type grower pigs. Optimal Thr:Lys ratio was above 0.60, both for weight gain and feed conversion efficiency in experiment of Sève et al. (1993) on growing pigs fed diets based on wheat with similar to Trial 2 protein level. In later experiment on net efficiency of digestible AA for protein deposition in growing pigs (20 to 50 kg of BW), Sève et al. (1995) estimated, that daily whole body retention of N and of most AA reached maximum response at 0.58% dietary true digestible Thr. This value corresponds with the level of digestible Thr of 0.56%; found to be adequate in the Thr supplemented diet in Trial 2. The estimates of Ettle et al. (2004) to optimize growth performance of meat-type pigs (35-65 kg of BW) correspond to similar dietary concentration of 0.57% true digestible Thr and at least 0.67% total Thr.

The supplemented diet in Trial 2 contained 0.69% total Thr. Similar level of this AA (0.68%) was found to be optimal for weight gain of barrows and gilts during the body weight range of approximately 20 to 40 kg (Schutte et al., 1990). Additionally, almost the same performance was achieved on the negative control diet supplemented with the limiting AA and containing 16% protein as compared to the positive control diet containing 18.5% protein. Comparing the higher (18%) and the lower protein (16.4%) diets used respectively in Trial 1 and Trial 2 of the nitrogen balance experiment, even better protein deposition and growth performance were obtained after feeding pigs with the lower protein, threonine supplemented diet. This can be explained by the reduction of net energy in the high-protein diet due to excess AA deamination and N elimination (van Lunen and Cole, 1996).

Due to the decrease dietary protein level, substantial reduction of N excretion was found in this study (on average 22%). The practice potentials, however, depend on the price relations between protein-bound AA in feed components and crystalline AA.

CONCLUSIONS

Our results suggest that the protein level in diets based mainly on wheat and soya feeds can be reduced to about 16% and supplemented with lysine and threonine for growing pigs. Our data also show that pigs with high lean gain potential, from 25 to 50 kg of body weight, can optimize their nitrogen retention and growth performance at a true ileal digestible threonine: lysine ratio of about

0.65. Corresponding dietary level for digestible threonine is 0.56% and for digestible lysine 0.86%.

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