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Effects of dietary crude protein level and meal frequency on energy utilization in growing pigs[1]

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SUMMARY – Three trials were conducted with 60 kg growing pigs in order to evaluate the effects of reducing the dietary crude protein (CP) content, in association with industrial amino acids supplementation (lysine, methionine, threonine, tryptophan, isoleucine and valine), on digestive and metabolic utilization of nitrogen and energy. In trial 1, four diets ranging in CP level between 18.9 and 12.3% were used, whereas in trials 2 and 3, two diets with 17.4 and 13.9% CP were used. In trials 1 and 2, the feed was given as 4 meals per day whereas in trial 3, 2 frequencies (2 and 7 meals/day) were compared. The results show that decreasing the CP content allows a reduction of nitrogen excretion by 40 (trials 2 and 3) or 60% (trial 1), without affecting the nitrogen retention. This reduction in nitrogen excretion was accompanied by both a reduction in urinary energy losses and of heat production equivalent to 3.5 and 7 kJ per gram decrease in protein intake, respectively. These data confirm the lower energy utilization of CP supplied above the pig's requirement and agree with the conclusions of the net energy system. Meal frequency (trial 3) had no effect on nitrogen and energy utilization.

Key words: Growing pigs, dietary protein, heat production, net energy.

RESUME – *"Effets du niveau de protéine brute alimentaire et de la fréquence des repas sur l'utilisation énergétique chez les porcs en croissance". Trois essais ont été menés sur des porcs en croissance de 60 kg afin d'évaluer les effets de la réduction de la teneur en protéine brute alimentaire (CP), associée à une supplémentation en acides aminés industriels (lysine, méthionine, thréonine, tryptophane, isoleucine et valine), sur l'utilisation digestive et métabolique de l'azote et l'énergie. Dans l'essai 1, on a mis en place quatre régimes dont le niveau de CP allait de 18,9 à 12,3%, tandis que dans les essais 2 et 3 on a utilisé deux régimes avec une CP de 17,4 et 13,9%. Dans les essais 1 et 2, l'aliment a été distribué à raison de 4 repas par jour tandis que dans l'essai 3, on a comparé 2 fréquences (2 et 7 repas/jour). Les résultats montrent qu'une diminution de la teneur en CP permet une réduction de l'excrétion d'azote de 40% (essais 2 et 3) ou 60% (essai 1), sans affecter la rétention d'azote. Cette réduction de l'excrétion d'azote était accompagnée d'une réduction des pertes énergétiques par l'urine et également d'une diminution de la production de chaleur équivalente à 3,5 et 7 kJ par gramme de diminution de l'ingestion protéique, respectivement. Ces données confirment l'utilisation énergétique plus faible de la CP apportée au-dessus des besoins des porcins et sont en accord avec les conclusions du système d'énergie nette. La fréquence des repas (essai 3) n'a pas eu d'effet sur l'utilisation de l'azote et de l'énergie.*

Mots-clés : *Porcs en croissance, protéine alimentaire, production de chaleur, énergie nette.*

Introduction

Recent advances in the determination of amino acid requirements for pigs and the increased availability of industrial amino acids allow to reduce the protein content in the feed with no negative effects on nitrogen retention and on animal performance but with marked reductions of nitrogen excreted in the environment (Bourdon *et al.*, 1995; Jondreville *et al.*, 1995; Canh *et al.*, 1998). It has been demonstrated that lowering the protein level of the feed reduces the energy losses in urine and as heat (Noblet *et al.*, 1987; Quiniou *et al.*, 1995). According to the net energy system proposed by Noblet *et al.* (1994), substitution of dietary proteins by starch and/or fat reduces heat production and increases the net energy value of the feed. However, the net energy system was established using higher crude protein levels (19.8% in average) than what is currently used for growing pigs. Therefore, the advantage of low protein diets on energy utilization need to be confirmed. Furthermore, a reduction of free amino acids utilisation has been reported in combination with low feeding frequencies (one or two meals per day), resulting in decreased animal performance (Batterham and Bayley, 1989).

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Three trials were conducted to measure the effect of reducing the crude protein level in the feed on heat production and energy utilisation according to meal frequency, in growing pigs. In the first trial, the objective was to measure the energy utilisation when part of dietary protein was substituted by starch. The objective of the second trial was to confirm the results of the first trial with diets formulated using conventional feedstuffs. In the third trial, the effect of meal frequency on protein and energy utilisation was tested.

Materials and methods

Diets

In trial 1, four diets were formulated in order to reduce the crude protein level from 18.9% (diet 1) to 12.3% (diet 4), with progressive substitution of soybean protein by corn starch (Table 1). Diets 2, 3 and 4 were supplemented with L-lysine, D/L-methionine, L-threonine, L-tryptophan, L-isoleucine and L-valine to maintain a constant digestible lysine/net energy ratio (0.76 g/MJ) and equilibrium between essential amino acids (Henry, 1993) (Table 2). In trials 2 and 3, two diets were formulated with conventional feedstuffs. The crude protein level was reduced from 17.4% (diet 1) to 13.9% (diet 2) with a partial substitution of soybean meal by wheat and corn. The two diets respected the same nutritional constraints as in trial 1.

Table 1. Composition of experimental diets

Composition (%)	Diets of trial 1				Diets of trials 2 and 3	
	1	2	3	4	1	2
Wheat	40.52	40.52	40.52	40.52	36.85	42.44
Corn	40.53	40.53	40.53	40.53	36.84	42.43
Soybean meal	7.00	7.00	7.00	7.00	23.00	11.00
Soybean proteins	8.70	5.80	2.90	–	–	–
Corn starch	–	2.54	4.98	7.28	–	–
L-lysine	–	0.20	0.39	0.58	0.06	0.43
D/L-methionine	–	0.05	0.11	0.18	–	0.11
L-threonine	–	0.08	0.16	0.25	–	0.16
L-tryptophane	–	0.03	0.05	0.08	–	0.05
L-isoleucine	–	–	0.03	0.14	–	0.04
L-valine	–	–	0.08	0.19	–	0.09
Minerals and vitamins	3.25	3.25	3.25	3.25	3.25	3.25

Animals and experimental design

Five to seven barrows per treatment weighing about 60 kg during the experimental period were used. In trial 1, the animals were allotted to treatments in order to balance live weight and ages between the 4 treatments. In trials 2 and 3, littermates were allotted across diets (trial 2) or across meal frequencies for a given diet (trial 3). In trials 1 and 2, the meal frequency was 4 meals/day (9 h, 13 h, 17 h and 21 h). In trial 3, the meal frequency was either 2 (9 h and 18 h) or 7 (9 h, 12 h, 15 h, 18 h, 21 h, 1 h and 5 h) meals/day. All animals were adapted to experimental conditions, diets and digestibility cages during 2 weeks and received about 1.90 MJ NE/kg of $BW^{0.60}$ after the adaptation period. Feces and urine were collected daily during 8 days. The energy balance was determined using indirect calorimetry (during the last 5 days of the collection period) as described by Noblet *et al.* (1993). Ambient temperature was kept at 24°C and a 13 h lighting schedule was used. Gas exchanges, feed intake and physical activity were measured continuously (van Milgen *et al.*, 1997). After the collection period, animals remained in the respiration chamber for two days to measure the fasting heat production.

Table 2. Chemical composition and nutritional values of experimental diets

	Diets of trial 1				Diets of trials 2 and 3	
	1	2	3	4	1	2
Chemical composition† (%)						
Crude protein	18.9	16.7	14.6	12.3	17.4	13.9
Starch	48.1	50.3	52.5	54.5	45.0	52.2
Crude fibre	1.7	1.7	1.7	1.8	2.4	1.9
Nutritional values††						
DE, MJ/kg	14.24	14.17	14.07	13.97	14.00	13.93
ME, MJ/kg	13.70	13.69	13.65	13.61	13.45	13.50
NE, MJ/kg	10.25	10.35	10.44	10.51	9.96	10.29
Digestible Lysine, g/MJ NE	0.76	0.76	0.76	0.76	0.76	0.76

†Measured values for an average 87.3% dry matter.
††DE: digestible energy; ME: metabolizable energy, calculated according to INRA tables (1989); NE: net energy according to Noblet *et al.* (1994); standardised digestible lysine content according to Eurolysine – ITCF tables (1995), for an average 87.3% dry matter.

Measurements

Animals were weighed at the beginning and at the end of the collection period and after measurement of the fasting heat production. During the total period, feed intake was measured daily. Feces and urine were daily collected and pooled for the total period. Samples of diets, feces and urine were constituted for analyses.

Samples of feed (one per diet) were analysed for energy, nitrogen, starch, fat and crude fibre contents (Table 2), as described by Noblet *et al.* (1989). Samples of feces and urine were analysed for energy and nitrogen contents.

Calculations

Heat production of the animals was calculated from dynamics of O_2 consumption, and CO_2 and CH_4 productions. The energy balance was determined as previously described by Noblet *et al.* (1989). Gas exchanges were analysed with a model developed by van Milgen *et al.* (1997, 2000). Other calculations, adjustments and statistical analyses were described by Le Bellego *et al.* (2000).

Results and discussion

Nitrogen utilization

Results of trial 1 show that a 6.5 points reduction of protein content of the feed resulted in a 60% reduction of nitrogen excretion while maintaining the same level of nitrogen retention (Table 3). The use of more conventional diets (trials 2 and 3) confirmed the possibility to reduce the nitrogen excretion (40% for 3.5 points of crude protein). These results agree with those of Dourmad and Henry (1994) and Canh *et al.* (1998), who proposed a 10% decrease of nitrogen excretion per point crude protein reduction in the feed.

Energy utilization

Results reported in Table 4 for trial 1 and in Table 5 for trials 2 and 3 show that the reduction of nitrogen excretion with the decrease of dietary protein content was associated with a reduction of the quantity of energy lost in urine. An overall analysis of data obtained in the 3 trials (n = 58) indicates that the quantity of energy lost in urine increased by 3.5 kJ per additional gram of protein intake.

Table 3. Effect of dietary crude protein level on nitrogen balance in growing pigs (trial 1)

	Crude protein (%)				RSD[†]	Effect[††]
	18.9	16.7	14.6	12.3		
Nitrogen intake (g/d)	69.7[a]	61.8[b]	54.6[c]	44.7[d]	2.0	D**
Nitrogen excreted (g/d)	37.0[a]	31.1[b]	24.8[c]	15.7[d]	2.0	D**
Nitrogen retained (g/d)	32.8	30.7	29.8	29.0	2.6	ns
Nitrogen retention coefficient	0.51[a]	0.54[a]	0.61[b]	0.72[c]	0.04	D**

[†]RSD: Residual standard deviation.
[††]Statistical significance: analysis of variance using "diet" (D) as variable. Statistical significance: *P < 0.05; **P < 0.01; ns: P > 0.10.
[a,b,c,d]Different superscripts indicate statistically different means (P < 0.05).

Table 4. Effect of dietary crude protein level on energy utilization in growing pigs (trial 1)

	Crude protein (%)				RSD[†]	Effect[††]
	18.9	16.7	14.6	12.3		
Urinary energy (% of DE)	3.80[a]	3.32[b]	2.87[c]	2.27[d]	0.20	D**
Energy balance[†††]						
ME intake	2.61	2.65	2.67	2.60	0.07	ns
Heat production	1.48[a]	1.46[a]	1.42[ab]	1.37[b]	0.06	D*
Retained energy	1.13[a]	1.19[ab]	1.25[b]	1.23[b]	0.06	D*
As proteins	0.39[a]	0.37[ab]	0.36[b]	0.35[b]	0.03	D*
As fat	0.74[a]	0.82[ab]	0.89[b]	0.88[b]	0.06	D*
Energy balance (adjusted for the same DE intake)[†††, ††††]						
Heat	1.27[a]	1.23[ab]	1.19[b]	1.19[b]	0.04	D*
Retained energy	1.13[a]	1.19[b]	1.23[bc]	1.25[c]	0.04	D**
Energy balance (adjusted for the same NEg intake)[†††, ††††, †††††]						
Heat	1.29[a]	1.23[b]	1.19[bc]	1.17[c]	0.04	D**
Retained energy	1.15	1.20	1.22	1.22	0.04	ns

[†]RSD: Residual standard deviation.
[††]See Table 3.
[†††]MJ/day/kg $BW^{0.60}$.
[††††]Adjusted data for zero activity and by analysis of covariance for the same digestible energy (DE) (2.507 MJ/day/kg $BW^{0.60}$) or the same net energy (NEg) (1.796 MJ/day/kg $BW^{0.60}$), using "diet" (D) as main effect. Levels of significance: *P < 0.05; **P < 0.01; ns: P > 0.10.
[†††††]NEg was calculated according to Noblet *et al.* (1994) and adjusted for zero activity.
[a,b,c,d]Different superscripts indicate statistically different means (P < 0.05).

Results reported in Tables 4 and 5 indicate a significant reduction of heat production as a result of the reduction of dietary protein. The lower heat production lead to a higher energy retention as fat. Covariance analysis carried out on heat production data of the 3 trials and adjusted for zero activity and using ME intake and protein intake as covariates showed that heat production increased by about 7 kJ per additional gram of protein intake. This confirms the negative effect of excess dietary protein level on efficiency of utilization of ME (Noblet *et al.*, 1994). The improved utilization of energy with low protein diets is confirmed when data are adjusted for zero activity and for the same energy intake.

Adjustment on a DE basis is associated with a significant effect of diet composition on energy gain while adjustment on a NE basis attenuates markedly the effect of diet. In other words, the DE system, which does not account for changes in energy loss in urine or as heat due to variation in dietary crude protein level, is unable to predict the energy retention by pigs. The use of a NE system is therefore preferable, even though our results suggest that for important reductions of dietary crude protein and high levels of amino acids supplementation, the NE value would be slightly underestimated according to equations proposed by Noblet *et al.* (1994).

Table 5. Effect of dietary crude protein level on energy utilization in growing pigs (trial 2)

	Crude protein (%)		RSD†	Effect
	17.4	13.9		
Urinary energy (% of DE††)	3.79	2.85	0.20	D**
Energy balance (adjusted for the same NEg intake)†††, ††††, †††††				
Heat production	1.25	1.18	0.02	D**
Retained energy	1.03	1.06	0.02	ns

†RSD: Residual standard deviation.
††Statistical significance: analysis of variance using "diet" (D), "replicate" (R) and interaction (D*R) as main effects. Levels of significance: *P < 0.05; **P < 0.01; ns: P > 0.10.
†††MJ/day/kg $BW^{0.60}$.
††††Adjusted data for zero activity and by analysis of covariance for the same digestible energy (DE) (2.349 MJ/day/kg $BW^{0.60}$ for trial 2 and 2.361 MJ/day/kg $BW^{0.60}$ for trial 3) or the same net energy (NEg) (1.678 MJ/day/kg $BW^{0.60}$ for trial 2 and 1.691 MJ/day/kg $BW^{0.60}$ for trial 3), using "diet" (D) and "replicate" (R) for trial 2 and "diet" (D) for trial 3 as main effects. Statistical significance: *P < 0.05; **P < 0.01; ns: P > 0.10.
†††††NEg was calculated according to Noblet *et al.* (1994) and adjusted for zero activity.

Results of Table 6 show that reducing meal frequency from 7 to 2 meals per day did not affect energy utilization and its partitioning between protein and fat deposition. Therefore, the reduced utilization of free amino acids is not confirmed in our study which means that, unlike conclusions of Batterham and Bayley (1989) utilization of amino acids for protein gain was not depressed by partial replacement of protein-bound essential amino acids by free amino acids.

Table 6. Effect of meal frequency on energy utilization of a 13.9% low protein diet in growing pigs (trial 3) (MJ/day/kg $BW^{0.60}$)

	Meals/day		RSD†	Effect††
	2	7		
ME intake	2.51	2.50	0.07	ns
Heat production	1.34	1.35	0.03	ns
Retained energy	1.17	1.16	0.07	ns
As proteins	0.33	0.34	0.02	ns
As fat	0.84	0.82	0.07	ns

†RSD: Residual Standard Deviation.
††Statistical significance: analysis of variance using "diet", "replicate", "meal frequency" and interactions as main effects. Levels of significance: *P < 0.05; **P < 0.01; ns: P > 0.10.

Conclusion

Our results confirm that reducing the crude protein level of the feed, while supplementing with industrial amino acids, allows an important reduction of the quantity of nitrogen excreted in the manure without affecting animals performance. The extent of the reduction depends directly on the commercial availability of amino acids that can be used in practical conditions. Low protein diets also result in a better utilization of energy due to a reduction of urinary energy losses and heat production. In the present study, this additional energy increased the energy retention (particularly fat deposition). These results are in full agreement with the net energy system. It can be recommended to formulate low protein diets on a net energy basis while ensuring optimal ratios between essential amino acids to prevent negative effects on fat deposition and on carcass composition. It was also demonstrated that with at least two meals per day, meal frequency has no effect on nitrogen and energy utilization of low protein diets.

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