

Self-selection of lysine by growing pigs: choice combinations between deficient or suboptimal and adequate or superoptimal dietary levels according to sex

Y Henry

INRA, Station de Recherches Porcines, 35590 Saint-Gilles, France

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Summary — Self-selection of dietary lysine was studied in one 42-d experiment involving 100 Large White growing pigs, with an equal number of females and castrated males, initially weighing 17.7 kg, within 5 treatments of 20 animals in each. A common basal diet (17% CP, 13.8 MJ DE/kg) based on maize, soybean meal and peanut meal, and containing a deficient level of lysine (0.61%), was used. Supplementary L-lysine was provided to obtain suboptimal (0.74%), optimal (0.85%) and superoptimal (1.21%) levels of total lysine for growth, as assumed from usual recommendations. In addition to treatment 1, a control where pigs were fed *ad libitum* a single diet with 0.85% lysine, 4 treatments with free choice of lysine within paired diets were compared. These included deficient or suboptimal levels of lysine on the one hand and optimal or superoptimal levels on the other according to a 2 x 2 factorial plan: 0.61 vs 0.85% (treatment 2), 0.74 vs 0.85% (treatment 3), 0.61 vs 1.21% (treatment 4), 0.74 vs 1.21% (treatment 5). The results showed sex difference in diet selection according to lysine content. Females had a distinct preference for the superoptimal level (1.21%) compared to the optimal level (0.85%), especially during the initial period of the trial, their requirement being higher than the presupposed 0.85% optimal level. For castrated males, the preference for lysine was restricted to the lower 0.85% level. The ability of females to self-select a greater proportion of the high lysine feed as opposed to castrated males was related to a higher potential for lean tissue growth. These results confirm that the growing pig is able to differentiate between diets differing only in their lysine contents. Complementary observations on plasma-free amino acids suggested the use of diets adequately balanced for amino acids when offered for free choice.

pig / growth / sex / lysine / appetite / feed preference

Résumé — Libre choix de la lysine par le porc en croissance : combinaisons de choix entre un taux déficient ou suboptimal et un taux optimal ou supra-optimal selon le sexe. L'étude du libre choix de lysine alimentaire a fait l'objet d'une expérience, d'une durée de 6 semaines, réalisée sur 100 porcs en croissance de race Large White, comprenant un nombre égal de femelles et de mâles castrés et d'un poids vif moyen initial de 17,7 kg, répartis entre 5 traitements de 20 animaux chacun. Un régime de base commun (17% de protéines brutes, 13,8 MJ d'énergie digestible/kg), composé de maïs, tourteau de soja et tourteau d'arachide, et déficient en lysine (0,61%), est utilisé. L'addition de L-lysine permet d'obtenir des teneurs en lysine suboptimale (0,74%), optimale (0,85%) et supra-optimale (1,21%) pour la croissance du porc au stade considéré, d'après les recommandations habituelles. En dehors d'un traitement témoin 1, dans lequel les animaux reçoivent à volonté un aliment unique contenant 0,85% de lysine, 4 traitements sont comparés sur la base du libre choix de lysine entre 2 régimes appariés, correspondant à une combinaison factorielle de type 2 x 2 oppo-

sant un taux déficient ou suboptimal à un taux optimal ou supra-optimal, 0,61 vs 0,85% (traitement 2), 0,74 vs 0,85% (traitement 3), 0,61 vs 1,21% (traitement 4), 0,74 vs 1,21% (traitement 5). Les résultats font apparaître une différence en fonction du type sexuel dans la préférence alimentaire pour la lysine. Les femelles manifestent une préférence marquée pour le taux supra-optimal (1,21%) de lysine par rapport à 0,85%, principalement pendant la phase initiale de l'expérience, avec un besoin supérieur au taux de 0,85% considéré initialement comme optimal. Par contre, chez les mâles castrés, cette préférence est limitée au taux de 0,85% de lysine. L'aptitude des femelles à ajuster leur consommation de lysine à un niveau supérieur à celui des mâles castrés peut être attribuée à un potentiel de dépôt de tissus maigres plus élevé. Ces résultats montrent que le porc en croissance est capable de discriminer les aliments qui lui sont offerts sur la base de leur teneur en lysine. Des observations complémentaires sur les concentrations des acides aminés libres dans le plasma suggèrent l'emploi de régimes équilibrés en acides aminés pour les études de libre choix en vue d'une croissance optimale.

porc / croissance / sexe / lysine / appétit / préférences alimentaires

INTRODUCTION

During recent years, with the advent of environmental constraints related to nitrogen pollution from feeding, self-selection of dietary protein and amino acids by growing pigs has become an attractive and alternative feeding strategy, compared to the conventional single-feed system, with the view that the animals could adjust their daily protein inputs to rapidly changing requirements over time and between individuals, and thus minimize nitrogen output. Several reports (Kyriazakis *et al*, 1990, 1991, 1993a,b; Kyriazakis and Emmans, 1991; Rose and Kyriazakis, 1991; Bradford and Gous, 1991, 1992) confirmed that when growing pigs are offered a choice between 2 feeds differing in their protein contents, they select a diet that meets their protein requirements, as assessed from growth rate and feed efficiency. In addition, they are able to match their changing requirements for dietary protein, either during growth or according to their genetic potential for growth (Kyriazakis *et al*, 1983b).

Considering the specific case of diets differing only in their contents of lysine as the limiting amino acid, we reported in a previous study (Henry, 1987) that growing pigs were still able to differentiate between 2 feeds on the basis of their lysine content. When offered a choice within a pair of

diets between a limiting level of lysine and the one meeting closely the requirement, they showed a preference for the adequate diet. The selected dietary protein content seemed to change with time, possibly in relation to change in requirement, with a preference for a higher amino-acid level during the initial period of growth. In addition, there was some indication that self-selection of lysine could depend on the range of concentration within diet pairs, according to their proximity to the optimum level for growth, with a risk of random selection when lysine levels are either too close or too distant from each other.

In order to bring experimental support to these assumptions, the objective of the present work was to compare self-selection of lysine by growing pigs when given a choice within diet pairs between deficient or suboptimal levels for growth on the one hand and optimal or superoptimal levels on the other, in relation to sex as an indicator of growth potential.

MATERIALS AND METHODS

Animal and diets

One hundred growing pigs of the Large White breed, averaging 17.7 ± 1.0 kg initial body

weight (BW), were assigned to 5 treatments, with 10 females and 10 castrated males on each treatment during a 42-d test period.

In treatment 1 (control) the animals were fed *ad libitum* a complete diet containing 0.85% lysine, which was supposed to meet the requirement for this amino acid at this stage of growth, as suggested by the usual recommendations (INRA, 1984). In the other treatments, the pigs were offered a choice between 2 diets, one either deficient (L1: 0.60%) or suboptimal (L2: 0.72%) in lysine, the other either optimal (L3: 0.85%) or superoptimal (L4: 1.20%) in lysine for growth. There were arranged according to a 2 x 2 factorial plan: 0.60 vs 0.85% (treatment 2), 0.72 vs 0.85% (treatment 3), 0.60 vs 1.20% (treatment 4), and 0.72 vs 1.20% (treatment 5).

A maize-soybean meal-peanut meal basal diet (table I) was used, containing 17% crude protein (CP: N x 6.25) and 0.60% total lysine. The lysine contents of the other diets were obtained by supplementation with L-lysine•HCl. Other limiting amino acids were added in the pure form (L-threonine and L-tryptophan), so that the contents of essential amino acids other than lysine were in accordance with the recommendations. The ratios between threonine, methionine, methionine + cystine, tryptophan and lysine, at 0.85% lysine level, were 0.65, 0.30, 0.64 and 0.20, respectively, meeting the usual recommendations (Henry, 1988). CP levels were equalized by adjustment with supplementary glycine as a source of non-essential nitrogen. The calculated energy content of the diets was 13.8 MJ digestible energy/kg.

The animals were housed individually in a closed room regulated at 16–18°C with free access to water. Their allotment to dietary treatments was made at random from complete blocks based upon age and weight, and nested within sex (split-plot design). They were allowed *ad libitum* access to a self-feeder with 2 compartments and 2 separate troughs for the self-selected diets. All diets were in pelleted form (5 mm diameter). The positions of diets in the feed boxes were switched each week. Feed intake was recorded weekly for each diet. The animals were weighed at weekly intervals. At the end of the trial (d 42), ultrasonic backfat measurements were made 6.5 cm from the midline at mid-back and rump.

Table I. Composition of basal diet.

Ingredients (%) ^a	%
Maize, 10.25% CP	76.54
Soybean meal, 44.2% CP	6.0
Peanut meal, 46.7%	13.0
Dicalcium phosphate	2.2
Calcium carbonate	1.2
Salt	0.5
Trace element and vitamin premix ^b	0.5
L-threonine	0.03
L-tryptophan	0.03
L-lysine•HCl ^c	—
Total	100.0

^a As-fed basis; ^b provided the following amounts of trace elements (mg/kg of complete diet): Cu (as CuSO₄•5 H₂O), 20; Fe (as FeSO₄•7 H₂O), 200; Mn (as MnO), 62; Zn (as ZnO), 160; I (as Ca (IO₃)₂), 0.5; Co (as CoSO₄•7 H₂O), 0.5; Se (as Na₂SeO₃), 0.22; and vitamins (units/kg of complete diet): vitamin A, 16 000 IU; vitamin D₃, 2 400; alpha tocopheryllacetate, 20 mg; vitamin K, 1.0 mg; thiamin, 1.0 mg; riboflavin, 5.0 mg; Ca pantothenate, 12 mg; pyridoxin, 2.0 mg; vitamin B₁₂, 20 µg; folic acid, 0.75 mg; biotin, 0.75 mg; choline, 1.6 g. Supplementary addition of ethoxyquin was made at 75 mg/kg diet. CaCO₃ (ground limestone) was added as carrier at 0.157% of diet, with talc (0.026%) and silica (25 mg/kg diet); ^c contained 78% lysine. The 4 experimental diets (L1, L2, L3 and L4) were obtained after supplementary addition of 0, 0.153, 0.318 and 0.764% L-lysine•HCl, respectively, at the expense of maize. N contents were equalized with addition of glycine at the levels of 0.612, 0.490, 0.357 and 0% in diets L1, L2, L3 and L4, respectively.

Blood sampling and plasma-amino-acid analysis

After recording their individual feed intake, the females of 4 blocks had their feed withdrawn on d 43 at 9 am. After a 5 h fast, 5–10 ml blood per animal were collected by *vena cava* puncture into heparinized tubes. Plasma was separated by centrifugation and deproteinized according to the procedure described by Sève *et al* (1991). Chromatographic separation of the amino acids was performed on a Biotronik LC 5001 analyzer.

Statistical analysis

Statistical analysis of experimental data was performed by using GLM procedure of SAS (1988). After taking into account the block effect nested within sex, the sex effect was tested against the mean square of the residual between blocks. Treatment effects were tested against the mean square of the residual within block variation. The proportion of selected high-lysine (0.85 or 1.21%: HL) diet, within each treatment and weekly period, was tested against the random proportion of 0.50, and contrasts between treatments were analyzed. For each treatment, the regression of average daily gain on daily feed or lysine intake was made, taking the sex effect into account.

RESULTS

The analyzed amino-acid contents of experimental diets (table II) were in close agreement with those initially estimated. Lysine contents in diets L1, L2, L3 and L4 were 0.61, 0.74, 0.85 and 1.21, respectively.

Growth performance and selected dietary lysine

Average pig performance is reported in table III. Except for self-selected dietary lysine content and gain/feed, there was no treatment x sex interaction, so that only treatment means are given. Daily live weight gain was lower ($P < 0.10$) on treatment 2 (0.61 vs 0.85) than on the other treatments, but there was no difference in average daily feed intake, in g/d or in g/kg BW^{0.75}. Daily lysine intake as well as selected lysine percentage in the total diet increased with the increase in lysine content of the diet offered. Treatment x sex interaction ($P < 0.10$) for selected lysine content was explained by a higher value for females than for castrated males in treatment 4 (0.96 vs 0.92%, respectively),

Table II. Proximate analyses and amino-acid contents of the experimental diets.

Diet ^a	L1	L2	L3	L4
Chemical analysis (%)				
Dry matter	86.6	86.7	86.4	86.9
Protein	17.3	17.5	17.1	17.4
Lysine ^b	0.61	0.74	0.85	1.21

^a As-fed basis. Calculated energy value: 13.8 MJ DE/kg; ^b the average contents of the other amino acids were the following: threonine, 0.57; methionine, 0.28; methionine + cystine, 0.57; tryptophan (calculated), 0.17; isoleucine, 0.71; leucine, 1.59; valine, 0.80; phenylalanine, 0.86; tyrosine, 0.69; histidine, 0.43; arginine, 1.27; glutamic acid, 3.19; aspartic acid, 1.61; alanine, 0.91; serine, 0.82; proline, 1.05. The contents of glycine in diets L1, L2, L3 and L4 were 1.36, 1.21, 1.08 and 0.75, respectively.

while similar values for both sexes were observed in the other treatments. The gain/feed ratio was lowest on treatment 2. A treatment x sex interaction ($P < 0.01$) originated from higher values for castrated males than for females, except in treatments 3 and 5 where gain/feed in castrated males was higher and similar, respectively, to that observed in females. At the end of the trial, there was no difference in backfat thickness between treatments. Backfat thickness at mid-back and rump was lower in females than in castrated males ($P < 0.05$ and $P < 0.10$, respectively). After covariance analysis on final BW, there was still a difference in backfat thickness at the rump ($P < 0.05$).

Treatment of diet preference data during weekly periods revealed a difference according to sex, and so the results are presented separately for females (table IV) and castrated males (table V). The proportion of high lysine diet (HL) over the whole experimental period in females, was only

Table III. Average performance of pigs given a choice of diets with different levels of lysine^a.

	Treatment					Sex		Treatment effect ^b	
	1	2	3	4	5	F	M	SE ^b	P-value
	Lysine level, %								
	0.85	0.61 vs 0.85	0.74 vs 0.85	0.61 vs 1.21	0.74 vs 1.21				
Initial BW, kg	17.7	17.7	17.7	17.7	17.7	17.4	18.0	0.23	
Final BW, kg	40.1	36.6	38.8	39.3	40.3	38.7	39.4	1.07	
Weight gain, g/d	534 ^a	452 ^b	503 ^{ab}	514 ^{ab}	540 ^a	507	510	24	0.076
Feed intake:									
kg/d	1.43	1.36	1.45	1.42	1.42	1.40	1.43	0.045	
g/kg BW ^{0.75} /d	114	115	118	115	114	115	115	2.3	
Lysine intake, g/d	12.1 ^a	10.0 ^b	11.6 ^a	13.4 ^c	14.1 ^c	12.1	12.3	0.39	0.0001
Average lysine content of consumed diet, % ^c	0.85	0.73 ^a	0.80 ^b	0.94 ^c	0.99 ^d	0.86	0.86	0.008	0.0001
Gain/feed ratio ^d	0.371 ^a	0.329 ^b	0.346 ^{ab}	0.356 ^{ab}	0.379 ^a	0.360	0.351	0.0085	0.001
Backfat thickness, mm:									
mid-back ^e	16.0	15.5	15.3	15.9	15.4	15.1	16.1	0.48	
rump ^f	20.1	20.5	21.2	21.5	21.4	20.4	21.5	0.74	

^a Twenty replicates per treatment: 10 females (F) and 10 castrated males (M); 42 d duration; 17.7 ± 1.0 kg average initial body weight (BW) to 39.0 ± 4.8 kg average final BW; ^b SE; standard error of the mean (20 animals per treatment). Means with different superscripts are different at $P < 0.10$ (Student-Newmann-Keuls test); ^c excluding treatment 1; treatment x sex interaction at $P < 0.10$; in treatment 4, 0.96 and 0.92% lysine for females and castrated males, respectively; similar values for both sexes in other treatments; ^d treatment x sex interaction at $P < 0.01$; higher values for females than for castrated males in treatments 1 and 4, and lower values in treatment 3; ^e sex effect at $P < 0.05$; after covariance analysis on final BW, $P < 0.05$; ^f sex effect at $P < 0.10$.

significantly greater than the random value 0.50 ($P < 0.05$) on treatment 4 (0.61 vs 1.21% lysine), and on treatment 2 (0.61 vs 0.85% lysine) in castrated males. In females offered a choice between the low lysine feed (0.61%) and a feed either optimal (0.85%: treatment 2) or superoptimal (1.21%: treatment 4) in lysine, the proportion of the high lysine feed (HL) was greater in treatment 4 than in treatment 2 in most periods. In contrast, when pigs were offered a choice between the suboptimal level of lysine (0.74%) and either the optimal (0.85%: treatment 3) or superoptimal (1.21%: treatment 5) contents, there was no difference in the proportion of high lysine feed (HL) between treatments 3 and 5. The overall contrast 2–3 vs 4–5 comparing 0.85 and 1.21% as the high lysine level was significant ($P < 0.05$) in weeks 1, 2 and 4, as well as for the whole experiment, indicating a sustained preference for the superoptimal compared with the opti-

mal level, especially during the initial period of the trial. In contrast to females, castrated males did not show a clear preference for either diet combination over the whole experimental period. The animals seemed to prefer ($P < 0.10$) 0.85% lysine to 1.21% in week 2. A similar tendency was observed over the whole experiment.

In order to study the respective contributions of daily selected lysine and overall feed intake to growth response, the regression of average daily gain (ADG, g) over the entire trial on mean daily feed intake (DFI, g) on the one hand and mean daily lysine intake (DLI, g) on the other was calculated for each treatment, taking sex into account. As shown in table VI, DFI was the first variable for explaining the variation in ADG with the exception of treatment pair 0.61 (deficient) and 0.85 (adequate), for which DLI was the first explanatory variable before DFI.

Table IV. Selection of diets according to lysine content and changes with time: proportion of self-selected high lysine (0.85 or 1.21%: HL) diet by females^a.

Week	Treatment				CV, %	Contrast 2–3 vs 4–5 ^a P-value
	2	3	4	5		
	Lysine level, %					
	0.61 vs 0.85	0.74 vs 0.85	0.61 vs 1.21	0.74 vs 1.21		
1 ^b	0.42	0.49	0.52	0.56	26.2	0.048
2 ^c	0.35	0.41	0.55	0.52	47.1	0.024
3 ^d	0.44	0.54	0.59	0.57	37.5	
4 ^e	0.49	0.46	0.63*	0.52	28.9	0.052
5 ^f	0.54	0.63†	0.66**	0.56	25.6	
6 ^g	0.47	0.60	0.54	0.54	27.0	
Total period ^h	0.46	0.54	0.59*	0.54	17.9	0.039

^a Probability levels: ** $P < 0.01$; * $P < 0.05$; † $P < 0.10$. The results of the comparison of the proportion of selected HL diet to the random value of 0.50 are given with their probability level: ^b 2 vs 4†; 2 vs 5†; ^c 2 vs 4*; 2 vs 5†; ^d 2 vs 4†; ^e 2 vs 4*; 3 vs 4*; ^f 2 vs 4†; ^g 2 vs 3*; ^h 2 vs 3†; 2 vs 4**; 2 vs 5†.

Table V. Selection of diets according to lysine content and changes with time, proportion of self-selected high lysine (0.85 or 1.21%: HL) diet selected by castrated males^a.

Week	Treatment				CV, %	Contrast 2-3 vs 4-5 ^a P-value
	2	3	4	5		
	Lysine level, %					
	0.61 vs 0.85	0.74 vs 0.85	0.61 vs 1.21	0.74 vs 1.21		
1	0.54	0.56	0.49	0.49	32.6	
2 ^b	0.55	0.56	0.40	0.51	32.9	0.075
3	0.52	0.56	0.60	0.56	35.3	
4 ^c	0.55	0.60*	0.49	0.57	22.1	
5 ^d	0.62*	0.56	0.62	0.50	29.1	
6	0.53	0.47	0.47	0.51	32.3	
Total period	0.55*	0.55	0.52	0.52	17.9	

^a Probability levels: * $P < 0.05$; † $P < 0.10$. The results of the comparison of the proportion of selected HL diet to the random value of 0.50 are given with their probability level; ^b2 vs 4*; 3 vs 4*; ^c3 vs 4*; ^d4 vs 5†.

Plasma-free amino acids

Plasma levels of free essential and non-essential amino acids are reported in table VII, along with average lysine intake during the 2 days prior to blood sampling. Lysine intake recording was restricted to 3 of the 4 female pigs that were sampled for plasma amino acids.

The lower performance of the animals in treatment 2, as evidenced by lysine deficiency, was associated with a low level of free lysine in the plasma, in mg/100 g or as a percentage of the sum of essential amino acids (Σ EAA), indicating an increased utilization of the limiting amino acid. At the same time, there was an accumulation of threonine, the second limiting amino acid.

Changes in plasma-amino-acid patterns between treatments were mainly ascribed to treatment 5 with self-selection of suboptimal (0.74%) and superoptimal (1.21%)

levels of lysine. Compared with the other pair-tests (treatments 2, 3 and 4), the plasma concentration of free threonine was lower. From the intake data at the time of blood sampling, this corresponded to a critical threonine/lysine ratio of 0.57, compared with the recommended value of 0.65. There was also a tendency to a decrease in methionine concentration, with a dietary methionine/lysine ratio 0.29, slightly lower than the recommended value of 0.30. These changes were associated with decreased concentrations of branched-chain amino acids (isoleucine, leucine and valine) compared with treatments 2 to 4 and close to control, and more generally with the decrease in the sum of essential amino acids (Σ EAA). Among the non-essential amino acids, the concentrations of serine and alanine were higher in treatment 5 than in treatments 2 to 4, while the content of free aspartic acid was higher. Urea and ornithine concentrations were also the lowest in treatment 5.

Table VI. Regression of average daily gain (ADG) on daily feed intake (DFI, kg) or daily lysine intake (DLI) over the entire trial.

Treatment ^a	Lysine % in diet pairs	Intercept (b0)	Slope of ADG on		R ²	CVr (%) ^b
			DFI, kg (b1)	DLI, g (b2)		
2	0.61 vs 0.85	-165.2	452.0	63.2	0.738	10.9
		-187.9			0.755	10.6
3	0.74 vs 0.85	-1.5	369.6	44.1	0.796	9.6
		23.7			0.781	9.9
4	0.61 vs 1.21	-333.6	562.1	66.0	0.816	13.0
		-399.9			0.815	13.0
5	0.74 vs 1.21	-185.6	506.7	46.9	0.865	8.0
		-124.1			0.834	8.9

^a Regression after covariance analysis taking sex effect into account; ^bCVr: residual coefficient of variation (%).

DISCUSSION

Our results are in agreement with those reported previously (Henry, 1987) showing that 18 to 40 kg growing pigs, when offered a choice between diets differing in their contents of lysine, as the first limiting amino acid, are able to show a preference for a diet better balanced for this amino acid compared to a deficient diet. They bring further experimental evidence of the ability of the growing pig to differentiate successfully between feeds differing not only in their overall protein content (Kyriazakis *et al*, 1990, 1991, 1993a; Kyriazakis and Emmans, 1991; Bradford and Gous, 1991), but also in the concentration of their limiting amino acid (Robinson, 1975; Henry, 1987; present study). As shown by the relationship between daily live-weight gain and voluntary feed or lysine intake, in agreement with earlier observations (Henry, 1987), there is also some evidence that the regulation of lysine intake predominates over the regulation of overall feed intake when pigs are offered a choice between lysine-deficient (0.61%) and lysine-

adequate (0.85%) diets. In the same way, we have shown (Henry, 1983) that the growing pig, when given a single diet containing a suboptimal level of the limiting amino acid (lysine or threonine), responds by eating more feed than with an amino-acid-adequate diet, in order to meet more closely its amino-acid requirement for growth.

Methodological aspects

In the present study, the pigs were given continuous access to a pair of feeds as a choice during 6-week period. Feed position in the troughs was changed once a week, in order to avoid habituation to the trough and therefore eventual interference with diet selection. Kyriazakis *et al* (1990) reported that an 8-d training period was necessary prior to selection of diets that differed in the kind and proportions of ingredients, so that the animals could learn the taste and nutritional characteristics of each of the feeds on offer. In our case, the diets had exactly the same composition ex-

Table VII. Free amino-acid concentrations in plasma (mg/100 g).

<i>Treatment</i>	1	2	3	4	5	
<i>Lysine, %</i>	0.85	0.61 vs 0.85	0.74 vs 0.85	0.61 vs 1.21	0.74 vs 1.21	<i>SE^a</i>
Lysine intake, g/d ^b	22.1	17.0	18.6	21.0	25.2	
<i>Essential amino acids (EAA):</i>						
Lysine	1.00 ^a	0.91 ^a	1.15 ^a	2.26 ^b	1.80 ^c	0.14
Threonine	1.13 ^{ab}	1.63 ^a	1.46 ^{ab}	1.38 ^{ab}	0.76 ^b	0.18
Methionine	0.70 ^a	0.59 ^a	0.70 ^a	0.69 ^a	0.42 ^a	0.07
Cystine	1.32 ^a	1.12 ^a	1.16 ^a	1.34 ^a	1.11 ^a	0.10
Isoleucine	0.94 ^a	1.20 ^b	1.38 ^b	1.38 ^b	0.87 ^a	0.08
Leucine	3.04 ^{ab}	2.34 ^b	3.33 ^b	3.31 ^b	2.40 ^a	0.20
Valine	2.70 ^{ab}	2.86 ^{ab}	3.05 ^{ab}	3.19 ^a	2.21 ^b	0.21
Phenylalanine	1.31 ^a	1.63 ^a	1.71 ^a	1.84 ^a	1.34 ^a	0.14
Tyrosine	1.42 ^a	1.65 ^a	1.59 ^a	1.88 ^a	1.32 ^a	0.15
Arginine	2.29 ^a	1.98 ^a	2.71 ^a	2.34 ^a	1.97 ^a	0.29
Σ EAA	17.25 ^{ab}	17.24 ^{ab}	19.66 ^a	21.03 ^a	15.25 ^b	1.03
<i>% Σ EAA:</i>						
Lysine	5.80 ^a	5.32 ^a	5.84 ^a	11.00 ^{ab}	12.54 ^b	1.43
Threonine	6.54 ^{ab}	9.33 ^a	7.41 ^{ab}	6.42 ^{ab}	4.81 ^b	0.88
Methionine	4.03 ^a	3.37 ^a	3.57 ^a	3.25 ^a	2.76 ^a	0.72
<i>Non-essential amino acids:</i>						
Glutamic acid	2.90 ^a	3.35 ^a	3.00 ^a	2.53 ^a	2.69 ^a	0.31
Glutamine	7.45 ^a	5.91 ^{ab}	7.10 ^a	5.82 ^{ab}	5.12 ^b	0.45
Aspartic acid	0.22 ^a	0.25 ^a	0.23 ^a	0.27 ^a	0.40 ^b	0.02
Asparagine	1.16 ^a	1.31 ^a	1.34 ^a	1.40 ^a	1.19 ^a	0.07
Serine	2.31 ^a	2.18 ^a	2.56 ^a	2.51 ^a	1.52 ^b	0.18
Glycine	10.96 ^a	8.23 ^a	11.19 ^a	10.88 ^a	9.34 ^a	0.75
Alanine	3.56 ^{ab}	3.88 ^{ab}	4.22 ^{ab}	4.70 ^a	3.35 ^b	0.28
Proline	6.22 ^a	4.84 ^a	6.04 ^a	7.15 ^a	5.19 ^a	0.69
Urea	31.4 ^a	40.2 ^b	26.5 ^{ac}	24.4 ^{ac}	22.1 ^c	2.1
Ornithine	1.45 ^{ab}	1.70 ^a	1.51 ^{ab}	1.58 ^{ab}	1.16 ^b	0.11

^aSE: standard error of the mean (4 female pigs per treatment). Means with different superscripts are different at $P < 0.05$ (Student–Newmann–Keuls test); ^bAverage lysine intake during 2 d prior to blood sampling (3 animals).

cept for lysine. Therefore, the pigs had only to learn to differentiate between lysine contents. It is difficult to know to what extent the weekly change of feed position could have affected feed preference in

comparison with continuous access to the same compartment of the self-feeder, but with the risk of a bias due to eventual positional preference. As shown by Robinson (1975), a sudden change of feed box may

alter immediate diet preference. According to Kyriazakis *et al* (1990), free-choice pigs offered diets differing in their protein contents took a few days before adjusting after reversal of feed troughs. However, in the case of selection between amino-acid-imbalanced diets and a control diet, Edmonds *et al* (1987) reported that the pig preference for the control diet could occur within hours with a daily rotation of feeders. Similarly, according to Mutsamura and Ishida (1982) and Mutsamura and Ohya (1982), growing rats could discriminate between diets differing in their contents of the limiting amino acid (lysine and methionine, respectively) from the first day of the experiment with daily change of the feed cup position. This is in agreement with Gietzen (1993), who reported that the rat can recognize a decreased concentration of the limiting amino acid in the test meal within a few hours. Similar response was observed in chucks by Picard *et al* (1993). This may explain the difference in the response of the animal to diet-selection whether it is offered diets manipulated in amino acids or in overall protein. The observed preference scores for specified lysine content in the feed on offer within weekly periods thus seems to corroborate the short time course that allows diet selection following a change in feed position.

Influence of sex and growth potential

An interesting contribution of this investigation was to show a clear differentiation of diet selection according to sex. Females, which were leaner than castrated males, had a sustained preference for high-lysine feed (1.21%), while castrated males did not exhibit any preference for this higher level. The higher lysine intake in treatment 5 (0.74 vs 1.21%) was associated with enhanced growth performance

compared with the other free-choice treatments. In comparison with the previous study (Henry, 1987), in which mostly females were used, there were some discrepancies in the animal response to similar treatments, but there was some indication that the pigs displayed a preference for the superoptimal level of lysine (1.21%) during the first part of the experiment. The absence of treatment x sex interaction in this investigation could be related to the choice of the levels of lysine in a non-factorial experimental plan. In another test, where pigs were offered a choice between protein and protein-free semi-purified feeds (Henry, 1968), female pigs had a higher voluntary protein intake than castrated males, in keeping with their higher requirement. Conversely, according to Kyriazakis *et al* (1990), diet selection did not differ between females and entire males, which have rather similar body composition. After comparing 2 different genotypes in a free-choice feeding system, Kyriazakis *et al* (1993a) reported a much lower protein consumption in an obese unimproved breed (Chinese Meishan) than in a fast-growing lean type of pig. These observations seem to validate the assumption that the leaner the genotype, the more closely it is able to adjust its self-selected protein or amino-acid intake to its requirement for lean tissue deposition.

Converging results from diet-selection tests (greater preference for 1.21% lysine than for 0.85%) and plasma-free amino acidemia (lower levels of threonine and methionine, the secondary limiting amino acids, in treatment 5 combining 0.74 and 1.21% lysine) indicate that the lysine requirement (% feed) in 18–40 kg female pigs is higher than the 0.85% level that was assumed as being optimal, particularly during the initial period when the pigs body weight was only 18 kg. This response to a higher lysine level than expected could also be partly explained by the lower ileal

amino-acid digestibility of the batch of peanut meal that had been overheated, according to the measurements made subsequently on a similar meal (Sève *et al*, 1993). Obviously, free-choice feeding diets with varying lysine content may be an interesting technique for assessing the requirement for this amino acid, as judged by growth performance and plasma amino-acid pattern.

Self-adjustment to time-related changes in requirements

The higher preference for the superoptimal (1.21%) lysine from the initial period of the trial in females brings further evidence that diet selection takes into account changes in requirement over time, with higher lysine need relative to energy, as stated previously (Henry, 1987). Since the experimental period was restricted to 6 wk and 18–40 kg BW range, this trend in preference change probably would have been more apparent over a wider range of BW intervals. In free choice between feeds differing in their overall protein contents, it is also well established (Kyriazakis *et al*, 1990, 1993a,b; Bradford and Gous, 1991, 1992) that the pig is able to select a protein level that decreases with time in a fashion corresponding to changing requirement relative to feed or energy, especially in lean genotypes (Kyriazakis *et al*, 1993b).

Differentiation between lysine contents in paired diets

Following our previous research (Henry, 1987), the assumption was made that the choice of dietary lysine could be influenced by the difference in concentrations within diet pairs, with more probability of random choice when the lysine contents of the

feeds are too close or too distant from each other. The dietary lysine combinations 0.74 vs 0.85% (treatment 3) and 0.61 vs 1.21% (treatment 4) were intended to reflect these situations of proximity and distance, respectively. In fact, since the superoptimal 1.21% lysine level was preferred to the supposedly optimal level of 0.85%, there was no indication of an unfavorable effect on diet selection of offering a wide range of lysine content, as was suggested previously (Henry, 1987), since the latter was preferred to the supposedly optimal level of 0.85%. On the other hand, from the comparison of 0.61 and 0.74% as the low lysine level in diet pairs (treatments 2 vs 3 and 4 vs 5), it follows that selection of feeds with relatively close lysine contents is made at random. Kyriazakis *et al* (1990) also noted a greater variation in the preference of individual pigs when they had a choice between 2 feeds that were not very different from each other.

Incidence of amino-acid balance in free-choice feeding

The present study was aimed at examining the ability of the growing pig to self-select lysine according to its changing requirements. Thus, the test diets contained the same contents of all essential amino acids except for lysine. Since lysine was offered for selection, the ratios between other dietary essential amino acids and lysine were closely associated with diet selection, their values decreasing with increased lysine intake and percentage above 0.85%. The low level of plasma-free threonine in the female pigs of treatment 5 (0.74 vs 1.21% lysine) means that the threonine/lysine ratio in the selected feed reached a critical value of 0.57 compared with higher recommended levels (Henry, 1988, 1993; Fuller, 1991), while the higher requirement for lysine than the presupposed 0.85% level

was reflected by lowered urea concentration. A similar but lesser trend was noted for methionine.

The necessity to raise the level of the second limiting amino acid, namely threonine, along with lysine, as evidenced by plasma-free amino-acid pattern, further justifies the use of diets adequately balanced for amino acids in free-choice feeding studies aimed at optimum performance. This implies the maintenance, within diet pairs, of similar ratios between lysine and other essential amino acids, at least for the secondary limiting amino acids (threonine, methionine, and tryptophan), corresponding to the recommended levels and for a specified dietary amino-acid pattern (lysine/protein ratio). In fact, self-selection of protein has generally been studied by comparing paired diets differing in their overall protein contents, without special concern for amino-acid balance that may exert specific effects on appetite (Henry and Sève, 1993). In some cases (Bradford and Gous, 1991, 1992), although the feeds offered for selection were intended to be balanced for amino acids, there were fluctuations in amino acid/lysine ratios, with some, like threonine, reaching critical levels in high protein diets. Similar constraints on amino-acid balance also hold in the case of continuous multiphase-feeding systems in which 2 feeds differing in their protein contents are mixed over a short-time basis to attain a close adjustment of daily protein and amino-acid supply to the requirements (Henry and Dourmad, 1993).

Limitations of free-choice feeding system

Although the amount of self-selected protein or lysine appears to reflect the requirement for growth, it is also known, from studies conducted with rats (Harper and

Peters, 1989), that protein or amino-acid intake in free-choice conditions is not precisely regulated. The ability of the animal to select diets would be mostly directed to avoiding both deficient and excessive levels of protein or amino acids. As reported previously (Henry, 1968, 1985), it would seem that under free-choice feeding the pig is only able to achieve a partial adjustment of dietary protein to its requirement according to its potential for muscular growth and dietary protein quality. In addition, the presence of unpalatable or harmful substances (Robinson, 1975; Bradford and Gous, 1992; Kyriazakis and Emmans, 1992, 1993) or excess amino acids (Edmons *et al*, 1987) may interfere with dietary protein selection, according to the different organoleptic properties of the feeds on offer. Following several practical feeding trials with cereals and a protein supplement (soybean meal) offered separately, recent findings from Batterham (1987) produced some evidence that choice-fed pigs generally eat less protein than those given complete diets, with great variability in the acceptance of the protein supplement, and have lower growth performance. Therefore, due to the complexity of the usual feeding conditions, there is still some uncertainty about the efficacy of free-choice feeding in pigs, especially in the usual group-feeding conditions, to achieve an adequate supply of protein or amino acid over time, according to growth potential.

CONCLUSIONS

The present work confirmed that 18–40 kg growing pigs, when offered a choice between paired diets differing only in their lysine contents, had a preference for diets containing adequate or superoptimal levels of lysine (0.85 and 1.21%, respectively) over diets either deficient or suboptimal in lysine (0.61 and 0.74%, respectively).

There was a sex difference in diet selection, with only females having a preference for superoptimal (1.21%) compared to adequate (0.85%) level, especially during the initial period of the trial. This greater preference of females for a higher lysine diet than castrated males is related to their higher potential for lean tissue growth and resulting increase in their requirement for lysine, as evidenced by their body composition and plasma-free amino-acid pattern.

More generally, the preference of the growing pig for dietary lysine, as the first limiting amino acid, in free-choice feeding, is an indication, as pointed out for different animal species (Boorman, 1979; Emmans, 1981; Henry, 1985) that growing animals, when given a suboptimal supply of a limiting amino acid in a single feed, attempt to eat more feed to meet more closely their requirements. Undoubtedly, further investigations are needed to validate the free-choice system as a promising feeding strategy in pigs for a self-adjustment of protein and amino-acid consumption to changing needs during growth, and lowered nitrogen output. This should be achieved by using diets properly balanced for essential amino acids, and apparently with greater chance of success by taking advantage of selection for lean tissue growth.

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