Effect of increasing lysine supply during last third of gestation on reproductive performance of Iberian sows

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Abstract

Ninety purebred Iberian (IB) sows in second or third parity were used to determine the effects of dietary lysine (Lys) concentration during last third of pregnancy on sow and litter performance. The sows were randomly assigned to one of three dietary treatments: 5.5 (LLys, Control), 7.4 (MLys) and 8.7 (HLys) g Lys kg^{-1} diet. Feed allowance was 2.30-2.33 kg d^{-1}. Close to farrowing a conventional lactation diet was used. Dietary Lys did not affect body-weight (BW) gain in late gestation of second-parity sows. However, in third-parity sows, a strong tendency was observed for BW gain to increase during late pregnancy with dietary Lys levels higher than 5.5 g kg^{-1} ($p = 0.061$). Body-weight losses during lactation were never influenced by Lys supply. A strong tendency ($p = 0.064$) for a lower ratio between piglets born alive and total piglets born was observed in second-parity sows fed the Control gestation diet. Litters and piglets from sows on this diet had the lowest weight at birth ($p < 0.05$). In contrast, third-parity sows on the MLys diet gave birth to piglets with the lowest BW ($p < 0.05$). Differences in piglets´ BW or litter weight at weaning were never significant ($p > 0.05$). In conclusion, under moderate energy supply, adequate reserves for subsequent lactation can be achieved in second- and third-parity IB sows with a daily provision of at least 17.2 g lysine over the last third of pregnancy, what implies a substantial increase in protein supply respect to traditional practices.

Additional key words: dietary lysine; dietary protein; Iberian pigs; lactation; late pregnancy.

Introduction

Body reserves play a key role on the reproductive performance of the sow. Restriction in feed intake is practiced to control weight gain in the gestating sow, as *ad libitum* intake during pregnancy may reduce milk production and appetite and increase maternal weight loss during lactation (Dourmad, 1991; Weldon *et al*., 1994; Pettigrew & Yang, 1997). Consequently, daily energy intakes have been recommended (NRC, 1998; BSAS, 2003) to achieve target gains for maternal tissues (partly in support of lactation) and for reproductive tissues (gravid uterus and mammary gland). In conventional sows, feed intake above 25 MJ ME d^{-1} in late gestation increases maternal gain (Dourmad *et al*., 1996) and body weight (BW) of the piglets at birth

(Cromwell *et al*., 1989; Close & Cole, 2000b). This effect might be more marked in multiparous than in primiparous sows (Close & Cole, 2000a). Under restricted supply of energy in late gestation, increased levels of dietary lysine (Lys) may be required to maximize milk production (Pettigrew & Yang, 1997). A moderate restriction in Lys supply during pregnancy seems to have little or no effect on litter BW at birth (King *et al*., 2006), but may reduce BW at weaning. Kusina *et al*. (1999) observed that an increase in Lys supply over requirements during pregnancy increased BW gain of the pigs. No data are available on this subject for the Iberian (IB) sow, whose mature size and traits for reproductive performance differ widely from those observed in the leaner breeds. Furthermore, mobilization of body protein in support of milk-protein

Abbreviations used: AA (amino acid); BW (body weight); CP (crude protein); DM (dry matter); GE (gross energy); IB (Iberian); Lys (lysine); ME (metabolizable energy); N (nitrogen).

synthesis in an obese genotype such as the IB sow may be an inefficient process. Consequently, under moderate feed restriction, a likely effect of Lys supply during late gestation on the growth rate of the small-size nursing litter of the IB sow cannot be discarded. To check this hypothesis, the present study was conducted in second-parity sows, which may not have reached mature body-weight, and in third-parity sows, probably of fully mature body size (with differences in requirements for maternal tissue accretion and amount of fat reserves for mobilization in support of fetal growth) fed diets differing in Lys supply over the last third of gestation.

Material and methods

The experimental protocol was approved by the Bioethical Committee of the Spanish National Research Council (CSIC, Spain). Sows and piglets were cared for following the guidelines of the Spanish Ministry of Agriculture (BOE, 2005).

Pigs, experimental design, diets and husbandry

Two consecutive trials were carried out with purebred IB sows of the Silvela strain at Montecastilla farm (Granada de Rio Tinto, Huelva, Spain), in their second or third parity. At mating sows were 15 and 20 month-old and had an average BW of 114 ± 10 and 127 ± 12 kg (mean $\pm$ SD), respectively. Within each parity 45-47 sows were selected from a group of approximately 90 sows 70 ± 2 days after the last insemination. Sows with extremely low or high BW or having been inseminated out of the 68 to 72 days before the beginning of the experiment were discarded. In each trial a different group of sows was used. A 5-month interval elapsed between the two trials. The sows were group-housed in open-air, fenced, 8 m × 25 m spaces and randomly assigned to one of three experimental treatments. The fenced areas had a shelter space (6 m × 4 m) and were equipped with 15 individual feeders placed on solid concrete floors and nipple drinkers opposite to the feeding area. The feeders were fixed to the fence within individual feeding spaces, separated by panels. Average outdoors minimum and maximum temperatures (mean $\pm$ SE) were 8.3 ± 0.42 and 20.0 ± 0.35°C in the trial carried out with second-parity sows, and 19.4 ± 0.26 and 34.4 ± 0.42°C, in the trial performed

with third-parity sows. From insemination to d 70 of gestation the sows were fed 1.8 kg d^{-1} of a conventional diet that contained 12.39 MJ ME, 5.5 g Lys and 108 g CP kg^{-1} (Table 1). This diet, frequently used for gestating IB sow, was used as one of the experimental diets (Control diet). A daily amount of 2.30 kg (28.5 MJ ME) of this diet was provided throughout the last third of gestation. Two additional isoenergetic gestation diets (12.49-12.51 MJ ME kg^{-1}) were also assayed. They were formulated to provide 7.4 (MLys diet) and 8.7 (HLys diet) g Lys kg^{-1} diet. These diets contained 101 and 120 g CP kg^{-1} (Table 1). The amino acid (AA) pattern of the dietary protein was formulated to follow the ideal protein concept (BSAS, 2003), although a marginal deficiency in tryptophan (Trp; 0.15 *vs.* 0.20 for Trp/Lys) and valine (Val; 0.65-0.66 *vs.* 0.74 for Val/Lys) was detected in the MLys and HLys diets, due to a slight excess of L-lysine sulphate. On the contrary, in the Control diet lysine might be limiting. An amount of 2.33 kg (29.1 MJ ME) of the MLys and HLys diets was offered daily to the gestating sows. To calculate daily feed allowance, the maintenance requirements of the gestating IB sow were assumed to be 422 kJ ME kg^{-1} BW$^{0.75}$ d^{-1}, a value obtained by Nieto *et al.* (2002) with castrated growing IB pigs. For an average IB sow in second and third parity (142.5 kg BW) a daily supply of 19.1 MJ for maintenance energy requirement was calculated, including provision for physical activity. The energy content of tissue gains (24.9 MJ kg^{-1}) was taken from the study of García-Valverde *et al.* (2008) for heavy IB pigs (150 kg BW). Once the contribution of reproductive tissue to total BW gain was accounted for, lactation reserves close to 250 g of maternal tissue were targeted. Such tissue gain would require the intake of 10.3 MJ ME d^{-1} respectively. From the study of Aguinaga *et al.* (2011) it can be calculated that between d 70 and d 112 of gestation 0.909 MJ of energy are retained daily in average litters of IB sow and consequently, foetus growth would require additionally 1.57 (0.909/0.58) MJ of ME d^{-1}. Therefore, for the gestating IB sows a daily ME intake close to 31.0 (19.1 + 10.3 + 1.57) MJ was calculated. The actual daily feed allowances provided 28.5-29.1 MJ ME, 181-215 g apparent digestible CP and 9.7-15.6 g apparent digestible lysine, according with the diet fed.

For a sow of 142.5 kg BW daily needs for protein main-tenance and protein accretion due to maternal growth were estimated to attain up to 51 ($142.5^{0.75} \cdot 0.196 \cdot 6.25$) and 205 [($250 \cdot 0.102 + 40.9 + 28.4$) / ($0.77 \cdot 0.60$)] g, respectively, assuming for N maintenance requirement

Table 1. Ingredients and chemical composition of the experimental diets (g kg^{-1} as-fed basis)

	Gestation diets[1]			Lactation diet
	LLys (Control)	MLys	HLys	
Ingredient				
Barley	600.0	962.4	911.3	854.5
Soybean meal, 47% CP	—	—	50.4	103.3
Wheat middlings	200.0	—	—	—
Rice bran, non-deffated	53.0	—	—	—
Sugarbeet pulp	101.4	—	—	—
Lard	10.0	—	—	—
Sodium chloride	5.0	5.0	5.0	5.0
Vitamin and mineral premix[2]	3.0	3.0	3.0	3.0
Calcium carbonate	17.5	14.7	14.7	13.6
Mono-calcium phosphate	8.4	5.6	5.9	11.7
L-Lysine sulphate, 50.7%	1.7	6.0	6.1	6.1
L-Threonine, 98%	—	2.1	2.2	1.9
DL-Methionine, 99%	—	1.2	1.4	0.9
Analysed composition[3]				
Crude protein (N·6.25), g kg^{-1}	108	101	120	148
Gross energy, MJ kg^{-1}	16.52	16.05	15.97	16.04
Calculated chemical composition[4] (g kg^{-1})				
Metabolizable energy (ME), MJ kg^{-1}	12.39	12.49	12.51	12.48
Digestible protein[5] ME^{-1}, g MJ^{-1}	6.71	6.23	7.39	9.13
Lysine	5.5	7.4	8.7	10.3
Methionine and cysteine	4.4	5.0	5.4	5.9
Histidine	2.6	2.1	2.6	3.3
Isoleucine	3.9	3.4	4.3	5.5
Leucine	7.1	6.5	7.8	9.8
Phenylalanine and tyrosine	8.1	7.4	8.8	11.0
Threonine	3.8	5.3	6.1	6.8
Tryptophan	1.4	1.1	1.3	1.7
Valine	5.4	4.8	5.7	7.0
Ether extract	33	19	19	19
Calcium	10.0	7.3	7.5	8.2
Phosphorus	6.9	4.6	4.8	6.2

[1] LLys, low-lysine gestation diet (Control); MLys, middle-lysine gestation diet; HLys, high-lysine gestation diet. [2] Provided (per kg of complete diet): retinol, 10,000 IU as retinyl acetate; cholecalciferol, 1,000 IU; DL-α-tocopherol, 20 mg as DL-α-tocopheryl acetate; menadione, 1.0 mg as menadione sodium bisulfite; thiamine, 1.0 mg; riboflavin, 3 mg; pyridoxine, 2.0 mg; cyanocobalamin, 15 µg; folic acid, 1 mg; nicotinic acid, 15 mg; D-pantothenic acid, 10 mg as calcium pantothenate; Mn, 25 mg as MnSO$_4$.4H$_2$O; Fe, 50 mg as FeSO$_4$.7H$_2$O; Zn, 50 mg as ZnO; I, 650 µg as KI; Cu, 5 mg as CuSO$_4$.5H$_2$O; Co, 200 µg as CoSO$_4$.7H$_2$O; Se, 100 µg as Na$_2$SeO$_3$.5H$_2$O. [3] Determined by laboratory analysis (n = 2). [4] Based on tabulated values (FEDNA, 2003). The AA composition was calculated from the analyses performed on barley and soybean meal, and from tabulated values (FEDNA, 2003) for wheat middlings, non-deffated rice bran and sugarbeet pulp. [5] Assuming a coefficient of apparent digestibility of the dietary protein of 0.770, obtained by Fernández-Fígares *et al.* (2008) in growing IB gilts.

the value of 0.196 g kg^{-1} BW$^{0.75}$ d^{-1} reported by Nieto *et al.* (2002) in the growing IB pig and for maternal growth a daily deposition of 25 g of protein (250 g · 102 g protein kg^{-1} BW gain; García-Valverde *et al.*, 2008) in the last 42 days of gestation. The protein requirements for the growth of the mammary gland and foetus in late gestation were estimated in 40.9 g d^{-1}, following Kim *et al.* (2009). Finally, protein

accreted daily in the body of IB foetuses throughout gestation was taken from Aguinaga *et al.* (2011). The new born IB piglet contains 128.9 g CP/kg BW and has an average empty BW of 1.36 kg. It was assumed that 92% of the protein accreted due to foetal growth (3.84 g d^{-1}) took place between d 70 and 112 of gestation (McPherson *et al.*, 2004). The average number of total piglets born per litter in IB sows is 7.4 (Laguna-Sanz,

1998). Accordingly, litter growth accounts for 28.4 g of protein accreted daily in late gestation (Aguinaga *et al*., 2011). In all, 95 g of protein were accreted daily in the growth and development of maternal and reproductive tissues. To calculate the total requirement of CP the apparent digestibility of the protein fraction of the experimental diets was fixed at 0.77, a value obtained in growing IB gilts by Fernández-Fígares *et al*. (2008). The efficiency of use of the apparent digestible N was assumed to be 0.60, as reported by Nieto *et al*. (2002) in growing pigs. Consequently, a daily allowance of 256 (51 + 205) g ideal CP (197 g apparent digestible (ideal) protein or 13.8 g apparent digestible Lys) should be provided during the last days of gestation. The actual feed intakes provided to the gestating sows digestible Lys amounts ranging from limiting to optimal, according to the dietary Lys content. Each diet was offered in a single meal at 09:00 h. Water was freely available. For each diet, a composite sample was obtained from aliquots taken daily throughout the experiment for analysis.

Approximately one week before the expected time of farrowing the sows were moved to an environmentally-controlled room (at $22 \pm 2°C$, in the second-parity trial, and $27 \pm 2°C$, in the third-parity trial), and housed individually in farrowing crates (1.90 m × 0.60 m) within pens (2.40 m × 1.60 m). The pens were equipped with a thermo-regulated surface (1.20 m × 0.40 m) at 33°C to 35°C during the first week of life and decreasing steadily to 25°C to 27°C at the end of the third week. Once in the farrowing room, the sows were fed a conventional lactation diet (Table 1) at a level of 1% of BW, offered in two equal meals at 08:00 h and 16:00 h. On the day of farrowing the sows were offered 1.5 kg of this diet, and then feed allowance was increased by 0.6 kg d^{-1} to reach 4.5 kg d^{-1} by d 5 of lactation and then kept at this level to weaning. Sows and litters had ad libitum access to water, but litters had no access to any feed. The light cycle was of continuous light. Farrowing took place within a 3-d period in all cases. Cross-fostering within treatment was practiced within 2 days after farrowing to standardize litters to six piglets each. Exceptionally, a few litters with 5 or 7 piglets remained in the study. Weaning took place at 35 ± 2 days of lactation.

Measurements

The sows were weighed on day 70, when moved to the farrowing room and at weaning. Also, back-fat depth was measured (Echoscan T-100, Import-Vet S.A., Barcelona, Spain) at the last rib (P2). Body weight of the sows at farrowing was estimated by extrapolation, assuming a linear change in BW gain between d 70 and farrowing. The weight loss of the sows due to delivery was estimated from the relative contribution of the foetus, placenta and fluids in gravid uterus calculated for sows of 110 d of pregnancy and 12 foetuses, adapted for litter size (Noblet *et al*., 1990). Litter weight accounted for 68.2% of gravid uterus content. Accordingly, total weight loss was estimated as litter weight × 1.465. The BW of the sows immediately after farrowing was estimated as BW at farrowing minus total weight loss due to farrowing. Total number of pigs born, born alive and weaned, and litter weight at birth and weans were recorded.

After weaning, sows were moved to an open-air, fenced yard of 3,000 m² surface, and fed 2.0 kg of the commercial gestation diet. Three days after weaning, sows were moved to an adjacent yard close to two boxes with a boar inside each of them, where they remained for about one hour before returning to the fenced area. This management was repeated the following day if needed. Oestrus was assumed when the sow showed a standing response and arched back induced by a back-pressure test in the presence of a boar. Sows in oestrus were inseminated on two consecutive days and then moved to a second 3,000 m² fenced yard. Fifteen days after the first insemination those sows that had not shown oestrum from d 5 to 9 after weaning were moved again near to the boars to detect oestrus. The day of mating for these sows was assumed to be the day of ovulation. Occurrence of oestrus during lactation and the three first days after weaning was not monitored.

Blood collection

Blood samples of approximately 10 mL were taken on d 70 and 105 ± 3 of gestation and 35 ± 2 d of lactation from the jugular vein, in heparinised vacutainer tubes before the morning meal, from 3 sows randomly chosen (6 sows at d 70 sampling) within each treatment. At each physiological stage sows sampled at random were used. Blood samples were placed in an ice bath immediately after collection and centrifuged at 1,400 *g* for 20 min. Plasma was transferred into polypropylene tubes and stored at –20°C until analysed.

Analytical techniques

Pooled composite samples of the experimental diets were analysed in duplicate according to the Association of Official Analytical Chemists (AOAC, 2003): dry matter (DM) content (method 934.01) and total N (method 984.13). Gross energy (GE) was measured in an isoperibolic bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Amino acids in barley and soya bean meal were determined by high-performance liquid chromatography (HPLC) after protein hydrolysis in 6M HCl plus 1% phenol in sealed, evacuated tubes at 110°C for 24 h (Pico Tag method; Waters, Milford, MA, USA; Cohen *et al.*, 1989) as described by Rivera-Ferre *et al.* (2006).

Plasma glucose, triglycerides, urea and creatinine, were determined colorimetrically using a Cobas Integra 400 analyser (Roche Diagnostics, NY, USA).

Statistical analyses

Data from each of the two parities were analyzed separately by a one-way ANOVA, using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The individual sow and the litter were used as the experimental unit. For sow weights (except for BW at farrowing) and back-fat changes covariates included the total number of piglets born per litter (in gestation) and the total number of weaned piglets (in lactation). Covariates for the analysis of piglet performance included total number of pigs born for BW at birth and total number of weaned pigs for BW changes at weaning.

Results

Second-parity sows

Two sows of the Control treatment group and one of the high-lysine treatment gave birth to only one or two piglets born-alive and were removed from the experiment.

Main results of sow performance appear in Table 2. No differences in BW changes during gestation or lactation were noticed among treatments ($p > 0.05$). Average values for BW gain and loss were 23.6 ± 0.7 and 21.8 ± 1.4 kg, respectively, the latter corresponding to an average daily BW loss of 635 ± 41 g.

There was a strong tendency ($p = 0.064$) for a lower Born-alive/Total born piglet ratio in the sows fed the Control gestation diet, in comparison with the sows on the other experimental diets. Litters and piglets from sows on the Control diet had the lowest weight at birth ($p < 0.05$) but not at weaning (mean values 47.3 ± 0.7 and 8.14 ± 0.12 kg, respectively; Table 2). A mean daily gain of 195 ± 3 g/piglet over the 35-d lactation was observed.

The Lys content of the diet did not affect plasma metabolites levels of the gestating sows ($p > 0.05$; Table 3). Neither in the lactating sow plasma metabolites were affected by dietary Lys during gestation ($p > 0.05$). Weaning-to-oestrus interval did not differ among treatment groups, being 6.4 ± 0.3 d on average.

Third-parity sows

Three sows, all of them on the high-lysine diet, gave birth to only one or two piglets born alive and they were eliminated from the experiment.

The effects of dietary Lys content on sows' performance are shown in Table 2. The MLys diet led to the highest BW gain in late gestation ($p = 0.061$), giving rise to the biggest difference in back-fat depth ($p < 0.001$). Corresponding values from the Control and HLys diets did not differ. Body-weight losses during lactation were not influenced by dietary Lys content and attained 22.9 ± 1.5 kg, corresponding to a daily BW loss of 631 ± 40 g.

The sows on the MLys diet gave birth to piglets with the lowest BW in comparison with piglets from sows in the Control and HLys group ($p < 0.05$). These sows had litters of lower weight at birth ($p < 0.05$). However, differences in piglets´ BW or litter weight at weaning among treatments were not significant ($p > 0.05$). A mean daily gain of 184 ± 4 g/piglet across the 35-d lactation was obtained.

Feeding the Control diet resulted in increased plasmatic levels of glucose and urea of the gestating sow ($p < 0.01$ and $p < 0.05$, respectively; Table 3). However, dietary Lys content did not affect plasma levels of triglycerides and creatinine ($p > 0.05$). No differences in plasma metabolites levels were observed among treatment groups in lactating sows, although a tendency for an increased level of plasma triglycerides in sows on the MLys diet in the previous treatment was noticed ($p = 0.082$).

Table 2. Effects of dietary protein supply in late gestation on body weight (BW), back-fat depth and litter performance of second- and third-parity Iberian sows

	Dietary treatments[1]			RSD[2]	p-value[3]
	LLys (Control)	MLys	HLys		
Second-parity sows[4]					
Number of sows	13	15	14		
BW, kg					
On d 70 of gestation[5]	136.5	131.3	137.0	12.9	0.438
At farrowing	159.1	155.8	160.8	13.2	0.590
Immediately after farrowing[5]	147.8	142.6	147.0	13.1	0.537
At weaning, after 35-d lactation[6]	122.5	125.2	125.2	13.1	0.832
BW gain during late pregnancy[5]	23.1	24.5	23.2	4.1	0.607
BW loss during lactation[6]	24.1	19.3	21.9	8.7	0.401
BW loss during lactation[6], g d^{-1}	707	559	638	261	0.374
Back-fat depth (mm, P2)					
On d 70 of gestation[5]	29.2	29.8	31.3	3.7	0.323
At farrowing[5]	30.9	32.4	34.0	4.8	0.286
At weaning[6]	22.1	21.7	23.5	3.3	0.341
Increase from d 70 to farrowing[5]	1.71	2.59	2.69	3.34	0.730
Decrease from farrowing to weaning[6]	9.01	11.0	10.1	4.4	0.544
Litter performance					
Total piglets born per litter[7]	7.62	7.93	8.15	1.65	0.707
Born-alive piglets/Total born	0.873[a]	0.972[b]	0.965[ab]	0.117	0.064
No. piglets after fostering	5.85	6.27	5.93	0.54	0.104
Weaned piglets/Total born-alive	0.976	0.975	0.966	0.123	0.947
Litter weight, kg					
At birth[5]	9.74[a]	11.12[b]	11.19[b]	1.47	0.026
At weaning[6]	45.40	49.04	47.35	4.65	0.160
Piglet weight, kg					
At birth[5]	1.25[a]	1.41[b]	1.43[b]	0.18	0.030
At weaning[6]	7.87	8.42	8.13	0.80	0.241
BW gain at weaning[6], kg	6.58	7.00	6.67	0.82	0.416
BW gain at weaning[6], g d^{-1}	191	203	192	20	0.246
Third-parity sows[4]					
Number of sows	16	16	13		
BW, kg					
On d 70 of gestation[5]	148.9	151.2	154.0	17.8	0.755
At farrowing	162.4	171.9	170.6	19.6	0.347
Immediately after farrowing[5]	150.0	159.1	157.5	19.5	0.397
At weaning, after 35-d lactation[6]	126.5	136.5	134.9	17.6	0.241
BW gain during late pregnancy[5]	14.2	20.1	16.6	6.7	0.061
BW loss during lactation[6]	23.4	23.0	22.2	10.0	0.953
BW loss during lactation[6], g d^{-1}	650	642	601	266	0.872
Back-fat depth (mm, P2)					
On d 70 of gestation[5]	30.4	27.4	30.1	4.2	0.111
At farrowing[5]	29.2	31.7	31.5	4.7	0.287
At weaning[6]	20.2	22.7	22.2	4.4	0.251
Increase from d 70 to farrowing[5]	−1.18[a]	4.28[b]	1.39[a]	3.74	0.001
Decrease from farrowing to weaning[6]	9.02	9.00	9.25	4.02	0.983

Table 2 (cont.). Effects of dietary protein supply in late gestation on body weight (BW), back-fat depth and litter performance of second- and third-parity Iberian sows

	Dietary treatments[1]			RSD[2]	*p*-value[3]
	LLys (Control)	MLys	HLys		
Litter performance					
Total piglets born per litter[7]	7.88	8.81	8.38	1.94	0.398
Born-alive piglets/Total born	0.942	0.909	0.954	0.129	0.610
No. piglets after fostering	6.44	6.56	6.31	0.83	0.712
Weaned piglets/Total born-alive	0.968	0.968	0.961	0.121	0.964
Litter weight, kg					
At birth[5]	11.11[a]	10.17[b]	11.03[a]	0.95	0.016
At weaning[6]	52.32	48.63	51.58	6.18	0.223
Piglet weight, kg					
At birth[5]	1.34[a]	1.21[b]	1.33[a]	0.12	0.013
At weaning[6]	8.16	7.54	8.07	0.96	0.164
BW gain at weaning[6], kg	6.82	6.33	6.74	0.90	0.284
BW gain at weaning[6], g d⁻¹	192	178	183	25	0.288

[1] Dietary treatments: LLys, low-lysine (Control) diet, (6.26 g Lys kg⁻¹ DM, fed at 28.5 MJ ME d⁻¹; MLys middle-lysine diet (8.43 g Lys kg⁻¹ DM, fed at 29.1 MJ ME d⁻¹; HLys, high lysine diet (9.7 g Lys kg⁻¹ DM, fed at 20.1 MJ ME d⁻¹). [2] RSD: residual standard deviation. [3] Within a row statistical differences are indicated by a different superscript (*p* < 0.05). [4] The second-parity sows were 15 months old at mating and weighed 114 kg on average; the third-parity sows were 20 months old at mating and weighed 127 kg on average. [5] Total number of piglets born per litter was used as covariate. [6] Total number of piglets at weaning was used as covariate. [7] Born-alive piglets plus stillborn piglets plus mummies.

Discussion

In the present study, a different group of sows was used in each of the two trials performed, each corresponding to a different reproductive cycle. In this way likely carryover effects were prevented. Moreover, five months elapsed between the two trials, what caused that environmental conditions outdoors were not the same for the two trials. Thus, some confounding parity-trial effect might happen which prevent direct comparison between trials.

Current recommendations on daily energy intakes (NRC, 1998; BSAS, 2003) are based on the recognition of the crucial role of the body reserves of the sow in reproductive performance. Restriction in nutrient supply is used to control BW gain of the gestating sow. Energy intake during pregnancy must be set within certain limits to avoid increased body fatness at farrowing, reduction of appetite and milk production (Revell *et al*., 1998) and enhanced maternal weight loss during the subsequent lactation (Dourmad, 1991; Weldon *et al*., 1994; Pettigrew & Yang, 1997). Nevertheless, there is evidence that foetal growth is relatively independent of maternal nutrition (Dourmad, 1991; Close & Cole, 2000a; King *et al*., 2006). In the

absence of specific nutrient recommendations for IB sows, in the present experiment daily feed allowance was calculated to provide energy or protein to achieve from d 70 of gestation to farrowing an increase in maternal tissue close to 10.5 kg (21 to 23 kg BW gain, according to parity). Actual feeding levels assayed corresponded to feed allowances of 2.30-2.33 kg that might be somewhat energy limiting (28.5-29.1 MJ ME) to achieve targeted gains. However, in the present experiment BW gain during late pregnancy was 23.6 ± 0.7 kg (562 g d⁻¹) on average, in second-parity sows, and ranged from 14.2 to 20.1 kg according to treatment, in third parity sows (338 to 479 g d⁻¹). So, in the multiparous sows ME intake was found to be too restricted to attain the target gain. Our diets contained 0.44, 0.59 and 0.70 g Lys MJ⁻¹ ME, providing 12.7, 17.2 and 20.3 g Lys d⁻¹. The feed allowance assayed supplied the gestating sows with limiting to surplus amounts of digestible lysine, according to the dietary Lys content. In second-parity sows there was no effect of dietary lysine content either on BW gain in late gestation or BW loss during the 35-d lactation. On the contrary, in third-parity sows a tendency for a lower BW gain was found in the gestating sows of the group fed the LLys, Control diet. However, the level of

Table 3. Effects of dietary protein supply in late gestation on plasma metabolites (mg dL^{−1}) of Iberian sows in second and third parity

	Dietary treatments[1]			RSD[2]	*p*-value[3]
	LLys (Control)	MLys	HLys		
Second-parity sows[4,5]					
Number of sows	3	3	3		
At farrowing					
Glucose	71.5	66.6	65.0	5.5	0.386
Triglycerides	71.5	73.8	61.9	26.6	0.847
Urea	14.6	14.8	14.5	2.7	0.993
Creatinine	1.08	1.36	1.17	0.16	0.150
At weaning, after 35-d lactation					
Glucose	48.8	46.6	55.6	11.6	0.637
Triglycerides	42.4	20.5	24.5	19.7	0.405
Urea	13.0	16.2	22.7	6.6	0.263
Creatinine	1.87	1.56	1.84	0.25	0.310
Third-parity sows[6,7]					
Number of sows	3	3	3		
At farrowing					
Glucose	95.8[a]	81.8[b]	76.3[b]	5.0	0.008
Triglycerides	59.8	63.4	73.8	23.2	0.757
Urea	16.7[a]	9.1[b]	10.5[b]	2.9	0.038
Creatinine	1.25	1.20	1.26	0.09	0.596
At weaning, after 35-d lactation					
Glucose	105	118	116	26	0.820
Triglycerides	11.5	21.7	11.4	5.2	0.082
Urea	15.8	14.7	11.4	5.2	0.584
Creatinine	1.18	1.37	1.03	0.16	0.106

[1] See Table 2. [2] RSD: residual standard deviation. [3] Within a row statistical differences are indicated by a different superscript (*p* < 0.05). [4] The sows were 15 month old at mating and weighed 114 kg on average. [5] Plasma levels of glucose, triglycerides, urea and creatinine at d 70 of gestation were (mg dL^{−1}) 77.4 ± 3.5, 110 ± 8, 20.8 ± 1.9 and 1.15 ± 0.08 (n = 6). [6] The sows were 20 month old at mating and weighed 127 kg on average. [7] Plasma levels of glucose, triglycerides, urea and creatinine at d 70 of gestation were (mg dL^{−1}) 63.4 ± 9.6, 38.6 ± 5.9, 23.8 ± 3.0 and 1.46 ± 0.08 respectively (n = 6).

dietary lysine in the previous treatment did not affect BW loss in lactation. Kusina *et al*. (1999) found in primiparous sows, given approximately 27 MJ ME d^{−1}, increased weight gain with increasing lysine intake from 4 to 16 g d^{−1} and no influence of lysine intake in gestation on total BW loss during lactation. Yang *et al*. (2009) reported that increasing dietary lysine during gestation (8.2 *vs*. 5.9 g kg^{−1} diet) augmented sow BW and backfat thickness, the effect being greater in multiparous than in primiparous sows.

In our study, there was a positive effect of the level of dietary lysine in gestation diet on litter and piglet BW at birth, for those born from sows in their second parity (*p* < 0.05). This fact suggests increased protein accretion in the products of conception with increased level of Lys at the moderate ME intake used, as suggested by Pettigrew & Yang (1997). In third-parity sows, however, this effect was not observed. Instead, new born piglets from sows on the MLys gestation diet were lighter than those from the other experimental treatments. Our findings are in agreement with the results found by Mahan (1998), comparing two levels of Lys in the gestation diet (5.5 and 7.5 g kg^{−1}) and Yang *et al*. (2009). Second-parity IB sows would have increased lysine requirements in comparison with multiparous sows, following the pattern described by Pettigrew & Yang (1997) for young and older gestating sows of conventional or lean genotypes. In the present experiment, dietary Lys content in gestation did not affect litter or piglets rate of gain, irrespective of parity. Our results are in contrast to those of Pettigrew & Yang (1997) who suggested that, at moderate energy supply, increased levels of dietary lysine may be required in late gestation to maximize subsequent milk production.

The small size of the litter of the IB sow may explain lower Lys needs to maximize milk yield. In our experiment, the marginal deficiency in Trp and Val of the dietary protein supplied in late gestation to sows on MLys and HLys treatments seems to have had a minor influence in view of the observed BW of their litters and piglets at birth, close to those reported by Aguinaga *et al.* (2011) from multiparous IB sows fed conventional gestation and lactation diets.

In the IB sow the mobilization of body protein in support of milk-protein synthesis may be rather inefficient compared to lean sows, mainly because of its comparatively lower body protein mass. Iberian-sow milk contains (kg^{-1}) 53.4 g CP and 4.626 MJ GE (Aguinaga *et al.*, 2011). A value of 0.195 for the piglets' BW daily gain-to-daily milk yield ratio has been measured (Aguinaga *et al.*, 2011). In our experiment, 6.02 ± 0.09 and 6.44 ± 0.12 piglets were suckled on average by sows in second- and third-parity respectively. Their average corresponding daily gains were 195 ± 3 and 184 ± 4 g. Consequently, the average milk yield can be calculated as being 6.02 and 6.08 kg d^{-1} in second- and third-parity sows, and therefore 321-325 g protein and 27.8-28.1 MJ energy were secreted daily in milk during the 35-d lactation period. During lactation, the IB sows were fed 4.5 kg d^{-1} of the lactation diet (56.2 MJ ME and 46.4 g Lys [666 g high-quality CP]). This feed allowance would meet total energy and lysine needs for daily milk production, estimated as 48.6 MJ ME and 36.5 g Lys on average (BSAS, 2003) and part of the maintenance requirements. Unexpectedly, daily BW losses during lactation reached 635 ± 41 and 631 ± 40 g in second- and third-parity sows, respectively. Such tissue mobilization seems to be surprisingly high, and would be indicative of poor efficiencies of conversion of dietary energy and protein to milk, a subject that merits future studies.

Apart from the great contrast between plasmatic levels of metabolites observed in gestation *vs.* lactation, as can be expected to find between an anabolic and a catabolic state (except for plasmatic urea, plasma level of all metabolites measured differed, $p < 0.01$-0.001), in our experiment no clear relationship could be established between plasma metabolite levels and the performance of either sows or piglets. This could be, unless in part, attributable to the low number of sows sampled at each physiological stage. In the anabolic state of the gestating sow, plasma urea level may indicate the extent of dietary or endogenous AA being oxidized. The higher plasma level of urea in the gestating third-parity sows on the LLys, Control diet suggests that lysine supply was limiting. Additional dietary Lys reduced urea in plasma in parallel with increased BW gain. On the other hand, the observed levels of urea in plasma are much lower than those reported for gestating sows of conventional breeds, usually fed diets containing higher concentrations of protein (Clowes *et al.*, 2003; Yang *et al.*, 2009). In the present study, lower levels of plasma creatinine have been observed in comparison with those found in leaner gestating sows by Clowes *et al.* (2003), who noticed that plasma creatinine concentrations were linearly related to total N retained. This fact may be indicative of a lower pool of protein being deposited daily both in maternal and reproductive tissues of the gestating IB sow. As an indicator of muscle catabolism, plasma creatinine may also increase in lactation compared with pregnancy, as we found in second-parity sows.

As final conclusions, adequate reserves for subsequent lactation can be attained in second-parity and multiparous IB sows given a moderate energy supply by providing them with at least 17.2 g lysine daily over the last third of pregnancy. This dietary regime implies a substantial increase in protein supply respect to traditional nutritional practices.

Acknowledgements

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