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## Dietary protein restriction during lactation in primiparous sows with different live weights at farrowing: II. Consequences on reproductive performance and interactions with metabolic status

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**Abstract** – The hypothesis that the restriction of dietary protein during lactation has different impacts on reproductive performance in light and heavy sows at farrowing was investigated, as well as the relationships between reproductive parameters and sow metabolic data. At farrowing, 38 primiparous sows were assigned to one of three groups: sows weighing 180 kg not restricted in dietary protein during lactation (180CP); sows weighing 180 or 240 kg restricted in protein (180LP and 240LP). Twenty-four sows were catheterized and serial blood samples were collected 1 d before and 1 d after weaning. The sows were inseminated at the first estrus after weaning and slaughtered at d 30 of gestation. Protein restriction reduced the proportion of sows that returned to estrus within 8 d after weaning in the 180LP sows ( $P < 0.03$ ), but not in the 240LP sows. It also induced a reduction in ovulation rate in the 180LP sows ( $P < 0.05$ ) and, to a lesser extent, in the 240LP sows ( $P = 0.12$ ). When the sows were categorized according to return to estrus (WOI  $\leq 8$  or  $> 8$  d), basal and mean concentrations of LH increased after weaning only in sows with a short WOI. Sows with a delayed estrus exhibited a higher ratio of plasma tyrosine to large neutral amino acids (AA,  $P < 0.01$ ). In conclusion, large body reserves at farrowing buffer, at least in part, the detrimental effect of a strongly negative nitrogen balance on reproduction. We suggest that the alteration of AA profiles induced by dietary protein restriction and body protein loss alters LH secretion via modifications of the neurotransmitters involved in GnRH secretion.

sow / rearing / lactation / protein intake / amino acid / reproductive performance

### 1. INTRODUCTION

Modern sows generally have a high milk yield ( $> 10 \text{ kg} \cdot \text{d}^{-1}$ ; review: [1]) and voluntary feed intake during lactation is often insufficient to meet the nutritional needs for milk

production, especially in primiparous sows [2]. As a consequence, sows must mobilize fat and protein reserves [3, 4]. The extent of body reserve mobilization depends on the amplitude and the nature of the nutritional deficit. Low protein supply during lactation

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amplifies muscle tissue mobilization [5–7]. In lactating primiparous sows, a severe deficit in dietary protein has been demonstrated to compromise lactation [6] and subsequent reproduction, especially the weaning-to-estrus interval (WOI) [5, 8, 9]. However, this detrimental effect on WOI or ovarian function at weaning was not observed in primiparous sows that had large body reserves at farrowing [10, 11]. Evidence exists that WOI is influenced not only by body reserve loss during lactation, but also by absolute amounts of reserves at farrowing and weaning [12–15]. Therefore we hypothesize that large protein reserves related to heavy body weight can play a protective role against the detrimental effects of protein restriction. The present experiment was designed to test this hypothesis in sows that had been fed differently during rearing and gestation, in order to achieve two different live weights at farrowing, and submitted to a dietary protein (lysine) restriction during lactation. The consequences of protein restriction on litter growth and sow's metabolic status are presented in a companion paper [16]. The present part of the study focuses on reproductive consequences. There is a large body of evidence showing that changes in metabolic status are transmitted to the reproductive axis through changes in concentrations of metabolites and metabolic hormones (reviews: [17–19]). Therefore relationships between metabolic and endocrine data and reproductive performance were also investigated.

## 2. MATERIALS AND METHODS

### 2.1. Animals and experimental design

The experimental design was extensively described in a companion paper [16]. The experiment was conducted in four replicates on Pietrain × (Landrace × Large White) crossbred gilts. Five-month old females were assigned to a group of “180 kg” or “240 kg” sows, depending on the objective of live weight at farrowing. After puberty, the estrous cycles were synchronized by a

progestagen treatment (Regumate<sup>®</sup>, Janssen-Cilag, Issy-les-Moulineaux, France). At the subsequent estrus, fifty-two gilts were inseminated at  $265 \pm 1$  d of age and  $136 \pm 11$  (group “180 kg”;  $n = 31$ ) or  $189 \pm 14$  kg live weight (group “240 kg”;  $n = 21$ ). On d  $104 \pm 1$  of gestation, the gilts were moved from the gestation to the farrowing facilities and were kept in individual farrowing crates. When necessary, parturition was induced by an i.m. injection of 2 mL of cloprostenol (Planate, Mallinckrodt veterinary, Meaux, France) on d 114 of gestation. Farrowing occurred on d 114 or 115 of gestation. Within 48 h after birth, the litters were standardised to 11 piglets and to 10 piglets 3 d later. Piglets had no access to creep feed and free access to the dam throughout lactation. They were weaned between 0830 and 0930 at  $28 \pm 1$  d of age. Water was freely available for the sows and piglets throughout the experimental period.

### 2.2. Diets

They were fed a standard growing pig diet until insemination. Feed allowance was calculated to meet 1.6 and 2.4× the energy requirements for maintenance. During gestation, all sows received a standard gestation diet containing 12.1 MJ of digestible energy (DE), 130 g of crude protein (CP) and 6 g of lysine per kg. The daily feed allowance was adjusted every three weeks in order to meet 110% of the energy requirements for gestation [20]. On the day of farrowing, the sows were provided 1 kg of the gestation diet. During lactation, the daily feed allowance was given in two equal meals provided at 0830 and 1430. One day after farrowing, 38 sows were allocated to one of three groups: (1) “180 kg” sows not restricted in dietary protein during lactation (180CP); (2) “180 kg” sows restricted in protein (180LP); and (3) “240 kg” sows restricted in protein (240LP). The low-protein diets were formulated on the basis of lysine being the first-limiting amino acid (AA) with other AA meeting or exceeding the suggested “ideal” ratio for each AA relative to lysine

**Table I.** Sow feed, energy and lysine intakes and changes in live weight during lactation (LSMEANS  $\pm$  SEM).

	Treatment <sup>1</sup>			
	180CP (n = 12)	180LP (n = 12)	240LP (n = 14)	
Feed intake, kg·d <sup>-1</sup>	4.1 $\pm$ 0.1 <sup>a</sup>	4.2 $\pm$ 0.1 <sup>a</sup>	3.5 $\pm$ 0.3 <sup>b</sup>	
ME <sup>2</sup> intake, MJ·d <sup>-1</sup>	53.1 $\pm$ 2.5 <sup>a</sup>	54.8 $\pm$ 2.5 <sup>a</sup>	45.6 $\pm$ 2.1 <sup>b</sup>	
Lysine intake, g·d <sup>-1</sup>	39.4 $\pm$ 1.1 <sup>a</sup>	21.6 $\pm$ 1.1 <sup>b</sup>	16.0 $\pm$ 1.0 <sup>c</sup>	
Sow live weight, kg				
At d 1	182.6 $\pm$ 6.6 <sup>a</sup>	181.1 $\pm$ 6.6 <sup>a</sup>	238.2 $\pm$ 6.4 <sup>b</sup>	
Loss during lactation	-19.4 $\pm$ 3.6 <sup>a</sup>	-22.1 $\pm$ 3.6 <sup>a</sup>	-37.6 $\pm$ 3.4 <sup>b</sup>	
Lipid <sup>3</sup> , kg				
d 1	29.1 $\pm$ 1.8 <sup>a</sup>	28.5 $\pm$ 1.8 <sup>a</sup>	53.6 $\pm$ 1.8 <sup>b</sup>	0.001
Loss during lactation	-8.5 $\pm$ 1.3 <sup>a</sup>	-7.4 $\pm$ 1.3 <sup>a</sup>	-15.3 $\pm$ 1.2 <sup>b</sup>	0.001
Protein <sup>3</sup> , kg				
d 1	29.6 $\pm$ 1.2 <sup>a</sup>	29.6 $\pm$ 1.2 <sup>a</sup>	36.4 $\pm$ 1.2 <sup>b</sup>	0.001
Loss during lactation	-2.4 $\pm$ 0.6 <sup>a</sup>	-3.3 $\pm$ 0.6 <sup>a</sup>	-4.9 $\pm$ 0.6 <sup>b</sup>	0.002

<sup>1</sup>180CP and 180LP: primiparous sows weighing 180 kg at farrowing and fed a control or low-protein diet during lactation; 240LP: sows weighing 240 kg at farrowing and fed a low-protein diet.

<sup>2</sup>Metabolizable energy.

<sup>3</sup>The chemical composition of sows was estimated from the body weight and backfat thickness measurements using the equations proposed by Dourmad et al. [40]: lipids (kg) =  $-26.4 + 0.221 \text{ EBW} + 1.331 \text{ P}_2$ , protein (kg) =  $2.28 + 0.178 \text{ EBW} - 0.333 \text{ P}_2$ , where EBW (kg) represents the sow empty live weight (EBW =  $0.905 \text{ BW}^{1.013}$ , BW = live weight in kg) and  $\text{P}_2$  (mm) = backfat thickness at the level of the last rib.

<sup>a,b,c</sup> Within a row, means without a common superscript letter differ ( $P < 0.05$ )

for lactating sows [21]. The diets provided similar amounts of metabolizable energy ( $13.0 \text{ MJ}\cdot\text{kg}^{-1}$ ; Tab. I). On d 1 and 2, all females received 2.5 and 3.5  $\text{kg}\cdot\text{d}^{-1}$  of the experimental diet, respectively. Thereafter, the amount of feed was restricted to  $4.3 \text{ kg}\cdot\text{d}^{-1}$  for the 180CP and 180LP and  $4.5 \text{ kg}\cdot\text{d}^{-1}$  for the 240LP sows to avoid differences in feed consumption and obtain the same ratio of energy ingested to energy requirements ( $\sim 70\%$ ) for all sows. Feed refusals were weighed daily before the morning meal and the actual feed intake was then calculated.

From the day of weaning (W) until the end of the experiment, all sows received a conventional gestation diet containing  $12.1 \text{ MJ DE}\cdot\text{kg}^{-1}$ , 130 g CP and 6 g lysine per kg, in two equal meals given at 0830 and

1430. At weaning, the sows remained in their farrowing crate for 2 d in order to facilitate serial blood sampling. Thereafter, they were moved and penned in individual gestation crates ( $0.7 \times 2.5 \text{ m}$ ) until the end of the experiment.

Feeding treatments during rearing and gestation produced sows that weighed around 180 (180CP and 180LP sows) or 240 kg (240LP sows) on the day after farrowing (Tab. I). Light sows (180CP and 180LP) had a similar body composition (the estimated contents of the sow's body lipid and protein were respectively 15.7% and 16.3% of live weight) but 240LP sows were fatter (lipid and protein contents represented 22.5% and 15.2% of live weight, respectively). Average feed intake during lactation was lower for heavy (240LP) than for light

sows, because they ate less than expected during the first 2 weeks of lactation. As a consequence, the 240LP were more severely restricted in dietary protein (lysine) than the 180LP sows. Lipid losses during lactation were relatively high and did not differ among treatments (~28% of lipid mass at the onset of lactation). Protein losses were significantly higher in the 240LP than in the 180CP sows and intermediate in the 180LP sows (Tab. I). They were estimated to represent 8, 11 and 13.5% of protein mass at farrowing for the 180CP, 180LP and 240LP sows, respectively (for details, see [16]).

### 2.3. Estrus, ovaries, ovulation rate and embryonic survival

The procedure was similar to that described by Mejia-Guadarrama et al. [10]. From 2 d after weaning (day W+2), the sows were monitored for estrus twice daily at 0800 and 1600 by exposing them to a fenceline contact with a mature boar for 15 min and using the back-pressure test. The sows were artificially inseminated twice with fresh diluted semen from Pietrain boars. When the first observation of standing estrus occurred in the morning, a first insemination was performed 8 h later, whereas insemination was performed 16 h later when estrus was first detected in the afternoon. The second insemination was performed 24 h after the onset of estrus in both cases. The doses of semen ( $3 \times 10^9$  spermatozoa·70 mL<sup>-1</sup>) consisted of pooled semen. The same pool was used for all sows belonging to a same replicate. Only sows that were detected in estrus within 8 d after weaning were inseminated. The sows were slaughtered  $36 \pm 1$  d after weaning and the genital tract was immediately collected after death. The weights of both ovaries were recorded. All corpora lutea were dissected and counted. The presence of corpora albicantia was also checked to correlate with records on estrus detection. Live and dead embryos were counted. An embryo was considered dead on the basis of size visual evaluation (markedly less than the mean of all embryos from

that sow) and/or by the observation of a necrotic process in situ. Embryo survival was expressed as the percentage of corpora lutea represented by live embryos.

### 2.4. Measurements and sampling

A subgroup of sows was randomly allocated to blood sampling (8/group). On d  $22 \pm 1$  of lactation, catheters were surgically inserted into the jugular vein of sows under general anaesthesia. Serial blood samples were collected via a catheter every 15 min from 0815 to 1615 on the day before (day W-1) and the day after weaning (day W+1). Single blood samples for IGF-I were taken at 1400 on day W and from day W+2 to day W+6. Blood samples were collected in heparinized tubes and immediately centrifuged at 4 °C for separation of plasma. Plasma samples were stored at -20 °C until assay.

The animals were reared in compliance with national regulations for the humane care and use of animals in research (certificate of authorization to experiment on living animals No. 7675).

### 2.5. Hormone assays

Plasma concentrations of LH were determined on days W-1 and W+1 using validated RIA [22]. The samples were run in duplicate in a single assay. Concentrations of LH were measured every 15 min from 0815 to 1615. The intraassay CV was 10% at 2 ng·mL<sup>-1</sup>, and average sensitivity, defined as 92% of total binding, was 0.5 ng·mL<sup>-1</sup>.

### 2.6. Calculations and statistical analyses

Profiles of LH were analyzed as previously described [23]. Characteristics of LH secretion (mean and basal concentrations, number of pulses) were calculated and used for statistical analyses.

Statistical analyses of sow performance were performed on data from the 38 sows allocated to experimental treatments and analyses of hormones on data from the

24 catheterized sows. The number of sows that returned to estrus within 8 d after weaning and the number of gestating sows were compared using the Cochran test. Other data were analyzed as a randomized complete block design, with three treatments in four replicates (blocks), by analysis of variance using the MIXED procedure of SAS [24]. All models included the effects of the treatment (fixed effect) and of the replicate (random effect). Data for LH were analyzed using repeated measures in MIXED procedures. The complete model included treatment, replicate, time and time  $\times$  treatment interaction as main effects, the sow was the experimental unit and significant differences among treatments were determined using sow within replicate  $\times$  treatment interaction as the error term. In the results, the least squares means and the standard errors of least squares means are given. Embryo survival data are expressed as a percentage and were analyzed after arcsine square root transformation.

To connect return to estrus after weaning and plasma metabolite and hormone concentrations, other analyses were performed on sows categorized according to their return to estrus ( $\text{WOI} \leq 8$  d or  $> 8$  d). The models included the effects of the WOI duration (fixed effect) and of the replicate (random effect).

Pearson correlation analysis was used to determine the relationships between reproductive characteristics and hormone data on days W-1 and W+1 for sows with catheters ( $n = 24$ ; SAS).

### 3. RESULTS

#### 3.1. Reproductive traits after weaning

Thirty sows returned to estrus within 8 d after weaning. Twenty-seven out of these 30 sows exhibited estrus 5 or 6 days after weaning. Among the sows with a delayed estrus, four were in anestrus at slaughter, one had a WOI of 10 d and 3 had a WOI of 30 d (this was confirmed by the presence of

only corpora lutea and no corpora albicantia in the ovaries at slaughter). The proportion of sows that returned into estrus within 8 d after weaning was lower ( $P < 0.05$ ) in the 180LP group than in the other groups (Tab. II). The number of corpora lutea (= ovulation rate) was reduced in the 180LP sows compared with the 180CP sows ( $P < 0.05$ ) and intermediate in the 240LP sows (Tab. II). The proportion of inseminated sows after weaning that were gestating did not significantly differ between the groups (Tab. II). In this subgroup of gestating sows, the ovulation rate followed the same trend as in the overall population, but differences between groups were not significant. The number of live and dead embryos and the embryonic survival did not differ ( $P > 0.1$ ).

Considering the sows from the endocrine data set, the proportion of sows in estrus within 8 days after weaning was lower in the 180LP sows compared with the other two groups. The number of corpora lutea and of live or dead embryos, as well as embryonic survival, did not significantly differ between treatments in this subgroup (Tab. II).

#### 3.2. Luteinizing hormone

Mean and basal concentrations of LH, as well as the number of LH pulses over 8 h, were not influenced by treatments, neither 1 d before nor 1 d after weaning ( $P > 0.1$ , Tab. III). Regardless of the treatment group, all criteria increased significantly after weaning (Tab. III).

When the sows were categorized according to their WOI, there was a significant interaction between treatment and day of sampling for the LH concentrations ( $P < 0.05$ , Tab. IV). Mean and basal LH concentrations increased from day W-1 to day W+1 in sows that returned into estrus within 8 d and not in sows with delayed estrus. As a consequence, LH concentrations on the day after weaning were higher in sows with a WOI in a "normal" range ( $P < 0.05$ ).

**Table II.** Reproductive traits of sows on treatment (LSMEANS  $\pm$  SEM).

	Treatment <sup>1</sup>			<i>P</i> -value
	180CP	180LP	240LP	
<b>All sow data (n = 38)</b>	12	12	14	
Number (%) <sup>2</sup>	11 (92) <sup>a</sup>	7 (58) <sup>b</sup>	12 (86) <sup>a</sup>	0.03
WOI, days	5.3 $\pm$ 0.3	5.7 $\pm$ 0.3	5.7 $\pm$ 0.3	0.46
No. corpora lutea	17.1 $\pm$ 0.6 <sup>a</sup>	14.0 $\pm$ 1.1 <sup>b</sup>	15.6 $\pm$ 0.7 <sup>ab</sup>	0.05
<b>Gestating sows (n = 24)</b>	10	5	9	
Gestation rate <sup>2,3</sup> %	91	71	75	0.72
No. corpora lutea	17.0 $\pm$ 0.7	14.0 $\pm$ 1.1	15.4 $\pm$ 0.7	0.10
No. embryos	13.0 $\pm$ 0.8	11.8 $\pm$ 1.2	12.4 $\pm$ 0.8	0.70
No. live embryos	12.8 $\pm$ 0.9	11.8 $\pm$ 1.3	12.1 $\pm$ 0.9	0.72
Embryonic survival <sup>4</sup> , %	75	84	79	0.54
<b>Endocrine data set (n = 24)</b>	8	8	8	
Females in estrus within 8 d after weaning:				
Number (%) <sup>2</sup>	7 (88) <sup>a</sup>	4 (50) <sup>b</sup>	8 (100) <sup>a</sup>	0.02
No. gestating females <sup>2</sup>	6	2	5	0.51
No. corpora lutea	17.0 $\pm$ 1.0	16.0 $\pm$ 2.0	16.0 $\pm$ 1.0	0.80
No. embryos	13.3 $\pm$ 1.0	13.5 $\pm$ 1.8	12.0 $\pm$ 1.1	0.66
No. viable embryos	13.2 $\pm$ 1.1	13.5 $\pm$ 1.9	11.6 $\pm$ 1.2	0.59
Embryonic survival <sup>4</sup> , %	78	84	75	0.92

<sup>1</sup>180CP and 180LP: primiparous sows weighing 180 kg at farrowing and fed a control or low-protein diet during lactation; 240LP: sows weighing 240 kg at farrowing and fed a low-protein diet.

<sup>2</sup>Analysis based on the Cochran test.

<sup>3</sup>Number of gestating sows/number of inseminated sows.

<sup>4</sup>Number of live embryos/No. of corpora lutea. Analysis based on arsin-transformed data.

<sup>a,b</sup> Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

### 3.3. Relationships between reproductive data and metabolic criteria

Sows with a short WOI exhibited a lower GH/IGF-I ratio than sows with an extended WOI ( $P < 0.001$ ; Tab. IV). Mean and basal concentrations of LH on day W+1 were negatively correlated with preprandial tyrosine concentrations on day W-1 ( $P = 0.09$  and  $P < 0.05$ , respectively) and with the tyrosine/LNAA ratio ( $P < 0.001$ , Tab. V). The frequency of LH pulses was negatively correlated with post-prandial concentrations

of serine and proline on day W-1 ( $r = -0.63$ ,  $P < 0.003$  and  $r = -0.46$ ,  $P < 0.05$ , respectively) and on day W+1 ( $r = -0.46$ ,  $P < 0.03$  and  $r = -0.55$ ,  $P < 0.007$ ).

Ovulation rate was negatively correlated with mean plasma GH before weaning and positively with post-prandial concentrations of methionine (Tab. VI). A positive correlation ( $P < 0.05$ ) also existed between ovulation rate and post-prandial concentrations of arginine, lysine and ornithine (data not shown).

**Table III.** Influence of treatment on the characteristics of LH secretion 1 d before (W-1) or after (W+1) weaning (LSMEANS  $\pm$  SEM).

	Treatment <sup>1</sup>			P-value <sup>2</sup>		
	180CP (n = 8)	180LP (n = 8)	240LP (n = 8)	T	D	T $\times$ D
Mean concentrations of LH, ng·mL <sup>-1</sup>						
W-1	1.4 $\pm$ 0.1	1.4 $\pm$ 0.1	1.4 $\pm$ 0.1	0.84	0.001	0.30
W+1	1.6 $\pm$ 0.1	1.5 $\pm$ 0.1	1.7 $\pm$ 0.1			
Basal concentration of LH, ng·mL <sup>-1</sup>						
W-1	1.3 $\pm$ 0.1	1.2 $\pm$ 0.1	1.3 $\pm$ 0.1	0.87	0.003	0.51
W+1	1.5 $\pm$ 0.13	1.4 $\pm$ 0.13	1.6 $\pm$ 0.1			
No. of LH pulses, /8 h						
W-1	1.9 $\pm$ 0.8	1.4 $\pm$ 0.8	2.0 $\pm$ 0.8	0.95	0.05	0.80
W+1	2.8 $\pm$ 0.7	2.8 $\pm$ 0.8	2.6 $\pm$ 0.7			

<sup>1</sup> 180CP and 180LP: sows weighing 180 kg at farrowing and fed a control or low-protein diet during lactation; 240LP: sows weighing 240 kg at farrowing and fed a low-protein diet.

<sup>2</sup> T = treatment effect; D = sampling day effect; T  $\times$  D = treatment  $\times$  sampling day interaction.

**Table IV.** Characteristics of LH secretion and GH/IGF-I ratio 1 d before (W-1) and after (W+1) weaning in sows showing a normal (2 to 8 d) or long (more than 8 d) weaning-to-estrus interval (LSMEANS  $\pm$  SEM).

Characteristics of LH	WOI		P-value <sup>1</sup>		
	$\leq$ 8 d (n = 19)	> 8 d (n = 5)	T	D	T $\times$ D
Mean concentration of LH, ng·mL <sup>-1</sup>					
W-1	1.4 $\pm$ 0.1 <sup>a</sup>	1.3 $\pm$ 0.1 <sup>a</sup>	0.187	0.024	0.031
W+1	1.7 $\pm$ 0.1 <sup>b</sup>	1.3 $\pm$ 0.1 <sup>a</sup>			
Basal concentration of LH, ng·mL <sup>-1</sup>					
W-1	1.3 $\pm$ 0.1 <sup>a</sup>	1.3 $\pm$ 0.2 <sup>a</sup>	0.478	0.097	0.003
W+1	1.5 $\pm$ 0.1 <sup>b</sup>	1.2 $\pm$ 0.1 <sup>a</sup>			
No. of pulses, /8 h					
W-1	1.8 $\pm$ 0.5	1.6 $\pm$ 1.0	0.733	0.106	0.951
W+1	2.8 $\pm$ 0.5	2.4 $\pm$ 1.0			
GH/IGF-I ratio					
W-1	0.06 $\pm$ 0.01	0.12 $\pm$ 0.03	0.001	0.001	0.307
W+1	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01			

<sup>1</sup> T = WOI effect; D = sampling day effect; T  $\times$  D = WOI  $\times$  sampling day interaction.

<sup>a,b</sup> Means without a common superscript letter differ ( $P < 0.001$ ).



**Table V.** Pearson correlation coefficients relating pre-prandial<sup>1</sup> plasma amino acid concentrations 1 d before weaning and plasma LH characteristics 1 d before (W-1) and after (W+1) weaning ( $n = 24$ ).

Amino acids	LH characteristics					
	on W-1			on W+1		
	Mean	Basal	Pulses	Mean	Basal	Pulses
Arginine	0.13	0.01	0.13	0.16	0.11	0.15
Tryptophan	0.25	0.30	0.10	0.29	0.17	0.29
Phenylalanine	-0.08	-0.19	0.15	0.15	0.09	0.05
Tyrosine	-0.26	-0.29	0.33	-0.36 <sup>T</sup>	-0.48*	0.11
LNAA <sup>2</sup>	-0.06	-0.15	0.34	0.10	0.03	0.09
Try/LNAA <sup>3</sup>	0.23	0.35	-0.26	0.19	0.15	0.11
Tyr/LNAA <sup>4</sup>	-0.28	-0.20	-0.03	-0.63***	-0.65***	-0.06

<sup>1</sup> For these AA, no correlation was found with post-prandial concentrations.

<sup>2</sup> Large neutral AA: isoleucine + leucine + valine + phenylalanine + tyrosine.

<sup>3</sup> Try/LNAA = tryptophan / LNAA.

<sup>4</sup> Tyr/LNAA = tyrosine / (isoleucine + leucine + valine + phenylalanine + tryptophan).

<sup>T</sup>  $P = 0.09$ ; \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .

**Table VI.** Pearson correlation coefficients relating hormone and amino acid concentrations and ovulation rate (OR,  $n = 16$ ).

OR	Threonine <sup>1</sup>	Methionine <sup>1</sup>	GH <sup>2</sup>	IGF-I <sup>2</sup>	Insulin <sup>3</sup>	GH <sup>2</sup>	IGF-I <sup>2</sup>	Insulin <sup>3</sup>
	on day W-1		on day W-1			on day W+1		
		0.50*	0.68**	-0.59*	-0.09	0.08	-0.22	-0.20

<sup>1,2,3</sup> Respectively post-prandial, mean and pre-prandial concentrations.

#### 4. DISCUSSION

Our findings show that large body reserves at farrowing attenuate the detrimental impact of low protein intake during lactation on reproductive performance after weaning.

The return to estrus after weaning was clearly impaired by dietary protein (lysine) restriction in the 180LP sows. This was not the case in the 240LP sows, despite a more severe restriction in protein and a greater mobilization of body protein during lactation (13.5% of initial protein mass in 240LP sows vs. 11% in 180LP sows). This suggests that the WOI is influenced not only by protein loss during lactation, but also by the absolute amount of protein reserves at far-

rowing and at weaning. However, the respective roles of fat and protein stores in the protective effect of large body reserves can be debated. Indeed, the 240LP sows were fatter than the 180LP sows and lost twice the amount of lipids as the 180LP sows. Lipid stores in the adipose tissue are extensively used when energy is lacking. Large fat reserves were used by the 240LP sows to cope with a more negative energy balance due to their reduced feed intake, as compared with the 180CP and 180LP sows [16]. Concerning the protein (lysine) restriction, muscle proteins are the only source to compensate for AA deficit. Therefore body protein reserves play the major role in the positive effect of large body reserves against protein deficit.

The influence of protein restriction during lactation on WOI has already been reported in primiparous sows [8, 9, 25] and it has been attributed to the inhibition of LH secretion during lactation or just after weaning [9, 25]. In the present experiment, characteristics of LH secretion around weaning did not differ among treatments. Nevertheless, LH secretion did not increase at weaning in sows with delayed estrus, as it did in sows with early return to estrus after weaning. It is therefore likely that the delayed estrus observed in the 180LP sows originated from LH inhibition around weaning.

Mechanisms by which negative energy or nutrient balance inhibits LH secretion are not yet fully understood. Many links between the metabolic status of females and the hypothalamo-pituitary unit have been proposed, the major one being glucose and insulin levels [17, 26]. We have recently proposed a role for AA availability [10], as suggested in rodents and ruminants [27]. Among the numerous neuropeptides potentially involved in the neuroendocrine regulation of GnRH secretion, some of them require, for their synthesis, AA precursors that are provided exclusively by dietary proteins. Serotonin is synthesized from tryptophan, norepinephrine and dopamine are synthesized directly from tyrosine or indirectly from phenylalanine. Moreover, several AA share a common AA transporter with large neutral AA (LNAA), and their uptake into the brain depends on the LNAA concentrations [28]. In pigs, brain serotonin concentrations are increased dose-dependently by dietary supplementation of tryptophan to a deficient ration [29], but it is decreased by additional crude protein or additional LNAA [29, 30] Barb et al. [31] reported that 48-h fasting reduced the tyrosine/LNAA ratio in the plasma of prepuberal gilts. These gilts exhibited an alteration in GH secretion but not in LH secretion. In the current experiment, the ratio of tyrosine to LNAA was strongly negatively correlated with mean and basal concentrations of LH on the day after weaning. A higher ratio likely favors the transportation of tyro-

sine into the brain, resulting in a greater availability of tyrosine for norepinephrine and dopamine synthesis. To our knowledge, there is no data in pigs on the influence of norepinephrine on LH secretion. The role of dopamine was first investigated in lactating sows by using a short-acting agonist, bromocryptine. The agonist induced either a decrease [32] or an increase [33] in LH secretion during the third week of lactation. In 1998, De Rensis et al. [34] reported that the administration of a long-acting dopamine agonist, cabergoline, increased LH secretion in late lactation. They suggested that dopamine may antagonize the inhibitory effect of endogenous opioids on GnRH secretion. Because of these findings, a positive correlation between tyrosine/LNAA ratio and LH secretion could have been expected, instead of a negative one. Yet, no data has been reported in weaned sows, when there is no longer an influence of endogenous opioids on LH secretion. Since neuropeptides interact together and with steroids to influence GnRH and LH secretion, the influence of dopamine on LH secretion may differ between late lactation and post-weaning days.

The physiological meaning of the relationships between serine or proline and the number of LH pulses is not clear. Either these AA play a role in the control of LH secretion via a cascade of events (AA synthesis) that remains to be established, or they only reflect the (protein) nitrogen status of the sows. Altogether, our findings show that an alteration of AA profiles at the end of lactation is likely to be involved in the delayed estrus after weaning in light protein-restricted sows.

The potential involvement of other mediators, insulin, IGF-I and GH, in LH secretion and WOI was also investigated. An interesting result is the higher ratio of circulating GH to IGF-I around weaning in sows with an extended WOI. A high ratio is likely to reflect the uncoupling between GH and IGF-I secretion, which characterizes a strong catabolic status [16]. This does

not imply a relation of cause to effect, since the influence of IGF-I and especially GH on the hypothalamo-pituitary unit has not yet been demonstrated in pigs (review: [18]). Nevertheless, it indicates that GH/IGF-I ratio may be a better indicator of the sows' metabolic status and reproductive performance than IGF-I alone or insulin and glucose circulating concentrations.

Ovulation rate in sows that returned to estrus within 8 d after weaning was reduced by protein (lysine) restriction in the 180LP sows, and, to a lesser extent, in the 240LP sows. The detrimental effect of protein restriction has already been reported on ovulation rate at the first estrus after weaning [10]. It is also consistent with data showing an impaired folliculogenesis at or after weaning in sows that lost a high amount of body protein during lactation compared with sows that lost a moderate amount of protein [11, 35, 36]. Such impairment was illustrated by a lower proportion of large follicles and reduced concentrations of oestradiol and/or IGF-I in follicular fluid. Clowes et al. [11] also reported that the sow's body mass at parturition has a greater effect on ovarian characteristics than protein loss during lactation. This was in agreement with the fact that heavy sows in the present experiment were less affected by protein loss than light sows, concerning ovulation rate.

Considering the well-known stimulatory role of insulin and IGF-I on folliculogenesis in mammals, it is plausible that low insulin and IGF-I peripheral concentrations in the 180LP sows, and insulin resistance in the 240LP (for details, see [16]), are responsible for folliculogenesis impairment. The lack of relationships between these hormones and ovulation rate in the present experiment can be related to the lack of treatment effect on ovulation rate in the subgroup of catheterized sows (sows from the endocrine data set). However, we previously reported this lack of relationship [10]. Similarly, the alteration in folliculogenesis observed in sows that lost a high amount of body protein did not seem to be related to pre-prandial insu-

lin and IGF-I concentrations during lactation [36]. However, it is possible that IGF-I concentrations in follicles would be more informative than peripheral IGF-I with respect to folliculogenesis. Secretion of IGF-I by growing follicles is stimulated by GH. Moreover, some *in vitro* data obtained on porcine follicular cells suggest that GH itself could have a stimulatory effect on folliculogenesis alone or in synergy with IGF-I and a direct inhibitory action on follicle atresia. Our previous data [10, 16] showed the uncoupling between IGF-I and GH peripheral concentration, likely related to GH resistance, in protein-restricted sows that have a reduced ovulation rate. If the GH resistance is not confined in the liver but extended to other tissues including the ovaries, it may impair follicular growth either directly or by reducing IGF-I production by follicles.

Clowes et al. [11] conjectured that the availability of certain AA in the peripheral circulation could directly influence ovarian function. This is supported in the present experiment by the positive relationship observed between several AA and ovulation rate. The strongest and most interesting relationship is that with the post-prandial concentration of methionine. Methionine can be used for the synthesis of S-adenosyl methionine, the universal donor of methyl groups for the methylation of DNA. Genomic DNA methylation can silence or activate gene expression, with consequences on cell growth and tissue-specific differentiation. Dietary restriction of methionine results in a decrease in DNA methylation in humans and animals [37]. Investigations are needed to determine whether low plasma concentrations of methionine, due to low AA intake and substantial AA needs for milk protein synthesis, may participate in the alteration of final follicular growth or follicular survival.

Delayed consequences of feed restriction during lactation or the luteal phase have been reported on subsequent embryo development and survival [38, 39]. Furthermore,

Yang et al. [35] provided evidence that lysine restriction throughout lactation impairs the ability of large follicles to support oocyte maturation before postweaning ovulation. In the current experiment, protein (lysine) restriction did not influence the number of embryos on d 30 of gestation and embryo survival, as previously observed [10]. It is possible that treatment effect on oocyte and follicle quality was obliterated by d 30 of gestation, as the uterine capacity becomes limiting.

In conclusion, these results confirm that metabolic and reproductive consequences of protein (lysine) restriction during lactation differ with sows' live weight at farrowing. Protein (lysine) restriction delayed postweaning estrus in sows that weighed between 170 and 190 kg at farrowing, but not in sows that weighed around 240 kg. Protein restriction also altered ovulation rate after weaning, but to a lesser extent in heavy sows. Large body reserves buffered, at least partially, the negative impact of a negative nitrogen balance on reproductive performance. From these results, we can confidently propose that the alteration in peripheral AA profiles at the end of lactation plays a role in the impaired activity of the reproductive axis around weaning.

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