

## Effects of Dietary Protein Content on Milk Composition of Mixed Parity Lactating Sows in a Tropical Humid Climate

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### Abstract

Eighteen multiparous Large White sows were used to determine the effects of dietary protein content and lactation stage on milk composition during a 28-d lactation under humid tropical climatic conditions. This study was conducted at the INRA facilities in Guadeloupe, French West Indies (latitude 16°N, longitude 61°W). The average minimum and maximum ambient temperatures and average daily relative humidity during the trial were 22.7 and 29.4°C, and 93.7%, respectively. The dietary experimental treatments were a normal protein (NP, 17.3%) diet and a low protein (LP, 14.1%) diet supplemented with essential amino acids. The ADFI tended to be higher for the sows fed the LP diets when compared with the NP treatment (i.e., +9%,  $P < 0.10$ ). Litter BW gain and mean BW of piglets at weaning were not affected by dietary protein level ( $P > 0.10$ ). The treatments did not influence ( $P > 0.10$ ) sow body weight loss during lactation. The sows fed LP diets tended to show lower backfat thickness losses when compared to the sows fed NP diets (2.4 vs. 6.3 mm, respectively;  $P < 0.10$ ). Milk production and composition were not affected by dietary treatments ( $P > 0.10$ ). Milk dry matter and ash contents linearly increased according to lactation stage (17.6 to 19.9%, and 0.72 vs. 0.97%, respectively from d 7 to d 27;  $P < 0.01$ ). Lactose content increased from d 7 to d 14 (3.95 vs. 4.91;  $P < 0.01$ ) and thereafter remained constant. Fat content did not change during lactation and averaged 7.5%. The amino acid concentrations in milk protein were affected by the lactation stage: methionine, threonine, tryptophan, valine, and alanine concentrations decreased ( $P < 0.05$ ) but glycine and glutamic acid contents increased ( $P < 0.05$ ) from d 7 to d 27. Fatty acids milk profile was not influenced ( $P > 0.10$ ) by lactation stage. Maternal BW loss during lactation was negatively correlated with the average daily feed intake ( $r = -0.76$ ;  $P < 0.05$ ) and positively correlated with backfat thickness loss ( $r = 0.55$ ;  $P < 0.05$ ). A positive correlation between milk production and body reserves mobilisation ( $r = 0.82$ ;  $P < 0.05$ ) was also observed. Polyunsaturated fatty acid content in milk fat was positively correlated with ADFI and negatively correlated to maternal BW loss ( $r = 0.62$  and  $r = -0.60$ ;  $P < 0.05$ ). In conclusion, reducing dietary protein content can be an alternative to attenuate the negative effects of heat stress by increasing ADFI. Milk composition changes significantly according to lactation stage and the ability of sows to produce milk will depend on their capacity to mobilize body reserve for providing milk precursors.

**Keywords:** Sow; Lactation; Milk; Amino acids; Fatty acids

### Implications

Heat stress is a constant problem in many tropical and subtropical areas. Under heat stress, sows reduce their appetite in order to reduce their metabolic heat production due to the thermic effect of feed. This reduced feed intake has negative consequences on body reserves mobilization and milk yield and composition. The way the sow's milk composition and production is affected by heat stress implicates in the need of new nutritional strategies to attend the current lactating sow's daily needs and a better understanding of the consequences of this stress towards metabolism.

### Introduction

The growth rate of nursing piglets is mainly determined by milk nutrient output by the dam. As a consequence, the quantity and the composition of milk produced by sows are key factors to reach a successful piglet production. Milk production appears to be highly variable and depends on many factors. It can be affected by sow characteristics (genotype, litter size, parity number, and lactation stage) and by environmental factors (feeding management, photoperiod, climatic parameters) [1]. Under tropical conditions, we estimated that milk yield was reduced by at least 30 to 50% in comparison with data obtained in temperate countries [2]. This result is mainly connected to the combined negative effect of high ambient temperature and relative

humidity resulting in a concomitant reduction of voluntary feed consumption and milk production combined with the reduced ability of the sow to mobilize maternal body reserves. In fact, under tropical conditions, because of opened or semi-opened buildings, animals are more directly exposed to nycthemeral variation of the outside climatic conditions [3,4]. While there is substantial information on milk composition from sows raised under temperate climate [5], data on milk composition obtained in tropical countries are scarce and limited to the general composition (total solids, protein, fat and ash contents) [3,6]. The objective of our study was to evaluate the amino acid and fatty acid profile of sow's milk composition according to the lactation stage and dietary protein content under tropical humid conditions. In

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the present paper, the relationship between sow's performance and milk composition are also analysed.

## Materials and Methods

Care and use of animals were performed according to the certificate of authorization to experiment on living animals issued by the French Ministry of Agriculture to the head of the experimental facilities.

### Animals and experimental procedure

The study was conducted during the year of 2007 at the INRA facilities in Guadeloupe, French West Indies (latitude 16°N, longitude 61°W) characterized as having a tropical humid climate. A total of eighteen Large White mixed parity sows from 3 successive replicates of 6 sows each were used in this study. Within each replicate, sows were distributed in a completely randomized experimental design between two dietary treatments according to parity order, BW and backfat thickness after farrowing.

The dietary experimental treatments were: a normal protein diet (NP) and a low protein diet (LP) supplemented with essential AA. The experimental diets (Table 1) were formulated using corn, wheat middlings, and soybean meal, which met or exceeded AA requirements of lactating sows (NRC, 1998). The NP and LP diets contained the same levels of standardized digestible lysine (i.e., 0.80 g/MJ of NE). The ratio between digestible essential amino acids and digestible lysine in the experimental diets were maintained by synthetic AA complementation (DL-methionine, L-threonine, L-tryptophan, L-isoleucine, L-valine) to ensure that they were not below that of the ideal protein recommended for this animal category [3]. Diets were offered as pellets. Diets were prepared for the three successive replicates and stored in a temperature-controlled room (24°C, 50 to 60% RH).

During the gestation period, sows were housed in open-fronted gestating pens in groups of 5 sows each and restrictively fed a conventional diet containing 13 MJ DE/kg, 140 g CP/kg, based on maize, wheat middling and soybean meal. Feed allowance during the first 30 d after mating was calculated to standardize body condition at farrowing, according to the model proposed by Dourmad et al. [7]. The feeding level was fixed at 2.5 kg/d from the 30<sup>th</sup> to the 114<sup>th</sup> of gestation. Ten days before parturition, sows were moved to open-fronted farrowing pens (2.1 × 2.2 m) on a slatted metal floor. Variations in ambient temperature, relative humidity, and photoperiod closely followed outdoor conditions. On d 1 postpartum, sows received 1 kg of the standard gestation diet and the allowance increased by 1 kg each day until d 4 of lactation to avoid over-consumption at the beginning of lactation and agalactia problems. The proportion of gestation diet decreased progressively over the 4-d postpartum (100, 75, 50 and 25% on d 1, 2, 3 and 4, respectively), and sows were fed only the lactation diet on d 5. From d 6 to 26 postpartum, sows were allowed to consume feed ad libitum. The day prior to weaning (i.e., d 27), sows were allowed 3 kg of feed (i.e., at least 1.5 kg lower than their usual feed intake) to standardize consumption for all sows for determination of sow BW at weaning.

After birth, piglets were handled for tooth cutting, umbilical cord treatment and ear tagged for labelling. On d 3, they received an intramuscular injection of 200 mg of iron dextran. When necessary, cross-fostering was conducted within the first 48 h after birth to standardize litter size at 11 piglets. On d 14, male piglets were castrated. After 21 d of lactation, piglets were offered creep feed containing 15.3 MJ of DE/kg, 20% CP, and 1.47% total lysine. Infrared lights provided supplemental heat for the piglets during the first 21 d of the lactation period.

### Measurements and chemical analysis

Sows were weighed after farrowing and at weaning. Backfat thickness measurements were taken ultrasonically (Agroscan, E.C.M., Angoulême, France) at 65 mm from the midline at the point beside the shoulder and at the last rib on each flank 2 d before farrowing and at weaning. The total number of piglets born, born alive, stillborn, and piglet deaths during lactation were recorded for each litter. Piglets were individually weighed at birth, at d 14 and 21 of lactation and at weaning. Every morning, feed refusals were collected, and fresh feed was immediately distributed once per day between 0700 and 0900. Feed consumption was determined as the difference between feed allowance and the refusals collected on the next morning. Every day, one sample of feed and feed refusals were collected daily for DM content measurement, and successive samples were pooled and stored at 4°C for further analyses.

At d 7, 14, 21 and 27 piglets were separated from the sows after suckling, and 50 min later (i.e., equivalent to average suckling interval) [8], the sow was injected with 10 IU of oxytocin (Intervet, Angers, France) in an ear vein and all functional mammary glands were hand milked. Samples (approximately 150 to 200 mL) were immediately stored at -20°C for further analyses. At the end of the experiment, all samples were freeze dried and analyzed for moisture, ash, and N contents according to AOAC methods [9]. Lactose content was measured using an enzymatic method (ENZYPLUS EZS784, BioControl Systems, Inc.). The amino acids contents were determined by ion-exchange liquid chromatography (Biochrom 20, Pharmacia, Saclay, France) after a 24 h-hydrolysis in HCl (6 mol/L). For sulfur AA, the hydrolysis was performed by a performic oxidation. Tryptophan was hydrolyzed only for feed and milk in barium hydroxide solution (1.5 mol/L) for 20 h, separated by HPLC, and detected fluorimetrically (Waters 600E, St Quentin en Yvelines, France). The total lipid content was determined following a chloroform/methanol (2:1) extraction method according to Folch et al. [10]. Fatty acid methyl esters were prepared with 20% boron trifluoride/methanol solution according to Morrison and Smith, the fatty methyl esters were separated on a gas chromatograph equipped with a SP-2330 capillary column (30 m × 0.25 mm internal diameter) with a non-bonded poly (80% biscyanopropyl/20% cyanopropylphenyl siloxane) stationary phase (a 0.20- μm film thickness) [11]. Furnace temperature was 180°C, and injector and detector temperatures were 240°C.

Feed (two samples per diet and per replicate) samples were analyzed for DM, ash, fat content (AOAC) and CP (N × 6.25 for feed and N × 6.38 for milk) according to Dumas method (AOAC) and analyzed for crude fiber and for cell wall components (NDF, ADF, and ADL) according to Van Soest and Wine [9]. Feed AA contents were analyzed by Ajinomoto Eurolysine (Amiens, France) using ion-exchange chromatography, except for tryptophan, which was analyzed using HPLC and fluorimetric detection (Waters 600E, St. Quentin en Yvelines, France).

### Calculation and statistical analysis

Daily maximum, minimum, mean, and variance of daily ambient temperatures and relative humidities were averaged for each replicate. Milk production was estimated from litter growth rate and litter size between d 1 and d 21, and milk DM using the equation from Noblet and Etienne [12]. The effects of diet composition, replicate, parity number, and their interactions on performance of sows and litters were tested the GLM procedure of SAS. The effect of lactation stage on daily feed intake was tested with a mixed linear model (Mixed procedure of SAS) for repeated measurements with diet composition, batch and parity

Ingredients, %	Normal protein	Low protein
Corn	59.5	67.4
Soybean meal	24.4	10.6
Wheat middlings	8.4	14.3
Soybean oil	3.4	2.4
L-lysine HCL	0.020	0.415
DL-methionine		0.109
L-threonine		0.175
L-tryptophan		0.064
L-isoleucine		0.127
L-valine		0.140
Monocalcium phosphate	1.0	1.0
Calcium carbonate	2.1	2.1
Salt	0.1	0.1
Minerals and vitamins <sup>2</sup>	1.1	1.1
<b>Analyzed composition</b>		
Crude protein	17.3	14.1
Starch	39.0	45.2
Ether extract	4.3	5.6
NDF	10.0	10.8
<b>Calculated composition</b>		
SID amino acids, % <sup>2</sup>		
Lysine	0.80	0.80
Methionine + cystine	0.49	0.48
Threonine	0.54	0.54
Tryptophan	0.18	0.17
Isoleucine	0.63	0.54
Leucine	1.36	1.07
Valine	0.71	0.65
<b>Fatty acids (FA), % total<sup>3</sup></b>		
Saturated FA		
C16	11.1	11.4
C18	2.9	2.6
Total	14.6	14.6
<b>Monounsaturated FA</b>		
C16:1	0.2	0.3
C18:1	23.4	23.6
Total	24.5	24.8
<b>Polyunsaturated FA</b>		
C18:2	54.6	55.1
C18:3	5.0	4.2
Total	60.9	60.6
<b>Calculated nutritional values<sup>3</sup></b>		
Net energy, MJ/kg	10.2	10.1
Digestible lysine, g/MJ of NE	0.80	0.80

<sup>1</sup>Adjusted for 88% DM; <sup>2</sup>Standardized ileal digestible (SID) AA contents were calculated from the analyzed AA content and estimated standardized digestibility coefficients of the raw materials from INRA Tables (Sauvant et al.); <sup>3</sup>Fatty acids composition and NE values was estimated from the chemical composition of the diet and the equation of Noblet et al.

**Table 1:** Composition of lactation diets, as fed basis and analyzed chemical composition of the lactation diets<sup>1</sup>.

number as main effects. The least square means procedure (PDIFF option) was used to compare means when a significant F-value was obtained. Milk composition data were submitted to a linear mixed model including the effect of diet, batch, and lactation stage as main effects. In this later model, the sow was considered as a random effect and the repeated measurement option of the mixed procedure of SAS was used with an autoregressive covariance structure to take into account the correlations between repeated measurements on the same animal. Means comparison was performed according to the Pdiff

	Diet		RSD <sup>1</sup>	Statistics <sup>2</sup>
	Normal protein	Low protein		
No. of sows	9	9		
Parity no.	2.5	3.0	-	
Body Weight (BW), kg				
At farrowing	224	226	31	
Loss during lactation	29	21	9	
Backfat thickness, mm				
At farrowing	19.1	17.2	4.5	
Loss during lactation	6.3	2.4	3.6	D <sup>1</sup>
ADFI, g/d	4162	4555	653	D <sup>1</sup>
Litter BW gain, g/d	2142	1967	349	B <sup>1</sup>
Milk production, g/d <sup>3</sup>	7800	7170	1450	B <sup>*</sup>

<sup>1</sup>Residual Standard Variation; <sup>2</sup>From an analysis of variance including the effect of diet (D), batch (B) and parity as main effects. Statistical significance: <sup>1</sup> P<0.10; <sup>\*</sup> P<0.05; <sup>3</sup>Milk production during lactation was calculated from litter BW gain, litter size between d 1 and 27 using the equation from Noblet and Etienne.

**Table 2:** Effects of dietary protein content on performance of lactating sows over a 28-d lactation (least square means).

option of SAS procedure using Tukey test for contrasts. Residual values were computed from the preceding models (without the random sow effect) and residual Pearson correlations between lactating performance and mean milk composition parameters were calculated using the CORR Procedure of SAS/STAT.

## Results and Discussion

Average minimum and maximum ambient temperatures and average daily relative humidity measured during the experimental period were 22.7 and 29.4°C, and 93.7%, respectively. After farrowing, sows were restrictively fed for 5 d according to the same feeding plan and the increase of ADFI was similar for both treatments until d 4. After d 4, ADFI tended to be higher for LP diet as compared to the NP diet during the lactation (i.e., 4.55 vs. 4.16 kg/d, respectively; P=0.08; Table 2).

Similarly to our findings, Renaudeau and Noblet evaluating the effect of protein reduction (14.2 vs. 17.6%) also reported a numerical increase of ADFI in heat stressed (29°C) sows (fed LP diet (+0.639 kg/d) [13]. Lynch also observed an increased feed consumption (+0.700 kg/d) in multiparous lactating sows fed a low CP diet (14 vs. 20%) under heat stress conditions (i.e., 28°C) [14]. In contrast, Quiniou and Noblet did not report any effect of diet on performance of lactating sows kept at 29°C when dietary protein content was reduced from 17 to 14% [15]. According to the later authors, the results could be due to the lack of interaction between temperature and diet to the low number of observations and (or) the deficiency in sulphur AA and Trp the low CP diet. The reduction of dietary protein content with a supplementation of industrial AA leads to an increase in the ratio between Trp and branched chain AA (LNAA: Leu, Ile, Val, Phe, Tyr) (i.e., 4.52 vs. 5.37% in NP and LP diet, respectively). According to Trotter and Easter, the reduction in the Trp:LNAA ratio through dietary addition of LNAA decreased feed intake of primiparous lactating sows [16]. Thus, it could be suggested that the increased ADFI in LP treatment may also be related to a reduced Trp:LNAA ratio. Tryptophan and LNAA share the same neutral carrier system to cross the blood-brain barrier, and they compete for uptake by the brain [17]. Serotonin and its precursor, Trp, are known to be involved in the control of feed intake; an increased ratio of Trp:LNAA is reported to increase appetite linearly in growing finishing pigs [18].

Litter BW gain, milk production and composition were not influenced by dietary CP content. Milk production from farrowing to d

27 and litter growth rate for the overall lactation period averaged 7,485 and 2,055 g/d, respectively. Similarly, Johnston et al. and Renaudeau et al. in lactating sows kept at 29°C, showed no change in litter BW gain when dietary CP level was decreased (from 16.7 to 13.3%; and from 17.6 to 14.2%, respectively) [13,19]. Lactation BW loss was not influenced statistically by treatments ( $P>0.10$ ), but numerically, the LP sows lost 8 kg less than the NP sows in agreement with previous results [13]. The LP sows also tended to show a numerically lower backfat loss than NP sows (2.4 vs. 6.3 mm;  $P<0.10$ ; Table 2). According to several authors these findings can be attributed to the higher feed intake observed for the LP sows, which probably contributed for the sow to maintain its body condition [8,13,15].

The macro composition of milk is shown in Table 3. According to our findings, dietary protein content did not affect general milk

composition and amino acids (AA) milk composition (Table 4). Similar results were reported by Dourmad et al. in primiparous lactating sows when dietary crude protein (CP) level was reduced from 17.1 to 15.5% without change in lysine concentration (0.77%) [7]. For a more severe restriction of dietary CP (15 to 5% and 23.8 to 6.3%, respectively), Elliott et al. and King et al. showed reduced fat and protein milk contents in low CP treatment [20,21]. In these latter studies, the milk AA composition expressed as a proportion of nitrogen content was slightly affected by protein supply. In particular, they reported a lower proportion of glutamic acid only in milk from sows receiving a diet with less than 10% dietary CP.

In the present study, milk DM and ash contents linearly increased with the advancement of lactation from 17.6 to 19.9% and from 0.72 to 0.97%, respectively between d 7 and d 27 ( $P<0.01$ ). Lactose content

No. obs.	Lactation day, d				RSD	Statistics
	7	14	21	27		
	18	18	18	18		
<b>Chemical composition, %</b>						
Dry matter	17.6 <sup>a</sup>	19.3 <sup>b</sup>	18.7 <sup>b</sup>	19.9 <sup>b</sup>	1.2	S**, B**
Ash	0.72 <sup>a</sup>	0.80 <sup>b</sup>	0.88 <sup>c</sup>	0.97 <sup>d</sup>	0.06	S**
Nitrogen	0.82 <sup>ab</sup>	0.80 <sup>a</sup>	0.83 <sup>ab</sup>	0.87 <sup>b</sup>	0.07	S*, B**
Lipids	7.12	8.00	7.07	7.65	1.06	
Lactose	3.95 <sup>a</sup>	4.91 <sup>b</sup>	4.88 <sup>b</sup>	4.90 <sup>b</sup>	0.36	S**

<sup>1</sup>Residual Standard Variation; <sup>a, b, c</sup>Within a line, means with different superscripts are significantly affected by treatment; <sup>2</sup>From an analysis of variance with a general linear model including the effect of diet (D) and batch (B), lactation stage (S) and their interactions as fixed effects. Repeated measurements of milk chemical composition were analysed using an unstructured covariance structure with sows within batch as a subject. Statistical significance: \* $P<0.05$ , \*\* $P<0.01$ .

**Table 3:** Effect of lactation stage on sows milk chemical composition (Least Square Means).

No. observations	Lactation day, d				RSD	Statistical analysis
	7	14	21	27		
	18	18	18	18		
<b>Essential amino acids, g/16 g N</b>						
Lysine	7.21	7.27	7.27	7.20	0.36	
Methionine	1.80	1.81	1.83	1.82	0.07	
Cystine	1.43 <sup>a</sup>	1.39 <sup>b</sup>	1.36 <sup>b</sup>	1.29 <sup>c</sup>	0.05	S***
Threonine	4.13 <sup>a</sup>	4.04 <sup>ab</sup>	4.01 <sup>ab</sup>	3.98 <sup>b</sup>	0.15	S*
Tryptophan	1.34 <sup>a</sup>	1.29 <sup>b</sup>	1.28 <sup>b</sup>	1.28 <sup>b</sup>	0.06	S*
Valine	5.24 <sup>a</sup>	5.14 <sup>b</sup>	5.12 <sup>b</sup>	5.08 <sup>b</sup>	0.12	S*
Leucine	8.43	8.38	8.36	8.32	0.17	B*
Isoleucine	4.00	3.98	3.97	3.92	0.19	
Histidine	2.58	2.59	2.58	2.57	0.10	
Tyrosine	4.18	4.14	4.13	4.11	0.23	
Phenylalanine	4.02	4.01	4.02	3.96	0.11	
Total	44.4	44.0	43.9	43.5	1.4	
<b>Non essential amino acids, g/16 g N</b>						
Arginine	4.64	4.59	4.58	4.65	0.11	B*
Glycine	3.08 <sup>a</sup>	3.07 <sup>a</sup>	3.11 <sup>ab</sup>	3.20 <sup>b</sup>	0.14	S*
Alanine	3.63 <sup>a</sup>	3.56 <sup>ab</sup>	3.54 <sup>ab</sup>	3.50 <sup>b</sup>	0.08	S**
Serine	5.14	5.11	5.14	5.12	0.15	
Aspartic acid	8.05	7.95	7.97	7.96	0.19	
Glutamic acid	19.2 <sup>a</sup>	19.6 <sup>ab</sup>	19.8 <sup>b</sup>	19.7 <sup>b</sup>	0.50	S*
Proline	10.1	10.3	10.5	10.7	0.53	
Total	53.8	54.2	54.6	54.8	1.3	

<sup>1</sup>Residual Standard Variation; <sup>a, b, c</sup>Within a line, means with different superscripts are significantly affected by treatment; <sup>2</sup>From an analysis of variance with a general linear model including the effect of diet (D) and batch (B), lactation stage (S) and their interactions as fixed effects. Repeated measurements of milk chemical composition were analysed using an unstructured covariance structure with sows within batch as a subject. Statistical significance: \*  $P<0.05$ , \*\*  $P<0.01$ .

**Table 4:** Effect of lactation stage on sows milk protein amino acid composition (Least Square Means).

increased from d 7 and d 14 (3.95 to 4.91%,  $P < 0.01$ ) and thereafter remained constant. Whatever the stage of lactation, the percentage of fat in milk was constant and averaged 7.46%. Nitrogen milk concentration was significantly affected by stage of lactation being minimum on d 14 and maximum on d 27. Our results for the overall milk composition are essentially the same as those reported by Salmon-Legagneur, Elliott et al., Klobasa et al. and Csapó et al. [20,22-24].

In our study, the AA composition of milk protein generally agreed with those presented by King et al. and Dourmad et al. (Table 4) [7,21]. Milk proteins were particularly rich in glutamic acid, proline, leucine, and aspartic acid (19.6, 10.4, 8.4, and 8.0 g/16 g N, respectively). In contrast, tryptophan, cystine, and methionine were present in a least amount in milk (1.3, 1.4 and 1.8 g/16 g N, respectively). The AA concentration in milk was affected by the stage of lactation: whereas sulfur AA, threonine, tryptophan, valine, and alanine concentrations decreased ( $P < 0.05$ ) but glycine and glutamic acid contents increased ( $P < 0.05$ ) from d 7 to d 27. Similar results were reported by Csapó et al. and Elliott et al. [21,24]. As the AA are derived from milk proteins, changes in AA patterns during lactation reflect a change in the relative distribution of milk proteins with different AA pattern. According to Klobasa et al. the relative proportion of caseins to whey proteins such as immunoglobulins and  $\alpha$ -lactalbumin increases during lactation in the sow's milk [23]. In fact, whey proteins in general have a lower concentration of glutamic acid, proline and methionine, and are richer in cysteine, threonine, and valine compared to caseins proteins [5]. From these results, it can be suggested that changes in AA pattern during lactation could be explained by the presence to some extent of immune proteins in mature milk produced after the colostrum stage. On average lysine milk content concentration was not affected by stage of lactation ( $P > 0.05$ ) and averaged 7.24 g/ 16 g N; this value is rather similar to the levels reported by Elliott et al., King et al., and Dourmad et al. (7.30, 6.95, and 7.39 g lysine / 16 g N, respectively) [7,20,21].

The fatty acids composition of milk fat is presented in Table 5. In agreement with data previously published in the literature, more than 80% of the fatty acids in sow's milk fat were palmitic (16:0), oleic (18:1) and linoleic acids (18:2) (Miller et al., Csapó et al. and Gerfault et al.) [24-26]. According to Darragh and Moughan, most of the fatty acids founds in milk reflect closely those in the blood triacylglycerol which in turn are influenced by the type of dietary fat ingested by the sow and/or the amount of mobilized maternal fat tissue [5]. In the present study, fatty acids composition in milk fat was not influenced ( $P > 0.05$ ) by the stage of lactation. Similarly, Bee did not report any change in fatty acids concentration in milk sampled on d 9, 16 or d 23 [27]. In contrast, Miller et al. and Csapó et al. showed that the proportion of linoleic acids (C18:2) was reduced whereas that of palmitoleic acid (C16:1) increased during lactation [24,25]. The discrepancy between the studies can be explained by differences in animal characteristics (genotype, milk production, ability to mobilize body reserves), in animal management (amount and FA composition of the diet; Rosero et al. [28]) or in the method of milk collection [5].

Residual correlations between sow performance and milk composition are presented in Table 6. Logically, the maternal BW loss during lactation was negatively correlated with ADFI ( $r = -0.76$ ) and positively correlated with backfat thickness loss ( $r = 0.55$ ). In addition, there was a positive correlation between milk production and body reserves mobilisation ( $r = 0.82$ ). This result would suggest that in our experimental conditions in which appetite was limited by the hot environment, the ability of sows to produce milk depends of their capacity to mobilize body reserve for providing milk precursors. The polyunsaturated FA (PUFA) content in milk fat was positively correlated with ADFI and negatively correlated to maternal BW loss ( $r = 0.62$  and  $r = -0.60$ ). The PUFA deposited in milk fat originated

	Lactation day, d				RSD	Statistical analysis
	7	14	21	27		
<b>No. observations</b>	18	18	18	18		
<b>Saturated fatty acids, mg/L</b>						
C12:0	14	17	17	15	4	
C14:0	178	195	201	179	36	
C16:0	1711	1814	1869	1783	295	
C18:0	287	262	258	246	50	
C20:0	13	16	16	15	7	
Total	2207	2307	2364	2240	362	
Total, %	37.1	38.0	38.9	38.0	2.6	
<b>Monounsaturated fatty acids, mg/L</b>						
C14:1	11	13	13	12	3	
C16:1	474	483	515	456	99	
C18:1	1799	1721	1751	1624	426	
C20:1	19	20	21	20	7	
Total	2313	2245	2308	2121	464	
Total, %	38.3	36.8	37.1	35.6	4.4	
<b>Polyunsaturated fatty acids, mg/L</b>						
C18:2	1274	1335	1306	1359	212	
C18:3	92	102	95	103	22	
C20:2	26	24	27	26	12	
C20:4	37	33	32	32	9	
Total	1410	1528	1497	1567	245	
Total, %	24.8	25.4	24.4	26.7	3.2	

<sup>1</sup>Residual Standard Variation; <sup>2</sup>From an analysis of variance with a general linear model including the effect of diet (D) and batch (B), lactation stage (S) and their interactions as fixed effects. Repeated measurements of milk chemical composition were analysed using an unstructured covariance structure with sows within batch as a subject. Statistical significance: \*  $P < 0.05$ , \*\*  $P < 0.01$ .

**Table 5:** Effect of lactation stage on sows milk fatty acid composition (Least Square Means).

	ADFI	dlys intake	BW loss	BT loss	Milk
ADFI, g/d	1.00	–	–	–	–
dlys intake, g/d	<b>0.92</b>	1.00	–	–	–
BW loss, g/d	<b>-0.76</b>	<b>-0.55</b>	1.00	–	–
BT loss, g/d	-0.39	-0.29	<b>0.62</b>	1.00	–
Milk, g/d	<b>-0.49</b>	-0.34	<b>0.82</b>	0.40	1.00
Dry matter, %	0.14	0.10	0.05	0.37	0.09
Ash, % DM	0.02	0.05	-0.33	<b>-0.51</b>	-0.42
Crude protein, % DM	-0.17	0.01	0.08	-0.43	0.19
Fat, % DM	0.16	0.12	0.13	0.39	0.21
Lactose, % DM	0.07	0.16	-0.37	-0.08	-0.26
SFA, mg/L	0.38	0.42	-0.02	0.22	-0.15
MUFA, mg/L	-0.41	-0.21	<b>0.78</b>	0.41	<b>0.73</b>
PUFA, mg/L	<b>0.62</b>	<b>0.47</b>	<b>-0.60</b>	-0.33	<b>-0.69</b>
Lysine, g/16 g N	-0.39	<b>-0.53</b>	-0.04	-0.31	-0.20
Sulfur AA, g/16 g N	-0.14	-0.25	-0.33	-0.39	<b>-0.61</b>
Threonine, g/16 g N	-0.16	-0.33	-0.31	-0.40	<b>-0.49</b>
Tryptophan, g/16 g N	-0.32	<b>-0.49</b>	-0.17	<b>-0.54</b>	-0.27
Leucine, g/16 g N	<b>0.58</b>	0.41	<b>-0.74</b>	-0.09	<b>-0.79</b>
Isoleucine, g/16 g N	-0.30	-0.41	-0.06	-0.24	-0.23
Valine, g/16 g N	0.05	-0.09	<b>-0.46</b>	<b>-0.30</b>	<b>-0.67</b>
Arginine, g/16 g N	0.01	-0.22	0.07	-0.07	0.39
Histidine, g/16 g N	<b>-0.65</b>	-0.31	-0.12	-0.21	-0.26

<sup>1</sup>Sow performance: ADFI (average daily feed intake), Dlys (digestible lysine) intake, BW (body weight) loss, BT (backfat thickness) loss and milk (milk production). SFA, MUFA, and PUFA for saturated fatty acids (FA), monounsaturated FA, and polyunsaturated FA, respectively. Correlation coefficient in bold was significantly different from 0 ( $P < 0.05$ ).

**Table 6:** Residual correlation coefficients between lactation performance and chemical composition of milk of sows over a 28-d lactation<sup>1</sup>.

mainly from dietary FA because animals cannot synthesize them, while saturated FA (SFA) and monounsaturated FA (MUFA) are derived from diet, mobilisation of fat tissue, or *de novo* synthesis. As a result, when sows are in a negative energy balance, a large amount of body reserves are mobilized and then exogenous PUFA are diluted with endogenous *de novo* synthesised fatty acids (SFA and MUFA). Finally, except for arginine, negative correlations were reported between milk production and AA concentration; the correlation coefficients were significantly different from zero only for sulphur AA, threonine, branched chain AA (leucine and valine). According to these results, changes in milk production would affect the AA composition of milk proteins. In conclusion, reducing dietary protein content can be an alternative to attenuate the negative effects of heat stress by increasing ADFI. Milk composition changes significantly according to lactation stage and the ability of sows to produce milk will depend on their capacity to mobilize body reserve for providing milk precursors.

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