

## Changes in the concentrations of glucose, non-esterified fatty acids, urea, insulin, cortisol and some mineral elements in the plasma of the primiparous sow before, during and after induced parturition

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(Received 7 July 1998; accepted 23 February 1999)

**Abstract** — The concentrations of selected metabolites, minerals and hormones relative to parturition were studied in 12 primiparous sows. Blood sampling was performed on days  $-5$ ,  $0$  and  $+5$  relative to the farrowing day. On the day of parturition (d 0), samples were taken every hour from 07.00 to 24.00 hours. All sows had an indwelling catheter in the jugular vein, and the evolution of glucose, insulin, urea, non-esterified fatty acids (NEFA), calcium (Ca), phosphorus (P), magnesium (Mg) and cortisol were studied. The concentrations of NEFA, cortisol and P were significantly higher at d 0 than at d  $-5$  or d  $+5$ , whereas the Mg level was lower. During the expulsion of foetuses, NEFA and cortisol levels increased ( $+18$  and  $+30$  %, respectively), and they decreased immediately after the birth of the last piglet, to reach the initial levels observed before farrowing (around  $700 \mu\text{Eq}\cdot\text{L}^{-1}$  and  $110 \text{ ng}\cdot\text{mL}^{-1}$ , respectively). Glucose and insulin levels remained unchanged during the expulsion of the piglets ( $105 \text{ ng}\cdot\text{dL}^{-1}$  and  $5 \mu\text{IU}\cdot\text{mL}^{-1}$ , respectively), but they both increased immediately after the birth of the last animal. During the expulsion of the foetuses, the Ca concentration remained unchanged ( $93 \text{ mg}\cdot\text{L}^{-1}$ ), whereas the P level increased ( $+9$  %) and the Mg concentration decreased ( $-7.4$  %). These data suggested that parturition induces large variations in the concentrations of plasma metabolites that may affect its normal process. © Inra/Elsevier, Paris.

sow / parturition / blood metabolites / cortisol / minerals

**Résumé** — Concentrations plasmatiques de glucose, d'acides gras libres, d'urée, d'insuline, de cortisol et de certains éléments minéraux avant, pendant et après la mise bas chez la truie primipare.

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Les concentrations plasmatiques de certains métabolites, hormones et minéraux sont étudiées aux alentours de la mise bas sur 12 truies primipares. Des prélèvements sanguins sont réalisés à 11 h, aux jours -5, 0 et +5, par rapport à la mise bas (J0). À J0, des prélèvements sanguins sont réalisés toutes les heures, à partir de 7 h et jusqu'à 24 h. Toutes les truies sont munies d'un cathéter jugulaire et les concentrations de glucose, d'insuline, d'urée, d'acides gras libres (AGL), de calcium, de phosphore, de magnésium et de cortisol sont déterminées. Les concentrations d'AGL, de cortisol et de phosphore sont significativement plus élevées à J0 qu'à J-5 ou J+5, tandis que celle du Mg est plus faible à J0. Pendant l'expulsion des fœtus, les concentrations d'AGL et de cortisol augmentent (+18 and +30 %), puis elles diminuent après la naissance du dernier porcelet, pour atteindre des valeurs similaires à celles observées avant le début de la mise bas (environ 700  $\mu\text{Eq}\cdot\text{L}^{-1}$  et 110  $\text{ng}\cdot\text{mL}^{-1}$ ). Au cours de la mise bas, les concentrations de glucose et d'insuline restent inchangées (105  $\text{ng}\cdot\text{dL}^{-1}$  et 5  $\mu\text{U}\cdot\text{mL}^{-1}$ ) et elles augmentent par la suite. La concentration en calcium reste stable (93  $\text{mg}\cdot\text{L}^{-1}$ ), tandis que celle du phosphore augmente (+9 %) et celle du magnésium diminue (-7,4 %). Ces résultats indiquent que des variations importantes de concentrations en métabolites sanguins ont lieu pendant la mise bas, ce qui peut affecter son bon déroulement. © Inra/ Elsevier, Paris.

**truie / mise bas / métabolites sanguins / cortisol / minéraux**

## 1. INTRODUCTION

Labour is the act of giving birth to young by the mother and it marks the termination of a normal pregnancy [7]. It is a part of the parturition process, which starts several hours before labour, and ends after the expulsion of the placenta [16]. Parturition is associated with changes in the concentration of reproductive hormones in the plasma of the mother and this has been used for the pharmaceutical control of the part itself [16]. In pigs, for instance, inducing parturition with an injection of prostaglandin  $\text{F2}\alpha$  or one of its analogues, such as cloprostenol, is a common practice and the percentage of induced parturition is estimated to be around 33 % of the total number of farrowings in France (Dagorn, pers. comm.). Inducing parturition facilitates supervision, which favourably affects the number of piglets born alive and consequently improves the economical performances of the herd [10]. The importance of parturition on both reproductive and economical performances explains why most of the studies on the periparturient sow have investigated the behavioural [12, 13] or the hormonal [6, 11, 18] aspects of this phenomenon. But, sur-

prisingly, little information is available on the general metabolism of the sow during this period.

In cows, mineral disorders at the beginning of lactation are related to milk fever [9]. This certainly explains the common practice of magnesium and calcium supplementation during the pre-term period in pigs, but the value of such mineral supplementation remains unclear. Parturition in sows is also considered as one of the most stressful situations in the animal's life [18]. Its onset is associated with an increased activity of the maternal pituitary-adrenal axis and with changes in the plasma concentration of cortisol [6]. The luteolytic process that ends gestation and initiates the start of parturition is under the control of the foetuses themselves [1]. The cortisol foetal release induces several changes in the hormonal plasma of the sow at the end of gestation and is one of the main factors involved in the occurrence of parturition [5]. But cortisol is also known to act on the general metabolism of the animal and it is a mediator in the changes of the concentration of several metabolites in the blood in response to stress [5]. To our knowledge, however, no information is available on such modifi-

cations in the sow during the expulsion of the foetuses, after induction of parturition.

The aim of this work was, then, to study the plasma profiles of selected metabolites, insulin, cortisol and some minerals in the peri-parturient sow and after induced parturition.

## 2. MATERIALS AND METHODS

### 2.1. Animals and management

Three weeks before the end of gestation, 18 pure Large White gilts from three different batches were selected according to their expected date of farrowing. They were fed individually twice a day, at 09.00 and 14.00 hours, a total amount of 2.6 kg·d<sup>-1</sup> of a standard gestation diet (12.6 MJ digestible energy (DE) per kg; 14.0 % crude protein (CP); 0.59 % lysine). Two weeks before the expected day of parturition, they were moved to a lactation unit, where they were individually penned in farrowing crates (1.9 × 2.6 m), on a concrete floor with straw bedding. On the expected day of parturition (day 0 of lactation), all sows received 1 kg of a commercial lactation diet (13.1 MJ DE·kg<sup>-1</sup>; 17.1 % CP; 0.90 % lysine), at 09.00 hours. Feed refused was removed and recorded 1 h after the beginning of the meal. From d 1–4 of lactation, the level of feed was progressively increased and sows had free access to feed from d 5 of lactation onwards.

### 2.2. Oestrus and farrowing management

At 260 days of age on average, oestrous cycles of the gilts were synchronized, with oral administration of allyl-trenbolones (Regumate®, Rousset-Uclaf, France). A daily dose of 20 mg as a top dressing on the feed was provided to each animal for 18 d. Gilts were inseminated twice with semen of pure Piétrain boars at the first detected oestrus following the last feeding of allyl-trenbolones. Parturition was induced on d 114 post-insemination, by a single intra-muscular injection of 2 mL of cloprostenol on d 113 of gestation (Planate®, Pitman-Moore, USA). Supervision of the piglet's birth and the sow's behaviour was provided during the expulsion of the foetuses.

### 2.3. Operation and sampling

On d 105 of gestation, an indwelling catheter (Silastic®, Dow Corning Corporation, Midland, USA) was surgically implanted into one of the jugular veins and exteriorized on the dorsal side of the neck under general anaesthesia. Every 2 d at 11.00 hours, catheters were rinsed with a 10-mL saline solution (0.9 % sodium chloride). The solution also contained heparin (20 IU·mL<sup>-1</sup>) and dihydrostreptomycin (0.02 g·mL<sup>-1</sup>) to prevent infections. At d -5, 0 and +5 relative to the farrowing day, 10 mL of blood were collected at 11.00 hours, to determine the levels of the non-esterified fatty acids (NEFA), glucose, urea, magnesium (Mg), calcium (Ca), phosphorus (P), insulin and cortisol before, during and after parturition. Blood samples were collected into heparinized tubes (two drops of 1 000 IU·mL<sup>-1</sup> of heparin per tube) and centrifuged at 2 500 rpm for 10 min. After centrifugation, the plasma was stored at -20 °C until the assay. On the expected day of farrowing, blood samples were collected every hour, from 07.00 to 24.00 hours, and treated as previously described.

### 2.4. Assays

Automated enzymatic methods using a Cobas Mira multichannel analyser (Roche, Basel, Switzerland) were performed to determine the concentrations of glucose, P, Ca, Mg (bioMérieux kits ref. 61273, 61571, 61041, 61411; Marcy-l'Étoile, France), NEFA (Wako Chemical NEFA C; Unipath, Dardilly, France), and urea (Urea unimate 5, ref. 07-3685-6; Roche, Neuilly-sur-Seine, France). Plasma levels of insulin were measured with a validated RIA method [22]. Sensitivity was 3 µIU·mL<sup>-1</sup>. Intra-assay CV of 10, 6 and 7 % and inter-assay CV of 14, 19 and 24 % were obtained from pool samples containing 3.8, 63.6 and 263.3 µIU·mL<sup>-1</sup> of insulin, respectively. Cortisol concentration was measured with a specific homologous double-antibody RIA, without extraction, in acid solution (pH = 3). The antiserum was prepared by J. Saumande (laboratoire de physiologie animale, Inra Tours) and obtained from a rabbit after immunization with 3 h carboxy-methoxime-cortisol, bound to bovine serum albumin. Cross-reactions were 70 % with cortisone and below 1 % with other C21 steroids. The tracer was <sup>3</sup>H-cortisol (TRK407, Amersham, Les Ulis, France) and cortisol (SERVA 25190, Sigma, Saint-Quentin Fallavier, France) was used for

the standard curve. Intra- and inter-assay variations were 4.8 and 14.3 % at 67 and 53.2 ng·mL<sup>-1</sup>, respectively. The assay sensitivity was 0.4 ng/tube.

## 2.5. Statistical analysis

Data were analysed by the use of the General Linear Model procedures of the Statistical Analysis System [24]. The models included the effect of animal, day of sampling and batch. For the results on metabolite concentrations at d -5, 0 and +5, the sow nested within the day of sampling was considered as the error term for testing the day of sampling effect, according to the SAS [24] specifications for repeated measure analyses. Similarly, during the expulsion of the foetuses, the sow nested within the time of sampling was considered as the error term for testing the time of sampling effect.

## 3. RESULTS

Results were available from 12 of the 18 catheterized sows, six of them did not farrow during the scheduled time, on d 114 of gestation. Two of them farrowed 1 day early (d 113) and the other four farrowed after d 114, despite the injection of cloprostenol. No feed refusal was observed the day of farrowing. The average age, BW and BF

(± standard deviation, SD) of the sows that farrowed at d 114 were 395 (± 5) days, 221 (± 7) kg and 20.5 (± 3.1) mm, respectively. The average duration of the expulsion of foetuses was 175 min, but ranged from 80 to 385 min. At birth, the total number of piglets and their average body weight (± SD) were 12.2 (± 1.4) and 1.5 (± 0.2) kg, respectively.

The plasma concentrations of metabolites, insulin and cortisol at d -5, 0 and +5 relative to the farrowing day are presented in *table I*. The levels of NEFA, cortisol and P were significantly higher at d 0 compared with those at d -5 and +5, whereas the level of Mg was significantly lower. At d 0, the level of plasma insulin was lower than at d -5 or +5 (6.1 versus 97 µUI·mL<sup>-1</sup>, respectively). The concentration of urea was lower at d -5 and d 0 than at d +5 (-39 %;  $P < 0.05$ ), whereas no difference in the concentrations of glucose and Ca was observed.

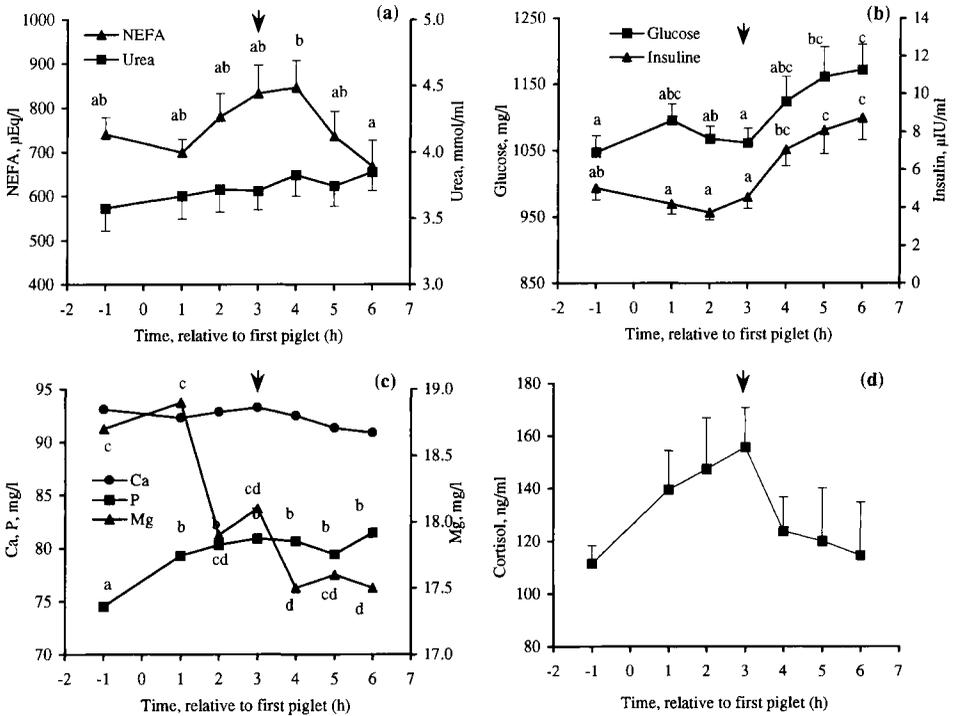
Only the results from the average samples during the expulsion of the foetuses are presented in *figure 1a-d*. The plasma concentration of NEFA increased in all sows until the birth of the last piglet (+18 %; *figure 1a*). It decreased thereafter and reached similar values as those measured at the beginning of expulsion (around 700 µEq·L<sup>-1</sup>). Although no significant difference was

**Table I.** Plasma concentrations of selected metabolites and cortisol relatively to the farrowing day in primiparous sows (mean values;  $n = 12$ ).

Item	Day relative to farrowing			
	-5	0	5	rsd*
NEFA, µEq·L <sup>-1</sup> ‡	123.1 <sup>a</sup>	576.7 <sup>b</sup>	97.5 <sup>a</sup>	134.0
Urea, mmol·mL <sup>-1</sup>	4.9 <sup>a</sup>	3.5 <sup>a</sup>	5.7 <sup>a</sup>	1.8
Glucose, mg·dL <sup>-1</sup>	114.3	102.1	107.2	23.1
Insulin, µIU·mL <sup>-1</sup>	103.0 <sup>a</sup>	6.1 <sup>b</sup>	90.8 <sup>a</sup>	54.5
Calcium, mg·L <sup>-1</sup>	98.1	95.0	91.2	7.8
Phosphorus, mg·L <sup>-1</sup>	63.6 <sup>a</sup>	73.2 <sup>b</sup>	62.0 <sup>a</sup>	5.1
Magnesium, mg·L <sup>-1</sup>	19.9 <sup>a</sup>	18.1 <sup>b</sup>	20.2 <sup>a</sup>	1.5
Cortisol, ng·mL <sup>-1</sup>	30.7 <sup>a</sup>	131.2 <sup>b</sup>	42.2 <sup>a</sup>	47.8

\* rsd: residual standard deviation; ‡ NEFA: non-esterified fatty acids.

<sup>a, b</sup> Within a row, values with different letters significantly differ at  $P < 0.05$ .



**Figure 1.** Plasma concentrations of metabolites and cortisol in the jugular vein of primiparous sows during the course of parturition (means  $\pm$  SEM). The arrow indicates the birth of the last piglet. For each profile of metabolites, values with different letters differ significantly at  $P < 0.05$ .

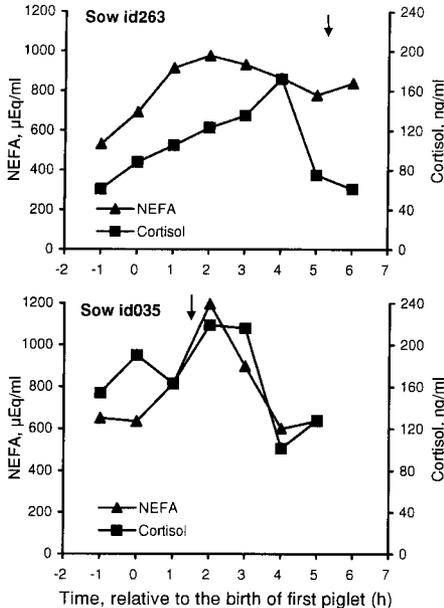
observed, the level of cortisol had a similar profile: it increased during the expulsion of the piglets (by  $45 \text{ ng}\cdot\text{mL}^{-1}$ ) and decreased after the birth of the last piglet to reach the values initially observed (around  $110 \text{ ng}\cdot\text{mL}^{-1}$ ; *figure 1d*). Insulin and glucose remained constant during the first 3 h of expulsion ( $105 \text{ ng}\cdot\text{dL}^{-1}$  and  $5 \text{ }\mu\text{IU}\cdot\text{mL}^{-1}$ , respectively), but increased after the birth of the last piglet ( $+10 \text{ ng}\cdot\text{dL}^{-1}$  and  $+5 \text{ }\mu\text{IU}\cdot\text{mL}^{-1}$ , respectively; *figure 1b*). During the expulsion of the foetuses, the concentration of Ca remained constant ( $93 \text{ mg}\cdot\text{L}^{-1}$ ), but a significant increase in the P level was observed ( $+9\%$ ; *figure 1c*), whereas the concentration of Mg tended to decrease during farrowing ( $-7.4\%$ ; *figure 1c*).

The concentrations of non-esterified fatty acids (NEFA) and cortisol during the course of parturition in two sows that had either a

short (80 min; id035) or a long (323 min; id263) expulsion time are presented in *figure 2*. In both cases, profiles are similar to those observed for average samples. The plasma concentration of NEFA increased at the birth of the first piglet, remained high during the duration of the expulsion of foetuses and decreased thereafter. The level of cortisol increased during the expulsion of piglets and decreased after the birth of the last piglet to reach the values initially observed.

#### 4. DISCUSSION

The average reproductive performances of the animals were slightly higher than those generally observed in primiparous



**Figure 2.** Concentrations of non-esterified fatty acids (NEFA) and cortisol in two primiparous sows that had either a short (80 min; id035) or a long (323 min; id263) farrowing (mean value). The arrow indicates the birth of the last piglet.

sows. This can be explained by old age and the high body weight of the animals at mating, and the use of Regumate® for the synchronization of the oestruses [14, 17]. After a single injection of prostaglandins F $2\alpha$  (PGF $2\alpha$ ) or its analogues, 90–95 % of the sows give birth within 48 h and the intervals between treatment and parturition generally vary between 20 and 40 h [2]. These authors also reported that 60 % of the treated sows farrowed during the working hours (08.00–18.00 hours) the day after treatment. This was close to the 67 % observed in the present study, between 07.00 and 23.00 hours.

As in other species, PGF $2\alpha$  are normally produced in sows and their concentration in the plasma rises around parturition, following the decrease in progesterone and the increase in oestrogen levels. They induce uterine contractions and, through the con-

trol of the secretion of relaxin, indirectly control the dilation of the cervix, through luteolytic processes [16]. Thus, their injection not only induces myometric contractions, but it also amplifies the luteolytic process, resulting in the fall in the progesterone level, which is required for the sustenance of pregnancy. But, PGF $2\alpha$  sometimes presents side effects, such as hyperpnea, increased salivation, urination, defecation, or, in farrowing crates, rooting, pawing and biting bars [21]. These side effects are generally short in duration, and, according to Pressing [21], prostaglandin analogues are essentially free of these side effects. As no negative effects are observed on the subsequent performances of sows when these analogues are used after d 110 of gestation [1, 21], they represent an attractive way to facilitate labour management and farrowing attendance. Indeed, between 20 and 36 h after their administration, more than 60 % of the sows commence parturition (see Pressing [21], for a review). When birth is induced too prematurely (before d 111 of gestation), however, post-natal survival is reduced and growth of the piglets is below that of the controls [2].

The higher concentration of NEFA and the lower level of insulin, on the day of farrowing (d 0) were partially the result of the lower level of feed received by the sows this day, in agreement with the results of Weldon et al. [27]. Indeed, on the morning of farrowing, the sows were fed only 1 kg of feed, following a 17-h fasting period, in comparison with 1.3 kg at d -5 and ad libitum at d +5. In addition, when the first piglet was born, all animals had been fasting for at least 4 h. The high level of circulating NEFA at parturition might also be amplified by the metabolic changes occurring with parturition itself and the start of lactogenesis [26]. In cows, for instance, Grum et al. [8] noted similar NEFA profiles on the day of farrowing as those observed in the present study and they suggested that a large proportion of the increase in NEFA level originated from the mobilization of the adipose

tissue needed for calving. In addition, Collier et al. [3] suggested that the increase in the adrenergic receptor population in adipose tissue when lactogenesis occurs results in an increase in epinephrine-stimulated fatty acid release, and consequently, in an increase in circulating plasma NEFA.

According to Neil [20] and Le Cozler et al. [14], the level of circulating urea mainly reflected the feed intake of the sow, rather than protein mobilization. Thus, the lower concentration of urea observed on the day of parturition compared with the values observed before or after farrowing, was, then, most likely the result of the low feed intake. Despite no significant difference being observed, the plasma concentrations of glucose and insulin were lower on the day of parturition than before or after, similarly to the results observed in cows [8]. This lower value of insulin was consistent with the greater rate of lipid mobilization and the lower level of circulating glucose, which also reflected the high energy demand for labour.

In cows, a high secretion of calcium in milk, associated with reduced plasma levels of calcium and phosphorus and increased levels of plasma magnesium, have been related to milk fever [9]. No mastitis or other problems around farrowing or later during lactation were observed in the present work, suggesting that changes in phosphorus and magnesium levels were not associated with health problems. With the onset of lactation, the evolution of these mineral elements might reflect their exportation in milk [4]. At d +5, however, their plasma concentrations were similar to the levels observed at the end of gestation. This result suggested that even if the exportation of these minerals in milk was high, their plasma levels remained almost constant and the changes observed during parturition were more likely associated with the labour itself.

Indeed, the high concentration of phosphorus on the farrowing day and during expulsion of the foetuses probably reflected

the intensity of the uterine contractions during labour. As this phenomenon consumes a lot of energy, it can be hypothesized that the high level of inorganic phosphorus observed in the present work might result from the intense de-phosphorylation of adenosine-tri-phosphate in the muscles. In the present study, the evolution of the levels of magnesium and phosphorus were opposite to each other on the day of parturition, and this might reflect the involvement of magnesium as a co-factor in the de-phosphorylation reactions [25]. Indeed, magnesium is known to play an important role in many enzymatic reactions, which involve phosphorus, and it has been reported to act either synergistically or antagonistically with calcium [25]. This might explain the apparent absence of changes in the levels of plasma calcium.

The value of calcium supplementation just before farrowing is not clearly shown in the present study, although it is widely used in practice, because no change in its plasma concentration was observed. Calcium is, however, never injected alone and is often coupled with magnesium. Thus, the decrease in the concentration of magnesium in the plasma during parturition suggests that its supplementation might prevent any deleterious effect of a too low level during the part itself. Nevertheless, even if no problems or disorders were observed in the present study, the value of mineral supplementation to prevent farrowing problems remains to be investigated in sows.

The higher concentration of plasma cortisol on the day of farrowing compared to the pre- and post-partum levels, and the evolution of maternal cortisol during the parturition, were in agreement with previous studies [5, 18, 28]. This rise in the concentration of plasma cortisol at parturition has already been described [5], and it has been associated with the rise in foetal cortisol [5, 23] and with maternal stress [19]. Thus, the profile of cortisol concentration and its decrease 3 h after the start of parturition

may reflect the end of cortisol production by the piglets when the last animal was born. As already observed by some authors [5, 18, 28], the level of plasma cortisol decreased rapidly after farrowing and, at the beginning of lactation, it was back to the concentrations observed at the end of gestation. However, in the present work the level of cortisol on the farrowing day was similar to the level previously reported by Randall [23], but higher than the concentration observed by Meunier-Salaün et al. [18]. This may partially be the result of differences between assays used in the laboratories, but also, between primiparous sows, which had never experienced parturition, and multiparous sows [18, 23].

Whitely et al. [28] noted a second peak of cortisol 12–18 h after the birth of the first piglet, and suggested that this second rise in cortisol might reflect the triggering of post-partum events such as lactogenesis. During stress, cortisol is known to increase mobilization of body reserves, and this may partially explain the similar evolution of cortisol and NEFA concentrations during parturition and their decrease after the birth of the last piglet. The decrease in both cortisol and NEFA levels at the end of parturition may also explain the increase in both glucose and insulin concentrations. However, the increase in the plasma glucose level at the end of farrowing, and consequently the increase in insulinaemia, may be the result in the occurrence of neoglucogenesis that generally follows a short fast, as reported in humans [15].

## 5. CONCLUSION

Parturition in pigs, as in many other species, is a stressful situation associated with a highly catabolic status. Despite the absence of any deleterious effects observed in the present study, the results suggested that the high demand for energy and nutrients, and the large variations in mineral concentrations could adversely affect perfor-

mances during parturition. The comparison of sows with normal parturition and sows with farrowing problems, as well as the comparison of the evolution of these elements in sows with non-induced and induced parturition, have to be investigated.

## ACKNOWLEDGEMENTS

The authors wish to thank J.C. Hulin, M. Masnard, M. Lefebvre and J. Gauthier of the Pig Research Station of Inra for technical assistance with animals; A.M. Mounier for cortisol analysis; and Dr U. Magnusson, of the Swedish University of Agricultural Sciences, for his advice during the preparation of the manuscript.

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