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Peripartal feeding strategy with different *n*-6:*n*-3 ratios in sows: effects on sows' performance, inflammatory and periparturient metabolic parameters

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The present study aimed to investigate the effects of two lactation sow feeds, differing in *n*-6:*n*-3 ratio, given to sows before parturition on body condition and feed intake, periparturient metabolism (leptin, insulin, triiodothyronine (T_3) and thyroxine (T_4)), inflammatory parameters (TNFα, IL-6, serum amyloid A (SAA)) and on piglet performance (birth weight, survivability). The feed contained either a low (supplemented with fish oil; f groups) or high (supplemented with sunflower-seed oil; s groups) *n*-6:*n*-3 ratio and was administered from 8 d (f8, s8) or 3 d (f3, s3) before parturition until weaning. The level of inclusion of the oil sources was 2 %. Seventy-two sows were randomly allocated 8 d before expected farrowing into four groups: f3, f8, s3, s8. Type of feed had a significant influence on the sows' feed intake during the first 2 d of lactation (s < f), leptin on days 4, 3 and 2 before parturition (f < s), insulin on day 1 after parturition (f < s), T_4 on the day before parturition (s < f) and rectal temperature on the day after parturition (f < s). Onset of administration of the feed (3 *v.* 8 d) had significant effects on leptin on day 2 before parturition (8 < 3), insulin on day 4 before parturition (3 < 8), T_3 on day 4 before parturition and on the day after parturition (3 < 8), SAA on day 3 after parturition (8 < 3) and piglet weight during the first days postpartum (3 < 8). In conclusion, under the present conditions, a lactation feed low in *n*-6:*n*-3 ratio administered from 8 d before farrowing ensures improved feed intake during the first days postpartum and was associated with a better metabolic change and inflammatory profile in sows in the periparturient period.

Lactating sows: *n*-6:*n*-3 ratio: Fatty acids: Leptin: Insulin

Commercial pig diets are based on cereals and protein feed which hardly contain long-chain *n*-3 fatty acids[1]. Actual requirements of the *n*-6 and *n*-3 families of fatty acids in pig diets and the ideal ratio of *n*-6:*n*-3[2] are still a matter of research. The *n*-6 and *n*-3 PUFA have distinct properties. It has been suggested that a high intake of *n*-6 PUFA, especially arachidonic acid, can contribute to inflammatory processes and predispose to or exacerbate inflammatory diseases[3,4]. Moreover, *n*-6 and *n*-3 PUFA are involved in glucose and lipid metabolism. A high *n*-6:*n*-3 ratio is considered to be a critical factor in both insulin resistance and atherosclerosis in studies in man and animal models[5].

A moderate replacement of safflower-seed oil with tuna oil prevented the development of insulin resistance at the whole-body level in Wistar rats[6]. Moreover, in rats the addition of long-chain *n*-3 PUFA to the diet resulted in the amelioration of insulin resistance[7]. Postpartum hypophagia in sows was shown to be related to decreased glucose tolerance and increased insulin resistance[8]. Therefore, it could be hypothesised that in the case of providing a diet for sows with a low *n*-6:*n*-3 ratio, insulin action and consequently postpartum feed intake would be improved, whereas diets with a high *n*-6:*n*-3 ratio would not exert these benefits.

During the past years, the effects of inclusion of *n*-3 and *n*-6 PUFA in sow diets on performance parameters and on accretion by the piglets have been studied. Piglet growth was improved when supplementing tuna oil at a level of 1·75 % at different periods during gestation[1]. Supplementation of salmon oil to the sow diets during gestation and lactation at a level of 1·65 % reduced pre-weaning mortality[9]. It was shown that the proportions of *n*-3 and *n*-6 PUFA in piglet brain and liver at birth depend on the fatty acid composition

Abbreviations: f, low *n*-3:*n*-6 ratio feed supplemented with fish oil; f3, group with feed f administered 3 d before parturition until weaning; f8, group with feed f administered 8 d before parturition until weaning; s, high *n*-3:*n*-6 ratio feed supplemented with sunflower-seed oil; s3, group with feed s administered 3 d before parturition until weaning; s8, group with feed s administered 8 d before parturition until weaning; SAA, serum amyloid A; T_3, triiodothyronine; T_4, thyroxine.

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of the maternal diet, when oil sources were supplemented at a level of 1·75 % during pregnancy[10]. The low *n*-6:*n*-3 ratio used in these and in other studies[11] ranged between 0·9 and 4·3, while the high ratio ranged from 6·6 to 15·3. For the present study an inclusion level of 2 % was decided, thus obtaining a low (2·1) and a high (10·1) *n*-6:*n*-3 ratio.

The periparturient period in sows ranges from 4 d before to 3 d after parturition[12]. Feeding sows *ad libitum* a lactation diet either from 4 d before the expected day of farrowing or from the farrowing day onwards, instead of the introduction of *ad libitum* feeding from 3 d after farrowing, resulted in higher daily feed intake and less mobilisation of body reserves by the sows[13]. Furthermore, elevated rectal temperatures and increased incidence of agalactia (now described as postpartum dysgalactia syndrome[14]) were reported in sows given feed *ad libitum* from farrowing compared with sows given the gestation feeding level until 3 d after farrowing[15]. Moreover, when sows were fed restrictively during the last 2 weeks of gestation, a higher feeding level was associated with elevated rectal temperatures and an increased incidence of agalactia[16]. The transition from a gestation to a lactation diet in sows commonly takes place when sows are transferred from gestation rooms to the farrowing crates. Depending on the study, the transition may occur at day 107[17,18], 108[19], 109[20], 110[21] or 111[22] of gestation.

It has not yet been investigated in sows, fed restrictively, whether the time point of the transition from a gestation to a lactation diet during late gestation (earlier: 8 d or closer: 3 d to parturition), in combination with lactation diets differing in the *n*-6:*n*-3 ratio, would have an effect on parameters in sows related to performance, metabolism and inflammatory processes. Based on the properties of *n*-6 and *n*-3 PUFA, it could be hypothesised that a lactation diet with a high *n*-6:*n*-3 ratio would increase inflammatory parameters, compared with a lactation diet with a low *n*-6:*n*-3 ratio. Leptin would be an interesting parameter for investigation, since it could be indicative of energy balance in pigs[23]. It has been shown in gilts that acute changes in feed intake can affect leptin secretion[24]. In addition, it is interesting to investigate the association between maternal levels of leptin and insulin during the periparturient period with progeny performance, as poor glucose tolerance of sows during late gestation was reported to be a risk factor for the survival of pigs after birth[25].

The present trial was set up to elucidate the consequences of the *n*-6:*n*-3 ratio of a lactation diet, a low *v.* a high ratio, in combination with the time point of transition from a gestation to a lactation diet in sows, either at 8 or 3 d before farrowing. Piglet viability and growth during lactation, sows' body condition and feed intake during lactation, parameters related to metabolism during the periparturient period and inflammatory parameters postpartum were monitored. Finally, associations between levels of leptin and insulin in sows during the periparturient period and progeny performance were also investigated.

Materials and methods

Study population and experimental design

The study was conducted in the Provimi Research Farm, Velddriel, The Netherlands at the end of 2006 and the beginning of 2007. In total, seventy-two pregnant hybrid sows (Topigs 20 breed; Dutch Landrace × Great York) were included. The experimental period started at day 107 of gestation and ended when the piglets were weaned at day 21 of lactation. The experimental protocol of the study was approved by the ethical committee of the Animal Sciences Group in Wageningen, The Netherlands.

Until day 107 of gestation, the sows were group-housed in a gestation unit and they were fed twice per d with a conventional gestation diet (4 kg/d). The composition (g/kg) of this feed was: DM, 892; ash, 67; crude protein, 126; crude fat, 34; neutral-detergent fibre, 197; metabolisable energy, 10 400 kJ/kg. Linoleic content was 11·53 g/kg, total *n*-3 PUFA was 1·66 g/kg, and DHA and EPA content was fairly low, i.e. $4{\cdot}09 \times 10^{-5}$ g/kg and $3{\cdot}47 \times 10^{-5}$ g/kg, respectively.

At day 107 of gestation, the pregnant sows were moved to the farrowing rooms, and they were randomly allocated using a 2 × 2 experimental design to four different treatment groups, namely f3, f8, s3, s8. Sows from the f and the s groups received a lactation diet that was supplemented with a fish oil product, or a sunflower-seed oil product, respectively. The final level of inclusion of fish oil was 2 %, while in the other group the level of inclusion of sunflower-seed oil was 1·5 % and soyabean oil was included at 0·5 % in order to achieve the final *n*-6:*n*-3 ratio. The *n*-6:*n*-3 ratios in these feeds were 2·09 and 10·13 for the f and s groups, respectively (Table 1). Half of the sows belonging either to the f group or the s group received the experimental lactation diets from day 107 of gestation (f8 and s8 groups) onwards (8 d before expected farrowing); the other half of the sows received the experimental lactation feed only from day 111 of gestation (f3 and s3 group) onwards, being 3 d before the expected day of farrowing (onset day groups). The latter sows continued to receive the conventional gestation feed between day 107 and day 111 of gestation. The allowance of feed before and after farrowing was according to the common practice in the herd. From day 107 of gestation onwards, the sows from all four groups received the same amount of feed, namely 3·3 kg/sow per d. An overview of energy and major nutrient supply in the four experimental groups before farrowing is given in Table 2. The herd practised a 3-week batch production system, and the seventy-two sows farrowed in three consecutive batches of twenty-four sows each. Within each batch, the sows were randomly assigned to the four experimental groups. The mean parity of sows per experimental group (f3 = 4·2; f8 = 4·4; s3 = 4·6; s8 = 4·3) was not significantly different between the groups (P = 0·570).

No feed was provided on the day of parturition (D0). From the first day of lactation onwards until weaning, the sows were fed twice per d, namely at 08.00 hours and again at 18.00 hours. The amount of feed that was provided daily was 3·0 kg on D1 and D2, 3·5 kg on D3, 4·0 kg on D4 and D5, 4·5 kg on D6 and D7, 5·0 kg on D8 and D9, and 5·5 kg from D10 until D21. The amount of feed consumed by every sow was recorded daily from D1 until D21. The feeding troughs were emptied and checked each evening.

Sows were weighed on a digital scale when they were placed in the farrowing crates (day 107 of gestation; D-8) and at weaning (D21). On these occasions, also backfat measurements using a digital backfat indicator (Renco Lean

Table 1. Chemical composition of the two different feeds for the sows*

	Diet supplement	
	Fish oil	Sunflower-seed oil
Diet (g/kg)		
DM	899	886
Ash	65	65
Crude protein	171	167
Crude fat	61	61
Neutral-detergent fibre	181	181
Metabolisable energy (kJ/kg)	12 300	12 300
Fatty acids (% total fatty acids)		
SFA	33·5	26·5
MUFA	33·4	29·7
PUFA	33·1	43·3
Total *n*-6 (% PUFA)		
18 : 2*n*-6	20·9	38·3
20 : 4*n*-6	0·2	0·2
Total *n*-3 (% PUFA)		
18 : 3*n*-3	2·1	2·4
18 : 4*n*-3	1·4	0·3
20 : 4*n*-3	0·2	ND
20 : 5*n*-3	2·8	0·4
22 : 5*n*-3	0·2	0·1
22 : 6*n*-3	3·4	0·6
n-6:*n*-3	2·09	10·13

ND, not detected.
*The feeds were supplemented either with fish oil or with sunflower-seed oil and differed in the *n*-6:*n*-3 ratio.

Meter) were made of every sow. Backfat levels were measured at three positions on each side of the sows: position A (position A left and position A right), between the crossing backside of the shoulder and the spine, 5 cm to the left and 5 cm to the right; position C (position C left and position C right), in the crossing between the last rib and the spine, 5 cm left of the spine and 5 cm right of the spine; position B (position B left and position B right), 5 cm left and 5 cm right of the spine in between points A and C.

Blood samples were taken from every sow daily from D-4 (day 110 of gestation) until parturition (D0). Two additional blood samples were taken after farrowing, namely at D1 and D3. The samples were taken before the morning meal by puncture of the jugular vein in vacuum tubes. The parameters analysed in serum were leptin, glucose, insulin, triiodothyronine (T_3), thyroxine (T_4), VLDL, while TNFα, IL-6 and serum amyloid A (SAA) were analysed in plasma. An overview of the examined parameters in blood is given in Table 3. The rectal temperature of the sows was recorded with a digital thermometer (accuracy 0·1°C) at the first day of lactation, before morning feeding and before blood sampling.

Piglets were individually identified and weighed within 1–2 h after completion of birth, or during the next morning when sows farrowed during the night. The piglets were individually weighed at D1, D2, D7, D14 and D21. The accuracy of the digital scale was 0·005 kg. The number of stillborn, the number of piglets that died until weaning and the number of piglets weaned was recorded. Cross-fostering of piglets was allowed only during D1 and only in between sows of the same experimental group. There was not an effort to equalise the litters.

Table 2. Overview of the energy and major nutrient supply (per d) in sows in every experimental group before farrowing*

	Between days 107 and 111 of gestation	Between day 111 of gestation and farrowing
Metabolisable energy (kJ/d)		
f3	34 300	40 600
f8	40 600	40 600
s3	34 300	40 600
s8	40 600	40 600
Protein (g/d)		
f3	415·8	564·3
f8	564·3	564·3
s3	415·8	551·1
s8	551·1	551·1
Fat (g/d)		
f3	112·2	201·3
f8	201·3	201·3
s3	112·2	201·3
s8	201·3	201·3
Neutral-detergent fibre (g/d)		
f3	605·1	597·3
f8	597·3	597·3
s3	605·1	597·3
s8	597·3	597·3

f, Low *n*-3:*n*-6 ratio feed supplemented with fish oil; f3, group with feed f administered 3 d before parturition until weaning; f8, group with feed f administered 8 d before parturition until weaning; s, high *n*-3:*n*-6 ratio feed supplemented with sunflower-seed oil; s3, group with feed s administered 3 d before parturition until weaning; s8, group with feed s administered 8 d before parturition until weaning.
*The feed contained either a low (supplemented with fish oil; f groups) or high (supplemented with sunflower-seed oil; s groups) *n*-6:*n*-3 ratio and was provided from 8 or 3 d before parturition (onset day 8 or 3).

Analytical procedures

Insulin was analysed by BioSource INS-IRMA KIP1251 and KIP1254 methods (BioSource Europe S.A., Nivelles, Belgium). Thyroid hormones (T_3 and T_4) were analysed by using a specific RIA. Leptin was determined using a commercially available RIA test kit (Multi-Species Leptin RIA Kit, catalogue number XL-85K; Linco Research, Inc., St Charles, MO, USA). Glucose was analysed by an enzymic method (Gluco-quant) on a Roche/Hitachi Modular. TAG were analysed with TAG GPO-PAP (Roche/Hitachi, Vilvoorde, Belgium) Reagent 1, 11876023 216. VLDL was determined by the Friedewald equation (TAG/5). TNFα was analysed by a porcine TNFα immunoassay (Quantikine; R&D Systems, Minneapolis, MN, USA). IL-6 was analysed by a porcine IL-6 quantitative sandwich enzyme immunoassay technique

Table 3. Overview of the different parameters that were analysed in blood from the seventy-two sows included in the study*

Parameters	D-4	D-3	D-2	D-1	D0	D1	D3
Insulin	×			×		×	×
T_3, T_4	×			×		×	×
Glucose	×			×		×	×
Leptin	×	×	×	×		×	×
VLDL	×			×		×	×
TAG	×			×		×	×
TNFα, IL-6, SAA						×	×

×, Parameter was analysed; T_3, triiodothyronine; T_4, thyroxine; SAA, serum amyloid A.
*Blood samples were taken daily from 4 d before parturition (D-4) until parturition (D0), and on D1 and D3 after parturition.

(Quantikine; R&D Systems). SAA was analysed by a solid-phase sandwich ELISA kit (Tridelta Phase™; Tridelta PLC, Bray, Co. Wicklow, Republic of Ireland). All samples were analysed in duplicate and in the same array, in order to avoid inter-array variation.

Statistical analyses

All examined parameters in blood, feed intake of the sows, litter weight and litter growth, piglet weight and piglet growth, backfat and weight of the sows were analysed with two-way ANOVA at each time point separately using SPSS 15.0 (SPSS, Inc., Chicago, IL, USA). This type of analysis was selected to enable a clear view at which time point significant differences of the examined parameters between the experimental groups occurred. Specifically, individual piglet weights were pooled per litter, and afterwards the mean individual piglet weight and mean individual piglet growth were used for the statistical procedures. Survival analysis, including the effect of mortality level in the different groups and the time of death, was used to analyse the difference in survivability of the piglets. The type of feed (fish oil *v.* sunflower-seed oil-supplemented) and the duration of providing the experimental feed (onset day 3 *v.* diet day 8) were included as fixed factors in the models. Correlations between the examined parameters were investigated using Pearson's correlations. Mean values with their standard errors were calculated for each experimental group separately (f3, f8, s3, s8). The level of significance was at $P \leq 0{\cdot}05$ (two-sided test).

Results

Piglets

Survivability of piglets between the four treatment groups is shown in Fig. 1.

Mean individual piglet weights, piglet growth, litter weights and litter growth in the four treatment groups are presented in Table 4. Piglet weight on D0, D1 and D2 was higher for the onset 8 groups than onset 3 groups ($P=0{\cdot}008$, $P=0{\cdot}004$, $P=0{\cdot}002$ at D0, D1 and D2, respectively). Piglet growth between D0 and D2 was higher in the onset 8 groups than in the onset 3 groups ($P=0{\cdot}048$).

Sow body condition and feed intake

The mean backfat levels at day 108 of gestation and at weaning were not statistically significant for all positions measured. At position A, f3 = 28·3 mm; f8 = 28·5 mm; s3 = 28·5 mm; s8 = 28·5 mm ($P=0{\cdot}921$); at position B, f3 = 21·7 mm; f8 = 22 mm; s3 = 22 mm; s8 = 22 mm ($P=0{\cdot}853$); at position C, f3 = 20·7 mm; f8 = 20·8 mm; s3 = 20·7 mm; s8 = 20·9 mm ($P=0{\cdot}908$). The differences in backfat levels between day 108 of gestation and weaning are indicated in Fig. 2.

The sows belonging in the s group consumed less feed than the sows belonging in the f group during the first 2 d of lactation (for D1, $P=0{\cdot}003$; for D2, $P=0{\cdot}014$) (Fig. 3).

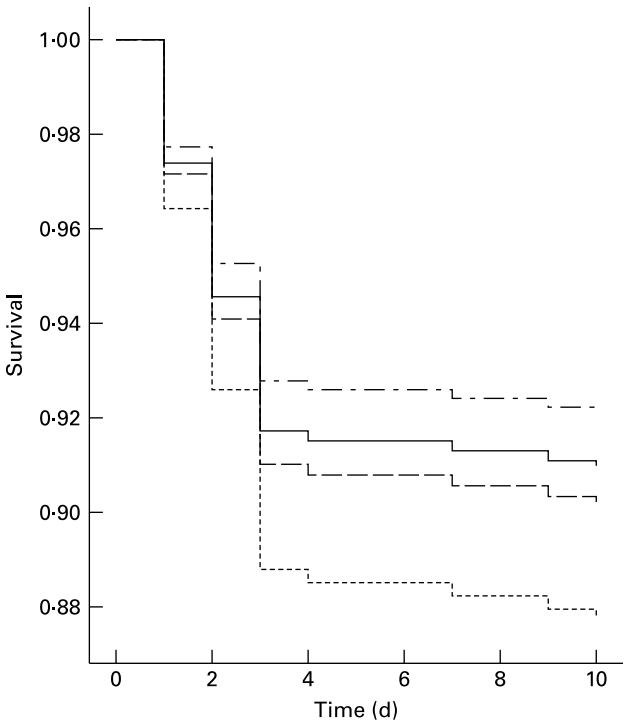

Fig. 1. Survival curves for piglet survivability during lactation from the four experimental diet groups in which sows were assigned. The feed contained either a low (supplemented with fish oil; f groups) or high (supplemented with sunflower-seed oil; s groups) *n*-6:*n*-3 ratio and was provided from 8 d (f8 (-·-·-), s8 (– – –)) or 3 d (f3 (—), s3 (- - -)) before parturition. Differences in survivability were not significant ($P>0{\cdot}05$). No losses of piglets occurred after day 10 of lactation and until weaning (day 21).

Sow metabolism and immunology parameters

The results of the parameters related to sow metabolism and immunology are presented in Table 5 and Figs. 4 and 5. On D-4, sows in the onset 8 groups had higher insulin levels than those in the onset 3 groups ($P=0{\cdot}021$). On D1, sows in the s group had higher insulin levels than the f group ($P=0{\cdot}029$) (Fig. 4). Insulin on D-1 was overall negatively correlated with litter weight (Table 6).

T_3 on D-4 was higher in the onset 8 than in the onset 3 group ($P<0{\cdot}001$). On D-1, the interaction between diet type and onset day was significant for T_3 levels: in the f group T_3 was higher in f3 than f8, but in the s group T_3 was higher in s8 than s3 ($P=0{\cdot}001$). On D1, T_3 was higher in the onset 8 than the onset 3 groups ($P=0{\cdot}001$). On D-4, T_4 concentrations were higher in the onset 3 than the onset 8 groups ($P=0{\cdot}046$). On D-1, T_4 levels were higher in the f than the s group ($P=0{\cdot}048$).

Leptin levels on D-4, D-3 and D-2 were higher in the s than the f groups ($P=0{\cdot}047$, $P=0{\cdot}005$, $P=0{\cdot}031$, respectively) (Fig. 4). Leptin on D-2 was higher in the onset 3 than in the onset 8 groups ($P=0{\cdot}003$) (Fig. 4). When leptin data were pooled across all treatment groups, significant negative correlations were found between leptin on D-4 and leptin on D-3 with litter weight and litter growth in different stages (Tables 6 and 7) . Furthermore, significant positive correlations existed between pooled leptin and insulin postpartum values on D1 (r 0·432; $P=0{\cdot}001$) and D3 (r 0·453; $P=0{\cdot}002$).

Table 4. Performance data (piglet and litter weight, piglet and litter growth) during lactation of the piglets from the sows of the four experimental groups†

(Mean values with standard errors from pooled data)

	Experimental group					
	f3	f8	s3	s8	SE	Significance
Piglet weight (kg)						
Day 0	1·30	1·41	1·28	1·43	0·024	**o
Day 1	1·40	1·53	1·38	1·55	0·026	**o
Day 2	1·57	1·71	1·53	1·74	0·029	**o
Day 7	2·43	2·58	2·45	2·52	0·052	NS
Day 14	4·37	4·56	4·38	4·56	0·076	NS
Day 21	6·19	6·53	6·22	6·54	0·099	NS
Piglet growth (g/d)						
Days 0–1	96	109	91	110	4·5	NS
Days 1–2	144	174	144	159	6·7	NS
Days 0–2	121	142	119	137	4·9	*o
Days 0–7	158	162	168	150	6·3	NS
Days 7–14	274	282	278	292	4·9	NS
Days 14–21	256	280	264	282	5·4	NS
Days 0–21	229	241	237	241	4·2	NS
Litter weight (kg)						
Day 0	14	16	16	16	0·4	NS
Day 1	15	17	17	17	0·5	NS
Day 2	17	19	18	18	0·5	NS
Day 7	26	29	29	26	0·9	NS
Day 14	47	50	51	48	1·6	NS
Day 21	65	71	73	68	2·1	NS
Litter growth (g/d)						
Days 0–1	807	1000	764	977	79·1	NS
Days 1–2	1327	1861	1303	1230	108·2	NS
Days 0–2	1067	1430	1033	1104	78·2	NS
Days 0–7	1620	1800	1821	1478	88·2	NS
Days 7–14	2946	3047	3233	3036	98·1	NS
Days 14–21	2731	3072	3131	2954	100·1	NS
Days 0–21	2432	2639	2729	2490	86·0	NS

f, Low *n*-3:*n*-6 ratio feed supplemented with fish oil; f3, group with feed f administered 3 d before parturition until weaning; f8, group with feed f administered 8 d before parturition until weaning; s, high *n*-3:*n*-6 ratio feed supplemented with sunflower-seed oil; s3, group with feed s administered 3 d before parturition until weaning; s8, group with feed s administered 8 d before parturition until weaning; o, onset day effect.

*$P<0·05$, **$P<0·01$.

† The feed contained either a low (supplemented with fish oil; f groups) or high (supplemented with sunflower-seed oil; s groups) *n*-6:*n*-3 ratio and was provided from 8 or 3 d before parturition (onset day 8 or 3).

On D-4, onset 3 groups had higher VLDL and TAG concentrations than the onset 8 groups ($P<0·001$ for both parameters). On D-1, s groups had higher VLDL and TAG concentrations than the f groups ($P=0·015$).

TNFα, IL-6 and SAA concentrations between the experimental groups on D1 and D3 are shown in Fig. 5. SAA on D3 was higher for the onset 3 group than the onset 8 group ($P=0·039$). Rectal temperature on D1 was higher in the s than the f group ($P=0·041$) (Fig. 5).

Discussion

The earlier transition from the gestation to a lactation diet (8 *v.* 3 d) led to an increase in neonatal piglet weight and subsequent growth. Although the increase in fetal weight during late gestation in sows reaches a plateau[26], it appears that the time point of diet change during the last days of gestation can affect piglet weight. This effect might be due to an increased transfer of energy and nutrients to the piglets *in utero*. The lactation diets used in the present study were superior in these elements comparing with the gestation diet.

In the present study, differences in feed consumption in sows during the first days of lactation were significant, even though sows of all groups were fed restrictively on an ascending scale and not on an *ad libitum* basis after farrowing. Feed consumption was lower when sows were fed the high *n*-6:*n*-3 ratio lactation diet. This may be attributed to the higher leptin levels during the periparturient period detected in this particular group in comparison with the other group. It has been described previously that intracerebroventricular treatment with leptin decreased feed intake in a dose-dependent manner in prepuberal gilts[27]. The role of insulin in this phenomenon should not be excluded. Central insulin action is catabolic (reducing feed intake and body weight), whereas its peripheral action is anabolic[28]. The high *n*-6:*n*-3 ratio diet induced higher insulin levels in sows at D1. Therefore, a peripheral insulin resistance may have occurred, while the central action of insulin may have remained unaltered.

After farrowing, an increase in insulin was triggered in all treatment groups. Increased insulin levels after parturition may play a role in partitioning nutrients to the mammary tissue[29] at the onset of milk production. Yet, as mentioned, insulin was higher at D1 when sows consumed the high

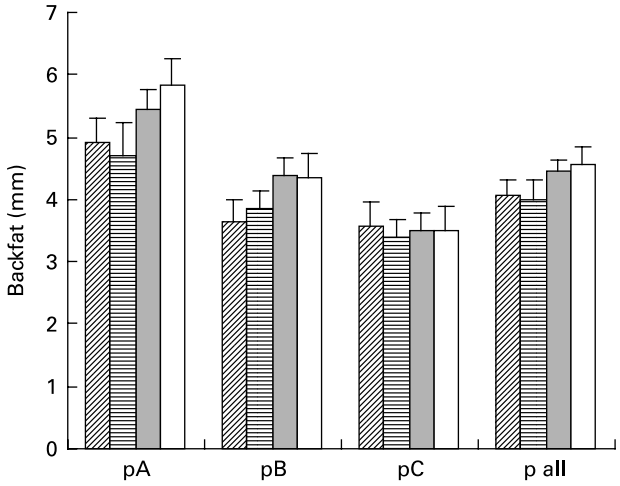

Fig. 2. Losses in backfat levels measured at day 108 of gestation and at weaning in the sows of the four experimental groups: (▨), f3; (▤), f8; (▧), s3; (□), s8. The feed contained either a low (supplemented with fish oil; f groups) or high (supplemented with sunflower-seed oil; s groups) *n*-6:*n*-3 ratio and was provided from 8 d (f8, s8) or 3 d (f3, s3) before parturition. Backfat was measured at three different positions: position A (pA) between the crossing backside shoulder and spine; position B (pB) at an equal distance between pA and position C (pC); pC in the crossing between the last rib and spine. p all, Average of backfat levels at the three positions. Values are means, with standard errors represented by vertical bars. No significant differences were found between the four experimental groups on the same position ($P>0{\cdot}05$).

n-6:*n*-3 ratio diet. Results from male miniature pigs suggest that substitution of *n*-3 for *n*-6 PUFA in dietary lipids is associated with enhanced insulin sensitivity[30]. In addition, fasting hyperinsulinaemia is present early in the process of diabetes and is thought to be a compensatory mechanism to maintain euglycaemia in the setting of insulin resistance[31]. Moreover, comparatively high levels of insulin in sows during lactation correspond to lower catabolic status and lower milk production[32]. It is therefore possible that in comparison with the low *n*-6:*n*-3 ratio groups, a short period of peripheral insulin resistance might have been developed after parturition in the high *n*-6:*n*-3 ratio groups.

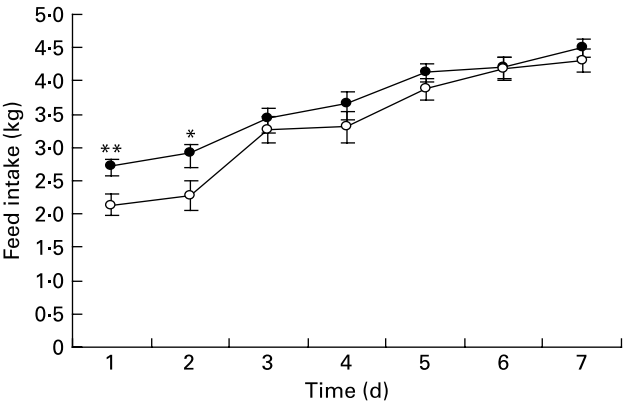

Fig. 3. Feed intake of sows during the first 7 d of lactation in the two diet-type groups. Sows were receiving two types of lactation diets differing in the *n*-6:*n*-3 ratio (the low ratio diet supplemented with fish oil; the high ratio diet supplemented with sunflower-seed oil), administered from two time points onwards before parturition. (○), Sunflower-seed oil-supplemented group; (●), fish oil-supplemented group. Values are means for each experimental group, with their standard errors represented by vertical bars. Mean value was significantly different from that of the sunflower-seed oil-supplemented group: *$P<0{\cdot}05$, **$P<0{\cdot}01$.

T_3 was increased and T_4 was decreased at D-4 in the groups that already have shifted to the lactation diets (onset 8 group). This indicated a higher conversion of T_4 into T_3. Again at D1, T_3 was higher in that experimental group. The secretions of the thyroid gland are involved with many metabolic functions including stimulating O_2 consumption and protein synthesis by the mammary gland and concomitantly increasing milk yield[33]. Impaired thyroid action had been speculated to be linked with the occurrence of the postpartum dysgalactia syndrome. Normal postpartum sows had significantly higher thyroid cell heights than either the agalactic or cyclic sows[34].

Leptin levels were affected by acute feeding events before farrowing, such as the transition from the gestation to the lactation diets. Between D-4 and D0, the time profile of leptin in sows receiving the lactation diets from 3 d pre-partum showed an increase and a subsequent spontaneous decrease. During the same period, the sows receiving the lactation diets from 8 d pre-partum already had the highest concentration of leptin. Of course, due to the absence of samples before D-4, such a profile in the sows in the 8 d group can only be hypothesised.

In the present study pre-farrowing levels of insulin and leptin in sows appeared to be associated with piglet performance. Similar findings were observed in rats[35], in which exogenous leptin given centrally in rats was associated with reduced litter weight gain. In addition, poor glucose tolerance of pregnant sows was reported previously[25] to be a risk factor for survival of pigs after birth. The precise mechanism underlying this relationship remains to be elucidated. In pigs, placental glucose transport occurs by a carrier-mediated facilitated diffusion involving several glucose transport isoforms, mainly GLUT1[36,37]. Most probably, pre-farrowing maternal levels of leptin and insulin affect these energy-dependent mechanisms and consequently affect litter weight at birth.

VLDL and TAG concentrations at D-4 were lower in the groups receiving already the lactation diets. This may indicate an increased rate of β-oxidation in the liver[37]. VLDL and TAG concentrations were increased at 1 d pre-partum by a high dietary *n*-6:*n*-3 ratio lactation diet. This suggests increased hepatic lipogenesis in these sows. Increased lipogenesis might not be related to changes in thyroid hormones, but might be more related to the lipogenic activity of the increased insulin.

The sows belonging to the high *n*-6:*n*-3 ratio group had significantly higher rectal temperatures. This can be attributed to the well-described pro-inflammatory properties of the *n*-6 fatty acids[3,4]. At D3, SAA levels were significantly higher in the onset 3 groups. Thus, SAA levels in sows postpartum can be affected by factors other than pathogenic challenges, as previously reported[38].

In conclusion, a lactation diet high in *n*-6:*n*-3 ratio was associated with higher leptin levels pre- and postpartum, a short-term peripheral insulin resistance on the first day of lactation and a decreased feed intake of sows on the first and second day of lactation. An increase in leptin concentration in the sows receiving the gestation diet until day 111 of gestation occurred after the acute elevation of the energy level, by offering these sows the lactation diet. Besides the effects

Table 5. Mean values of glucose, triiodothyronine (T_3), thyroxine (T_4), VLDL and TAG in the sows of the four experimental groups around parturition (D0)† (Mean values with standard errors from pooled data)

	Experimental group					
Time of measurement	f3	f8	s3	s8	SE	Significance
Glucose (mmol/l)						
D-4	3·882	4·003	3·975	3·715	0·052	NS
D-1	3·996	3·802	3·975	3·940	0·045	NS
D1	3·750	4·380	4·240	4·127	0·116	NS
D3	4·130	3·908	3·957	3·958	0·101	NS
T_3 (ng/ml)						
D-4	0·155	0·237	0·140	0·215	0·008	***o
D-1	0·186	0·150	0·117	0·176	0·007	***to
D1	0·252	0·414	0·281	0·340	0·017	***o
D3	0·306	0·343	0·285	0·317	0·012	NS
T_4 (ng/ml)						
D-4	16·066	13·020	14·516	12·768	0·596	*o
D-1	10·856	10·999	7·859	10·449	0·455	*t
D1	18·167	20·574	18·278	19·932	0·749	NS
D3	14·470	15·224	13·002	15·548	0·551	NS
VLDL (mg/l)						
D-4	166·83	125·17	214·36	124·00	9·19	***o
D-1	61·00	61·33	96·80	69·20	4·71	*t
D1	72·70	66·00	89·90	70·70	4·31	NS
D3	58·62	55·47	48·88	55·20	2·70	NS
TAG (mg/l)						
D-4	834·20	625·80	1071·80	620·00	45·97	***o
D-1	305·00	306·70	484·00	346·00	23·57	*t
D1	363·60	330·00	449·30	353·30	21·57	NS
D3	293·10	277·30	275·90	276·00	15·75	NS

f, Low *n*-3:*n*-6 ratio feed supplemented with fish oil; f3, group with feed f administered 3 d before parturition until weaning; f8, group with feed f administered 8 d before parturition until weaning; s, high *n*-3:*n*-6 ratio feed supplemented with sunflower-seed oil; s3, group with feed s administered 3 d before parturition until weaning; s8, group with feed s administered 8 d before parturition until weaning; o, onset day effect; t, diet type effect; to, interaction between diet type and onset day.
*$P<0·05$, **$P<0·01$, ***$P<0·001$.
† The feed contained either a low (supplemented with fish oil; f groups) or high (supplemented with sunflower-seed oil; s groups) *n*-6:*n*-3 ratio and was provided from 8 or 3 d before parturition (onset day 8 or 3).

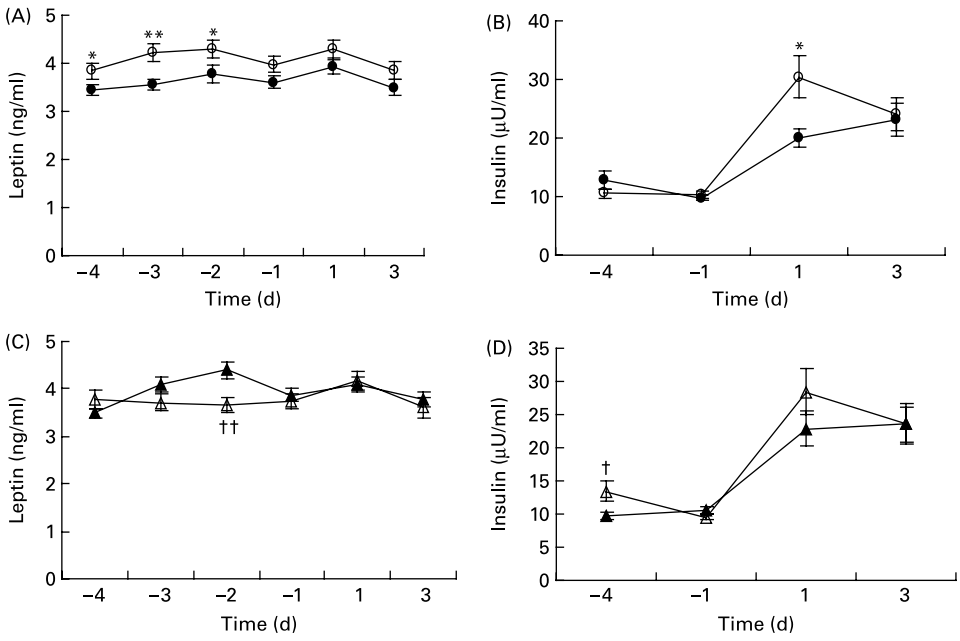

Fig. 4. Leptin (A, C) and insulin (B, D) in sows (*n* 71) during the periparturient period of −4 d before farrowing to 3 d after farrowing. Sows received two lactation diets differing in the *n*-6:*n*-3 ratio (the low ratio diet supplemented with fish oil (●); the high ratio diet supplemented with sunflower-seed oil (○)), administered from two time points onwards before parturition (8 d (△) or 3 d (▲) before farrowing; onset 8 or 3 groups). (A) Leptin in the two diet-type groups. (B) Insulin in the two diet-type groups. (C) Leptin in the two diet-day groups. (D) Insulin in the two diet-day groups. Values are means, with standard errors represented by vertical bars. Mean value was significantly different from that of the fish oil-supplemented group: *$P<0·05$, **$P<0·01$. Mean value was significantly different from that of the onset 3 group: †$P<0·005$, ††$P<0·01$.

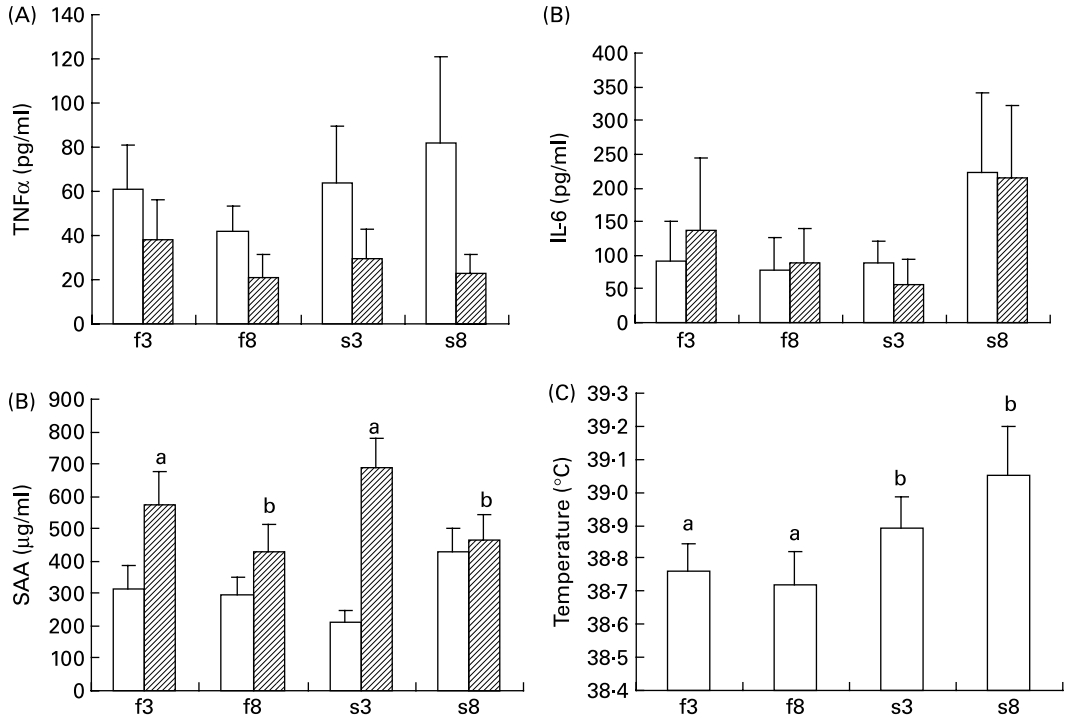

Fig. 5. TNFα (A), IL-6 (B), serum amyloid A (SAA; (C)) and rectal temperature (D) of the sows (*n* 71) at the first (□) and third day (▨) of lactation. Sows were receiving two types of lactation diets differing in the *n*-6:*n*-3 ratio (the low ratio diet supplemented with fish oil; the high ratio diet supplemented with sunflower-seed oil), administered from two time points onwards before parturition (8 d or 3 d before farrowing; onset 3 or 8 groups). f3, Fish oil–onset 3; f8, fish oil–onset 8; s3, sunflower-seed oil–onset 3; s8, sunflower-seed oil–onset 8. Values are means, with standard errors represented by vertical bars. [a,b] Mean values within the same day of measurement with unlike letters were significantly different ($P<0·05$).

Table 6. Associations (Pearson's correlation; *r*) between sow insulin levels 1 d before farrowing (D-1) and leptin levels on the fourth and third day before farrowing (D-4 and D-3 respectively) from the four experimental groups with litter weight at birth (Lw 0) and on days of lactation (Lw 1, 2, 7, 14 and 21)

	Lw 0	Lw 1	Lw 2	Lw 7	Lw 14	Lw 21
Insulin D-1	−0·420**	−0·441**	−0·412**	−0·304*	−0·280*	−0·263
Leptin D-4	−0·196	−0·201	−0·221	−0·355**	−0·336**	−0·302*
Leptin D-3	−0·182	−0·220	−0·265*	−0·345**	−0·319*	−0·314*

*$P<0·05$, **$P<0·01$.

on leptin, the time point of transition from a gestation to a lactation diet profoundly influenced piglet weight at birth and at subsequent stages. Any separate effect of *n*-6 and *n*-3 PUFA and pre-partum feeding strategy on insulin, thyroid hormones, leptin, rectal temperature, feed intake of sows in the periparturient period and the piglet growth in the early days of lactation should not be disregarded, but these effects should be considered as a whole. This approach should be a prerequisite to a better understanding of the pathogenesis of postpartum dysgalactia syndrome from a clinical nutrition approach. According to the present results, a diet low in the *n*-6:*n*-3 ratio (fish oil-supplemented diet) provided 8 d before the expected farrowing was the most appropriate combination.

Table 7. Associations (Pearson's correlation; *r*) between sow leptin levels from all experimental groups on the fourth and third day before farrowing (D-4 and D-3 respectively) with litter growth (Lgr) between different stages of lactation

	Lgr 1–2	Lgr 0–2	Lgr 0–7	Lgr 7–14	Lgr 14–21	Lgr 0–21
Leptin D-4	−0·197	−0·165	−0·395**	−0·278*	−0·192	−0·316**
Leptin D-3	−0·335**	−0·362**	−0·405**	−0·255*	−0·262*	−0·334**

*$P<0·05$, **$P<0·01$.

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