

Effects of L-carnitine supplementation on milk production, litter gains and back-fat thickness in sows with a low energy and protein intake during lactation

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The present study investigated the effect of L-carnitine supplementation during pregnancy (125 mg/d) and lactation (250 mg/d) on milk production, litter gains and back-fat thickness in sows fed a low-energy and low-protein diet during lactation. Sows supplemented with L-carnitine produced more milk on days 11 and 18 of lactation (+18%; $P < 0.05$) and had higher litter gains during suckling (+20%; $P < 0.01$) than control sows. Loss of body weight during lactation was similar in both groups, but sows supplemented with L-carnitine had a greater reduction of back-fat thickness (+45%; $P < 0.05$) during lactation than control sows. In conclusion, this study shows that L-carnitine increases milk production and litter gains in sows in a strongly negative energy and N balance, and enhances body fat mobilisation.

L-Carnitine: Sow: Lactation: Milk production: Energy balance

Recent studies have shown that supplementing sow diets with L-carnitine during pregnancy and lactation improves their reproductive performance. In particular, sows fed diets supplemented with L-carnitine had heavier litters than control sows (Musser *et al.* 1999; Eder *et al.* 2001b; Ramanau *et al.* 2002). Moreover, the piglets of sows supplemented with L-carnitine grew faster during the suckling period than those of control sows (Musser *et al.* 1999; Eder *et al.* 2001b; Ramanau *et al.* 2002, 2004). This effect is due to an increased milk yield in sows treated with L-carnitine compared with control sows (Ramanau *et al.* 2004).

The biochemical mechanisms underlying these effects of L-carnitine in sows are not fully understood. Owing to its function (Bremer, 1963), it seems plausible that the effects of L-carnitine in sows might be due to increased β -oxidation of fatty acids. In growing pigs, L-carnitine supplementation reduced body fat deposition and increased protein accretion (Owen *et al.* 1996, 2001b; Heo *et al.* 2000). These effects are due to an increased rate of β -oxidation and an increased reutilisation of waste N for protein synthesis by dietary L-carnitine (Owen *et al.* 2001a). Lactating sows are usually unable to consume sufficient feed to meet the heavy demand for nutrients needed for milk production. They therefore mobilise energy and amino acids from body stores, i.e. body fat and body protein, which are used for milk production (Rozeboom *et al.* 1996; Van den Brand *et al.* 2000). Based on studies in growing pigs, we hypothesise that L-carnitine supplementation promotes the mobilisation of energy from adipose tissue, which can be used for the production of surplus milk. To test this hypothesis, we conducted an experiment with sows fed a low-energy/low-protein diet during lactation to induce a strongly negative energy and N balance. In order to draw inferences for milk production and fat mobilisation, we measured milk output on days 11 and 18, weight gains of

the suckling piglets during lactation and back-fat thickness of the sows at the beginning and end of lactation.

Practical feeding strategies for lactating sows aim to minimise weight loss during lactation (Aherne & Williams, 1992). The low-energy and low-protein diets used in the present study do not reflect the practical feeding of lactating sows that are low in energy and protein. This study should therefore be regarded as a model to help to ascertain the effects of L-carnitine in lactating sows in negative energy and N balance.

Methods

Animals and housing

Crossbred gilts (German Landrace \times Large White; n 24) in their third reproductive cycle were used. They were assigned to two groups of twelve sows each. The sows were artificially inseminated with sperm from Pietrain boars. In each group, ten of the twelve sows conceived. Sows that failed to conceive were removed from the experiment. The sows were kept in single crates until day 30 of pregnancy. From day 30 to day 110 of pregnancy, the sows were kept in groups of eight in pens measuring 45 m² that had fully slatted floors, nipple-drinkers and electronic feeding stations. On day 110 of pregnancy, they were moved to the farrowing accommodation, where they were housed in single farrowing pens. Prior to farrowing, rubber mats were put down as a lying surface for the piglets. An IR heater was suspended above each rubber mat to keep the temperature for the newborn piglets at a constant 35°C. Piglets were suckled for 29 d and weaned on day 30 of lactation. The climate in the dry sow accommodation and the farrowing unit was maintained at a

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temperature of $19 \pm 2^\circ\text{C}$ and 60–80% relative humidity by means of an air-conditioning system. A light–dark cycle (12 h light, 12 h dark) was applied. All animal procedures described followed established guidelines for the care and handling of laboratory animals and were approved by the regional council of Saxony-Anhalt.

Diets and feeding

One basal diet was used throughout pregnancy and lactation. The diet consisted of (g/kg diet): dried sugar beet pulp (303); barley (299); wheat bran (150); alfalfa meal (67.5); oat bran (40); extracted sunflower meal (50); malt sprouts (50); soyabean hulls (20); molasses (10) premix including minerals, vitamins and L-lysine (10.5). The diet contained 9.0 MJ metabolisable energy per kg. Nutrient concentrations in the diets were (g/kg diet): crude protein (144); crude fibre (131); crude fat (27); starch (182); sugar (64); lysine (6.4); methionine (2.1); threonine (4.7) tryptophan (1.5). Nutrient concentrations conformed to recommendations for pregnant sows; concentrations of energy and essential amino acids were considerably below the levels recommended for lactating sows (National Research Council, 1998). The concentration of L-carnitine in the diet was 20 mg/kg.

From the beginning of the experiment until day 30 of pregnancy, the sows were given 3.0 kg of this diet per day; from day 30 to day 110, the diet was offered for consumption *ad libitum*. The daily feed intake of the sows from day 30 to day 110 of pregnancy was recorded by means of an electronic sow feeding station (Type IVOG 2FR VH; HohoFarm, Insentec BV, Marknesse, The Netherlands). From day 110 to farrowing, each sow was fed 2.5 kg of this diet. On the day of farrowing, the sows were fed 1.5 kg/d, which was then successively increased (3 kg/d on day 1 and day 2 of lactation; 4.5 kg/d on day 3 and day 4 of lactation; consumption *ad libitum* from day 5 of lactation to weaning). The feed intake was determined by weighing the amount of unconsumed diet. Water was provided from nipple-drinker systems.

Supplementation with L-carnitine

Sows in the treatment group were supplemented with 125 mg L-carnitine/d during pregnancy and 250 mg L-carnitine/d during lactation. L-Carnitine was supplied as tablets containing L-carnitine (125 mg/tablet), lactose and dextrose, supplied by Lohmann Animal Health (Cuxhaven, Germany). The tablets were administered once daily in the morning (09.00 hours) by hand. Each sow of the treatment group was given one tablet per d during pregnancy and two tablets during lactation. The levels of L-carnitine supplementation during pregnancy and lactation were selected according to our recent studies (Ramanau *et al.* 2002, 2004). Control animals were given the same tablets without L-carnitine.

Data recording

Body weights (using scales with an accuracy of ± 100 g) and back-fat thickness (by ultrasound) were recorded on day 1 of pregnancy, after farrowing and on the day of weaning. Back-fat thickness was measured by placing the probe of the ultrasound machine (Type SSD500; Aloka, Meerbusch, Germany) vertically 5 cm left of the spinal column at the level of the thirteenth/fourteenth rib. To minimise the measuring error, the measurement

was repeated 5 cm cranial and 5 cm caudal to the first measuring site. The three readings were combined to form the mean.

The number of piglets born (total, number born alive and number stillborn) was recorded. Individual piglets were weighed at birth (not later than 6 h after birth) and at weaning on day 30 using scales with an accuracy of ± 10 g. All the sows that conceived (ten in each group) were evaluated for number of piglets born, piglet and litter weights at birth and plasma L-carnitine concentration. The eight sows whose litters were standardised to ten piglets/litter (see 'Determination of milk output', later) were used for measuring milk output and milk nutrients, litter gains and back-fat thickness, and for estimating energy balance.

Determination of nutrients in the diets

Concentrations of crude nutrients, starch and sugar in the diets were analysed according to the official German Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten methodology (Bassler & Buchholz, 1993). Metabolisable dietary energy was calculated as recommended by the German Nutrition Society (Gesellschaft für Ernährungsphysiologie, 1987). The amino acid concentrations of the diet were also determined according to the official Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten method (Bassler & Buchholz, 1993). Samples were oxidised and subsequently hydrolysed with 6M-HCl. The separation and quantification of amino acids was performed by ion exchange chromatography following post-column derivatisation in an amino acid analyser (Biotronic LC 3000; Eppendorf, Hamburg, Germany). For determination of tryptophan, the diet was digested with $\text{Ba}(\text{OH})_2$ (Fontaine *et al.* 1998). The tryptophan concentration was determined by reverse-phase HPLC (Eder *et al.* 2001a).

Determination of milk output

Milk output was measured on days 11 and 18 of lactation in eight of the ten sows of each group. In order to eliminate the effect of litter size on milk production, the litter size of these sows was standardised to ten piglets/litter within 2 d of farrowing. Sows with more than ten piglets had the surplus piglets taken away, and sows with fewer than ten piglets were given piglets from other sows of the same group. Piglets removed from sows and piglets given to sows were selected on the basis of their body weight. The average weight of the piglets of each sow after litter standardisation was matched to that before litter standardisation. Surplus piglets were nursed by the remaining two sows of each group, which were not considered for milk output determination. Piglets that dropped out before day 18 of lactation were immediately replaced by equivalent piglets with a similar body weight that had previously been nursed by the remaining two control or treated sows.

Milk output was measured by the 'weigh–suckle–weigh' method (Kirchgessner *et al.* 1992). On the day of the milk recording procedure, the piglets were separated from the sow by means of a barrier from 06.00 hours to 16.00 hours and allowed supervised access to the sow only at 1 h intervals for the duration of suckling. The first two weighings (07.00 hours and 08.00 hours) were carried out to allow sow and piglets to become accustomed to the procedure; the calculation of the daily milk production was based on the last seven measurements. In order to minimise losses

through faeces and urine, the piglets were weighed rapidly; the accuracy of the scales was ± 1 g.

Analysis of milk constituents

On day 11 of lactation, after completion of the milk output determination, the sows were given 15 IU oxytocin (Atarost GmbH&Co, Twistringen, Germany) by intramuscular injection. Milk samples (80–100 ml from each sow) were collected by hand from all the functional nipples. The concentration of lactose in the milk was determined using an enzymatic kit reagent from Boehringer (Cat. No. 0176303; Mannheim, Germany). The concentration of protein in the milk was determined by the Kjeldahl procedure using the IDF-ISO-AOAC method (Association of Official Analytical Chemists, 1990), and the concentration of fat in the milk was determined by the ether extraction method (Association of Official Analytical Chemists, 1990). The energy content of the milk was calculated from the concentrations of protein, fat and lactose, the following energy concentrations being used: lactose 16.4 kJ/g; fat 39.4 kJ/g; protein 23.5 kJ/g (National Research Council, 1998). The amounts of fat, protein, lactose and energy secreted with the milk on day 11 of lactation were calculated by multiplying the daily milk yield by the concentrations of these nutrients or energy, respectively, in the milk.

Analysis of L-carnitine in plasma, milk and diet

Sows were bled by puncturing the jugular fossa 6 h after feeding on days 95 of pregnancy and 21 of lactation. Plasma was obtained by centrifugation of the blood (1100 g, 10 min) and stored at -20°C pending analysis. The concentration of total carnitine in plasma, milk and diet was determined by a radiochemical method, which is based on conversion of carnitine into [^3H]acetylcarnitine by carnitine-O-acetyltransferase (McGarry & Foster, 1976).

Estimations of energy balance of the sows

The sows' energy balance during lactation was estimated using the following equations:

- (1) Energy balance = Metabolisable energy from diet consumed during lactation – (Energy for sow maintenance + Energy for milk production).
- (2) Energy requirement for maintenance = $0.44 \text{ MJ metabolisable energy} \times \text{kg}^{0.75}$ per d (National Research Council, 1998; body weights used were: $(\text{Body weight day 1} + \text{Body weight day 30})/2$)
- (3) Energy requirement for milk production = Milk energy/Efficiency of use of energy from diet and body stores for milk production (assuming that the efficiency of dietary energy for milk production is 0.72 and that of energy mobilised from tissue is 0.88; National Research Council, 1998)
- (4) Milk energy (MJ gross energy/d) = $[(4.92 \times \text{average litter gain in g/d}) - (90 \times \text{number of pigs})] \times 0.00419$ (National Research Council, 1998).

Statistical analysis

The means of the two groups of sows were compared by a Student's *t* test. The milk output of the sows, which was measured on days 11 and 18 of lactation, was additionally analysed by

two-way ANOVA using the Minitab statistical software (Release 13; Minitab Inc., State College, PA, USA) with treatment, day of lactation and their interaction as classification factors. The results are expressed as means with their standard errors. Means were considered significantly different for $P < 0.05$.

Results

Feed intake, body weights of the sows and back-fat thickness

Feed intake during pregnancy and lactation did not differ between control sows and sows supplemented with L-carnitine: pregnancy 3.5 (SE 0.1) v. 3.9 (SE 0.3) kg/d, lactation 4.6 (SE 0.3) v. 4.7 (SE 0.3) kg/d, in control sows and sows supplemented with L-carnitine, respectively (eight for each group). Body weights on day 1 of pregnancy, after farrowing and at weaning did not differ between control sows and sows supplemented with L-carnitine: day 1 of pregnancy 202 (SE 7) v. 211 (SE 6) kg, after farrowing 257 (SE 5) v. 260 (SE 5) kg, at weaning 194 (SE 8) v. 194 (SE 3) kg, in control sows and sows supplemented with L-carnitine, respectively (eight for each group). The sows of both groups lost much weight during lactation, but the losses were similar in both groups: 62.4 (SE 5.2) in control sows v. 65.8 (SE 5.1) kg in sows supplemented with L-carnitine (eight for each group).

Back-fat thickness of the sows on day 1 of pregnancy and on the day of farrowing did not differ between control sows and sows supplemented with L-carnitine: day 1 of pregnancy 17.9 (SE 1.0) v. 18.6 (SE 1.4) mm, after farrowing 23.4 (SE 1.2) v. 25.3 (SE 1.7) mm, in control sows and sows supplemented with L-carnitine, respectively; eight for each group. Back-fat thickness at weaning was, however, lower in sows treated with L-carnitine than in control sows: 9.6 (SE 0.9) v. 12.4 (SE 1.1) mm (eight; $P < 0.05$). The reduction of back-fat thickness from the day of farrowing to weaning was greater in sows supplemented with L-carnitine than in control sows: 16.0 (SE 1.5) v. 11.0 (SE 1.8) mm (eight; $P < 0.05$).

Number and birth weights of piglets

The total litter size and number of piglets born alive did not differ between control sows and sows supplemented with L-carnitine: number of piglets born 12.2 (SE 1.4) v. 12.3 (SE 0.9), number of piglets born alive 12.1 (SE 1.4) v. 12.3 (SE 0.9), in control sows and sows supplemented with L-carnitine, respectively (eight for each group). There was also no difference in the birth weights of piglets and litters between the two groups of sows: weights of piglets 1.55 (SE 0.13) v. 1.65 (SE 0.06) kg, weights of litters 17.9 (SE 1.7) v. 20.1 (SE 0.9) kg, in control sows and sows supplemented with L-carnitine, respectively (eight for each group).

Weights of litters after standardisation

After standardisation of the litter sizes to ten piglets/litter, mean piglets weights were similar to those before standardisation: 1.59 (SE 0.07) kg for control sows and 1.69 (SE 0.05) kg for sows supplemented with L-carnitine (eight). Litter weights at the beginning of the suckling period did not differ between control sows and sows supplemented with L-carnitine: 15.9 (SE 0.7) kg for control sows and 16.9 (SE 0.5) kg for sows supplemented with L-carnitine (n 8). During the 29 d suckling period, the litters of sows supplemented with L-carnitine gained more weight (91.2

(SE 3.2) v. 76.1 (SE 3.0) kg; n 8; $P < 0.01$) and were heavier at weaning (108.1 (SE 3.7) v. 92.0 (SE 3.1) kg; n 8; $P < 0.01$) than those of control sows.

Milk output and milk nutrients

Bifactorial analysis showed significant ($P < 0.05$) effects of treatment and time of lactation on the milk output of the sows. Milk production on day 18 was higher than on day 11. Sows supplemented with L-carnitine produced 18% more milk than control sows (Table 1). The interaction between the two factors was not significant. Concentrations of fat, protein and lactose and the amount of gross energy in the milk on day 11 of lactation did not differ between sows treated with L-carnitine and control sows (Table 1). The amounts of fat, protein, lactose and energy secreted with the milk were 15–18% higher in sows supplemented with L-carnitine than in control sows. The differences were not, however, statistically significant.

Concentrations of L-carnitine in plasma and milk

Sows treated with L-carnitine had higher concentrations of total L-carnitine in the plasma on day 95 of pregnancy and on day 21 of lactation, and in the milk on day 11 of lactation: plasma, day 95 of pregnancy 7.5 (SE 0.3) v. 11.1 (SE 1.0) $\mu\text{mol/l}$, $P < 0.05$; plasma, day 21 of lactation 9.0 (SE 0.6) v. 14.1 (SE 1.7) $\mu\text{mol/l}$, $P < 0.05$; milk, day 11 of lactation 130 (SE 9) v. 170 (SE 9) $\mu\text{mol/l}$, $P < 0.05$; in control sows and sows supplemented with L-carnitine, respectively (eight for each group).

Estimated energy balance of the sows

The dietary energy intake and the estimated energy requirement for maintenance during the whole lactation period did not differ between the two groups of sows (Table 2). However, sows supplemented with L-carnitine had a higher energy requirement for milk production and a higher total energy requirement. Both groups were in a strongly negative energy balance, although

Table 1. Milk production at day 11 and day 18, concentrations of nutrients in the milk and amounts of nutrients secreted with milk at day 11 of lactation in control sows and sows supplemented with L-carnitine*

(Mean values and standard errors for eight sows per group)

Treatment	Control		+ L-carnitine	
	Mean	SE	Mean	SE
Milk production (kg/d)				
Day 11	7.76	0.72	9.06	0.65
Day 18	8.74	0.74	10.43	0.50
Nutrients in the milk at day 11				
Fat (g/kg)	94.9	4.3	95.7	5.8
Protein (g/kg)	50.1	1.7	49.2	1.1
Lactose (g/kg)	55.1	0.5	55.5	0.8
Gross energy (MJ/kg)	5.77	0.13	5.78	0.20
Nutrients secreted with milk on day 11 (g)				
Fat (g)	745	84	865	79
Protein (g)	385	30	442	25
Lactose (g)	428	42	504	38
Gross energy (MJ)	45.0	1.6	52.2	1.5

† For details of diets and procedure, see p. 718.

Table 2. Estimated energy balance of control sows and sows supplemented with L-carnitine during the lactation period †

(Mean values and standard errors for eight sows per group)

Treatment	Control		+ L-carnitine	
	Mean	SE	Mean	SE
Energy intake by diet (MJ metabolisable energy)	1192	81	1236	75
Energy requirement for sow maintenance (MJ)	742	14	746	9
Energy requirement for milk production (MJ)	1740	73	2100**	83
Energy balance (MJ)	-1290	89	-1610**	71

Mean values were significantly different from those of the control group: * $P < 0.01$.

† For details of diets and procedure, see p. 718.

this was greater in the sows supplemented with L-carnitine than in the control sows.

Discussion

In the present study, sows were supplemented with L-carnitine during pregnancy and lactation. The observation that supplementing the sows during pregnancy and lactation increases the concentrations of total L-carnitine in plasma and milk agrees with other studies (Musser *et al.* 1999; Ramanau *et al.* 2004) and suggests that L-carnitine supplementation improved the sows' L-carnitine status. The finding that L-carnitine supplementation did not improve piglet number and birth weights is in disagreement with our recent studies, in which L-carnitine supplementation increased the number of piglets born and the litter weights in sows under similar feeding conditions during pregnancy (Ramanau *et al.* 2004). The reason for the contradiction is unknown. We are, however, aware that because of the small number of animals used, this study is not suitable for investigating the effects of L-carnitine on litter parameters at birth.

This study shows that sows whose diet is supplemented with L-carnitine produce more milk during lactation than control sows, even in a strongly negative energy and protein balance. The higher milk production of the L-carnitine-supplemented sows, which was demonstrated by the 'weigh-suckle-weigh' procedure on days 11 and 18 of lactation, was presumably responsible for the increased weight gains of their suckling piglets compared with those of control sows. It has been shown that a linear relationship exists between feeding level during lactation and milk yield (Pettigrew, 1995; Noblet *et al.* 1998). Even a mild restriction of energy intake can cause a considerable reduction in milk yield (Van den Brand *et al.* 2000). It is noteworthy that sows supplemented with L-carnitine had high milk yields and fast-growing litters despite the strongly negative energy balance.

Estimation of energy balance using the National Research Council (1998) model (Table 2) shows that the L-carnitine-supplemented sows were in a more strongly negative energy balance than the control sows because of their higher milk yield. The observation that the back-fat thickness of L-carnitine-treated sows decreased far more sharply during lactation than that of control sows suggests that the L-carnitine-supplemented sows additionally mobilised adipose tissue that could be used in the production of surplus milk.

It has previously been shown that L-carnitine supplementation reduces body fat deposition in piglets and growing-finishing

pigs (Owen *et al.* 1996, 2001b; Heo *et al.* 2000). Studies of the hepatocytes of L-carnitine-supplemented pigs have shown that L-carnitine enhances β -oxidation by increasing the activity of carnitine palmitoyl transferase I (Owen *et al.* 2001a). These studies suggest that L-carnitine enhances the utilisation of fatty acids in pigs. Our study indicates that L-carnitine might also enhance the utilisation of body fat by sows in a strongly negative energy balance.

It is clear that the results of this study cannot be readily transposed to practical sow feeding situations because the energy and protein deficit during lactation was much more severe than would have been the case under practical feeding conditions. When feeding lactating sows, the aim is to minimise the loss of body mass by providing them with sufficient energy and nutrients. Nevertheless, a considerable energy deficit and loss of body mass during lactation can also occur in practical feeding situations, particularly in primiparous sows (Rozeboom *et al.* 1996; Van den Brand *et al.* 2000). This study suggests that, in such situations, dietary L-carnitine may help sows to maintain a high milk yield and fast growth of suckling litters.

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