



Effect of carnitine feed supplementation on biochemical parameters and the relative levels of inflammatory markers in suckling and weaned piglets

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Abstract

The early postnatal period and the weaning period are the most critical stages in the life of piglets, characterized by high mortality rates and the need to identify effective approaches to improve viability. The aim of this study was to determine the effect of dietary carnitine supplementation in suckling piglets on biochemical parameters and the relative levels of inflammatory markers before and after weaning. After farrowing, two groups of crossbred piglets (Yorkshire × Landrace × Duroc) were formed: 188 piglets in the control group and 190 piglets in the experimental group. During the suckling period, starting from the fifth day of life, piglets in both groups were fed a prestarter compound feed. Piglets were weaned at 28 days of age. The experimental group received a feed supplemented with 50 % L-carnitine at a dose of 500 g/t of compound feed. Blood samples were collected from 10 piglets in each group three days before and three days after weaning, prior to morning feeding, from the orbital sinus. The obtained blood serum was frozen and stored at -20°C until analysis. Biochemical parameters in blood serum were determined using an automatic biochemical analyzer, Miura 200 (Italy), with commercial reagent kits. The relative content of molecular markers in blood serum was determined by Western blot analysis. Carnitine supplementation during the suckling period had a positive effect on liver condition, as evidenced by increased albumin synthesis and decreased levels of biochemical markers of liver damage. In particular, a decrease in ALT activity and, to a lesser extent, AST activity was observed. A higher glucose level under the influence of the supplement was also established, which may result from redistribution of energy substrates with predominance of fatty acid oxidation processes. The effect of carnitine on the relative levels of molecular inflammatory markers was characterized by a decrease in the relative content of pro-inflammatory cytokines and an increase in anti-inflammatory cytokines. These patterns persisted under weaning stress conditions.

Keywords: liver synthetic function; albumins; glucose; molecular markers; glucose.

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1. Introduction

High mortality of newborn piglets before weaning remains a major economic and animal welfare problem in pig production worldwide. This problem is primarily associated with starvation and hypothermia in piglets. In addition, the high mortality rate results from the underdeveloped immune system of piglets. Therefore, considerable attention is focused on improving piglet welfare both through management practices and by stimulating immune responses and enhancing the energy capacity of the organism.

Newborn piglets have a limited ability to oxidize milk fat and produce ketone bodies, as well as a higher rate of fatty acid esterification compared with newborns of other species (Adams et al., 1997). One of the causes of insufficient fatty acid oxidation is L-carnitine deficiency (Virmani & Cirulli, 2022).

Carnitine is a water-soluble nutrient whose primary function is the transport of long-chain fatty acids into mitochondria for β -oxidation in order to support ATP production as the cellular energy currency. It plays a crucial role in the metabolic functions associated with normal growth and development of newborns, particularly in energy production and detoxification through the removal of acyl residues (Lin et al., 2024). The main source of carnitine for fetuses and newborns is maternal supply via placental transfer and milk uptake, due to the limited capacity of newborns to synthesize carnitine de novo (Kendler, 1986). Low carnitine levels have also been reported in newborn animals such as rats (Lin et al., 2005) and pigs (Kerner et al., 1984), suggesting that endogenous carnitine reserves may be insufficient to meet the optimal metabolic demands of newborns. The concentration of carnitine in sow milk decreases quadratically with the progression of lactation days (Pike et al., 2025).

Adequate carnitine intake is therefore critically important for suckling piglets to obtain sufficient energy from milk fat metabolism, and disturbances in this process may contribute to high piglet mortality.

After weaning, adipose tissue, similarly to the neonatal period, also serves as a major energy source for piglets (Zhao et al., 2023). This is explained by reduced feed intake (Fabà et al., 2023), as well as increased production of pro-inflammatory cytokines and impaired intestinal immune status (Tang et al., 2022). Therefore, the search for feed additives capable of improving feed intake and enhancing the physiological status of piglets during the weaning period remains highly relevant (Yefimov et al., 2017; Zhao et al., 2024).

The aim of the present study was to determine the effect of dietary carnitine supplementation in suckling piglets on biochemical parameters and the relative levels of inflammatory markers before and after weaning.

2. Materials and methods

Experimental Design. Upon transfer to the farrowing unit, two groups of 18 sows each were formed according to the principle of analogs, taking into account breed, body weight, and number of farrowings. After farrowing, nearly equal numbers of crossbred piglets (Yorkshire × Landrace × Duroc) were obtained from both groups: 188 piglets in the control group and 190 piglets in the experimental group. During the suckling period, starting from the fifth day of life, piglets in both groups were fed a prestarter compound feed. Piglets were weaned at 28 days of age.

Piglets in the experimental group received “Carniking” (LAH, Germany), containing 50 % L-carnitine, incorporated into the compound feed at a dose of 500 g/t of feed. The second group served as the control. Feeding and housing conditions complied with established animal husbandry requirements.

Sample Collection. Blood samples were collected from 10 piglets in each group three days before and three days after weaning, prior to morning feeding, from the orbital sinus. The obtained blood serum was frozen and stored at –20 °C until analysis.

Biochemical Analysis. Blood serum was analyzed for total protein, albumin, urea, creatinine, total calcium, inorganic phosphorus, glucose concentrations, as well as ALT and AST activities using an automatic biochemical analyzer Miura 200 (Italy) with commercial reagent kits.

Determination of Relative Levels of Molecular Markers. The relative content of molecular markers in blood serum was determined by immunoblotting.

Sample Preparation. Blood serum samples were diluted 1:10 with Tris-buffered saline, pH 7.2, containing 0.1 % Tween-20 (TBS-T), and mixed with reducing sample buffer (Laemmli buffer) at a sample-to-buffer ratio of 1:1. The mixtures were thoroughly homogenized and incubated in a water bath at 95 °C for 5 min. Protein samples fixed in this buffer were frozen and stored at –80 °C until Western blot analysis.

Western Blot Analysis. Serum proteins were separated by polyacrylamide gel electrophoresis (PAGE) using a 5–20 % acrylamide gradient gel. Polypeptides separated according to molecular weight were transferred from the gel onto a poly-

vinylidene difluoride (PVDF) membrane using an external electric field at 150 mA for 120 min.

After transfer, PVDF membranes were washed three times for 5 min with TBS-T. Membranes were then blocked in 1 % bovine serum albumin (BSA) solution in TBS-T. The blocked membranes were incubated with primary antibodies for 14 h at 4 °C.

To determine the relative content of molecular markers, the following monoclonal antibodies were used: anti-interferon- γ (Santa Cruz Biotechnology, sc-74104), anti-IL-1 β antibody (Santa Cruz Biotechnology, sc-52012), anti-IL-10 (Santa Cruz Biotechnology, sc-32815), and anti-fibronectin (Santa Cruz Biotechnology, sc-8422), all at a working dilution of 1:1000.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a normalization marker. Relative GAPDH content was assessed using anti-GAPDH primary antibodies (Santa Cruz Biotechnology, sc-365062) at a working dilution of 1:2000.

Following incubation with primary antibodies, membranes were washed three times for 5 min with TBS-T and subsequently incubated with horseradish peroxidase-conjugated mouse IgG secondary antibodies at room temperature for 60 min (Abcam, ab6721) at a working dilution of 1:10000.

After incubation with secondary antibodies, membranes were again washed three times with TBS-T for 5 min, and peroxidase activity was visualized using the enhanced chemiluminescence method. Immediately after washing, membranes were incubated for 1 min in a luminol–coumaric acid–hydrogen peroxide solution and exposed to X-ray film for 30–120 s (Konica Minolta, Japan).

Densitometric analysis of Western blot results was performed using TotalLab TL120 software (USA). The staining intensity values obtained by scanning each individual band were normalized to the intensity of the corresponding GAPDH band from the same sample.

All experimental studies were conducted in accordance with the “General Ethical Principles of Animal Experiments” (Ukraine, 2001), in compliance with the Law of Ukraine “On the Protection of Animals from Cruel Treatment,” as well as the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985).

Statistical Analysis. Statistical analysis was performed using Student’s t-test. Data are presented as follows: M – mean value; m – standard error of the mean (SEM).

3. Results and discussion

3.1. Results

When comparing the biochemical parameters of piglet blood serum before weaning (Table 1), a higher level of albumins by 18.8 % ($P < 0.05$) and urea by 76.1 % ($P < 0.01$) was observed in the serum of experimental piglets.

The blood serum of experimental piglets showed a 35.7% higher glucose concentration ($P < 0.001$) compared with the control group. The obtained data also indicated reduced AST and ALT activities. In addition, the experimental animals demonstrated a lower calcium level, which nevertheless remained within physiological limits. At the same time, no significant differences in inorganic phospho-

rus concentration between control and experimental animals were detected.

Under the conditions of weaning stress, the blood serum of piglets in the experimental group (Table 2) demonstrated a 10.5% higher level of total protein ($P < 0.05$) and a 20.5% higher level of albumins ($P < 0.01$).

Table 1

Biochemical parameters in the blood serum of piglets before weaning under the influence of L-carnitine, day 25 of life ($M \pm m, n = 10$)

Parameters	Control group	Experimental group
Total protein, g/L	55.07 ± 1.47	57.39 ± 2.79
Albumins, g/L	27.89 ± 0.96	33.13 ± 1.74**
Globulins, g/L	27.18 ± 1.43	24.26 ± 3.08
AST, U/L	51.9 ± 5.01	40.0 ± 6.50
ALT, U/L	54.0 ± 2.04	26.4 ± 5.52***
Glucose, mmol/L	4.85 ± 0.10	6.58 ± 0.24***
Total calcium, mmol/L	2.49 ± 0.05	2.14 ± 0.05**
Inorganic phosphorus, mmol/L	2.78 ± 0.26	3.38 ± 0.13
Urea, mmol/L	1.84 ± 0.11	3.24 ± 0.54*
Creatinine, μmol/L	101.51 ± 2.69	108.96 ± 3.21

Notes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with the control group

Table 2

Biochemical parameters in the blood serum of piglets after weaning under the influence of L-carnitine, day 31 of life ($M \pm m, n = 10$)

Parameters	Control group	Experimental group
Total protein, g/L	56.25 ± 0.71	62.18 ± 2.73*
Albumins, g/L	30.08 ± 1.40	36.24 ± 0.99**
Globulins, g/L	26.17 ± 1.28	25.94 ± 3.34
AST, U/L	62.8 ± 5.16	58.1 ± 4.22
ALT, U/L	76.3 ± 4.59	63.9 ± 3.27*
Glucose, mmol/L	3.54 ± 0.33	4.75 ± 0.32**
Total calcium, mmol/L	2.51 ± 0.03	2.47 ± 0.03
Inorganic phosphorus, mmol/L	2.88 ± 0.11	2.37 ± 0.15*
Urea, mmol/L	2.43 ± 0.23	4.26 ± 0.27
Creatinine, μmol/L	118.06 ± 4.27	95.29 ± 6.84*

Notes: * $P < 0.05$; ** $P < 0.01$ compared with the control group

Table 3

Relative content of molecular markers in the blood serum of piglets under the influence of L-carnitine, arbitrary units ($M \pm m, n = 10$)

Group and age of piglets	IFN-γ	IL-1	IL-10	Fibronectin
Control 25 day	4.17 ± 0.53	3.41 ± 0.43	1.27 ± 0.19	1.58 ± 0.23
Control 31 day	7.39 ± 1.08	6.45 ± 0.89	3.12 ± 0.48	4.75 ± 0.53
Experimental 25 day	1.37 ± 0.23***	1.46 ± 0.15***	5.42 ± 0.43***	0.75 ± 0.08**
Experimental 31 day	2.74 ± 0.51***	2.15 ± 0.41***	7.18 ± 0.86***	0.58 ± 0.06***

Notes: *** $P \leq 0.01$; **** $P \leq 0.001$ compared with the control group

Carnitine is also known to reduce oxidative stress in the liver and prevent fatty steatosis (Pradhany et al., 2022). L-carnitine increases hepatic glycogen content and enhances the expression of AMPKα1 and PGC-1α genes in the liver and muscles. It reduces oxidative stress by lowering MDA levels and increasing SOD activity in rats subjected to physical exercise, although it does not affect blood glucose concentration (Posrdee et al., 2025). According to our data, L-

Weaning resulted in increased activity of the studied transamination enzymes in the blood serum of piglets from both groups. At the same time, a significantly higher glucose level (by 34.2 %, $P < 0.01$) was observed in the blood of piglets receiving L-carnitine supplementation.

The results of our study indicate that weaning stress stimulates protein catabolism in piglets, as evidenced by increased urea levels after weaning by 32.1 % in the control group and by 31.5% in the experimental group. At the same time, creatinine levels were lower in the serum of piglets receiving the L-carnitine feed additive (by 19.3 %, $P < 0.05$).

Biochemical changes in blood serum were also accompanied by alterations in molecular inflammatory markers (Table 3).

Feeding piglets with the L-carnitine feed additive resulted in a substantial decrease in the relative content of IFN-γ, IL-1, and fibronectin. At the same time, IL-10 levels were 2.3–4.3 times higher in experimental piglets both at the end of the suckling period and three days after weaning.

3.2. Discussion

The search for effective approaches to improve the functional status of piglets during the suckling and early post-weaning periods remains an urgent issue in pig production (Yefimov et al., 2017; Masiuk et al., 2025). Carnitine appears to be a promising agent for this purpose. In particular, carnitine supplementation in piglets resulted in increased albumin fractions both before and after weaning. Carnitine deficiency may impair albumin biosynthesis in the liver, potentially leading to hypoalbuminemia (Hanai et al., 2020). Therefore, we consider these changes to reflect enhanced synthetic activity of hepatocytes. Similar findings were reported in cows affected by myocardiosis (Vus et al., 2025).

Under the influence of L-carnitine, reduced activities of both AST and ALT were observed in piglet blood serum. However, these changes were more pronounced for ALT, whose activity was significantly lower in piglets both before and after weaning. No significant differences in AST activity were detected, which is also consistent with findings reported in humans (Li et al., 2023). Thus, the supplement may be considered an effective means of preventing hepatodystrophy in weaned piglets, as also indicated by other researchers (Oh et al., 2022).

carnitine maintained higher blood glucose levels in piglets both during the suckling period and after weaning. This is likely associated with greater utilization of fatty acids in energy metabolism. Therefore, supplementation may provide greater metabolic flexibility, defined as the ability to regulate substrate oxidation (primarily glucose and fatty acids) depending on nutrient availability and accessibility (Shahouzehi et al., 2023).

In weaned piglets, liver function deteriorates, and oxidative stress may lead to hepatic necrosis. However, the most pronounced disorders occur in the intestine (Szczepanik et al., 2023). Weaning induces a number of inflammatory reactions in the intestine, which negatively affect the entire organism (Suppi et al., 2025).

Fibronectin is an adhesion molecule widely distributed within the interstitial matrix and contains multiple functional domains. It participates in cell adhesion, migration, proliferation, and differentiation, including in intestinal cells (Adams, 2013; Masiuk et al., 2023). IFN- γ is a cytokine that plays a crucial role in numerous immune responses, including enhancement of antiviral activity, regulation of cellular apoptosis, and proliferation (Ng et al., 2023). At the same time, it is a pro-inflammatory cytokine responsible for pro-inflammatory activation of macrophages (Fu et al., 2023). Other pro-inflammatory markers include a range of cytokines, among which IL-1 is one of the most potent (Dinarello, 2000). In contrast to IFN- γ , IL-1, and other pro-inflammatory cytokines that negatively affect the general condition of the organism and the course of inflammatory reactions, IL-10 acts as an anti-inflammatory cytokine with immunoregulatory functions and suppresses the expression of inflammatory cytokines such as TNF- α , IL-6, and IL-1 (Kumar et al., 2017).

The L-carnitine feed additive reduced IFN- γ and IL-1 levels. This is likely due to a lower level of oxidative stress in the organism of experimental piglets in general, including in the liver. The lower fibronectin level under these conditions may result from reduced release of this molecule into the bloodstream from the intercellular space. In contrast, IL-10 levels were higher in piglets of the experimental group, indicating enhancement of anti-inflammatory mechanisms under the influence of carnitine and possibly stimulation of humoral defense mechanisms. Indeed, IL-10 secretion in pigs is known to occur intensively in B lymphocytes (Milburn et al., 2022).

The positive effects of carnitine supplementation on molecular inflammatory markers in piglets were observed both during the suckling period and after weaning, despite the pronounced pattern of changes characteristic of adaptive processes occurring during weaning.

4. Conclusions

Carnitine supplementation as a feed additive to suckling piglets has demonstrated a beneficial effect on liver functioning, that accompanied by increased albumin synthesis and reduced levels of biochemical markers of liver damaging. It was established that the effect of carnitine on the relative levels of molecular inflammatory markers was characterized by a decrease in the relative content of pro-inflammatory cytokines and an increase in anti-inflammatory cytokines.

Conflict of interest

The authors declare no conflict of interest.

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