



## Features of piglet metabolism under the application of monoglycerides

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**Abstract.** Optimising piglet metabolism in the early postnatal period is an important task in modern animal husbandry. The use of functional additives such as SCFA-M can have a positive effect on protein and energy metabolism, reduce stress, and improve the productivity and health of piglets. The aim of the study was to determine the effect of SCFA-M on protein and energy metabolism and the general physiological condition of animals in the early rearing stage. The study was conducted on 42-day-old piglets divided into control and experimental groups; the animals in the experimental group received SCFA-M as part of their diet. The evaluation was carried out using biochemical methods of blood analysis to determine the levels of major metabolites and the activity of key enzymes; statistical processing was performed with a check of the normality of distribution and the corresponding parametric/non-parametric criteria. The use of SCFA-M significantly modulated protein metabolism: total protein increased by 9.1% ( $P > 0.05$ ), globulins by 31.8% ( $P > 0.001$ ), while the protein coefficient decreased by 16.5% ( $P > 0.001$ ). Aminotransferase activity was lower, indicating less stress on the liver. Blood urea and urea nitrogen levels decreased by 16.5% and 20%, respectively, indicating more efficient nitrogen utilisation. Carbohydrate and mineral metabolism also improved: glucose increased to 6.48 mmol/L (+47.9%;  $P > 0.001$ ), calcium absorption increased by 10%, and phosphorus absorption increased by 28.9% ( $P > 0.001$ ). The results obtained expand understanding of the mechanisms of action of monoglycerides and confirm the feasibility of their use in pig farming; at the same time, they require further research with longer monitoring, performance evaluation and intestinal homeostasis indicators. The practical significance lies

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in the possibility of using SCFA-M as part of support programmes for piglets in the post-weaning period, with a possible reduction in the need for antibacterial agents and an improvement in animal welfare

**Keywords:** SCFA-M; protein metabolism; enzymes; energy metabolism; mineral metabolism

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## Introduction

The post-weaning period in pig farming was accompanied by a high risk of metabolic destabilisation, diarrhoea and growth suppression caused by stress, diet changes and intestinal microbiome restructuring. The need for antibiotic-free strategies to support barrier function and metabolism is driving demand for functional lipid supplements. During this period, it is particularly important to maintain the stability of the intestinal environment and control opportunistic microflora, as these factors determine the animal's ability to effectively absorb nutrients. In addition, the increased susceptibility of young animals to infectious agents, combined with restrictions on the use of antibiotics, stimulates the search for new approaches to feeding.

C.S. White *et al.* (2024) noted that short- and medium-chain fatty acid monoglycerides (SCFA/MCFA) combine antimicrobial and metabolic-modulating properties. They emphasised that, unlike free acids, monoglycerides are more stable in the intestinal lumen, are better tolerated and act over a wider pH range; they are capable of disrupting the lipid membranes of pathogens while serving as an energy substrate for enterocytes and commensal bacteria. The authors also emphasised that these properties create the conditions for strengthening the barrier function of the intestine and improving the absorption of nutrients. Z. Cui *et al.* (2020) found that under standard housing conditions, SCFA/MCFA monoglycerides had no significant effect on the average performance of weaned piglets, but there were positive signals regarding intestinal health and metabolic markers. The researchers also emphasised that the effects may be more pronounced under conditions of stress or infection, indicating the potential of such supplements in specific scenarios.

Researchers L. Li *et al.* (2022) showed that in multifactorial feeding schemes, the effects of monoglycerides on growth and feed conversion were variable but were accompanied by improvements in selected indicators of intestinal homeostasis and inflammatory response. They added that monoglycerides can modulate metabolic processes and immune responses even in the absence of a significant impact on overall production performance. L.L. Thomas *et al.* (2020) determined that adding a mixture of monoglycerides to piglets reduced the gain/feed ratio in ETEC-infected pigs and reduced the severity of intestinal disorders while maintaining the local immune response. The authors noted that these properties make monoglycerides a promising tool for reducing antibiotic use in intensive farming programmes.

A. Kumar *et al.* (2022) showed that in a model of necrotic enteritis in poultry, monoglyceride mixtures demonstrated barrier-strengthening effects but did not always translate into improved performance. They further emphasised that the effectiveness of such additives is highly dependent on the composition of the mixture and the specific conditions of the disease, which is important for the adaptation of feeding programmes. L. Li *et al.* (2023) investigated the effect of  $\alpha$ -GML in sows during late gestation and lactation and found an increase in piglet weight at weaning and a change in nutrient digestibility, indicating an indirect effect on the early postnatal period of piglets. The authors emphasised that maintaining the health of sows can have long-term consequences for the productivity and sustainability of offspring.

Researchers M. Yang *et al.* (2024) concluded that the effectiveness of monoglycerides is largely determined by the dose, diet matrix, age and physiological condition of the animals, as well as the

epizootic situation. They added that combining monoglycerides with other functional ingredients can significantly enhance their positive effects on gut health and immune response. S. Park *et al.* (2024) emphasised that the results of monoglyceride use depend on the dose, diet composition and housing conditions, adding that combinations with other functional components can significantly enhance the positive effects on performance and gut health. The authors also noted that such strategies may be useful for optimising antibiotic-free programmes in pig and poultry farming.

The aim of the study was to determine the corrective effect of adding SCFA-M to the diet of 42-day-old piglets on nitrogen metabolism, markers of hepatocellular load, and indicators of carbohydrate and mineral homeostasis.

## Material and Methods

The study was conducted in 2022-2023 at a commercial pig farm located in the Dnipropetrovsk district, Dnipropetrovsk region. For the experiment, 100 three-breed hybrid piglets of DanBred genetics aged 42 days were selected; animals “analogous” in age and live weight after stratification by weight were randomly divided into two groups: control and experimental (50 heads in each). The piglets were kept in group pens in accordance with the stocking density standards established in the EU: 0.20 m<sup>2</sup>/head for a weight range of 10-20 kg and 0.30 m<sup>2</sup>/head for 20-30 kg; actually provided with 0.22-0.24 and 0.33-0.36 m<sup>2</sup>/head, respectively. The piglets had free access to water and were fed a complete feed ration without restrictions according to the same schemes in both groups, with the exception of the addition of an experimental supplement in the experimental group.

The microclimate was maintained within stable limits: temperature 22-26 °C, relative humidity 55-65 %, ventilation – mechanical; a fixed photoperiod of 16L:8D was maintained. Before the start of the experiment, the animals were clinically examined; only clinically healthy piglets were allowed to participate. Weaning from sows

was carried out on the 26th day of life; the experiment began at 42 days of age. The experimental group aged 42-77 days received a diet containing a composition of short- and medium-chain fatty acid monoglycerides (SCFA-M, C3-C12), manufactured to order by PARTNERAGRO 2016 LLC at the production facilities of NETAG B.V. (The Netherlands), at a dose of 1.0 kg/t of compound feed. According to the quality certificate, the ratio of glyceride fractions (mass %) was as follows: monoglycerides – 31%, diglycerides – 18%, triglycerides – 1%, free glycerol – 14%.

The glycerol-esterified acids included: propionic (C3), butyric (C4), caprylic (C8), capric (C10) and lauric (C12) acids. The addition was carried out in a production mixer with technological control of batch homogeneity. The control group received an identical basic diet without SCFA-M. To assess the metabolic status of piglets in both groups, biochemical studies were conducted, for which whole blood without anticoagulant was collected from 10 piglets from the experimental and control groups at 42, 56 and 77 days of age for further serum extraction. In blood serum samples, the Miura-200 automatic biochemical analyser (Italy) was used with ready-made reagent kits manufactured by Spinreakt (Spain), Dialab (Austria), Cormay (Poland) and HTI (USA) to determine the content of total protein (with biuret reagent), albumin (with bromocresol green), urea (enzymatically by Berthelot's reaction), creatinine (by the rate of formation of the creatinine-picrate complex in the Jaffe reaction), glucose (by the glucose oxidase method), total calcium (with arsenazo III) and inorganic phosphorus (with ammonium molybdate).

The activity of transaminase enzymes (ALT and AST) was determined kinetically using a set of Spinreakt reagents (Spain), and alkaline phosphatase (ALP) activity was determined by the rate of 4-nitrophenol formation (Cormay, Poland). Laboratory studies were conducted at the Biosafety-Center Research Centre for Biosafety and Environmental Control of Agricultural Resources at Dnipro State Agrarian and Economic

University. Data processing was performed in GraphPad Prism 9. The normality of distribution was evaluated using the Shapiro-Wilk test separately for each group and time point; the homogeneity of variances was evaluated using the Brown-Forsyth/Levene test. The main approach for intergroup comparisons was two-factor ANOVA (factors: "group" and "age") with post-hoc Tukey. For violations of the assumptions of normality/homogeneity, non-parametric analogues were used: Kruskal-Wallis with post-hoc Dunn or paired Mann-Whitney comparisons within individual time points. Multiple comparisons were corrected using the Benjamini-Hochberg procedure (FDR 5%). Data are presented as  $\bar{X} \pm SD$ ; statistical significance was accepted at  $P < 0.05$ . The study was conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council (2010) and ARRIVE 2.0 guidelines (Percie du Sert *et al.*, 2020).

## Results and Discussion

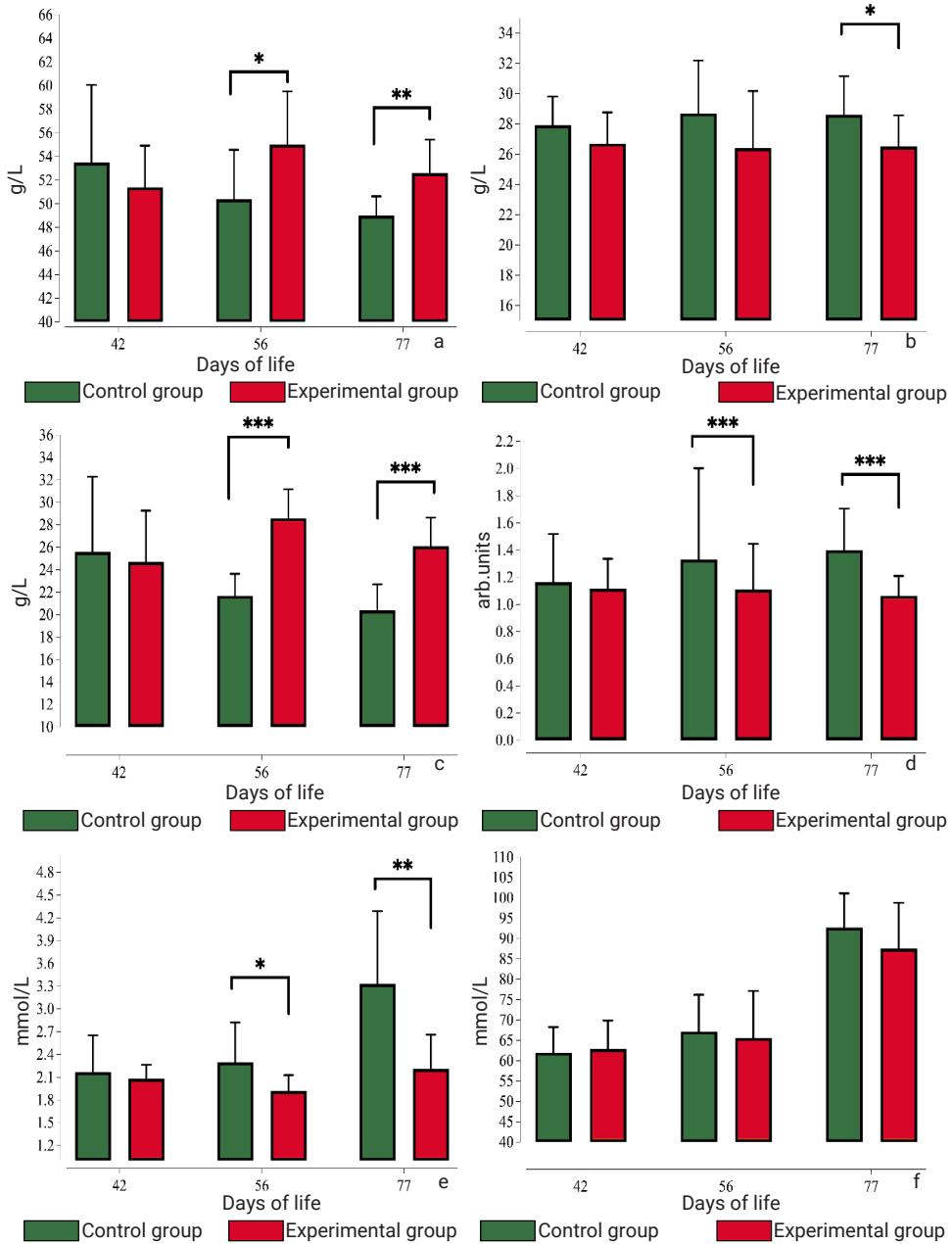
The study analysed the effect of SCFA-M on protein metabolism indicators in piglets at different stages of growth. The levels of total protein, albumin, globulin, protein coefficient, as well as urea and creatinine concentrations in blood serum were evaluated (Fig. 1). It was found that on the 42nd day of the study, the total protein level in the blood serum of pigs in the control group, which were not fed SCFA-M, was 53.5 g/L (SD = 6.57), while in pigs in the experimental group, which received SCFA-M, it was 51.4 g/L (SD = 3.53). The albumin content in the blood serum of piglets in the control group was 27.9 g/L (SD = 1.9), while in the experimental group it was 26.7 g/L (SD = 2.1). The globulin level in the blood serum of the control group piglets was 25.6 g/L (SD = 6.7), while in the experimental group it was 24.7 g/L (SD = 4.6). The protein coefficient was almost the same in both groups (1.12-1.16 conventional units), and the urea level was 2.17 mmol/l in the control group versus 2.08 mmol/l in the experimental group. Thus, at the initial stage of the study, the difference between the groups was insignificant.

On day 56, the total protein level in the blood serum of the control group piglets decreased to 50.4 g/L (SD = 4.17), while in the experimental group receiving SCFA-M, it increased to 55.0 g/L (SD = 4.52), which was 9.1% higher ( $P < 0.05$ ). The albumin content in the blood serum of piglets in the control group increased to 28.7 g/L (SD = 3.5), while in the experimental group it remained lower – 26.4 g/L (SD = 3.8), i.e., 8% lower. The most pronounced changes were observed in the globulin content: in the control group, its amount decreased to 21.7 g/L (SD = 1.95), while in the experimental group, it increased to 28.6 g/L (SD = 2.59), a difference of 31.8% ( $P < 0.001$ ). The protein coefficient in the blood serum of piglets in the control group rose to 1.33 conventional units (SD = 0.67), while in the experimental group it decreased to 1.11 conventional units (SD = 0.34), reflecting the predominance of globulin fractions under the action of SCFA-M ( $P < 0.001$ ). The urea level in the control group was 2.3 mmol/L, while in the experimental group it decreased to 1.92 mmol/L (-16.5%;  $P < 0.05$ ).

By day 77, the total protein level in the blood serum of the control group piglets decreased to 49.0 g/L (SD = 1.63), while in the experimental group it remained high at 52.6 g/L (SD = 2.84), which was 7.3% higher ( $P < 0.01$ ). The albumin content in the control group was 28.6 g/L (SD = 2.5), and in the experimental group it was 26.5 g/L (SD = 2.1), which is 7.3% lower ( $P < 0.05$ ). In contrast, the globulin content in the control group decreased to 20.4 g/L (SD = 2.31), while in the experimental group it remained significantly higher – 26.1 g/L (SD = 2.56), with a difference of 27.9% ( $P < 0.001$ ). The protein coefficient in the blood serum of piglets in the control group rose to 1.40 conventional units (SD = 0.31), while in the experimental group it remained low – 1.06 conventional units (SD = 0.15), a difference of 24.3% ( $P < 0.001$ ). At the same time, the urea level in the control group increased to 3.33 mmol/L (SD = 0.96), while in the experimental group it increased only to 2.21 mmol/L (SD = 0.45), which is 33.6% lower ( $P < 0.01$ ). Creatinine levels increased in both groups, but remained lower in

the experimental group (87.5 mg/100 ml vs. 92.7 mg/100 ml). The data obtained indicate that feeding SCFA-M contributed to maintaining higher

levels of total protein and globulins, reducing the protein coefficient, and limiting the growth of nitrogenous metabolites in the blood of piglets.



**Figure 1.** Indices of protein metabolism in piglet blood serum under the influence of SCFA-M

**Note:** a – total protein content, g/L; b – albumins, g/L; c – globulins, g/L; d – protein coefficient, arb. units; e – urea, mmol/L; f – creatinine, mmol/L; M ± SD; n = 10; \* – P < 0.05; \*\* – P < 0.01; \*\*\* – P < 0.001 – compared with the control group

**Source:** developed by the authors

The changes in the protein coefficient recorded in the study are consistent with the data of L.E. Duvall *et al.* (2023), who noted that such changes may indicate a shift in the balance between protein synthesis and immune response. The decrease in urea and urea nitrogen levels in the blood of piglets in the current study under the influence of short- and medium-chain fatty acid monoglycerides (by 16.5% and 20%, respectively,  $P < 0.05$ ) indicates an improvement in protein metabolism and a decrease in protein catabolism. These observations partly contrast with the results of other scientific studies. In particular, K.A. Barbosa *et al.* (2023) observed an increase in urea levels with monoglycerides/tributyryl ( $P < 0.05$ ). Similarly, researchers J.A. Santos-Marcos *et al.* (2019) noted an increase in urea concentration ( $P < 0.05$ ) in the plasma of starter piglets fed monoglycerides. The discrepancies in the results can be explained by various factors. The study by K.A. Barbosa *et al.* (2023) included tributyrin, which is a source of butyrate and is actively metabolized in intestinal cells, which may affect overall protein metabolism. At the same time, differences in the growth phase of piglets, diet matrix, and epizootic background could modify the metabolic response of animals to the supplement.

The second direction of the study was to investigate changes in enzymatic activity and mineral metabolism in piglets under the influence of SCFA-M. The study examined the dynamics of ALT, AST, alkaline phosphatase, as well as serum glucose, calcium, and phosphorus levels (Fig. 2). On day 42, ALT activity in the blood serum of pigs in the control group was 87.2 U/L (SD = 17.65), while in animals receiving SCFA-M it was 81.7 U/L (SD = 13.84). A similar trend was observed for AST: 87.2 U/L (SD = 15.72) in the control group versus 81.6 U/L (SD = 16.55) in the experimental group. At the same time, alkaline phosphatase activity was significantly higher in the experimental group of piglets – 358.68 U/L (SD = 53.41) versus 254.19 U/L (SD = 61.15) in the control group (+41.1%;  $P < 0.001$ ). Glucose levels also tended to increase with SCFA-M – 5.38 mmol/L (SD = 0.63) versus 4.72 mmol/L (SD = 0.94) in the control group,

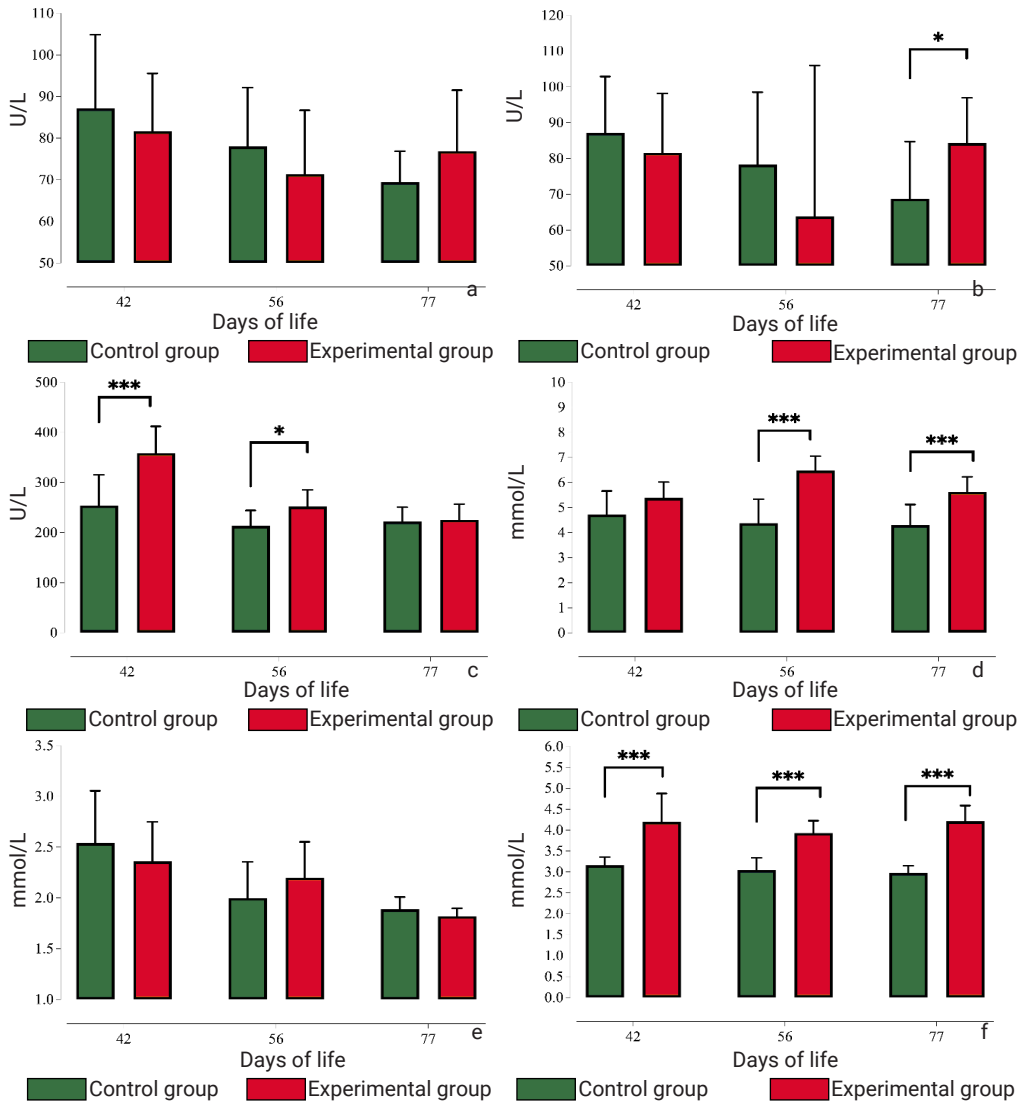
while calcium concentration was slightly lower – 2.36 mmol/L (SD = 0.39) versus 2.54 mmol/L (SD = 0.51). At the same time, the phosphorus level in the experimental group exceeded the control by 32.9% – 4.2 mmol/L (SD = 0.67) versus 3.16 mmol/L (SD = 0.20;  $P < 0.001$ ).

By day 56, enzyme activity in the blood serum of piglets in both groups had decreased, but with varying intensity. Thus, ALT in the control group was 78.0 U/L (SD = 14.17), while in the experimental group it was 71.4 U/L (SD = 15.33), which is 8.5% lower. AST decreased to 78.3 U/L (SD = 20.20) in the control group and to 63.8 U/L (SD = 42.16) in the experimental group, a difference of 18.5%. Alkaline phosphatase activity decreased to 213.23 U/L (SD = 31.19) in the control group and to 251.76 U/L (SD = 33.71) in the experimental group, i.e. by 18.1% higher (the difference is not significant). A decrease in the activity of these enzymes in the blood serum of piglets under the action of SCFA-M until the 55th day of life may indicate a reduction in the load on the liver and a decrease in the level of hepatocellular damage. The glucose concentration in the control group decreased to 4.38 mmol/L (SD = 0.96), while in the experimental group it increased sharply to 6.48 mmol/L (SD = 0.57), which was 47.9% higher ( $P < 0.001$ ). Calcium levels in the control group decreased to 2.00 mmol/L (SD = 0.36), while in the experimental group they increased to 2.20 mmol/L (SD = 0.35), exceeding the control by 10%. Phosphorus levels were 3.05 mmol/L (SD = 0.29) in the control group and 3.93 mmol/L (SD = 0.29) in the experimental group, which was 28.9% higher ( $P < 0.001$ ).

On day 77, stabilisation or even opposite dynamics of individual indicators were noted. In the blood serum of piglets in the control group, ALT decreased to 69.5 U/L (SD = 7.40), while in the experimental group it increased to 76.9 U/L (SD = 14.63), which was 10.6% higher. A similar trend was observed for AST: 68.8 U/L (SD = 15.89) in the control group versus 84.3 U/L (SD = 12.65) in the experimental group (+22.5%;  $P < 0.05$ ). Alkaline phosphatase activity remained virtually unchanged – 222.69 U/L (SD = 28.11) in the control group and

225.64 U/L (SD = 31.32) in the experimental group. The glucose concentration in the control group was low – 4.31 mmol/L (SD = 0.81), while in the experimental group it was 5.62 mmol/L (SD = 0.60), which is 30.4% higher ( $P < 0.05$ ), indicating activation of gluconeogenesis and improved energy metabolism. This may provide animals with the additional energy needed for growth. The

calcium level in the control group decreased to 1.89 mmol/L (SD = 0.12), and in the experimental group to 1.82 mmol/L (SD = 0.08). The most pronounced change was in phosphorus: 2.98 mmol/L (SD = 0.17) in the control group versus 4.21 mmol/L (SD = 0.38) in the experimental group, which is 41.3% higher ( $P < 0.001$ ), indicating a prolonged effect of SCFA-M on mineral metabolism in piglets.



**Figure 2.** Biochemical parameters of piglet blood serum under the influence of SCFA-M

**Note:** a – ALT activity, U/L; b – AST activity, U/L; c – ALP activity, U/L; d – glucose content, mmol/L; e – calcium content, mmol/L; f – phosphorus content, mmol/L; \* –  $P < 0.05$ ; \*\* –  $P < 0.01$ ; \*\*\* –  $P < 0.001$  – compared to the control group

**Source:** developed by the authors

Researchers J.L. Genova *et al.* (2020) used alkaline phosphatase (ALP) to study intestinal disorders such as intestinal dysbiosis. In the current study, no effect of SCFA-M supplementation on ALP activity in the blood serum of piglets was found, which indirectly indicates a normal balance of the microbiome in the digestive tract. This fact is particularly important because ALP is often used as a sensitive biomarker of intestinal mucosal damage; its stable values confirm the absence of pathological changes and allow to conclude that the barrier function of the intestine is preserved even during the stressful post-weaning period. Thus, it can be assumed that SCFA-M creates favourable conditions for maintaining the homeostasis of the intestinal ecosystem. Evaluating the effect of combined monoglyceride supplements on blood metabolites, a promising effect on health, metabolism, and nutrient absorption has been reported. Such effects were observed by P. Nowak *et al.* (2019) through blood analysis, which revealed an increase in the concentration of total protein, albumin, glucose and triglycerides in combination with a low concentration of urea. The results of the current study partially correspond with these data: in the experimental group of piglets receiving SCFA-M, an increase in total protein and glucose in the blood was also observed, indicating an improvement in nitrogen balance and activation of energy metabolism. At the same time, lower albumin levels combined with higher globulin fractions may indicate

a shift in metabolism towards strengthening the immune response, which is an adaptive reaction of the body during weaning. Thus, SCFA-M provides a more comprehensive immunometabolic effect than individual classic supplements.

Improvement of intestinal trophic status in pigs, antibacterial activity against various bacteria when monoglycerides are administered. Monoglycerides are accompanied by modulation of blood metabolites (e.g., glucose and urea) in weaned piglets. SCFA-M can improve the absorption of minerals such as calcium and phosphorus, which is reflected in an increase in their blood levels. This is important for bone growth and meeting the structural needs of the body. Higher blood phosphorus levels were observed in the experimental group of piglets throughout the experiment ( $P < 0.001$ ).

To assess the relationships between protein, nitrogen, enzyme, and mineral-carbohydrate metabolism circuits, a correlation analysis of blood biochemical parameters was performed in 56-day-old piglets in the control group and under the influence of SCFA-M (Table 1). It was found that in 56-day-old piglets of the control group, there was a close positive correlation between total protein content and albumin content in blood serum ( $r = 0.89$ ;  $P < 0.001$ ) and between the protein coefficient and albumin content ( $r = 0.77$ ;  $P < 0.01$ ), while urea content directly correlated with calcium content ( $r = 0.76$ ;  $P < 0.05$ ) and inversely correlated with the protein coefficient ( $r = -0.65$ ;  $P < 0.05$ ).

**Table 1.** Correlation matrix of biochemical blood parameters in 56-day-old piglets exposed to SCFA-M

Indicators	Total protein	Albumin	Globulin	Urea	Creatinine	AST	ALT	ALP	Glucose	Ca
Control group										
Albumin	0.89	-	-	-	-	-	-	-	-	-
Globulin	0.55	0.10	-	-	-	-	-	-	-	-
Urea	-0.15	-0.43	0.45	-	-	-	-	-	-	-
Creatinine	0.23	0.16	0.21	0.53	-	-	-	-	-	-
AST	-0.21	-0.37	0.22	0.27	-0.26	-	-	-	-	-
ALT	-0.24	-0.16	-0.22	-0.48	-0.20	-0.22	-	-	-	-
ALP	0.04	0.02	0.05	-0.58	-0.55	0.03	0.56	-	-	-
Glucose	0.49	0.60	-0.04	-0.53	0.05	-0.67	0.25	0.45	-	-
Ca	-0.30	-0.40	0.08	0.76	0.48	0.48	-0.42	-0.41	-0.45	-
P	-0.1	-0.01	-0.21	-0.58	-0.22	0.00	0.60	0.23	-0.13	-0.59

Table 1. Continued

Indicators	Total protein	Albumin	Globulin	Urea	Creatinine	AST	ALT	ALP	Glucose	Ca
Experimental group										
Albumin	0.82	-	-	-	-	-	-	-	-	-
Globulin	0.55	-0.03	-	-	-	-	-	-	-	-
Urea	0.47	0.59	-0.04	-	-	-	-	-	-	-
Creatinine	0.27	0.54	-0.33	0.46	-	-	-	-	-	-
AST	0.17	0.03	0.26	0.29	0.33	-	-	-	-	-
ALT	0.05	-0.10	0.23	0.14	0.00	0.66	-	-	-	-
ALP	-0.06	0.32	-0.57	0.35	0.53	0.52	0.35	-	-	-
Glucose	-0.09	0.16	-0.40	0.07	0.01	-0.70	-0.1	-0.17	-	-
Ca	0.24	0.14	0.22	-0.42	-0.28	-0.4	-0.21	-0.35	0.11	-
P	0.38	0.61	-0.23	0.53	0.52	0.33	-0.19	0.45	-0.26	0.01

**Note:** values are reliable:  $P < 0.05$  –  $r = 0.63$ - $0.76$ ;  $P < 0.01$  –  $r = 0.77$ - $0.86$ ;  $P < 0.001$  –  $r = \geq 0.87$ ;  $n = 10$

**Source:** compiled by the authors

A negative correlation between glucose content and AST activity was established ( $r = -0.67$ ;  $P < 0.05$ ). Under the action of SCFA-M, a strong correlation between total protein content and albumin was maintained ( $r = 0.82$ ;  $P < 0.01$ ), and the negative correlation between glucose and AST remained ( $r = -0.70$ ;  $P < 0.05$ ), while the relationships between urea and Ca content and between urea and the protein coefficient, which were characteristic of the control group, became reversed and unreliable ( $P > 0.05$ ). In addition, the contribution of albumin to the protein coefficient value increased ( $r = 0.86$ ;  $P < 0.01$ ), and a positive association of creatinine with this protein coefficient appeared ( $r = 0.63$ ;  $P < 0.05$ ). Taken together, this indicates a strengthening of “albumin determinism” of the protein coefficient indicator and a weakening/decoupling of nitrogen-mineral associations, which is consistent with a possible redistribution of nitrogen from catabolism to deposition in tissues and a less pronounced enzymatic load on the liver.

Correlation analysis of blood biochemical parameters in 77-day-old piglets in the control group showed the preservation of “structural” protein bonds (Table 2): the protein coefficient indicator closely correlated with albumin content ( $r = 0.90$ ,  $P < 0.001$ ) and inversely with globulin

content ( $r = -0.96$ ,  $P < 0.001$ ), while albumin and globulin were negatively correlated ( $r = -0.78$ ,  $P < 0.01$ ); there was also a positive correlation between the liver enzymes ALT and AST ( $r = 0.63$ ,  $P < 0.05$ ) and positive associations between phosphorus content and albumin ( $r = 0.81$ ,  $P < 0.01$ ) and protein coefficient ( $r = 0.71$ ,  $P < 0.05$ ). At the same time, in the experimental group (SCFA-M), the protein profile shifted towards the globulin fraction: total protein directly correlated with globulin content ( $r = 0.71$ ,  $P < 0.05$ ), while maintaining the “structural” links between the protein coefficient and albumins ( $r = 0.69$ ,  $P < 0.05$ ) and globulins ( $r = -0.88$ ,  $P < 0.001$ ). Additionally, there were significant relationships between alkaline phosphatase activity and total protein ( $r = 0.67$ ,  $P < 0.05$ ) and globulin content ( $r = 0.71$ ,  $P < 0.05$ ), which may reflect enhanced tissue remodelling/intestinal growth component. The negative correlation between glucose content and AST activity remained ( $r = -0.68$ ,  $P < 0.05$ ), while the ALT and AST correlation characteristic of the control disappeared in the experiment ( $r = 0.09$ ;  $P > 0.05$ ), as did the relationship between phosphorus and albumin and the protein coefficient. Taken together, this indicates that on day 77, SCFA-M is associated with a predominance of globulin-dependent contribution to total protein and the appearance

of ALP associations, while decoupling the phosphate circuit from the protein ratio and weakening the relationship between transaminases; This

can be interpreted as signs of a redistribution of protein/mineral metabolism without increasing the hepatocellular load.

**Table 2.** Correlation matrix of biochemical blood parameters in 77-day-old piglets exposed to SCFA-M

Indicators	Total protein	Albumin	Globulin	Urea	Creatinine	AST	ALT	ALP	Glucose	Ca
Control group										
Albumin	0.45	-	-	-	-	-	-	-	-	-
Globulin	0.21	-0.78	-	-	-	-	-	-	-	-
Urea	0.08	-0.14	0.21	-	-	-	-	-	-	-
Creatinine	-0.25	-0.07	-0.10	0.43	-	-	-	-	-	-
AST	0.09	0.2	-0.15	-0.22	-0.45	-	-	-	-	-
ALT	0.46	0.33	-0.04	0.35	-0.03	0.63	-	-	-	-
ALP	0.00	0.15	-0.17	-0.25	0.12	-0.32	-0.21	-	-	-
Glucose	0.04	0.3	-0.30	-0.51	0.09	0.16	-0.14	-0.07	-	-
Ca	0.00	-0.45	0.50	0.03	-0.53	-0.32	-0.48	-0.14	-0.51	-
P	0.40	0.81	-0.60	0.25	0.07	-0.09	0.28	0.29	0.03	-0.34
Experimental group										
Albumin	0.49	-	-	-	-	-	-	-	-	-
Globulin	0.71	-0.26	-	-	-	-	-	-	-	-
Urea	0.31	0.02	0.33	-	-	-	-	-	-	-
Creatinine	0.61	0.15	0.55	0.05	-	-	-	-	-	-
AST	-0.14	-0.09	-0.09	0.28	0.43	-	-	-	-	-
ALT	-0.33	-0.21	-0.19	-0.02	0.00	0.09	-	-	-	-
ALP	0.67	0.04	0.71	0.44	0.37	0.06	0.00	-	-	-
Glucose	-0.03	0.27	-0.25	-0.34	-0.54	-0.68	-0.4	-0.28	-	-
Ca	-0.01	0.14	-0.12	0.40	-0.57	-0.18	-0.27	0.15	0.57	-
P	0.04	-0.29	0.28	-0.04	0.26	0.17	-0.17	0.09	0.25	0.26

**Note:** values are reliable:  $P < 0.05 - r = 0.63-0.76$ ;  $P < 0.01 - r = 0.77-0.86$ ;  $P < 0.001 - r = \geq 0.87$ ;  $n = 10$

**Source:** compiled by the authors

Thus, the introduction of SCFA-M into the diet of piglets was accompanied by controlled modulation of protein, nitrogen and energy metabolism. In 56-day-old piglets, an increase in the protein fraction due to globulins and a decrease in the protein coefficient were noted against the background of a less pronounced enzymatic load on the liver and signs of lower protein catabolism. Enzyme markers of intestinal pathology did not change, which is consistent with the absence of enteropathies. At the same time, an improvement in energy status and higher mineral supply were observed. Until the end of the study, the trend persisted with a more pronounced "globulin"

profile. An increase in AST activity without a synchronous increase in ALT is considered to be extrahepatic adaptation associated with muscle tissue growth rather than liver damage. These effects are consistent with the literature data on the barrier, immunomodulatory and metabolic effects of SCFA/MCFA monoglycerides. A comparison of the results obtained with the data of C. Neath *et al.* (2022) allows to trace both common features and differences. In their *in vitro* study, the authors found that pig pathogens were highly sensitive to certain active ingredients in feed additives, particularly monoglycerides, which determines their potential as an alternative to antibiotics in

the fight against pathogens such as *Escherichia coli* and *Clostridium perfringens*. The current *in vivo* study demonstrated a broader spectrum of action of SCFA-M: in addition to the possible antibacterial effect confirmed by stable ALP values, there was an improvement in protein metabolism (increase in total protein and globulins), a decrease in urea levels, activation of the energy circuit (increase in glucose) and optimisation of mineral supply (higher phosphorus levels). This indicates that under physiological conditions, monoglycerides are capable of exerting not only the direct antimicrobial effect noted by C. Neath *et al.* (2022), but also indirect immunomodulatory and metabolic effects. Thus, the results of the current study extend the conclusions of *in vitro* studies, emphasising the practical significance of SCFA-M as a functional feed additive in the post-weaning period.

Additionally, the results of J. Lan *et al.* (2021), obtained on broilers, confirm the immunomodulatory potential of  $\alpha$ -glycerol monolaurate, which manifested itself in increased immune function and positive changes in the intestinal microbiota. Despite the difference in the object of study, the similarity of the effects indicates a universal mechanism of action of monoglycerides associated with the modulation of the immune response and maintenance of intestinal homeostasis. In the current experiment, similar effects were observed through an increase in the globulin fraction in the protein profile of piglets and a stable association between protein metabolism and enzyme markers. This suggests that SCFA-M may combine the properties described in both pig and bird studies, providing a multi-component effect on metabolic and immune status.

Analysing the results in the context of the review by D.H. Nguyen *et al.* (2020), it should be noted that these authors emphasised the multifaceted effect of organic acids and monoglycerides, which simultaneously act as antimicrobial agents, regulators of the intestinal environment and modifiers of metabolism. Such additives have been shown to maintain the stability of the microbiota, improve the digestibility of nutrients,

and increase energy efficiency. In the current study, these conclusions are confirmed by practical results: the stability of enzyme markers, increased glucose, and improved mineral absorption are consistent with the concept of the systemic action of SCFA/MCFA. Thus, the data from D.H. Nguyen *et al.* (2020) provide a theoretical basis for explaining the mechanisms that have been confirmed experimentally.

There are no publicly available studies on the effect of SCFA-M supplementation on protein profile, nitrogenous metabolites, enzymes, and mineral-carbohydrate balance in piglets. Most similar studies focus on productivity, intestinal morphology, immunomarkers, and selective serum indicators, which allows to reasonably position this study as unique in terms of the range of indicators. A summary of the data obtained shows that a significant increase in total protein and globulins in the blood serum of piglets receiving SCFA-M was accompanied by a decrease in the protein coefficient and urea concentration. This dynamic indicates a reorientation of protein metabolism towards activation of the immune response and a decrease in the intensity of protein catabolism, which is consistent with the ideas of Y. Saco & A. Bassols (2023) about the role of protein fractions in the formation of immune reactivity. Thus, the results confirm the immunomodulatory effect of SCFA-M and emphasise its difference from mixtures with tributyrin, for which an increase in urea levels has been described in the literature, which may indicate other mechanisms of action and a different direction of nitrogen metabolism.

At the midpoint of the study, a decrease in ALT and AST activity was observed in the SCFA-M group, corresponding to a decrease in hepatocellular load (Egeli *et al.*, 1998). Similar effects with normalisation of liver biochemical markers and restoration of intestinal barrier function are described by Q. Zhang *et al.* (2022) when using monolaurin in PEDV-infected piglets. At the final stage of the experiment, an increase in AST was recorded without a parallel increase in ALT, which is interpreted as extrahepatic adaptation

associated with muscle mass growth, similar to the reports in the works of C.S. White *et al.* (2024) on the maintenance of intestinal integrity and immune responses. At the same time, glucose concentration in the SCFA-M group increased significantly, indicating activation of energy metabolism; this is consistent with reviews of the functional properties of medium-chain fatty acid monoglycerides. The absence of significant differences in ALP levels indicates the absence of enteropathies, which is consistent with the findings of J.L. Genova *et al.* (2020), where ALP is used as an indicator of intestinal disorders.

Throughout the experiment, significantly higher levels of inorganic phosphorus were found in the blood of piglets receiving SCFA-M, while calcium levels changed less markedly. Given the impact of water quality on homeostasis (Ahmadi *et al.*, 2024), it is advisable to interpret changes in glucose and mineral profile indicators taking this potential confounder into account. This dynamic is consistent with the mechanistic models of bone tissue growth and mineral requirements in pigs proposed by M. Lautrou *et al.* (2020), which emphasise the key role of the optimal phosphorus-calcium ratio for mineralisation processes. An increase in phosphorus levels against the background of SCFA-M feeding may indicate its more efficient absorption and a potentially positive effect on bone tissue formation.

Thus, the results of the experiment show that SCFA-M is an effective tool for supporting metabolic processes, including mineral metabolism, and is of direct importance for the growth and structural development of young animals. The identified changes in the protein profile, decreased protein catabolism, and improved energy and phosphorus supply reflect the multifactorial effect of the supplement on the physiological state of animals. The data obtained are consistent with literature reports on the role of monoglycerides in maintaining homeostasis, but at the same time expand on them through comprehensive biochemical analysis, creating a scientific basis for the introduction of SCFA-M into antibiotic-free pig farming technologies.

## Conclusions

The use of SCFA-M in piglet diets has a positive effect on protein metabolism, as evidenced by an increase in total protein and globulin levels in the blood, as well as a decrease in the protein coefficient, indicating an improvement in the balance between protein synthesis and immune response. In particular, the administration of SCFA-M in the 42-77-day period was accompanied by an increase in total protein content by 9.1% ( $P < 0.05$ ) and globulins by 31.8% ( $P < 0.001$ ) on day 56 with a decrease in the protein coefficient by 16.5% ( $P < 0.001$ ), a decrease in urea content by 16.5% ( $P < 0.05$ ) and urea nitrogen by 20%, an increase in glucose by 47.9% ( $P < 0.001$ ), an increase in calcium content by 10% and phosphorus by 28.9% ( $P < 0.001$ ), as well as a decrease in ALT activity by 8.5% and AST by 18.5% in the blood serum of animals. On day 77, the effects persisted with a "globulin" profile: total protein was 7.3% higher ( $P < 0.01$ ), globulin content was 27.9% higher ( $P < 0.001$ ), albumin was 7.3% lower ( $P < 0.05$ ), urea was 33.6% lower ( $P < 0.01$ ), glucose was 30.4% higher ( $P < 0.05$ ), while AST activity increased by 22.5% ( $P < 0.05$ ) without a parallel increase in ALT.

Correlation analysis confirmed the strengthening of "albumin determinism" of the protein coefficient indicator on day 56 ( $r = 0.86$ ;  $P < 0.01$ ) and the predominance of globulin contribution to total protein on day 77 of the piglet's life ( $r = 0.71$ ;  $P < 0.05$ ) with a stable negative association of glucose with AST activity at both points ( $P < 0.05$ ), which in general indicates a redistribution of protein and nitrogen metabolism towards a decrease in catabolism and maintenance of energy and mineral status without signs of increased hepatocellular load. The activation of energy metabolism is manifested by an increase in glucose levels, which provides animals with the additional energy necessary for growth. SCFA-M contributes to the improvement of mineral absorption, in particular calcium and phosphorus, which has a positive effect on bone tissue growth and the overall development of piglets. The established effect of

SCFA-M on metabolic parameters confirms their effectiveness as a functional feed additive aimed at improving animal productivity and health. Further research should focus on studying the long-term effects of SCFA-M, their combination with other functional additives, as well as the mechanisms of immune response and digestive tract microbiome regulation.

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## Conflict of Interest

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## Особливості метаболізму поросят за умов застосування моногліцеридів

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**Анотація.** Оптимізація метаболізму поросят у ранньому постнатальному періоді є важливим завданням сучасного тваринництва. Використання функціональних добавок, таких як SCFA-M, здатне позитивно впливати на білковий та енергетичний обмін, сприяти зниженню стресу, поліпшенню продуктивності та здоров'я поросят. Мета дослідження полягала у визначенні впливу SCFA-M на білковий і енергетичний метаболізм та загальний фізіологічний стан тварин на етапі раннього вирощування. Дослідження проводили на поросятах віком 42 дні, розподілених на контрольну та дослідну групи; тварини дослідної групи отримували SCFA-M у складі раціону. Оцінювання здійснювали біохімічними методами аналізу крові з визначенням рівнів основних метаболітів і активності ключових ензимів; статистичну обробку виконували з перевіркою нормальності розподілу та відповідними параметричними/непараметричними критеріями. Застосування SCFA-M достовірно модулювало білковий обмін: загальний білок зріс на 9,1 % ( $P > 0,05$ ), глобуліни – на 31,8 % ( $P > 0,001$ ), тоді як білковий коефіцієнт знизився на 16,5 % ( $P > 0,001$ ). Активність амінотрансфераз була нижчою, що свідчить про менше навантаження на печінку. Рівні сечовини та азоту сечовини у крові зменшилися на 16,5 % і 20 % відповідно, вказуючи на ефективніше використання азоту. Вуглеводний і мінеральний обміни також покращилися: глюкоза підвищилася до 6,48 ммоль/л (+47,9 %;  $P > 0,001$ ), засвоєння кальцію – на 10 %, фосфору – на 28,9 % ( $P > 0,001$ ). Отримані результати розширюють уявлення про механізми дії моногліцеридів і підтверджують доцільність їх використання у практиці свинарства; водночас потребують подальших досліджень із тривалішим моніторингом, оцінкою продуктивності та показників інтестинального гомеостазу. Практична значущість полягає у можливості використання SCFA-M як елемента програм підтримки поросят у поствідлучний період, з потенційним зменшенням потреби в антибактеріальних препаратах та покращенням благополуччя

**Ключові слова:** SCFA-M; білковий обмін; ферменти; енергетичний обмін; мінеральний обмін