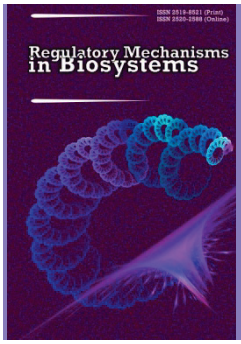

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2023, 14(4), 680–686
doi: 10.15421/022397

Effect of calcium propionate on rats with a high-fat hypercaloric diet

M. A. Lieshchova*, M. V. Bilan*, R. V. Mylostyvyi*, M. V. Kravtsova*, V. V. Brygadyrenko*, **

**Dnipro State Agrarian and Economic University, Dnipro, Ukraine*
***Oles Honchar Dnipro National University, Dnipro, Ukraine*

Article info

Received 12.09.2023
Received in revised form 28.10.2023
Accepted 16.11.2023

Dnipro State Agrarian and Economic University, Serhii Efremov st., 25, Dnipro, 49600, Ukraine. Tel.: +38-067-256-24-86. E-mail: lieshchova.m.o@dsau.dp.ua

Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010, Ukraine. Tel.: +38-050-93-90-788. E-mail: brigad@ua.fm

Lieshchova, M. A., Bilan, M. V., Mylostyvyi, R. V., Kravtsova, M. V., & Brygadyrenko, V. V. (2023). Effect of calcium propionate on rats with a high-fat hypercaloric diet. Regulatory Mechanisms in Biosystems, 14(4), 680–686. doi:10.15421/022397

Calcium propionate, as an approved food additive, is widely used as a mold inhibitor in food and feed. It is recognized as safe, but when metabolized in the gastrointestinal tract, it can affect the body's metabolism both directly and indirectly through the intestinal microbiota. The effect of various doses of calcium propionate on the body of model animals, with the study of the quantitative and qualitative composition of their intestinal microbiome, was investigated in this experiment. Four groups of male laboratory rats were formed, which for 20 days consumed: a high-fat diet with the addition of 0.0%, 0.5%, 1.0%, 2.0% calcium propionoate. We determined changes in body weight, the condition and mass indices of the internal organs, biochemical blood parameters, the functional state of the nervous system using the "open field" method, as well as changes in the intestinal microbiota. Adding calcium propionate to a high-fat diet does not affect the rate of animals' weight gain, nor the amount of feed and water consumed. High dose consumption of calcium propionate caused a decrease in the relative weight of the spleen and an increase in the relative weight of the kidneys, without affecting the functional state of the nervous system. With the addition of calcium propionate to a high-fat diet, only minor changes in some biochemical blood parameters were observed (increased De Ritis ratio and Ca/P ratio, moderate dyslipidemia). Calcium propionate had the most significant changes in the quantitative and qualitative composition of the intestinal microbiota in laboratory rats. Among the representatives of the microflora, the most sensitive to this substance against the background of a high-fat diet were obligate microorganisms from the genera *Bifidobacterium* and *Lactobacillus*. The antimicrobial action of calcium propionate was also demonstrated by the pathogenic *Staphylococcus aureus*. Amid a deficiency of obligate microflora, proliferation of transient microflora was found – facultative anaerobic and aerobic microorganisms (bacteria of the genera *Klebsiella*, *Enterococcus*, *Clostridium*, fungi of the genus *Candida*). Further research will be aimed at studying the effect of calcium propionate in various doses on the biological systems of different age group laboratory animals in a long-term experiment.

Keywords: propionates; E282; high-fat diet; relative mass of organs; organ mass index; blood biochemical parameters; bodyweight gain; gut microbiota.

Introduction

Propionates are substances found in the normal diet. Propionic acid is produced by certain bacteria and is found in various foods and feeds as a result of microbial production. Calcium propionate (CP) is an organic compound, a salt of calcium and propionic acid, $Ca(C_2H_5COO)_2$, formed as a result of the reaction between calcium hydroxide and propionic acid. It appears as colorless crystals that are highly soluble in water (Pongsavee, 2019). CP is an approved food additive, listed under number 282 in the Codex Alimentarius, and is one of the derivatives of the food preservative E280 (propionic acid). CP (E 282) is obtained by the reaction of propionic acid and calcium oxide in water with the presence of a flocculant. The product is filtered, spray dried, sieved and packaged. It is used in industrial baking as a preservative for food, feed, and cosmetics. CP is used in baked goods as a mold inhibitor (usually in an amount of 0.1–0.4% by weight of the product). It is also used as a preservative in a wide variety of products, including various baked goods, processed meats, and dairy products. CP is 'generally recognized as safe' according to the FDA and is widely used in food products without any restrictions other than current good manufacturing practice (EFSA, 2014).

CP is a strong preservative with little or no taste in normal use that can be effective against mold and bacteria and is widely used in food, feed and pharmaceuticals. It has the ability to inhibit the growth of mold and other microorganisms without apparent inhibiting yeast. CP does not have teratogenic activity and reproductive toxicity, and propionic acid can be excreted in the urine; thus, there is no risk of accumulation in the human body even at high doses (Sequeira et al., 2017; Zhang, et al., 2020).

Garcia et al. (2021) determined the comparative effectiveness of two preservatives (CP and potassium sorbate) against the three most common fungal strains responsible for bread spoilage. Potassium sorbate was more effective in inhibiting fungal growth. CP does not inhibit yeast growth, making it one of the most useful antimicrobial preservatives in the fermented food industry. CP strongly reduces the amount of fungus *Aspergillus flavus*, which produces carcinogenic aflatoxins (Bintvihok & Kositcharoenkul, 2006). In aqueous solutions, it can dissociate into propionic acid (the active antifungal ingredient) and calcium ions (Sequeira et al., 2017). The antimicrobial effect of CP depends on the pH value of the product, since its undissociated form has a better antimicrobial effect than its dissociated form (Suhr & Nielsen, 2004).

The antibacterial activity of CP has been studied very well. It can reduce *Escherichia coli* O157:H7, *Salmonella enterica typhimurium* (Kwak et al., 2011), *Clostridium* spp. (Wen et al., 2017) and other types of bacteria. The combined use of CP with biological preservatives increases their effectiveness in prolonging the shelf life of food products. The use of 0.3% CP by weight of the product and *Lactobacillus plantarum* significantly reduces the amount of mold and yeast compared to the use of CP alone (Bahmanpour et al., 2023).

CP is used for paper preservation (Sequeira et al., 2017), as a fungicide for fruits (Quiles et al., 2006), to prevent bread spoilage (Ryan et al., 2008), as an additive to silage and total mixed rations (TMR) for dairy

cows (Zhang et al., 2020). CP is also used as a preservative in cosmetics and personal care products. Propionic acid and its salts are capable of accelerating the locomotor activity of some predatory invertebrate species (Moshkin & Brygadyrenko, 2023), and reduce the degree of survival in nematodes – parasites of mammals' intestines (Boyko & Brygadyrenko, 2017, 2019, 2022).

Once in the body, CP dissociates in the gastrointestinal tract into propionic acid and calcium, while in rats the absorption degree of propionic acid is at least 77%. Propionic acid itself is a potent stimulator of endogenous glucose production (EGP) in various mammals. Considering this, CP is widely used in feed for dairy cows and sheep to increase the glucose concentration in milk (Aschenbach et al., 2010). In agriculture, CP is used in the production of animal feed and as a feed additive (Dahiya et al., 2016; Rathert-Williams et al., 2021). It is metabolized and absorbed by animals, providing them with calcium and glucose precursors, a benefit that other mold control products do not have. Therefore, CP is widely used in dairy cows as an antimicrobial agent, glucose precursor, and calcium source (Zhang et al., 2022a).

The effect of propionic acid salts (sodium propionate) on metabolism was studied experimentally. Acute oral administration of sodium propionate stimulates resting energy expenditure (REE) and lipid oxidation in humans (Chambers et al., 2019). Similar results were obtained in studies on mice: chronic administration of sodium propionate improves body weight and glucose homeostasis (den Besten et al., 2015).

Oral administration of propionate (propionic acid) to patients with hypertension and high cardiovascular risk reduced cardiac hypertrophy and fibrosis, susceptibility to cardiac arrhythmias, and atherosclerotic lesions (Bartolomaeus et al., 2019).

The EAE (experimental autoimmune encephalomyelitis) animal model is often used to study certain pathophysiological aspects relevant to multiple sclerosis. With its help, the effectiveness of oral propionate administration was studied by measuring clinical assessments of the body based on muscle function in model animals (tail tone, partial or complete paralysis of the limbs, death). Patients with multiple sclerosis are recommended to take 1 g of propionate daily as an addition to their usual therapy (Tobin et al., 2021).

Although CP is approved as a dietary supplement and is considered safe, studies in mice have shown its negative effects on their metabolism: increased concentrations of glucagon, norepinephrine, and endogenous glucose (Tirosh et al., 2019). A randomized, placebo-controlled, crossover study in humans found that CP, compared with placebo, significantly increased concentrations of glucagon and norepinephrine under normal conditions, glucagon, norepinephrine, and epinephrine under euglycemic conditions, and norepinephrine and epinephrine under hypoglycemic conditions (Adler et al., 2021). Propionate can activate catecholamine-mediated enhancement of insulin counter-regulatory signaling, leading to insulin resistance and hyperinsulinemia, which may contribute to obesity and metabolic disorders over time (Tirosh et al., 2019).

Propionates are widely used in agriculture (Bedford et al., 2018; Arrazola et al., 2019; Li et al., 2021). Calcium propionate can be used in silage and general mixed rations to prevent the accumulation of mycotoxins in feed (Alam et al., 2014). Bintvihok & Kositcharoenkul (2006) shows that the addition of CP to broiler diets containing aflatoxin B_1 is effective in reducing its toxicity. During the perinatal period, many cows fail to adapt to metabolic, endocrine and physiological changes, resulting in ketosis and fatty liver due to negative energy balance or postpartum hypocalcaemia, which damages their health and reduces productivity (Zhang et al., 2020, 2022a). CP supplements can be used in calf feeds as a rumen growth promoter (Diao et al., 2019). CP can be used as a feed preservative, growth promoter, gut microbiota enhancer, or appetite suppressant in animal nutrition (Arrazola & Torrey, 2019).

Pongsavee (2014) reports the ability of CP to suppress the growth of mold and other microorganisms without obvious inhibition of yeast in compound feeds. Yuan et al. (2017) noted that the addition of CP to alfalfa silage when it was prepared for an hour (after 30 days) resulted in a greater concentration of lactic, acetic, propionic and total amount of organic acids as well as microbial populations of lactic acid bacteria, but also changed the population of enterobacteria, mould and clostridia. Similar results were reported by Dong et al. (2017): addition of calcium propionate during ensiling of alfalfa can suppress unwanted enterobacteria ($P = 0.029$), mould ($P < 0.001$) and clostridia ($P < 0.001$), while the quantity of lactic acid bacteria in silage increases. Kwak et al. (2017) found that combined thermal ultrasound treatment and 2% CP effectively inactivated (reduced by more than 100,000) *E. coli* O157:H7 and *S. typhimurium* on freshly cut celery. Wen et al. (2017) also assume that when silage is prepared, the lower amount of the mold and clostridia may be associated with the antimicrobial effect of calcium propionate. But the influence of CP on the intestinal microbiome has not been studied enough (Agus et al., 2021). Besides, CP, through its effects on the hormonal system, is a potential metabolic disruptor in humans, highlighting the need to carefully evaluate the long-term metabolic effects of this widely used food preservative.

The aim of our study is to determine the effect of calcium propionate different doses on changes in body weight and internal organs, blood biochemical parameters, nervous system functional state and intestinal microbiota of model animals consuming a high-fat diet.

Materials and methods

The choice of animals for the experiment, research protocols, and withdrawal of animals from the experiment was approved by the local ethical committee of Dnipro State Agrarian and Economic University (Dnipro, Ukraine). The maintenance, nutrition, care of animals and their withdrawal from the experiment were carried out in accordance with the principles set forth in the "European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes" (Strasbourg, France, March 18, 1986, ETS No. 123) and in Law of Ukraine "On protection of animals from cruel treatment" (Kyiv, February 21, 2006, No. 3447-IV).

The experiment used 20 outbred male laboratory rats, 6 months old, with an average weight of 350 ± 20 g. Four groups were formed: one control and three experimental ones (5 animals in each). Animals were kept in polycarbonate cages in a temperature-controlled room (20–22 °C), with a 12/12-hour light/dark cycle, with free access to food and water. For 20 days, animals of all groups received a diet containing a high percentage of fat. It was prepared on the basis of a standard diet with the addition of 15% sunflower oil. The rats in the control group simply consumed a high-fat diet throughout the experiment. Rats in the experimental groups were fed a high-fat diet supplemented with calcium propionate in different doses: I experimental group – 0.5% CP, II – 1.0% CP, III – 2.0% CP by weight of food. During the experiment, the amount of food and water consumed by each group was taken into account. Each animal was observed and weighed, followed by calculation of the total increase in the weight of the animals and daily increases in live weight (Lieshchova & Brygadyrenko, 2021, 2023a).

Animals were slaughtered on the 20th day of the experiment under general anaesthesia (80 mg/kg of ketamine and 12 mg/kg of xylazine, intraperitoneally) by total bloodletting from the heart. After the autopsy, the condition of the internal organs was visually assessed for the presence of pathological changes. The selection of organs (heart, liver, lungs, thymus, spleen, stomach, small intestine, caecum, colon, rectum, kidneys, brain) was carried out with surgical instruments. The mass of internal organs was determined to an accuracy of 10 mg. Blood sampling was carried out during the euthanasia of rats, followed by biochemical and morphological analysis. After injecting the rats with anaesthetic agents, blood was drawn directly from the heart with a syringe into two test tubes. In the first, whole blood (1.0–1.5 mL) was collected to obtain serum and to perform further biochemical studies. In the second, 0.5–1.0 mL of blood was collected, adding an anticoagulant (potassium EDTA) for further automatic calculation of complete blood cell count and preparation of smears for a leukogram. Biochemical parameters were determined using a Miura 200 automatic analyser (I.S.E. Srl, Rome, Italy), and High Technology reagent kits (High Technology Inc, North Attleborough, MA, USA), PZ Cormay S.A. (Cormay Diagnostics, Lublin, Poland) and Spinreact S.A. (Spinreact, Girona, Spain). Red blood cell and white blood cell count in the rats' stabilized blood were determined using an automatic haematology analyser, BC-2800Vet (Mindray, Shenzhen, China). For the leukogram, blood smears were prepared according to Pappenheim with their further staining according to Romanovsky-Giemsa.

At the beginning (days 1–4) and at the end (days 16–20) of the experiment, the functional state of the nervous system was determined by indicators of orientation-physical activity and the emotional state of experimental animals in the "open field" test (Lieshchova et al., 2018, 2023b; Brygadyrenko et al., 2019; Lieshchova & Brygadyrenko, 2022).

To determine the effect of CP supplementation on the rats' intestinal microbiota, microbiological research was carried out. Faecal samples were collected individually from the rectum after the slaughter of the animals in accordance with the established rules of asepsis. Serial tenfold dilutions were carried out to 10^{-10} and the corresponding dilutions were sowing on selective nutrient media (*Bifidobacterium agar*, *Lactobacillus agar*, *Enterococcus agar)*, Endo's medium, bismuth sulphite agar, Wilson & Blair medium, Baird-Parker agar, Sabouraud dextrose agar (HiMedia, India), and 5% blood agar (Biomerieux, France) and incubated in a thermostat at 24, 37 and 42 °C. After 24–72 hours, the results were obtained, by identifying CFU/g, counting the number of colonies that grew. Anaerobic microorganisms were grown in an anaerostat (7 L), using GENbox anaeropacks (Biomerieux, France). Control anaerobiosis was carried out using the anaerobic indicator (Biomerieux, France). Visual confirmation of microorganisms was carried out using an additional light microscope MICROmed XS-3330 by examining gram-stained smears.

Identification and differentiation of microorganisms was carried out using highly enzymatic methods on Hiss's media with various sugars, Olkenitskyi, Christensen, Simmons citrate agar, malonat agar, etc. (Farmaktiv, Ukraine), as well as using tests API 20 REF 20 600, API Staph REF 10 20 500, API 20 E REF 20 100 / 20 160, API 20 NE REF 20 050, API Candida REF 10 500 (Biomerieux, France) based on the Bergey's Manual of Systematic Bacteriology (1986, 2009) (Bilan et al., 2019).

All the data were analyzed using Statistica 8.0 program (StatSoft Inc., USA). Results in the tables are demonstrated as x ± SE (mean ± standard error). Differences between the control and experimental groups values were determined by using the Tukey test (with consideration of Bonferroni's correction), where the differences were considered significant at $P < 0.05$.

Results

Consumption of calcium propionate by rats in different dosages fed a high-fat diet did not lead to a significant change in the body weight of animals in all three experimental groups (Table 1). Analyzing food and water consumption by animals during the experiment, we determined that the highest daily feed consumption was in the group that received 0.5% CP, and the minimum in the group receiving 2.0% CP. Daily water consumption was higher in rats receiving CP, and lowest in rats consuming only a high-fat diet. A daily increase in body weight was detected in the control group rats, as well as in rats receiving 1.0% CP; at the same time, rats of two experimental groups receiving 0.5% and 2.0% CP decreased in weight. The indicators of body length and the length of the small intestine did not differ significantly, but there was a tendency to increase in the length of the small intestine in rats of the experimental groups.

Table 1

Change in the body weight of young male rats under the influence of calcium propionate diet supplementation (x ± SD, n = 5, duration of experiment – 20 days)

Experiment day	0%	0.5%	1.0%	2.0%
1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
11	101.9 ± 2.6	98.6 ± 3.2	100.9 ± 3.2	103.1 ± 2.9
12	101.6 ± 2.1	98.0 ± 3.7	96.4 ± 10.4	101.7 ± 2.8
13	100.1 ± 3.0	99.7 ± 2.2	94.7 ± 6.9	101.8 ± 1.3
14	99.0 ± 3.8	100.3 ± 3.6	92.9 ± 6.8	101.4 ± 4.6
15	100.7 ± 2.2	101.7 ± 4.9	94.1 ± 5.5	101.0 ± 3.7
16	100.1 ± 1.8	101.2 ± 2.4	94.2 ± 3.5	101.9 ± 4.3
17	101.2 ± 2.0	100.4 ± 1.8	94.8 ± 3.4	99.2 ± 3.1
18	102.3 ± 2.8	101.7 ± 1.9	95.5 ± 3.7	98.3 ± 2.2
19	101.4 ± 2.5	100.5 ± 3.4	98.0 ± 3.9	96.5 ± 3.0
20	100.8 ± 2.7	99.9 ± 2.5	97.0 ± 3.9	94.5 ± 2.9

Note: no significant differences were found within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction.

The relative weight of most organs of animals did not change significantly when using different doses of CP in food (Table 3). A significantly low relative spleen weight was detected in animals consuming 1.0% and 2.0% CP. The addition of CP to a high-fat diet caused a gradual increase in the kidneys' relative weight, and the increase was proportional to the dose of the substance consumed.

To determine the general state of metabolism in rats, blood biochemical parameters were examined before the start of the experiment. After a 20-day experiment, the metabolic state was also analyzed by changes in blood biochemical parameters.

Table 2

Change in the young male rats' body weight and food consumption under the influence of calcium propionate supplementation to their diet (x ± SD, n = 5, duration of experiment – 20 days)

Parameter	0.0%	0.5%	1.0%	2.0%
Food consumption by rats, g/day	16.8	21.9	14.4	15.7
Water consumption by rats, g/day	13.8	16.3	19.7	16.4
Body weight change, mg/day	100 ± 454[a]	–50 ± 481[a]	550 ± 647[a]	–950 ± 542[a]
Length of small intestine, cm	137.6 ± 19.3	141.3 ± 16.2	139.8 ± 17.0	154.2 ± 13.2
Body length, cm	22.5 ± 0.6	24.3 ± 1.9	23.5 ± 0.5	24.3 ± 0.6

Note: see Table 1.

Table 3

Change in relative mass (%) of organs of male rats under the influence of calcium propionate supplementation to their diet (x ± SE, n = 5, duration of experiment – 20 days)

Organ	0.0%	0.5%	1.0%	2.0%
Heart	0.356 ± 0.035	0.352 ± 0.039	0.361 ± 0.042	0.330 ± 0.019
Liver	3.602 ± 0.233	3.674 ± 0.308	3.420 ± 0.271	3.878 ± 0.222
Lungs	0.830 ± 0.172	0.982 ± 0.395	1.171 ± 0.230	1.064 ± 0.136
Spleen	0.220 ± 0.045[a]	0.221 ± 0.022[a]	0.173 ± 0.014[ab]	0.164 ± 0.029[b]
Stomach	0.554 ± 0.131	0.474 ± 0.080	0.510 ± 0.076	0.586 ± 0.039
Small intestine	2.016 ± 0.378	1.721 ± 0.146	1.762 ± 0.320	1.693 ± 0.130
Cecum	0.253 ± 0.063	0.241 ± 0.066	0.204 ± 0.116	0.225 ± 0.034
Colon	0.306 ± 0.135	0.217 ± 0.069	0.284 ± 0.051	0.234 ± 0.042
Rectum	0.241 ± 0.076	0.197 ± 0.061	0.238 ± 0.049	0.252 ± 0.064
Right kidney	0.304 ± 0.022[a]	0.315 ± 0.024[a]	0.325 ± 0.016[ab]	0.362 ± 0.033[b]
Left kidney	0.308 ± 0.031[a]	0.305 ± 0.030[a]	0.322 ± 0.016[ab]	0.363 ± 0.032[b]
Right testis	0.461 ± 0.036	0.412 ± 0.279	0.494 ± 0.044	0.588 ± 0.097
Left testis	0.479 ± 0.045	0.416 ± 0.280	0.505 ± 0.054	0.539 ± 0.105
Brain	0.581 ± 0.053	0.509 ± 0.096	0.594 ± 0.045	0.621 ± 0.036

Note: different letters indicate values that differed one from another reliably within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction; if within a line the numbers do not have letter indices, then no significant differences were found within one line of the table.

Most of the blood biochemical parameters in experimental animals did not change when consuming CP with food (Table 4). But the consumption of a high-fat diet and the additional introduction of CP into the diet led to a disruption of the blood enzymatic activity, which was expressed by a significant change in the De Ritis ratio (AST/ALT). This indicator significantly increased when the rats ate a high-fat diet (from 1.53 ± 0.55 to 1.90 ± 0.65 relative units) and consumed CP 0.5% and 1.0% of the feed weight (Table 4). The experiment also revealed a change in mineral metabolism, which was expressed by a change in the Ca/P ratio. Thus, in the rats' blood before the start of the experiment, this indicator was 1.73 ± 0.32 relative units; when eating a high-fat diet, it significantly decreased to 1.14 ± 0.55 units, and when adding 0.5% CP, to 0.90 ± 0.14 units. Adding CP to a high-fat diet also caused changes in the amount of HDL in the blood: when consuming 0.5% CP, the HDL level increased dramatically, and a dose of CP 1.0% caused a sharp decrease in this indicator, almost halved.

When using different concentrations of CP, there were no changes in physical activity, orientation activity and emotional state (Table 5).

Table 4

Change in blood biochemical parameters of male rats under the influence of calcium propionate (x ± SE, n = 5, duration of experiment – 20 days)

Parameters	Before the experiment	0.0%	0.5%	1.0%	2.0%
Total protein, g/L	70.3 ± 8.4	69.8 ± 3.0	76.8 ± 4.6	71.4 ± 5.2	71.6 ± 6.9
Albumins, g/L	31.3 ± 4.0	32.6 ± 2.3	37.8 ± 1.3	35.2 ± 1.6	34.4 ± 3.9
Globulins, g/L	39.0 ± 12.3	37.2 ± 2.4	39.0 ± 5.1	35.8 ± 5.1	37.2 ± 8.1
Protein coefficient, U	0.87 ± 0.35	0.85 ± 0.10	0.98 ± 0.17	1.02 ± 0.15	0.98 ± 0.24
Urea, mmol/L	3.70 ± 1.39	4.06 ± 0.85	3.35 ± 0.66	3.32 ± 0.61	3.36 ± 0.86
Blood urea nitrogen (BUN), mg/100 g	7.07 ± 2.63	7.76 ± 1.62	6.40 ± 1.24	6.36 ± 1.15	6.42 ± 1.66
Creatinine, μmol/L	58.7 ± 0.6	53.4 ± 14.2	56.3 ± 4.9	56.8 ± 8.6	57.4 ± 6.4
Aspartate aminotransferase (AST), U/L	103.0 ± 9.2	193.8 ± 37.9	182.3 ± 13.6	225.0 ± 31.1	177.8 ± 34.9
Alanine aminotransferase (ALT), U/L	76.7 ± 33.8	134.0 ± 65.9	86.8 ± 17.5	101.4 ± 18.8	72.2 ± 10.5
De Ritis ratio (AST/ALT), U	1.53 ± 0.55[a]	1.90 ± 0.65[ab]	2.18 ± 0.47[ab]	2.28 ± 0.57[b]	2.48 ± 0.27[b]
Alkaline phosphatase, U/L	481 ± 300	685 ± 210	620 ± 209	637 ± 366	745 ± 386
Alpha amylase, U/L	1028 ± 83	1055 ± 212	1098 ± 191	1130 ± 220	1091 ± 147
Total bilirubin, μmol/L	5.87 ± 0.60	1.80 ± 1.00	1.95 ± 0.24	3.48 ± 1.72	2.86 ± 1.16
Glucose, mmol/L	6.07 ± 0.60	3.72 ± 1.12	4.00 ± 0.53	3.86 ± 0.55	3.82 ± 0.51
Total calcium, mmol/L	2.63 ± 0.12	2.34 ± 0.32	2.50 ± 0.29	2.42 ± 0.22	2.26 ± 0.11
Non-organic phosphorus, mmol/L	1.57 ± 0.23	2.62 ± 1.60	2.75 ± 0.47	2.14 ± 0.53	1.96 ± 0.44
Ca/P	1.73 ± 0.32[a]	1.14 ± 0.55[ab]	0.90 ± 0.14[b]	1.22 ± 0.33[ab]	1.18 ± 0.23[b]
Gamma-glutamyl transferase (GGT), U/L	6.00 ± 1.00	5.00 ± 0.82	4.00 ± 2.45	4.00 ± 1.73	4.80 ± 1.10
Cholesterol, mmol/L	1.77 ± 0.32	1.84 ± 0.26	1.85 ± 0.24	1.92 ± 0.13	2.08 ± 0.34
Blood triglycerides, mmol/L	0.69 ± 0.12	0.86 ± 0.36	0.88 ± 0.24	0.88 ± 0.36	0.92 ± 0.38
Low density lipoprotein (LDL) cholesterol, mmol/L	0.34 ± 0.12	0.85 ± 0.61	0.69 ± 0.37	1.13 ± 0.50	0.98 ± 0.60
High density lipoprotein (HDL) cholesterol, mmol/L	0.57 ± 0.32[ab]	0.59 ± 0.38[ab]	0.68 ± 0.13[b]	0.30 ± 0.10[a]	0.46 ± 0.34[ab]

Note: see Table 3.

Table 5

Change in behavioral characteristics of three rats groups during 2 minutes of the experiment, in whose diet calcium propionate was added (x ± SE, n = 15, duration of the experiment was 20 days)

Characteristic	0.0%, 1st day	0.0%, 20th day	0.5%, 1st day	0.5%, 20th day	1.0%, 1st day	1.0%, 20th day	2.0%, 1st day	2.0%, 20th day
Number of visited peripheral squares	12.93 ± 2.40	18.00 ± 2.36	9.60 ± 2.02	9.07 ± 2.89	13.67 ± 3.00	20.47 ± 3.28	6.53 ± 2.25	8.93 ± 3.23
Number of visited central squares	0.60 ± 0.38	0.53 ± 0.27	0.27 ± 0.12	0.20 ± 0.20	0.73 ± 0.43	0.47 ± 0.27	0.67 ± 0.36	0.13 ± 0.13
Number of upright stands in peripheral squares	2.93 ± 0.54	3.47 ± 0.31	3.13 ± 0.58	2.40 ± 0.70	2.67 ± 0.77	4.20 ± 0.75	2.20 ± 0.71	3.07 ± 0.48
Number of upright stands in central squares	0.80 ± 0.42	0.47 ± 0.27	1.13 ± 0.41	0.40 ± 0.16	1.67 ± 0.50	1.57 ± 0.47	0.00 ± 0.00	0.60 ± 0.24
Number of grooming acts	1.00 ± 0.29	2.13 ± 0.24	1.13 ± 0.42	0.87 ± 0.24	0.87 ± 0.29	0.67 ± 0.19	0.60 ± 0.24	2.33 ± 0.40
Number of faecal boluses	0.20 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.09	0.20 ± 0.11	0.20 ± 0.14	0.00 ± 0.00
Physical activity	13.53 ± 2.29	18.53 ± 2.44	9.87 ± 2.03	9.27 ± 3.01	14.40 ± 3.27	20.93 ± 3.31	7.20 ± 2.31	9.07 ± 3.22
Orientation activity	3.73 ± 0.67	3.93 ± 0.48	4.27 ± 0.83	2.80 ± 0.83	4.33 ± 1.02	5.67 ± 1.01	2.20 ± 0.71	3.67 ± 0.57

Note: physical activity – the number of visited squares of the open field, for orientation activity – the number of upright stands; there were no significant differences between the groups for most of the parameters studied; within a line, the numbers do not have letter indices, since no significant ($P < 0.05$) differences were found within one line of the table according to the Tukey test results with Bonferroni correction.

In animals that were given the highest concentration of CP in addition to a high-fat diet, the intestine content had a liquid consistency. Also, CP in the diet affected the number of representatives of the genera *Bifidobacterium* and *Lactobacillus*, which form the basic bacterial flora for all groups of rats. The number of *Bifidobacterium* spp. under the conditions of CP consumption, significantly decreased (Table 6), but in all four groups it remained within the normal range. Similar, but much more intense changes were observed for *Lactobacillus* spp. under the influence of CP: 1.0% and 2.0% concentration of the total weight of feed in the rats' diet caused significant $\log_{10}$ changes in the number of lactobacilli from 7.77 to 6.93 and 5.27 CFU/g, respectively. The typical form of *Escherichia coli* at high concentrations of this food additive in the diet did not significantly change its number compared to the number in the control group of rats. Weakly fermenting *E. coli* dramatically increased their number ($\log_{10}$) from 4.39 in the control group to 6.25 and 6.94 CFU/g in groups with a concentration of 1.0% and 2.0% CP in the diet. The number of lactose-negative *E. coli* did not significantly change under the influence of CP.

A reliable change in the bacterial number of the genus *Clostridium* is a cause for concern. The logarithm of the number of these bacteria increased under the conditions of CP consumption from 2.04 in the control group to 2.64 and 2.97 CFU/g for 1.0% and 2.0% of this food additive by weight of feed in the diet of rats.

It should be noted that no opportunistic enterobacteria of the genus *Citrobacter* were found in any of the experimental animals, but a probable increase in the number of bacteria from the genus *Klebsiella*, *Enterococcus*, and *Clostridium* was observed. Even at a low concentration (0.5% of the feed mass), CP stimulated the growth of the number of *Candida* fungi ($\log_{10}$ of their number increases from 4.90 to 5.64 CFU/g).

Amid an insignificant increase in the number of *Staphylococcus epidermidis*, CP showed an antibacterial effect and caused a noticeable inhibition of *Staphylococcus aureus* bacterial growth ($\log_{10}$ of their number decreased from 5.13 to 3.91 CFU/g).

Discussion

In this study, we assessed the effect of various doses of calcium propionate as dietary supplement on the body of white laboratory rats fed a high-fat diet during a 20-day experiment for indicators of food and water consumption, changes in body weight and organs, blood biochemical parameters, the functional state of the nervous system and changes in gut

microbiome composition. It is known that the body weight of animals and its changes under the influence of various factors are important indicators that are used in determining the toxicity of drugs and food additives (Lieshchova et al., 2021; Lieshchova & Brygadyrenko, 2023b). Also, in laboratory animals, one of the important indicators that can be used to determine their well-being and health is the amount of food and water consumed. A decrease in body weight may indicate an adverse effect of the test substances (Lieshchova et al., 2023a).

Table 6

Number of microorganisms (lg_{10} CFU/gram of feces) in four groups of rats fed with calcium propionate (x ± SE, n = 5, duration of the experiment was 20 days, BD – basic diet)

Intestinal microflora	Norm	0.0%	0.5%	1.0%	2.0%
Bifidobacterium spp.	7–10	9.40 ± 0.24^{a}	9.27 ± 0.19^{ab}	8.80 ± 0.37^{ab}	8.80 ± 0.20^{b}
Lactobacillus spp.	5–8	7.77 ± 0.23^{a}	6.96 ± 0.47^{ab}	6.93 ± 0.15^{b}	5.27 ± 0.15^{c}
Escherichia coli typical	6–8	6.28 ± 0.14^{a}	4.97 ± 0.32^{b}	6.62 ± 0.75^{a}	6.82 ± 0.45^{a}
Escherichia coli weakly fermenting	<25 %	4.39 ± 0.13^{a}	5.87 ± 0.33^{b}	6.25 ± 0.44^{bc}	6.94 ± 0.39^{c}
Escherichia coli lactose-negative	2	2.22 ± 0.09^{a}	2.56 ± 0.08^{b}	2.49 ± 0.15^{ab}	2.67 ± 0.03^{bc}
Clostridium spp.	2	2.04 ± 0.07^{a}	1.74 ± 0.47^{a}	2.64 ± 0.04^{b}	2.97 ± 0.04^{c}
Enterococcus spp.	5–7	5.31 ± 0.42^{ab}	5.87 ± 0.14^{b}	5.02 ± 0.31^{a}	6.03 ± 0.16^{b}
Proteus spp.	2–4	4.45 ± 0.04^{b}	4.75 ± 0.19^{c}	4.69 ± 0.20^{bc}	3.41 ± 0.46^{a}
Enterobacter spp.	2–4	3.62 ± 0.44^{a}	4.64 ± 0.25^{c}	4.34 ± 0.06^{b}	3.61 ± 0.27^{a}
Citrobacter spp.	2–4	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Klebsiella spp.	2–4	3.00 ± 0.42^{a}	3.60 ± 0.63^{ab}	4.40 ± 0.61^{b}	3.75 ± 0.79^{ab}
Staphylococcus epidermidis	2–4	4.08 ± 0.07^{a}	4.21 ± 0.09^{ab}	4.22 ± 0.07^{b}	4.20 ± 0.14^{ab}
Staphylococcus aureus	3–5	5.13 ± 0.46^{b}	4.89 ± 0.21^{b}	4.60 ± 0.13^{b}	3.91 ± 0.13^{a}
Candida spp.	2–5	4.90 ± 0.17^{a}	5.64 ± 0.22^{b}	5.75 ± 0.23^{b}	5.72 ± 0.11^{b}

Note: see Table 3.

Chronic exposure of rats to a dose of CP equivalent to that used for food preservation resulted in a gradual increase in body weight (Tirosh et al., 2019). In our experiment, we did not detect the effect of CP on rats' body weight, and the amount of consumed food and water did not change significantly.

Zhang et al. (2017) showed that CP supplementation for 160 days improved intestinal development in calves. However, Bunting et al. (2000) found that supplementation with 6.4% CP for 42 days did not improve intestinal development. In our experiment, we determined the total length and relative weight of the small intestine in rats receiving different doses of CP for 20 days, as well as the relative weight of the stomach and individual sections of the large intestine. By the end of the experiment, we did not observe any significant differences in these indicators between the groups, so CP consumption on the background of a high-fat diet does not have an effect on the morphofunctional state of the GI tract in model animals. It is also known that high doses of propionic acid can cause local lesions of the GI tract epithelium at the site of where the substance was first in contact with the body in the form of acanthosis, hyperkeratosis and proliferative processes (Harrison et al., 1991). In our study, we did not find any pathomorphological changes in the GI tract mucous membranes of experimental animals.

The relative weight of internal organs reflects the growth, health and general condition of the body. Of interest is a significant decrease in the relative weight of the spleen in animal groups receiving 1.0% and 2.0% CP, and at the same time an increase in the relative weight of their kidneys. This can be explained by a possible increase in blood flow (blood leaving the depot – a decrease in the weight of the spleen) and activation of the blood filtration function (increase in the relative weight of the kidneys). At the same time, the functional state of the kidneys, which can be assessed by the concentration of creatinine in the blood, worsened in animals receiving different doses of CP, since this indicator was higher than the reference values for this age group of animals (Shayakhmetova et al., 2020). The effect of CP on body weight gain and the weight of some internal organs of Jersey calves was studied by Zhang et al. (2017). They determined that CP promotes weight gain and increases average daily weight gain. Also, by the end of the experiment (160 days), calves that received CP had the greatest spleen weight, significantly higher liver and rumen weight, while CP consumption had no effect on the weight of the heart and kidneys. In rats fed a diet containing varying amounts of propionic acid (25–45,000 mg/kg body weight) for three months, no deaths or clinical toxicities were observed. At the same time, a decrease in the amount of food consumed and a decrease in average body weight were observed only when consuming the highest dose of the substance. Regardless the dose of propionic acid consumption, no changes in morphological and biochemical blood parameters, as well as the absolute weight of organs, were observed in experimental animals (EFSA, 2014). Similar studies were conducted with dogs, where they found that even the consumption of the highest tested dose of propionic acid (2084 mg/kg body weight per day) in the diet did not cause mortality, slightly reduced appetite, but did not affect body weight and weight gain (BASF, 1988). Feeding rats sodium propionate (2%) for 104 weeks showed no mortality and no adverse effects on body and organ weight, food intake, or blood counts, but did increase water intake and urine and sodium excretion. Also, a dose of 5.13% sodium propionate in the diet of Beagle dogs did not cause adverse effects on their body weight, amount of food consumed, general condition, or blood counts (EFSA, 2014).

The effect of propionic acid and its derivatives on lipid metabolism in the body has long been known. Haghikia et al. (2022) showed in a study on mice that treatment with exogenous propionate (given orally via gavage) reduced total and low-density lipoprotein cholesterol levels in the blood. In mice fed a high-fat diet, propionate reduced the absorption of cholesterol in the intestine and reduced the area of atherosclerotic lesions in the aorta. In our study, different doses of CP amid a high-fat diet consumed by rats for 20 days also caused changes in lipid metabolism. Cholesterol concentrations were at minimal levels in animals prior to the study; consumption of a high-fat diet and the smallest dose of CP (0.5%) caused a slight increase in this indicator, and higher doses of CP (1.0 and 2.0%) caused a further increase in the concentration of cholesterol in animals' blood plasma. It should be noted that a high-fat diet together with CP caused an increase in cholesterol levels above reference values (Shayakhmetova et al., 2020). The concentration of blood triglycerides also increased in rats after consumption of a high-fat diet and the addition of various doses of CP (it was also higher than the reference normal values). CP consumption also caused a significant increase in low density lipoprotein (LDL) cholesterol levels regardless of CP dose. The concentration of high density lipoprotein (HDL) cholesterol in rats consuming a high-fat diet remained virtually unchanged compared to the values of this indicator before the experiment.

Intestinal eubiosis is supported by representatives of the main phylotypes: Bacteroides, Firmicutes, Proteobacteria, Actinobacteria, etc., which, forming a balanced ecological system with the macroorganism, play a decisive role in maintaining the immune status, metabolic homeostasis, and protection against pathogens of infectious diseases (Lieshchova et al., 2020). Thus, in an experiment on mice with a microbiota depleted by treatment with antibiotics, which consumed a normal diet and a diet with a high fat content for six weeks, an increase in the concentration of very low density lipoproteins and low density lipoproteins was found compared to those in mice with a normal microbiota. These results confirm the functional role of metabolic pathways dependent on gut microbiota in controlling blood cholesterol levels (Haghikia et al., 2022).

Lipopolysaccharides (LPS) in the cell wall of Gram-negative bacteria and harmful metabolites such as trimethylamine (TMA) can enter the

systemic circulation and induce chronic inflammatory responses *in vivo* (Yan et al., 2022). This can result in vascular endothelium dysfunction, atherosclerosis, vascular calcification, heart failure, and hypertension. These researchers found that propionate, administered orally and rectally to laboratory animals, was able to enrich components of the microbiota that promote the production of short-chain fatty acids (SCFA) and maintain a healthy gut barrier, reduce inflammation, and reverse vitamin D and nicotinamide induced vascular calcification (VDN).

Zhang et al. (2022b) reported that CP as a dietary supplement prevents ketosis and metabolic disturbances in postpartum hypocalcemia in early lactation dairy cows, increases milk yield in early lactation dairy cows, and reduces gut inflammatory response by improving the relative abundance of Bacteroidetes, including Rikenellaceae RC9 gut group and Alistipes. A significant increase in the number of Bacteroidetes in rats' feces with arthritis was also indicated by Fan et al. (2021).

Cao et al. (2020) found that CP supplementation decreased the relative abundance of the phylum Bacteroidetes but tended to increase the abundance of Proteobacteria. In addition, CP supplementation reduced the diversity of bacteria and archaea in the rumen compared to calves fed the control diet, both pre- and post-weaning.

Conclusions

The supplementation with different doses of calcium propionate to the high-fat diet of model animals did not not have a significant effect on the intensity of body weight gain, feed and water consumption. With the consumption of 1% and 2% CP, the relative mass of the spleen significantly decreased and the mass of the kidneys increased. Most of the blood biochemical parameters of experimental animals did not change when CP was used with food. The revealed changes in some biochemical parameters of the blood (increased De Ritis ratio, Ca/P ratio and moderate dyslipidemia) do not allow us to strate a significant positive effect of CP amid a high-fat diet. When using different concentrations of CP, there were no changes in physical activity, orientation activity, and emotional state, which suggests that there is no influence of this substance on the functional state of the nervous system.

Among the representatives of the intestinal microbiota, obligate microflora microorganisms of the genera *Bifidobacterium* and *Lactobacillus* turned out to be the most sensitive to CP amid a high-fat diet. The antimicrobial effect of calcium propionate was also manifested against opportunistic *Staphylococcus aureus*. Against the background of an obligate microflora deficiency, the reproduction of transient microflora representatives – facultative anaerobic and aerobic microorganisms (bacteria of the genera *Klebsiella*, *Enterococcus*, *Clostridium*, and fungi of the genus *Candida*) was established.

Further research will be aimed at studying the influence of different doses of CP on the biosystems of the body of laboratory animals in a long-term experiment on different age groups.

This research was funded by the Ministry of Education and Science of Ukraine within the topic "Modeling of metabolic processes and immune status of animals by drugs that are based on medicinal herbs with a high-calorie diet", grant number 0122U000975.

The authors declare no conflict of interest.

References

Adler, G. K., Hornik, E. S., Murray, G., Bhandari, S., Yadav, Y., Heydarpour, M., Basu, R., Garg, R., & Tirosh, A. (2021). Acute effects of the food preservative propionic acid on glucose metabolism in humans. BMJ Open Diabetes Research and Care, 9(1), e002336.

Agus, A., Richard, D., Faïs, T., Vazeille, E., Chervy, M., Bonnin, V., Dalmasso, G., Denizot, J., Billard, E., Bonnet, R., Buisson, A., Barnich, N., & Delmas, J. (2021). Propionate catabolism by CD-associated adherent-invasive *E. coli* counteracts its anti-inflammatory effect. Gut Microbes, 13(1), 1–18.

Alam, S., Shah, H. U., Afzal, M., & Magan, N. (2014). Influence of calcium propionate, water activity and storage time on mold incidence and aflatoxins production in broiler starter feed. Animal Feed Science and Technology, 188, 137–144.

Arrazola, A., & Torrey, S. (2019). Conditioned place avoidance using encapsulated calcium propionate as an appetite suppressant for broiler breeders. PLoS One, 14(7), e0206271.

Arrazola, A., Mosco, E., Widowski, T. M., Guerin, M. T., Kiarie, E. G., & Torrey, S. (2019). The effect of alternative feeding strategies for broiler breeder pullets: 1. Welfare and performance during rearing. Poultry Science, 98(9), 3377–3390.

Aschenbach, J. R., Kristensen, N. B., Donkin, S. S., Hammon, H. M., & Penner, G. B. (2010). Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. IUBMB Life, 62(12), 869–877.

Bahmanpour, H., Sowti Khiabani, M., & Pirsa, S. (2023). Improving the microbial and physicochemical shelf life of yufka paste using *Lactobacillus plantarum* and calcium propionate. Food Science and Nutrition, 2023, in press.

Bartolomaeus, H., Balogh, A., Yakoub, M., Homann, S., Markó, L., Höges, S., Tsvetkov, D., Krannich, A., Wundersitz, S., Avery, E. G., Haase, N., Kräker, K., Hering, L., Maase, M., Kusche-Vihrog, K., Grandoch, M., Fielitz, J., Kempa, S., Gollasch, M., Zhumadilov, Z., & Wilck, N. (2019). Short-chain fatty acid propionate protects from hypertensive cardiovascular damage. Circulation, 139(11), 1407–1421.

BASF (1988). Study of the toxicity of propionic acid in beagle dogs. Project No. 31D0473/8590. BASF Abteilung Toxikologie Department of toxicology. Ludwigsfen, Germany.

Bedford, A., Beckett, L., Hardin, K., Dias, N. W., Davis, T., Mercadante, V. R. G., Ealy, A. D., & White, R. R. (2018). Propionate affects insulin signaling and progesterone profiles in dairy heifers. Scientific Reports, 8(1), 17629.

Bilan, M. V., Lieshchova, M. A., Tishkina, N. M., & Brygadyrenko, V. V. (2019). Combined effect of glyphosate, saccharin and sodium benzoate on the gut microbiota of rats. Regulatory Mechanisms in Biosystems, 10(2), 228–232.

Bintvihok, A., & Kositcharoenkul, S. (2006). Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon, 47(1), 41–46.

Boyko, A. A., & Brygadyrenko, V. V. (2017). Changes in the viability of the eggs of *Ascaris suum* under the influence of flavourings and source materials approved for use in and on foods. Biosystems Diversity, 25(2), 162–166.

Boyko, O. O., & Brygadyrenko, V. V. (2019). The impact of acids approved for use in foods on the vitality of *Haemonchus contortus* and *Strongyloides papillosus* (Nematoda) larvae. Helminthologia, 56(3), 202–210.

Boyko, O., & Brygadyrenko, V. (2022). Nematicidal activity of organic food additives. Diversity, 14, 615.

Brygadyrenko, V. V., Lieshchova, M. A., Bilan, M. V., Tishkina, N. M., & Horchanok, A. V. (2019). Effect of alcohol tincture of *Aralia elata* on the organism of rats and their gut microbiota against the background of excessive fat diet. Regulatory Mechanisms in Biosystems, 10(4), 497–506.

Bunting, L. D., Tarifa, T. A., Crochet, B. T., Fernandez, J. M., Depew, C. L., & Lovejoy, J. C. (2000). Effects of dietary inclusion of chromium propionate and calcium propionate on glucose disposal and gastrointestinal development in dairy calves. Journal of Dairy Science, 83(11), 2491–2498.

Cao, N., Wu, H., Zhang, X. Z., Meng, Q. X., & Zhou, Z. M. (2020). Calcium propionate supplementation alters the ruminal bacterial and archaeal communities in pre- and postweaning calves. Journal of Dairy Science, 103(4), 3204–3218.

Chambers, E. S., Byrne, C. S., Aspey, K., Chen, Y., Khan, S., Morrison, D. J., & Frost, G. (2018). Acute oral sodium propionate supplementation raises resting energy expenditure and lipid oxidation in fasted humans. Diabetes, Obesity and Metabolism, 20(4), 1034–1039.

Dahiya, R., Berwal, R. S., Sihag, S., Patil, C. S., & Lalit (2016). The effect of dietary supplementation of salts of organic acid on production performance of laying hens. Veterinary World, 9(12), 1478–1484.

den Besten, G., Bleeker, A., Gerding, A., van Eunen, K., Havinga, R., van Dijk, T. H., Oosterveer, M. H., Jonker, J. W., Groen, A. K., Reijngoud, D.-J., & Bakker, B. M. (2015). Short-chain fatty acids protect against high-fat diet–induced obesity via a PPARγ-dependent switch from lipogenesis to fat oxidation. Diabetes, 64(7), 2398–2408.

Diao, Q., Zhang, R., & Fu, T. (2019). Review of strategies to promote rumen development in calves. Animals, 9(8), 490.

Dong, Z., Yuan, X., Wen, A., Desta, S. T., & Shao, T. (2017). Effects of calcium propionate on the fermentation quality and aerobic stability of alfalfa silage. Asian-Australasian Journal of Animal Sciences, 30(9), 1278–1284.

EFSA (2014). Scientific opinion on the re-evaluation of propionic acid (E 280), sodium propionate (E 281), calcium propionate (E 282) and potassium propionate (E 283) as food additives. EFSA Journal, 12(7), 3779.

Fan, Z., Yang, B., Paul Ross, R., Stanton, C., Zhang, F., Sun, J., Zhao, J., Zhang, H., & Chen, W. (2021). Propionate restores disturbed gut microbiota induced by methotrexate in rheumatoid arthritis: From clinic to experiments. Journal of King Saud University – Science, 33(6), 101545.

Garcia, M. V., Garcia-Cela, E., Magan, N., Copetti, M. V., & Medina, A. (2021). Comparative growth inhibition of bread spoilage fungi by different preservative concentrations using a rapid turbidimetric assay system. Frontiers in Microbiology, 12, 678406.

Haghikia, A., Zimmermann, F., Schumann, P., Jasina, A., Roessler, J., Schmidt, D., Heinze, P., Kaisler, J., Nageswaran, V., Aigner, A., Ceglarek, U., Cineus, R., Hegazy, A. N., van der Vorst, E. P. C., Döring, Y., Strauch, C. M., Nemet, I., Tremaroli, V., Dwibedi, C., Kränkel, N., … Landmesser, U. (2022). Propionate attenuates atherosclerosis by immune-dependent regulation of intestinal cholesterol metabolism. European Heart Journal, 43(6), 518–533.

Harrison, P. T. C., Grasso, P., & Badescu, V. (1991). Early changes in the forestomach of rats, mice and hamsters exposed to dietary propionic and butyric acid. Food and Chemical Toxicology, 29(6), 367–371.

Kwak, T. Y., Kim, N. H., & Rhee, M. S. (2011). Response surface methodology-based optimization of decontamination conditions for *Escherichia coli* O157:H7 and *Salmonella typhimurium* on fresh-cut celery using thermoultrasound and calcium propionate. International Journal of Food Microbiology, 150(2–3), 128–135.

Li, H., Zhao, L., Liu, S., Zhang, Z., Wang, X., & Lin, H. (2021). Propionate inhibits fat deposition via affecting feed intake and modulating gut microbiota in broilers. Poultry Science, 100(1), 235–245.

Lieshchova, M. A., & Brygadyrenko, V. V. (2021). Influence of *Lavandula angustifolia*, *Melissa officinalis* and *Vitex angus-castus* on the organism of rats fed with excessive fat-containing diet. Regulatory Mechanisms in Biosystems, 12(1), 169–180.

Lieshchova, M. A., & Brygadyrenko, V. V. (2023b). Effect of *Echinacea purpurea* and *Silybum marianum* seeds on the body of rats with an excessive fat diet. Biosystems Diversity, 31(1), 90–99.

Lieshchova, M. A., Bilan, M. V., Bohomaz, A. A., Tishkina, N. M., & Brygadyrenko, V. V. (2020). Effect of succinic acid on the organism of mice and their intestinal microbiota against the background of excessive fat consumption. Regulatory Mechanisms in Biosystems, 11(2), 153–161.

Lieshchova, M. A., Bohomaz, A. A., & Brygadyrenko, V. V. (2021). Effect of *Salvia officinalis* and *S. sclarea* on rats with a high-fat hypercaloric diet. Regulatory Mechanisms in Biosystems, 12(3), 554–563.

Lieshchova, M. A., Tishkina, N. M., Bohomaz, A. A., Gavrilin, P. M., & Brygadyrenko, V. V. (2018). Combined effect of glyphosphate, saccharin and sodium benzoate on rats. Regulatory Mechanisms in Biosystems, 9(4), 591–597.

Lieshchova, M., & Brygadyrenko, V. (2022). Effects of *Origanum vulgare* and *Scutellaria baicalensis* on the physiological activity and biochemical parameters of the blood in rats on a high-fat diet. Scientia Pharmaceutica, 90(3), 49.

Lieshchova, M., & Brygadyrenko, V. (2023a). Effect of *Rhodiola rosea* rhizomes and *Punica granatum* fruit peel on the metabolic processes and physiological activity of rats fed with excessive fat diet. Food Technology and Biotechnology, 61(2), 202–211.

Lieshchova, M., Logvinova, V., Bilan, M., Bohomaz, A., & Brygadyrenko, V. (2023). Effect of azodicarbonamide on rats with a high-fat hypercaloric diet. Acta Fytotechnica et Zootechnica, 16(1), 15–27.

Moshkin, V. S., & Brygadyrenko, V. V. (2023). Influence of organic acids on locomotor activity of *Stratiolaelaps scimitus* (Mesostigmata, Laelapidae). Biosystems Diversity, 31(3), 401–409.

Pongsavee M. (2019). Effects of 3300 del A-1061 Ter BRCA1 frameshift mutation and calcium propionate on oxidative stress and breast carcinogenesis. International Journal of Molecular Epidemiology and Genetics, 10(3), 47–52.

Quiles, A., Hernando, I., Pérez-Munuera, I., & Lluch, M. Á. (2006). Effect of calcium propionate on the microstructure and pectin methylesterase activity in the parenchyma of fresh-cut Fuji apples. Journal of the Science of Food and Agriculture, 87(3), 511–519.

Rathert-Williams, A. R., Salisbury, C. M., Lindholm-Perry, A. K., Pezeshki, A., Lalman, D. L., & Foote, A. P. (2021). Effects of increasing calcium propionate in a finishing diet on dry matter intake and glucose metabolism in steers. Journal of Animal Science, 99(12), skab314.

Sequeira, S. O., Phillips, A. J. L., Cabrita, E. J., & Macedo, M. F. (2017). Antifungal treatment of paper with calcium propionate and parabens: Short-term and long-term effects. International Biodeterioration and Biodegradation, 120, 203–215.

Shayakhmetova, G. M., Kovalenko, V. M., Basovska, O. G., & Vozna, A. V. (2020). Clinical biochemical parameters of healthy adult white male rats blood serum (retrospective assessment). Pharmacology and Drug Toxicology, 2019, 13(6), 428–433.

Suhr, K. I., & Nielsen, P. V. (2004). Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. International Journal of Food Microbiology, 95(1), 67–78.

Tirosh, A., Calay, E. S., Tuncman, G., Claiborn, K. C., Inouye, K. E., Eguchi, K., Alcala, M., Rathaus, M., Hollander, K. S., Ron, I., Livne, R., Heianza, Y., Qi, L., Shai, I., Garg, R., & Hotamisligil, G. S. (2019). The short-chain fatty acid propionate increases glucagon and FABP4 production, impairing insulin action in mice and humans. Science Translational Medicine, 11, eaav0120.

Tobin, D., Vige, R., & Calder, P. C. (2021). The nutritional management of multiple sclerosis with propionate. Frontiers in Immunology, 12, 676016.

Wen, A., Yuan, X., Wang, J., Desta, S. T., & Shao, T. (2017). Effects of four short-chain fatty acids or salts on dynamics of fermentation and microbial characteristics of alfalfa silage. Animal Feed Science and Technology, 223, 141–148.

Yuan, X., Wen, A., Desta, S. T., Dong, Z., & Shao, T. (2016). Effects of four short-chain fatty acids or salts on the dynamics of nitrogen transformations and intrinsic protease activity of alfalfa silage. Journal of the Science of Food and Agriculture, 97(9), 2759–2766.

Zhang, F., Nan, X., Wang, H., Guo, Y., & Xiong, B. (2020). Research on the applications of calcium propionate in dairy cows: A review. Animals, 10(8), 1336.

Zhang, F., Wang, Y., Wang, H., Nan, X., Guo, Y., & Xiong, B. (2022a). Calcium propionate supplementation has minor effects on major ruminal bacterial community composition of early lactation dairy cows. Frontiers in Microbiology, 13, 847488.

Zhang, F., Zhao, Y., Wang, Y., Wang, H., Guo, Y., & Xiong, B. (2022b). Effects of calcium propionate on milk performance and serum metabolome of dairy cows in early lactation. Animal Feed Science and Technology, 283, 115185.

Zhang, X., Wu, X., Chen, W., Zhang, Y., Jiang, Y., Meng, Q., & Zhou, Z. (2017). Growth performance and development of internal organ, and gastrointestinal tract of calf supplementation with calcium propionate at various stages of growth period. PloS One, 12(7), e0179940.