ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 17(2), 67-84
Received 07.01.2026 Revised 22.04.2026
Accepted 21.05.2026 Published 29.05.2026

UDC 636.087.7:615.9:615.454.1
DOI: 10.31548/animal.2.2026.67

ISSN 2706-8331
e-ISSN 2706-834X

Determination of the acute toxicity and irritant effects of a stabilised coenzyme-containing feed additive

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Abstract. The relevance of this study stems from the need to develop and provide toxicological justification for safe feed additives with antioxidant properties for use in animal husbandry. The aim of the study was to determine the acute toxicity and irritant effects of a stabilised coenzyme-containing feed additive supplemented with α-lipoic acid. The acute toxicity of the feed additive was investigated in Wistar rats following intragastric administration at doses ranging from 100 to 6,000 mg/kg, with a 14-day observation period. The irritant effect was evaluated in rabbits by instilling a suspension into the conjunctival sac of the eye, followed by the assessment of the main haematological and biochemical blood parameters. The results demonstrated the absence of mortality in rats administered the feed additive at doses up to 6,000 mg/kg body weight. The LD_{50} value exceeded 5,000 mg/kg, allowing the tested additive to be classified as a slightly toxic or practically non-toxic substance. At the highest doses, some animals exhibited transient gastrointestinal disturbances and mild depression, which resolved spontaneously without intervention. Haematological and biochemical blood parameters remained within the physiological reference range. No evidence of pronounced haemotoxic, hepatotoxic, or nephrotoxic effects was

Suggested Citation:
Mishchenko, V., & Omelian, A. (2026). Determination of the acute toxicity and irritant effects of a stabilised coenzyme-containing feed additive. *Animal Science and Food Technology*, 17(2), 67-84. doi: 10.31548/animal.2.2026.67.

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observed. Sulphydryl group levels indicated preservation of the antioxidant status of the organism. Evaluation of the irritant effect in rabbits revealed only slight transient ocular discharge and mild oedema of the conjunctival mucosa, both of which completely resolved by the second day of the study. No signs of hyperaemia or prolonged irritation were detected. The findings confirm the safety of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid and support its potential for further application in animal husbandry. The obtained results may be used to substantiate the safety and facilitate the introduction of the stabilised coenzyme-containing feed additive with α-lipoic acid into animal husbandry practice, as well as in the development of new functional feed products with antioxidant properties

Keywords: α-lipoic acid; laboratory animals; haemotoxicity; hepatotoxicity; sulphydryl groups; biochemical blood parameters; toxicological assessment; carbohydrate metabolism

Introduction

The intensive development of modern animal husbandry has been accompanied by an increasing demand for safe feed additives capable of supporting metabolic processes, enhancing the resistance of animals to adverse factors, and reducing the detrimental effects of oxidative stress (Hamlin *et al*., 2021). Particular attention has been directed towards biologically active compounds with antioxidant properties, among which α-lipoic acid occupies a prominent position. This naturally occurring sulphur-containing compound participates in the function of mitochondrial enzyme complexes and cellular energy metabolism. According to F. Superti & R. Russo (2024), α-lipoic acid exhibits pronounced antioxidant activity, influences redox processes, and contributes to the regulation of metabolic homeostasis.

Recent studies have identified α-lipoic acid as a compound capable of reducing oxidative stress and protecting cellular structures against damage induced by reactive oxygen species. F. Mozaffarian *et al*. (2022) demonstrated that α-lipoic acid exerted a protective effect against arsenic-induced oxidative stress in isolated rat liver mitochondria, highlighting its important role in maintaining mitochondrial function and protecting cells against the toxic effects of xenobiotics. Similarly, M. Nema *et al*. (2024) reported that α-lipoic acid alleviated the manifestations of acute deltamethrin toxicity in rats by improving biochemical parameters and reducing oxidative stress. The relevance of investigating α-lipoic acid as a component of feed additives is attributable to its potential to maintain the antioxidant status of the organism. In animal husbandry, antioxidant compounds play an important role in preserving the physiological condition of animals, particularly under the influence of technological, nutritional, and environmental stressors (Doboşi *et al*., 2022). O.E. Oke *et al*. (2024) emphasised that dietary modulation of the antioxidant defence system represents an important strategy for maintaining productivity, immune competence, and the health of poultry, especially under technological and environmental stress conditions.

The importance of α-lipoic acid in animal nutrition has also been highlighted by R.M. Anthony *et al*. (2021), who reviewed current evidence regarding its application as a nutraceutical feed supplement for dogs. The authors concluded that α-lipoic acid possesses high biological activity, participates in cellular energy metabolism, and contributes to the maintenance of antioxidant status in animals. These findings support the potential use of α-lipoic acid as a component of feed additives for various animal species. Nevertheless, the inclusion of biologically active substances in

feed additives requires comprehensive toxicological evaluation. Even naturally occurring compounds may induce functional alterations when administered at high doses or under specific conditions of use. C.M. Zwickl *et al.* (2022) emphasised that acute toxicity assessment is an essential stage in determining the hazard profile of chemical substances and that modern toxicological evaluation should integrate both experimental and predictive approaches. Consequently, the determination of the acute toxicity of feed additives is a necessary prerequisite for their practical application.

Particular importance should also be attached to evaluating the effects of the investigated preparations on haematological and biochemical blood parameters, as these reflect the functional status of the liver, kidneys, haematopoietic system, and protein metabolism. Accurate interpretation of these parameters in laboratory animals requires consideration of species-, age-, and sex-related differences. Recent studies have shown that the establishment of species-specific reference intervals is essential for the correct assessment of the physiological status of rabbits and for the interpretation of toxicological findings (Strong-Townsend *et al.*, 2024). Clinical investigations have likewise reinforced the scientific interest in α-lipoic acid as an antioxidant compound. A. Divković *et al.* (2023) evaluated the effects of α-lipoic acid on oxidative stress and lipid metabolism in women with low-grade cervical intraepithelial lesions. Although this clinical study was conducted in humans and is not directly related to feed additives, it further supports the scientific interest in α-lipoic acid as a biologically active compound with potential antioxidant properties.

Nevertheless, information regarding the toxicological characteristics of stabilised coenzyme-containing feed additives based on α-lipoic acid remains limited. In particular, insufficient data are available on their acute toxicity, effects on the clinical condition of animals, haematological, biochemical, and antioxidant blood parameters, as well as their potential irritant effects on mucous membranes. Thus, a comprehensive evaluation of the acute toxicity and local irritant effects of such a feed additive is of considerable importance for both toxicology and animal nutrition. An essential aspect of the safety assessment of novel feed additives is the investigation of their effects on the oxidative status of the organism. It is well established that disruption of the balance between the generation of reactive oxygen species and the activity of the antioxidant defence system may result in damage to cellular membranes, proteins, and nucleic acids. According to A. Divković *et al.* (2023), administration of α-lipoic acid was associated with improved antioxidant defence and a reduction in oxidative stress, further confirming its biological activity and potential for wider application. The authors also reported that α-lipoic acid is capable of modulating lipid peroxidation and maintaining the cellular redox balance.

Furthermore, contemporary toxicological studies emphasise the importance of evaluating the effects of biologically active substances not only on the clinical condition of animals but also on biochemical blood parameters, which serve as sensitive indicators of the functional status of the organism. Consequently, the determination of total protein concentration, transaminase activities, and indicators of nitrogen and carbohydrate metabolism constitutes an important component of the toxicological characterisation of novel feed additives. Such investigations provide a more objective assessment of the safety of these preparations and facilitate the prediction of potential risks associated with their practical application in animal husbandry. A new feed additive containing stabilised α-lipoic acid has been developed at the Research Institute of Food Technologies and Animal Husbandry of Bila Tserkva National Agrarian University. In accordance with current regulatory requirements, the introduction of new feed additives requires evaluation oftheir

toxic effects on animals (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) *et al*., 2017).

The aim of the present study was to determine the level of acute toxicity and assess the irritant potential of a stabilised coenzyme-containing feed additive based on α-lipoic acid. The scientific novelty of the study lies in the comprehensive evaluation of the toxicological properties of this stabilised coenzyme-containing feed additive, including its acute toxicity, local irritant effects, effects on the clinical condition of laboratory animals, and its influence on haematological and biochemical blood parameters.

Materials and Methods

Preclinical studies evaluating the toxicity of the stabilised coenzyme-containing feed additive were conducted in the vivarium and laboratories of the Institute of Animal Husbandry and Food Technologies at Bila Tserkva National Agrarian University during 2024-2025. The acute toxicity of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid was investigated using 11-week-old female Wistar rats (non-pregnant) with a body weight of 192.0-197.0 g. The irritant effects of the feed additive were evaluated in male California rabbits weighing 2.6-2.7 kg. During the experiments, the air temperature in the quarantine and procedure rooms for laboratory animals was maintained at 17-18 °C, relative humidity at 57-64%, and illumination at cage level ranged from 305 to 320 lux.

The acute toxicity study of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid consisted of two stages: a preliminary experiment involving three groups of animals and an extended experiment involving five groups. Each group comprised five rats. Animals were allocated to experimental groups by random selection. Before the experiments, all laboratory animals underwent a quarantine period in accordance with the relevant requirements. With unrestricted access to fresh drinking water, the animals were fasted for 18 hours prior to the start of the experiment. Each rat was weighed individually before treatment. A suspension of the feed additive containing α-lipoic acid was administered as a single intragastric dose, either as a single administration or divided into fractions. The suspension was prepared using a freshly prepared 1% starch solution, with a final concentration of the active substance of 30 mg/cm^3. A stainless-steel gastric gavage tube was used for intragastric administration. The acute toxicity study was performed in two consecutive stages.

During the first stage (the preliminary experiment), the rats received the feed additive intragastrically at doses of 100, 500, and 5,000 mg/kg body weight. Progression to the second stage of the study was permitted only in the absence of mortality and pronounced clinical signs of toxicity, including prolonged depression, convulsions, impaired motor coordination, or persistent gastrointestinal dysfunction, within 24-48 hours after administration of the highest dose. Under these conditions, the maximum dose was considered sufficiently safe for the extended study, in which doses of 2,000, 3,000, 4,000, 5,000, and 6,000 mg/kg body weight were evaluated. The rats were observed for 14 days following intragastric administration of the feed additive. Throughout the observation period, the animals were examined daily for general clinical condition, locomotor activity, behavioural responses, motor coordination, responsiveness to external stimuli, feed and water consumption, coat condition, respiratory rate, the occurrence of diarrhoea, convulsions, paresis, and any other signs of intoxication. Body weight was recorded immediately before treatment and again at the end of the observation period. During the first 24 hours, the animals were monitored continuously, after which observations were conducted at 8-hour intervals. Standard compound feed was returned to the cages 6 hours after administration of the feed additive, while fresh drinking water was available throughout the study.

The assessment of the irritant effects of the feed additive suspension was performed using four clinically healthy rabbits that had completed the quarantine period. Two drops of the suspension were instilled into the conjunctival sac of the left eye of each animal using sterile pipettes. Following instillation, the nasolacrimal duct of each rabbit was occluded for 85-90 seconds. The right eye of each rabbit served as the untreated control. Ocular irritation was evaluated one hour after instillation based on the severity of ocular changes, including conjunctival hyperaemia, oedema (chemosis), ocular discharge, and corneal lesions. Each parameter was scored according to the recommendations of OECD Test Guideline 405 (OECD, 2023): 0 = no changes; 1 = slight changes; 2 = moderate changes; and 3-4 = marked or severe changes, depending on the parameter assessed. Ocular examinations were subsequently performed once daily for the following 13 days.

The acute toxicity of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid was determined in accordance with OECD Test Guideline 423, Acute Oral Toxicity – Acute Toxic Class Method (OECD, 2002). On Day 14 of the experiment, blood samples were collected from the animals for haematological and biochemical analyses. Blood sampling was carried out in the morning (08:00-10:00). In rats, blood was collected from the tail vein, whereas in rabbits it was obtained from the marginal ear vein. Between 2 and 4 cm^3 of blood was collected into sterile tubes. Following centrifugation, the serum was separated and used for subsequent biochemical analyses. All determinations were performed using standard reagent kits and laboratory equipment according to the manufacturers' protocols.

The total protein content in the animals' blood serum was determined using the method of O.H. Lowry *et al*. (1951). The content of protein, low-molecular-weight and total thiol groups in the blood serum was determined using the method of G.L. Ellman (1959). The urea content in blood serum was determined by the enzymatic urease method using the Humalyzer biochemical analyser, BioSystems, Cobas. Glucose levels were determined using the glucose oxidase-peroxidase method of R. Trinder (1969), whilst the activity of aminotransferases in the blood serum of the animals was investigated according to the method of S. Reitman & S. Frankel (1957). The haemoglobin content in the blood of rats and rabbits was determined by spectrophotometry in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI), specifically document H15, which specifies the cyanmethaemoglobin reference method as the standard for the quantitative determination of haemoglobin in blood. The method is based on the conversion of all forms of haemoglobin into stable cyanmethaemoglobin, followed by photometric measurement of the solution's optical density at a wavelength of 540 nm. Calibration and quality control were carried out in accordance with the requirements of CLSI C46 (CLSI, 2009), which ensures the standardisation of measurements and the reproducibility of results in clinical laboratory studies.

All experimental studies were conducted in accordance with current methodological approaches and in compliance with the relevant requirements and standards. Animal husbandry and all procedures were carried out in accordance with the provisions of the Regulations on the Conduct of Animal Experiments by Scientific Institutions (Law of Ukraine No. 249, 2012) and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986).

Statistical analysis of the research results was carried out using Microsoft Excel 2021 and Statistica 10.0 software. The arithmetic mean (M) and the standard error of the mean (m) were determined. The significance of differences between indicators was assessed using the Student's t-test. A difference was considered statistically significant at $p < 0.05$.

Results and Discussion

The study was conducted in two stages – a preliminary experiment and a full-scale experiment – which allowed the potential toxicity of the feed additive to be assessed across a wide range of concentrations. According to the preliminary experiment, it was found that the administration of the feed additive at doses of 100 and 500 mg/kg body weight did not result in mortality among the experimental animals (Table 1).

Table 1. *Observation data for rats during the determination of the acute toxicity of the α-lipoic acid feed additive (preliminary experiment, n = 15)*

Number of rats in the group	Dose, mg/kg body weight	Number of rats that died		
		total	Expressed as %	Time of death recorded
5	100	0	0	-
5	500	0	0	-
5	5,000	0	0	-

Source: compiled by the authors

When rats were fed a dietary supplement at a dose of 100 t 500 mg/kg body weight, their behaviour was consistent with physiological norms. After the feed was placed in the cages, the animals consumed the feed and drank water periodically. Their response to external stimuli was appropriate. No disturbances in gastrointestinal function were observed.

Throughout the entire observation period, no clinical signs of intoxication, behavioural abnormalities or changes in physiological status were recorded in the rats. Nor was any time of death recorded for the animals, confirming the absence of acute toxic effects of the preparation even at the maximum dose used in the preliminary experiment – 5,000 mg/kg body weight. Gastrointestinal disturbances were observed in these rats during the first 24 hours of the study. Feed intake returned to normal on the second day of the experiment. The results of this preliminary experiment formed the basis for a full-scale study using even higher doses of the feed additive. In the full-scale experiment, α-lipoic acid was administered intragastrically to rats at doses of 2,000, 3,000, 4,000, 5,000 and 6,000 mg/kg body weight (Table 2).

Table 2. *Observation data for rats during the determination of the acute toxicity of the α-lipoic acid feed additive, (extensive experiment n = 25)*

Number of rats in the group	Dose, mg/kg body weight	Number of rats that died		
		total	Expressed as %	Time of death recorded
5	2,000	0	0	-
5	3,000	0	0	-
5	4,000	0	0	-
5	5,000	0	0	-
5	6,000	0	0	-

Source: compiled by the authors

The observational findings demonstrated that none of the rats died following administration of the investigated feed additive, irrespective of the dose administered. The mortality rate was 0% in all experimental groups, and no deaths were recorded throughout the observation period. These findings indicate that even the highest dose of 6,000 mg/kg body weight did not induce acute toxic effects in the laboratory animals. No behavioural or ethological

changes were observed in rats receiving doses of 2,000-3,000 mg/kg during the 14-day observation period. Clinical examination following administration of the feed additive revealed no alterations in behavioural responses, motor coordination, coat condition, respiration, or feed and water consumption. The animals remained active, responded appropriately to external stimuli, and maintained normal locomotor activity. The absence of central nervous system depression, convulsions, paresis, diarrhoea, or other signs of intoxication indicates good tolerability of the feed additive in rats. Gastrointestinal disturbances were observed in three animals that received 4,000 mg/kg of the feed additive. All rats administered doses of 5,000 or 6,000 mg/kg developed diarrhoea accompanied by mild depression during the first 24 hours of the experiment.

The absence of mortality in all experimental groups indicates that the median lethal dose (LD_{50}) of the investigated feed additive exceeds 5,000 mg/kg body weight. According to widely accepted toxicological classifications, these findings indicate that the preparation has a low level of toxicity and may be classified as a slightly toxic or practically non-toxic substance. Thus, the results of both the preliminary and extended experiments confirm the safety of the α-lipoic acid-based feed additive following a single intragastric administration to albino rats. The findings demonstrate the absence of acute toxic effects, which represents an important criterion in the safety assessment of feed additives and supports their potential for future application in animal husbandry. Following administration of the feed additive at a dose of 2,000 mg/kg body weight, the haemoglobin concentration in the blood of the rats was 138 g/L, which was within the physiological reference range. Administration of the feed additive at a dose of 3,000 mg/kg body weight did not result in a reduction in haemoglobin concentration compared with the animals receiving 2,000 mg/kg (Fig. 1).

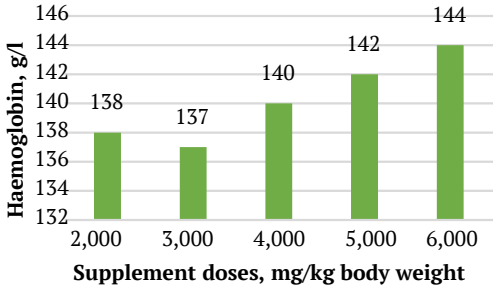

Figure 1. Haemoglobin concentration in the blood of rats administered different doses of the coenzyme-containing feed additive, n = 25
Source: compiled by the authors

Following administration of the feed additive at a dose of 4,000 mg/kg body weight, the haemoglobin concentration in the blood of the rats remained within the physiological reference range. The highest dose of the feed additive (6,000 mg/kg body weight) resulted in a 4.3% increase in blood haemoglobin concentration compared with the group receiving the lowest dose in the extended experiment. Overall, haemoglobin concentrations remained within the physiological range, indicating the absence of suppression of erythropoiesis, anaemic changes, or pronounced haemotoxic effects of the additive. The slight increase in haemoglobin observed at doses of 5,000-6,000 mg/kg may be regarded as a physiological variation rather than evidence of a toxic effect.

The indicators of protein metabolism likewise showed no signs of marked toxic injury. The total protein concentration increased gradually with increasing doses of the feed additive administered intragastrically to the rats. At a dose of 2,000 mg/kg body weight, the total protein concentration in the blood serum was 62.3 g/dm^3. Administration of 3,000 mg/kg resulted in a 2.0% increase in total protein concentration compared with the 2,000 mg/kg group; however, the difference was within the margin of experimental error. No statistically

significant increase in serum total protein concentration was observed in rats receiving 4,000 mg/kg of the feed additive. A tendency towards increased blood total protein concentration was recorded in animals administered 5,000 mg/kg of the feed additive (Table 3).

Table 3. *Protein metabolism parameters in the blood serum of rats during the determination of the acute toxicity of the coenzyme-containing feed additive (α-lipoic acid), n = 25*

Administered dose of the feed additive, mg/kg body weight	Total protein concentration, g/dm³	AST activity, μmol/h/mL	ALT activity, μmol/h/mL	Urea concentration, mmol/dm³
2,000	62.3 ± 2.31	1.02 ± 0.078	0.81 ± 0.0	4.8 ± 0.18
3,000	63.3 ± 1.43	1.05 ± 0.065	0.82 ± 0.0	4.7 ± 0.07
4,000	65.2 ± 1.56	1.12 ± 0.056	0.84 ± 0.0	5.0 ± 0.24
5,000	70.2 ± 2.45	1.21 ± 0.088	0.92 ± 0.0	5.1 ± 0.21
6,000	70.6 ± 2.65*	1.20 ± 0.094	0.93 ± 0.0	5.3 ± 0.28

Note: * refers to the lowest dose of the feed additive

Source: developed by the authors

At the dose of 6,000 mg/kg body weight, a statistically significant increase in serum total protein concentration was observed compared with rats receiving 2,000 mg/kg. However, the increase remained within the physiological reference range and should not be regarded as an adverse finding, as it was not accompanied by marked elevations in liver enzyme activities or serum urea concentration. AST activity in the serum of rats receiving the lowest dose of the feed additive was within the physiological range. Increasing the dose to 3,000 mg/kg body weight produced virtually no change in AST activity. Administration of the feed additive at doses of 5,000 and 6,000 mg/kg body weight resulted in a tendency towards increased AST activity compared with rats receiving 2,000 mg/kg. A dose-dependent trend was also observed for ALT activity, with higher doses of the feed additive being associated with increased serum ALT activity. Nevertheless, these increases remained within the physiological reference range and were not statistically significant.

This moderate increase in enzyme activity at the highest doses may reflect a degree of metabolic loading on the liver; however, it does not indicate pronounced hepatotoxicity, as the observed changes were minor and showed only a moderate dose-dependent pattern. Serum urea concentrations ranged from 4.7 to 5.3 mmol/dm³, indicating no substantial impairment of protein catabolism or renal excretory function. The slight increase in urea concentration observed at the dose of 6,000 mg/kg was not statistically significant compared with animals receiving the lowest dose of the feed additive and may have been associated with increased protein metabolism rather than representing an acute toxic response.

Serum sulphydryl group concentrations remained stable throughout the study. A dose-dependent increase in the concentration of total sulphydryl groups was observed with increasing doses of the feed additive. Administration of the feed additive at doses of 5,000 and 6,000 mg/kg body weight resulted in increases in total thiol group concentrations of 6.4% and 7.1%, respectively, compared with rats receiving 2,000 mg/kg. The differences between the groups represented a trend rather than a statistically significant effect (Table 4).

Table 4. *Sulphydryl group concentrations in the blood serum of rats, μg/cm3, n = 25*

Administered dose of the feed additive, mg/kg body weight	Thiol groups		
	total	protein-bound	free
2,000	15.4 ± 0.58	13.1 ± 0.36	2.3 ± 0.05
3,000	15.6 ± 0.36	13.5 ± 0.13	2.1 ± 0.03
4,000	16.1 ± 0.42	13.7 ± 0.23	2.4 ± 0.04
5,000	16.4 ± 0.28	14.0 ± 0.34	2.4 ± 0.03
6,000	16.5 ± 0.31	14.2 ± 0.38	2.3 ± 0.02

Source: compiled by the authors

Evaluation of the concentration of protein-bound thiol groups in the blood serum of the rats demonstrated a gradual increase with increasing doses of the feed additive. However, no statistically significant differences in sulphydryl (SH) group concentrations were observed between the experimental groups. The concentration of free sulphydryl groups ranged from 2.1 to 2.4 μg/cm^3 and remained essentially unchanged throughout the study. These findings suggest preservation of the antioxidant capacity of the blood serum and the absence of pronounced oxidative stress.

Blood glucose concentration decreased progressively with increasing doses of the feed additive. Following administration of the feed additive at a dose of 2,000 mg/kg body weight, the serum glucose concentration was 6.5 mmol/dm^3. Increasing the dose to 3,000 mg/kg resulted in a 1.5% reduction in serum glucose concentration compared with the group receiving 2,000 mg/kg. In rats administered 4,000 and 5,000 mg/kg of the feed additive, serum glucose concentrations decreased by 9.2% and 12.3%, respectively. Compared with the group receiving the lowest dose, these differences represented a trend rather than a statistically significant effect. A similar trend towards reduced serum glucose concentration was observed in rats receiving the highest dose of the feed additive. Nevertheless, glucose concentrations remained within the physiological reference range at all dose levels (Fig. 2). This trend may be attributable to the metabolic effects of α-lipoic acid, which is known to participate in the regulation of carbohydrate metabolism. As the reduction in glucose concentration was modest, it does not indicate the occurrence of an acute toxic response.

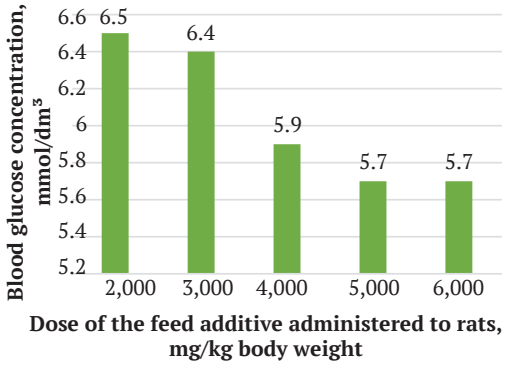

Figure 2. Blood glucose concentration in rats administered different doses of the coenzyme-containing feed additive supplemented with α-lipoic acid, n = 25

Source: compiled by the authors

The findings indicate that administration of the coenzyme-containing feed additive supplemented with α-lipoic acid to rats at doses of 2,000-6,000 mg/kg body weight did not result in marked pathological alterations in haematological or biochemical blood parameters. Thus, administration of the coenzyme-containing feed additive at doses of up to 6,000 mg/kg body weight did not induce pronounced adverse changes in the blood of the rats. These findings are consistent with those reported by R.M. Anthony *et al.* (2021), who described the

good tolerability of α-lipoic acid in animals and the absence of adverse effects on the principal physiological parameters when it is used as a component of feed additives. The evaluated haematological, protein metabolism, enzymatic, antioxidant, and carbohydrate metabolism parameters all remained within the physiological reference range. Collectively, these results indicate that, under conditions of acute administration, the investigated feed additive does not exhibit pronounced haemotoxic, hepatotoxic, or nephrotoxic effects.

Given the absence of evidence of systemic toxicity in the acute toxicity study in rats, the next stage of the investigation focused on assessing the local irritant effects of the coenzyme-containing feed additive. For this purpose, an experiment was conducted in rabbits, which are a widely accepted model for evaluating the local safety of novel feed additives and veterinary medicinal products. Following instillation of the investigated feed additive, all animals exhibited slight ocular discharge during the first day, which was assigned a score of 1 (Table 5).

Table 5. *Harmful effects of the feed additive on the ocular mucosa of rabbits, expressed as scores (n = 4)*

Effect of the feed additive	Eye examination during the experimental period					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 14
			Rabbit I			
Discharge	1	0	0	0	0	0
Oedema	0	0	0	0	0	0
Hyperaemia	0	0	0	0	0	0
			Rabbit II			
Discharge	1	0	0	0	0	0
Oedema	1	0	0	0	0	0
Hyperaemia	0	0	0	0	0	0
			Rabbit III			
Discharge	1	0	0	0	0	0
Oedema	0	0	0	0	0	0
Hyperaemia	0	0	0	0	0	0
			Rabbit IV			
Discharge	1	0	0	0	0	0
Oedema	0	0	0	0	0	0
Hyperaemia	0	0	0	0	0	0

Source: compiled by the authors

A slight oedema of the conjunctival mucosa, corresponding to a score of 1, was also observed in one rabbit. No signs of hyperaemia were detected in any animal throughout the study period. By the second day of the experiment, all clinical signs of irritation had completely resolved. During the subsequent observation period – on Days 3, 4, 5, and 14 – no pathological changes of the ocular mucosa were observed. No ocular discharge, oedema, or redness was detected, indicating complete tissue recovery and the absence of prolonged irritant effects of the feed additive. The rabbits exhibited normal physiological behaviour throughout the study. Feed and water consumption resumed on the first day of the experiment after the animals had settled. The rabbits responded appropriately to sound, light, and tactile stimuli, and their respiratory rate remained within the normal physiological range while at rest.

These findings indicate that the α-lipoic acid-containing feed additive does not exert a pronounced irritant effect on the ocular mucosa of rabbits. The transient changes observed, consisting of slight ocular discharge and mild oedema, resolved spontaneously without intervention. Accordingly, the investigated feed additive may be regarded as safe with respect to its local effects on mucous membranes. On Day 14 of the experiment, haemoglobin concentration was determined in the blood of the rabbits. The highest value was recorded in Rabbit I (153 g/L), whereas the lowest was observed in Rabbit II (137 g/L). These findings indicate normal haematopoiesis and an adequate capacity for oxygen transport to the tissues (Fig. 3).

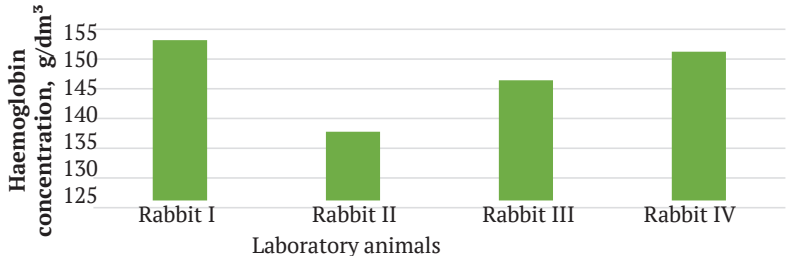

Figure 3. Haemoglobin concentration in the blood of rabbits following exposure to the irritant effects of the coenzyme-containing feed additive (n = 4)

Source: compiled by the authors

The absence of marked fluctuations in haemoglobin concentration confirms that the feed additive did not suppress erythropoiesis or induce the development of anaemic conditions. Analysis of the protein metabolism parameters showed that the highest total protein concentration was recorded in Rabbit I, with values remaining within the physiological reference range. The lowest value was observed in Rabbit IV (52.9 ± 2.32 g/dm³). The difference in total protein concentration between these animals was 7.5% and was not statistically significant (Table 6). These findings indicate a stable protein-synthesising function and the absence of disturbances in metabolic processes.

Table 6. *Protein metabolism parameters in the blood serum of rabbits during the assessment of the irritant effects of the coenzyme-containing feed additive supplemented with α-lipoic acid (n = 4)*

Laboratory animal	Total protein concentration, g/dm³	AST activity, μmol/h/cm³	ALT activity, μmol/h/cm³	Urea concentration, mmol/dm³
Rabbit I	57.2 ± 1.98	0.96 ± 0.054	0.86 ± 0.032	5.3 ± 0.23
Rabbit II	53.2 ± 2.24	1.05 ± 0.043	0.91 ± 0.025	5.4 ± 0.29
Rabbit III	56.3 ± 3.11	1.07 ± 0.054	0.91 ± 0.027	5.6 ± 0.19
Rabbit IV	52.9 ± 2.32	0.95 ± 0.013	0.88 ± 0.036	5.2 ± 0.07

Source: compiled by the authors

The activities of the enzymes AST and ALT also remained stable. AST activity ranged from 0.95 to 1.07 μmol/h/cm³, while ALT activity ranged from 0.86 to 0.91 μmol/h/cm³. The absence of increased transaminase activity indicates that the feed additive did not induce hepatotoxicity or impair the functional integrity of hepatocyte cell membranes. The serum urea concentration in the rabbits ranged from 5.2 to 5.6 mmol/dm³, corresponding to physiological

reference values and indicating normal protein metabolism and the absence of renal dysfunction. The highest value was recorded in Rabbit III (5.6 ± 0.19 mmol/dm³); however, it remained within the physiological range, and the difference from the lowest value was not statistically significant. The concentration of sulphydryl (thiol) groups in the blood serum was likewise stable. Total thiol group concentrations ranged from 16.2 to 17.1 μg/cm³. The difference between the maximum and minimum values was 5.5% and was not statistically significant (Table 7).

Table 7. *Sulphydryl group concentrations in the blood serum of rabbits, μg/cm³ (n = 4)*

Laboratory animal	Thiol groups		
	total	protein-bound	free
Rabbit I	17.1 ± 0.56	15.3 ± 0.43	1.8 ± 0.16
Rabbit II	16.9 ± 0.78	15.0 ± 0.39	1.9 ± 0.14
Rabbit III	16.2 ± 0.73	14.7 ± 0.29	1.5 ± 0.12
Rabbit IV	17.0 ± 0.43	15.1 ± 0.22	1.9 ± 0.09

Source: compiled by the authors

The mean concentration of protein-bound sulphydryl groups in the rabbits was 15.02 μg/cm³. Deviations from the mean value did not exceed 2.5%. The concentration of free thiol groups was within the physiological reference range. These findings indicate preservation of the antioxidant status of the organism and the absence of pronounced oxidative stress following exposure to α-lipoic acid. Blood glucose concentrations in the rabbits ranged from 5.9 to 6.5 mmol/dm³. These values reflect a stable state of carbohydrate metabolism and indicate that the feed additive had no adverse effect on the energy metabolism of the animals (Fig. 4)

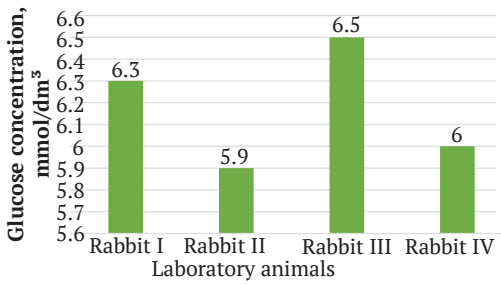

Figure 4. Blood glucose concentration in rabbits during the assessment of the irritant effects of the coenzyme-containing feed additive supplemented with α-lipoic acid (n = 4)

Source: compiled by the authors

The results of the haematological and biochemical analyses of rabbit blood performed during the assessment of the irritant effects of the coenzyme-containing feed additive supplemented with α-lipoic acid indicate that the investigated preparation had no adverse effect on the general physiological condition of the animals. The parameters measured were within the physiological normal range for rabbits and did not indicate the development of pathological processes or toxic injury to the organism. The results of the study into the irritant effect of a feed supplement containing α-lipoic acid on the mucous membrane of the rabbits' eyes indicate a low level of local toxicity. Thus, the results of biochemical and haematological studies confirm that the coenzyme-containing feed supplement with α-lipoic acid does not exert a toxic or pronounced irritant effect on the rabbits' bodies.

The results obtained indicate a low level of acute toxicity of the stabilised coenzyme-containing feed supplement with α-lipoic acid. The absence of fatalities in rats, even when administered the maximum dose of 6,000 mg/kg body weight, indicates good tolerability of the preparation by laboratory animals and is consistent with current understanding of the safety of α-lipoic acid. According to the data

from C.M. Zwickl *et al.* (2022), the assessment of acute toxicity is one of the key stages in the toxicological evaluation of new biologically active substances, and the absence of mortality at high doses indicates a low level of toxic risk associated with the test substance. These findings are consistent with the studies by E.J. Calabrese *et al.* (2023), which showed that α-lipoic acid is capable of reducing the toxic effects of deltamethrin in rats and helping to stabilise biochemical blood parameters. The authors attribute this to the antioxidant properties of α-lipoic acid and its ability to maintain the functional integrity of cell membranes. Similar results were also obtained by S. Rezaei Zonooz *et al*. (2021) and B. Skibska *et al.* (2023), who noted the protective effect of α-lipoic acid under conditions of oxidative stress induced by cypermethrin. The absence of marked changes in haematological parameters in the studies conducted indicates that the feed additive does not have a negative effect on the haematopoietic system. Haemoglobin levels in the blood of rats and rabbits remained within the physiological range, and minor fluctuations in these parameters showed no signs of haemotoxic effects. These results are consistent with the findings of M. Nema *et al*. (2024) and F. Superti & R. Russo (2024), which indicate that α-lipoic acid helps to normalise haematological parameters and mitigates oxidative stress-induced blood disorders caused by toxic agents.

The parameters of protein metabolism and the levels of AST and ALT in the blood serum of rats also did not indicate the development of any pronounced hepatotoxic effects. A slight increase in transaminase activity following the administration of high doses of the supplement was dose-dependent in nature, but did not exceed the limits of physiological variation. This is consistent with the findings of E.M. El-Mancy *et al*. (2022), who demonstrated that α-lipoic acid exhibits hepatoprotective properties and reduces the severity of oxidative stress and apoptosis in the livers of rats following exposure to cyclosporin A. Similarly, F. Mozaffarian *et al.* (2022) found that α-lipoic acid protects the mitochondria of rat livers from arsenic-induced oxidative damage.

The results obtained regarding the stability of sulphhydryl group levels in blood serum indicate that the antioxidant status of the laboratory animals' organisms is maintained. The observed trend towards an increase in the content of total thiol groups when high doses of the feed additive were used may be associated with the activation of the body's antioxidant system under the influence of α-lipoic acid. M. Nema *et al.* (2024) note that α-lipoic acid is a versatile antioxidant capable of functioning in both the aqueous and lipid environments of the cell, as well as participating in the regeneration of glutathione and other antioxidant compounds. The studies conducted revealed a trend towards a reduction in serum glucose levels in rats as the dose of the feed supplement increased. These findings can be explained by the role of α-lipoic acid in regulating carbohydrate metabolism and increasing tissue sensitivity to insulin. Similar properties of α-lipoic acid are described in the work of E.J. Calabrese *et al*. (2023) and A. Gezer *et al*. (2023), where the authors emphasise its important role in the regulation of energy metabolism and cellular respiration processes.

The absence of any marked irritant effect of the feed additive on the rabbits' ocular mucosa confirms its low local toxicity. Short-term manifestations in the form of mild discharge and slight oedema, which had completely disappeared by the second day of the experiment, do not indicate the development of persistent pathological changes. The biochemical parameters measured in the rabbits' blood were within the physiological reference ranges reported in recent studies on healthy rabbits (Strong-Townsend *et al.*, 2024; Khedr *et al.*, 2025), confirming the absence of any adverse effect of the feed additive on the animals' functional status.

The results obtained are consistent with current understanding of the biological properties of α-lipoic acid as a compound with antioxidant and cytoprotective effects. A. Divković *et al*. (2023) report that the use of α-lipoic acid helps to reduce the manifestations of oxidative stress and improve metabolic parameters, confirming the potential for using this substance in biologically active preparations. Thus, the results of the studies confirm the low toxicity and good tolerability of a stabilised coenzyme-containing feed supplement containing α-lipoic acid. The absence of marked pathological changes in clinical status, haematological, biochemical and antioxidant blood parameters indicates the potential for further study and practical application of the feed additive under investigation in animal husbandry.

Conclusions

The results of the present study demonstrated that the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid is characterised by a low level of acute toxicity. Following a single intragastric administration to albino rats at doses ranging from 100 to 6,000 mg/kg body weight, no mortality was recorded, and the median lethal dose (LD_{50}) exceeded 5,000 mg/kg body weight. These findings support the classification of the investigated feed additive as a slightly toxic or practically non-toxic substance according to current toxicological classifications. Clinical observations of the laboratory animals showed that administration of the feed additive was not associated with significant disturbances in general physiological condition, behavioural responses, motor coordination, or central nervous system function. The animals remained active, responded appropriately to external stimuli, and maintained normal feed and water intake. Only at the highest doses of the additive (4,000-6,000 mg/kg body weight) did some rats exhibit transient gastrointestinal disturbances and mild depression during the first 24 hours of the experiment. These effects were temporary and resolved spontaneously without intervention.

The haematological findings indicate that the feed additive had no adverse effect on the haematopoietic system. Haemoglobin concentrations in both rats and rabbits remained within the physiological reference range, and the observed variations showed no evidence of haemotoxicity or the development of anaemia. The slight increase in haemoglobin concentration observed at the highest doses of the additive remained within physiological limits and was most likely associated with adaptive responses of the organism. Biochemical analyses of the blood serum likewise confirmed the absence of marked toxic effects of the investigated additive. Total protein concentration, AST and ALT activities, and urea concentration all remained within the physiological reference range. Although a tendency towards a moderate increase in transaminase activity was observed at the highest doses of α-lipoic acid, these changes were not accompanied by pathological alterations and did not indicate the development of significant hepatotoxic or nephrotoxic effects. Overall, the findings demonstrate good tolerability of the feed additive in laboratory animals.

Assessment of the antioxidant status showed that the concentrations of total, protein-bound, and free sulphydryl groups in the blood serum remained stable throughout the study. The moderate increase in thiol group concentrations observed following administration of the highest doses of the feed additive may indicate activation of the antioxidant defence system and further supports the antioxidant properties of α-lipoic acid. The absence of marked changes in sulphydryl group concentrations suggests preservation of cellular homeostasis and the absence of pronounced oxidative stress. Administration of the feed additive to rats did not cause significant disturbances in carbohydrate metabolism. Blood serum glucose

concentrations remained within the physiological reference range, although a tendency towards a moderate reduction was observed with increasing doses of α-lipoic acid. This finding is likely attributable to the well-established metabolic properties of α-lipoic acid and its role in the regulation of energy metabolism.

The results of the assessment of the irritant effects of the feed additive on the ocular mucosa of rabbits indicate a low level of local toxicity. During the first day following application, slight ocular discharge and mild oedema of the conjunctival mucosa were observed in some animals, each receiving a score of 1. These changes resolved completely by the second day of the experiment. No pathological alterations of the ocular mucosa were detected during the subsequent observation period. The absence of hyperaemia, prolonged irritation, or impairment of the general condition of the animals indicates good local tolerability of the investigated feed additive. Thus, the results of the toxicological, haematological, and biochemical investigations confirm the safety of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid following acute administration to laboratory animals. The investigated additive exhibited no evidence of pronounced haemotoxic, hepatotoxic, nephrotoxic, or irritant effects, supporting its potential for practical application in animal husbandry. Further studies should focus on the evaluation of the chronic toxicity of the feed additive, its effects on the productivity and antioxidant status of farm animals, and the assessment of its efficacy under different livestock production systems.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

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Встановлення гострої токсичності та подразнювальної дії стабілізованої коензимвмісної кормової добавки

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Анотація. Актуальність роботи зумовлена необхідністю розробки та токсикологічного обґрунтування безпечних кормових добавок з антиоксидантними властивостями для використання у тваринництві. Метою роботи було встановлення гострої токсичності та подразнювальної дії стабілізованої коензимвмісної кормової добавки з α-ліпоєвою кислотою. Гостру токсичність кормової добавки досліджували на щурах лінії Wistar після внутрішньошлункового введення у дозах 100-6000 мг/кг із 14-добовим спостереженням, а подразнювальну дію – на кролях шляхом внесення суспензії у кон'юнктивальний мішок ока з подальшим визначенням основних гематологічних і біохімічних показників крові. У результаті досліджень встановлено відсутність летальних випадків у щурів за введення кормової добавки у дозах до 6000 мг/кг маси тіла. Показник LD_{50} перевищував 5000 мг/кг, що дозволяє віднести досліджувану добавку до малотоксичних або практично нетоксичних речовин. За використання високих доз у окремих тварин спостерігали короткочасні розлади функції шлунково-кишкового каналу та незначне пригнічення, які самостійно зникали. Гематологічні та біохімічні показники крові залишалися у межах фізіологічної норми. Встановлено відсутність вираженої гемотоксичної, гепатотоксичної та нефротоксичної дії. Показники сульфогідрильних груп свідчили про збереження антиоксидантного статусу організму. Під час оцінки подразнювальної дії у кролів виявлено лише слабкі короткочасні виділення з ока та незначний набряк слизової оболонки, які повністю зникали на другу добу дослідження. Ознак гіперемії чи пролонгованого подразнення не встановлено. Результати дослідження підтверджують безпечність стабілізованої коензимвмісної кормової добавки з α-ліпоєвою кислотою та перспективність її подальшого використання у тваринництві. Отримані результати можуть бути використані для обґрунтування безпечності та впровадження стабілізованої коензимвмісної кормової добавки з α-ліпоєвою кислотою у практику тваринництва та при розробленні нових функціональних кормових засобів з антиоксидантними властивостями

Ключові слова: α-ліпоєва кислота; лабораторні тварини; гемотоксичність; гепатотоксичність; сульфогідрильні групи; біохімічні показники крові; токсикологічна оцінка; вуглеводний обмін