The effect of a new combined feed on some physiological parameters of laboratory rats

Yurii Boiko¹^{*}, and *Irina* Boiko²

¹Odesa State Agrarian University, Department of Physiology, Pathophysiology and Biochemistry, 65000, Krasnova st., 3a, Odesa, Ukraine

²Odesa National Medical University, Department of Pharmacology and Pharmacognosy, 65082, Valikhovsky Lane 2, Odesa, Ukraine

> **Abstract.** For normal growth, development and vital activity, laboratory animals must receive a complete feed ration. A new feeding ration for rats was proposed, which is based on the use of combined feed PC 120-1. A comparison was made with a standard vivarium ration, which included mostly natural, unprocessed components. To establish the differences between the two rations, changes in the following physiological parameters were studied in experimental animals: body weight, blood composition, blood pressure, and heart rate. It was established that the use of a ration with PC 120-1 combined feed accelerates body weight gain by 5.5%, increases the number of erythrocytes by 23.8%, and hemoglobin by 12.0% compared to animals of the control group. At the same time, under the influence of the applied experimental ration, there was a decrease in blood pressure (by 28%) and heart rate (by 16.6%) in comparison with animals of the control group. Such effects of mixed feed can be explained by a more optimal protein composition and additional addition of a mixture of vitamins and trace elements. Combined fodder is better digested and animals like it better. Recommend further investigation of compound feed PC 120-1 as the main compound feed of vivarium for rats.

1 Introduction

Modern medical and biological research is not possible without the use of laboratory animals. This makes the task of maintaining them a part of experimental scientific work. Feeding laboratory animals is an important component of the process of their maintenance. The nutritional quality of feed for laboratory animals affects their ability to realize their genetically determined potential for growth, reproduction and longevity. Affects the level of resistance to pathogenic microorganisms and environmental stresses. A balanced ration is necessary for the normal life of laboratory animals, and it is important for the researcher to make sure that the experimental results are not influenced by factors caused by nutrition [1, 2]. The nature of the feeding diet of laboratory animals is one of the most uncontrolled parameters that affects the experimental work and leads to differences between the data obtained by different authors in similar studies [3].

⁻ * Corresponding author: yuriyalexb@gmail.com

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Regardless of the significance of the feeding diet of laboratory animals for the final results of research, in scientific works that were performed using biological models, the most common description of the diet is "standard" or "adopted in a vivarium". Laboratory animals need about 50 nutrients in physiological concentrations. The necessary nutrients must not only be contained in the feed, but also be available for assimilation by the animal. The level of assimilation of nutrients will be influenced by the quality of the food, the level of breakdown and absorption in the gastrointestinal tract. Digestion processes depend on the physicochemical characteristics of feed, such as shape, particle size, moisture, contamination, organoleptic qualities and storage conditions.

The amount of nutrients that an animal needs to consume will be influenced by biological features. These include genetic differences between animal species, breed and sex characteristics, and individual qualities that affect nutrient requirements. An example is guinea pigs and monkeys, which lost the ability to synthesize vitamin C due to a genetic mutation due to the absence of L-gulonolactone oxidase, an enzyme that oxidizes L-gulono-1,4-lactone to L -xylo-hex-3-gulonolactone [4, 5]. The activity and expression of Lgulonolactone oxidase does not match both within different species of rodents and within the same species. Enzymatic features differ in different lines of rats and depend on sex [6]. Studies have shown differences in the needs of B vitamins in mice of different lines. Genetically determined differences in growth rate, body size, and intensity of metabolism in different lines and breeds of animals lead to differences in the required amount of protein and amino acids. Nutrient requirements and the nature of the ration change during ontogenesis: they depend on the age of the animal, its physiological status (pregnancy, lactation, level of physical activity). The building of the body's own tissues requires glucose, amino acids, fatty acids, microelements, and a number of other substances, which must be supplied in sufficient quantities with food. For highly productive farm animals, the nature of the ration is the key to obtaining the maximum amount of milk or meat [7, 8]. This principle can be applied to laboratory animals as well, but the corresponding studies have not been carried out so far. Because of this, it is not possible to form scientifically based rations for laboratory animals that would meet their needs at different stages of life, taking into account individual characteristics.

In the generally accepted practice of keeping laboratory animals, it is customary to feed them *ad libitum*, but this type of feeding can lead to the development of obesity, endocrine diseases, pathologies of the cardiovascular system, pathologies of the digestive system, increase mortality and reduce life expectancy [9-12]. The ration of laboratory animals should be dietary. It should be noted that the term "dietary" means not only restrictions on energy nutrition, but also balancing on chemical composition and availability of sufficient quantities of substances necessary for the animal's vital activity. A significant number of studies have shown that the use of dietary rations can reduce the risk of developing neoplasia's, diseases of the excretory system, and endocrine disorders [13-15]. Also, diets with reduced calorie content weaken the effect of stress factors, reduce the development of inflammatory processes and intracellular damage by active forms of oxygen [16-18].

Over the past several decades, there has been a marked segregation of the life spans of laboratory rats. The decrease in life expectancy and increased mortality of laboratory animals seriously complicates long-term research. A low-calorie diet in the case of rats can lead to an increase in the average lifespan of rats by 14-45%, while a similar diet can extend the life of laboratory mice by only 4-27% [19]. In the line of SPF Fischer 344 rats, it was shown that animals maintained on a diet with 60% of calories from *ad libitum* had not only a longer average life span, but also greater absolute life span indicators. Kidney pathologies, interstitial tumours, bile duct hyperplasia, and pathological changes in the myocardium later developed or did not develop in such animals [20].

Despite the convincing data on the positive effect of a low-calorie diet on the vital indicators of laboratory rats, there are some technical difficulties that inhibit the wide implementation of appropriate diets in the practice of vivarium's. Rats are highly social animals and are mostly kept in groups. The unequal status of rats in the formed social group leads to an uneven distribution of feed and nullifies the positive qualities of a low-calorie diet. Part of this problem can be solved by the use of complex automatic feed dispensers, but rats quickly learn to bypass the feed delivery algorithms built into them. A preliminary analysis of the available compound feeds showed that the PC 120-1 compound feed, which was selected for further research, was the most optimal in terms of its chemical composition.

The purpose of this work was to study the effect of the developed ration for laboratory rats on some physiological parameters: body weight, cellular and biochemical composition of blood, arterial blood pressure and heart rate.

2 Materials and methods

2.1 Laboratory animals

All studies were conducted on the basis of the experimental vivarium of the Department of Physiology, Pathophysiology and Biochemistry, Odesa State Agrarian University. Laboratory animals are white male Wistar rats, 3-4 months old, weighing 200-220 grams. Animals were kept at a temperature of 23-24 \degree C, relative humidity 55 \pm 15%, in individual cages measuring 48 x 28 x 20 cm.

There was free access to water. Water was in graduate's polycarbonate drinking dish for rodents. The animals were randomly divided into two groups of 10 rats per group. The first group was an experimental one and received an experimental ration. The second group was a control and received a standard vivarium ration. The period of observation of animals was 2 months.

All the studies corresponded to the regulatory documents adopted at the Odessa State Agrarian University ("Regulations on the maintenance of laboratory animals", order No. 553, dated 02.07.2021), the national legislation of Ukraine and international regulatory documents (European Convention for Protection of Vertebrates Strasbourg, 1986; European Union Directive 2010/63/EU of September 22, 2010, entitled "On protection of animals used for scientific purposes").

2.2 Feed and rations

Two feeding rations were used in the study: an experimental and a standard vivarium ration.

The standard feeding ration included: wheat grain (2.88 g), corn grain (1.15 g), sunflower seeds (1.15 g), peas (0.57 g), oat grain (0.57 g), hay (1.33 g), fish (5 g), carrots (1 g) , beets (0.78 g) , a mixture of white and black bread 1:1 (4.33 g) , sodium chloride (0.13 g) g). The experimental feeding ration included: 15 g of compound feed PC 120-1, carrots (1 g), beets (0.78 g). The daily portion sizes are indicated for both rations. Food was given twice a day, in equal portions. The total calorie content of both rations reached 22.4 kcal/animal.

Combined feed PC 120-1 was obtained from the company "Rezon-1" and was made from: grain, soybean cake, sunflower cake, wheat cake, wheat bran, milk powder, vegetable oil, fodder yeast, fish meal, vitamin and mineral complex (manufacturer's data). In terms of chemical composition, compound feed PC 120-1 included Table 1. The appearance of the feed is presented in Fig. 1.

are per 100 g of feed (manufacturer's data).

Fig. 1. Appearance and size of PC 120-1 feed granules.

2 2.3 Study of physiologic al parameter rs

All animals were weighed every week throughout the study period. TVE-1.5-0.01 scales, manufacturer "Technovag", Ukraine, were used for weighing. Cytological analysis of blood composition was performed by microscopy of blood smears. Blood smears were obtained by applying peripheral blood from tail capillaries to glass, followed by staining with a ready-made set of dyes "LDF 200", produced by Erba Lachema s.r.o., Germany.

glucose) were determined on a BA-88A biochemical analyzer, manufactured by Mindray, China. Test systems manufactured by DAC-Spectromed SRL, Moldova, were used for determination. Heart rate were measured using a non-invasive blood pressure system, CODA monitor, Kent Scientific, USA. Determination of the amount of total daily water consumption was carried out by measuring the change in the volume of water in drinking dish. The relative daily water consumption was determined by dividing the total daily water c consumption b by the animal's body weight. Biochemical indicators of blood plasma (total protein, albumins, urea, total cholesterol,

2 2.4 Statistica al analysis

average values. Checking the data for normality of distribution was performed using the Kolmogorov-Smirnov test and the Shapiro–Wilk test, since the data distribution was normal, parametric methods of analysis were subsequently applied. The significance of the differences between the mean values was assessed by Student's t test. Means were considered to be different at $P<0$. 05. Calculations about vehicles in the Excel application, M Microsoft 365 . Statistical processing was carried out with the calculation of average values and errors of

3 3 Results a and discus ssion

The set of live weight is one of the main indicators, thanks to which it is possible to evaluate the nutritional quality of feed and its compliance with the animal's physiological needs. Data on changes in the live weight of animals of the experimental and control groups a are shown in F Fig. 2.

Fig. 2. Parameters of live weight of animals during the experiment. Measurements were made at the end of each day of the experiment $(* - P < 0.05; ** - P < 0.01; *** - P < 0.001).$

During the entire period of the experiment, there was a progressive set of live weight by the animals of both groups. Animals of the experimental group increased their weight during the experiment by an average of 55%, animals of the control group by 49.5%. On the fourth day of the experiment, live weight gain was faster in animals of the control group $(268.1\pm1.91 \text{ g}; 255.7\pm3.45 \text{ g}; P < 0.01)$, but later, starting from the sixth week, more a rapid increase in live weight was observed in the experimental group. At the end of the experiment, the average difference in live weight between the animals of the experimental and control groups was 5.5% (P < 0.001).

All animals belonged to young animals in the active phase of growth, which with proper feeding quickly gain body weight with the formation of mainly connective tissues (muscle, bone, fat). Nutrient characteristics of feed are important for ensuring the processes of growth and development, gain of live weight. At the same time, the wrong ration can lead to the development of obesity and a decrease in the percentage of muscle tissue [21, 22].

Faster body weight gain by the animals of the experimental group compared to the animals of the experimental group with the same energy value of the feed indicates a more effective assimilation of nutrients from the combined feed of the experimental ration. A sufficient intake of vitamins is also necessary for the growth and development of the animal organism. It was established that vitamins A and E [23, 24] affect cell division and overall growth of the organism, and vitamin B12 is also necessary for the processes of cell replication, which affects not only the growth of young rats, but is also a factor in their survival [25]. The standard ration of the vivarium is balanced in terms of the amount of vitamin substances, but due to the use of natural components, the chemical composition of which is variable, the final amount of vitamins in it may vary. A decrease in the amount of vitamins can become a factor that negatively affects the development of animals and, as a result, on gaining live weight. The experimental compound feed is free from this drawback, since a complex of necessary vitamins is artificially added to it in the required amount.

The amount of poorly or indigestible substances that form the basis of roughage, such as cellulose, hemicelluloses, lignins, is reduced in compound feed, which can increase its bioavailability and positively affect the level of digestibility. Rough components of food for rats are not as important as for other rodents, such as rabbits or guinea pigs, so their proportion can be reduced.

For the nature of gaining live weight, the ratio between caloric content and the amount of proteins is important. Increased caloric content with a decrease in protein intake leads to increased deposition of adipose tissue [26]. With the same energy value of rations, a ration with a lower level of protein absorption can lead to obesity against the background of a decrease in the percentage of muscle tissue in the body structure.

The high nutritional quality of the ration can have a positive effect on the physiological parameters of the animal, only when the animal likes the food and does not refuse its use. It was noticed that uneaten fragments of coarse feed regularly remained in the feeders of animals of the control group, less often a small amount of grain feed remained. In the animals of the experimental group, no uneaten remains of the experimental combined feed were observed. From this it is possible to conclude that the animals liked the combined feed more than the standard ration, this was an additional factor that increased its nutritional value for the animals.

The blood system is the most dynamic of all physiological systems of the body. The blood system quickly reacts to changes in the influence of external and internal factors, which gives it a special status in the diagnostic process. Haematological and biochemical parameters of the blood of rats are given in Table 2 and Table 3.

 $*$ - P < 0.05

Table 3. Biochemical parameters of blood serum samples of rats at the beginning and at the end of the experimental period.

Parameter	Before the start of the experiment		At the end of the experiment	
	Experimental	Control	Experimental	Control
Total protein, g/L	63.4 ± 4.13	65.2 ± 5.63	64.7 ± 3.26	$75.1 \pm 2.87*$
Albumins, g/L	42.8 ± 2.71	44.7 ± 3.67	41.6 ± 2.91	$51.9 \pm 2.54*$
Urea, mmol $/L$	3.56 ± 0.31	3.72 ± 0.44	3.69 ± 0.16	3.64 ± 0.23
Total cholesterol,	1.56 ± 0.13	1.44 ± 0.23	1.63 ± 0.16	1.59 ± 0.21
mmol/L				
Glucose, mmol/L	5.48 ± 0.08	5.31 ± 0.02	5.62 ± 0.06	5.55 ± 0.11

 $*$ - P < 0.05

Before the start of the experiment, there was no difference in the blood parameters of the animals of both groups. After the end of the experiment, significant differences in the number of leukocytes and erythrocytes were recorded between the animals of the experimental and control groups. An increase in erythrocytes was recorded in the animals of the experimental group by 23.8% (P<0.05) compared to the animals of the experimental group, the number of leukocytes was reduced by 22.5% (P<0.05), respectively. Haemoglobin content in animals of the experimental group was 12.0% (P<0.05) higher than in animals of the control group. Among the biochemical indicators, an increase in total protein (by 16.0%, $P \le 0.05$) and albumins (24.76%, $P \le 0.05$) of blood serum was recorded in animals of the experimental group compared to animals of the control group.

The authors [27] indicate the difference in the number of erythrocytes and haemoglobin content, which may be due to the different age and sex of the animals, as well as to which line they belong. At the same time, the work shows the dependence of the indicated blood parameters on the nature and ration of feeding. Frequent cases of the development of anaemic states are noted due to alimentary insufficiency of general protein or vitamins or microelements, or a combination of the lack of these feed components. The authors note that it is the alimentary factor that is the cause of many literary discrepancies in indicators of blood composition of laboratory animals [27]. The experimental ration used by us led to an increase in the number of red blood cells in rats. This may be associated to the strengthening of erythropoietic processes in the red bone marrow against the background of balanced feeding. An increase in the haemoglobin content of the blood was also recorded in the animals of the research group, which may be evidence of increased intake and assimilation of calcium and iron by the body of rats.

In work [28] it was shown that calcium and iron deficiency are the main causes of alimentary anaemia and a decrease in haemoglobin content. Other trace elements did not have a significant effect on the production of erythrocytes and haemoglobin in the bone marrow, so they are not considered critical for the process of haematopoiesis.

The increased protein value of the experimental ration is confirmed by the increase in the total protein of the blood serum and the increase in the albumin content. It should be noted that in addition to the total amount of proteins supplied with feed, their qualitative composition is important for further protein metabolism. Feed proteins must contain a sufficient amount of essential amino acids. The standard ration of the vivarium included fodder of animal origin in the amount of \sim 25% by weight, but their digestibility was lower than the protein components of the combined feed from the experimental ration.

Both applied diets did not lead to an increase in the serum concentration of glucose. The importance of the connection between the diet and the level of glucose in the blood is now beyond doubt [29]. A significant number of diets have been developed that can provoke the development of diabetes or worsen the condition of animals that already suffer from this pathology [30, 31]. The formation of type 2 diabetes in rats is possible with diets containing a high percentage of fats (60%) and a low percentage of carbohydrates (10%). A decrease in glucose tolerance with such a diet is observed after 2 months, and the development of diabetes mellitus is possible after 16 months [32]. A high-protein, low-carbohydrate diet can, conversely, reduce glycaemic tolerance and body weight in both healthy and diabetic rats [33]. The concentration of glucose in the blood of animals of both groups, both at the beginning and at the end of the experiment, did not exceed 5.2-5.7 mmol/L, which can be evidence of the optimal ratio between carbohydrates, fats and proteins in the proposed diets.

An elevated level of cholesterol in blood plasma is a factor that increases the risk of developing cardiovascular diseases [34], diabetes [35], obesity [36], gallstone disease [37]. The concentration of cholesterol in the blood serum of animals of both groups was in the range from 1.44 to 1.63 mmol/L and did not change under the influence of the studied diets. Maintaining the serum cholesterol concentration within physiological limits is a significant positive feature of both diets. The pathogenesis of diseases that develop as a result of hypercholesterolemia is a complex multistage process. In the case of cardiovascular diseases, elevated cholesterol levels can directly cause the development of atherosclerosis [38] and hypertension [39], or contribute to the development of diseases (diabetes, metabolic syndrome, obesity), which will later trigger the development of these cardiovascular pathologies. An increase in the level of total cholesterol and a decrease in the level of high-density lipoprotein cholesterol are components of hypercholesterolemia. Hypercholesterolemia can be primary, which is caused by internal metabolic processes, and secondary, which occurs in response to the action of external factors. It is possible to correct secondary hypercholesterolemia thanks to a properly selected diet.

The use of both diets keeps the serum concentrations of glucose and total cholesterol within the physiological norm, this allows you to avoid the development of a number of diseases: atherosclerotic disease, hypertension, metabolic syndrome, obesity, diabetes, gallstone disease.

The amount of sodium chloride in the ration is a factor that significantly affects the level of blood pressure and, with excessive intake, can lead to the development of hypertension. But different animals, even if they belong to the same line, can have significantly different susceptibility to the hypertonic effect of salt [40]. When preparing the ration, the upper threshold was chosen for the salt content in the feed. Thus, the possible development of hypertension would be determined not only by the individual properties of the body, but also by other components of the ration. Indicators of arterial blood pressure and heart rate in animals of both groups are presented in Table 4.

 $*$ - P < 0.05

Before the start of the experiment, there were no differences in blood pressure and heart rate between the animals of the experimental and control groups. At the end of the experiment, an increase in blood pressure was recorded in the animals of the control group (by 23.6% systolic, 33.6% diastolic, P<0.05) in comparison with the animals of the experimental group. The frequency of heart contractions in animals of the control group exceeded the corresponding indicator in animals of the experimental group by 16.6% $(P<0.05)$.

Since both diets contained the same amount of salt, and the animals belonged to the same line and were randomly divided into groups, it is possible to exclude the influence of individual sensitivity on the development of hypertension and tachycardia. It is believed that an increase in the amount of salt above 0.4% in the ration will lead to hypertensive phenomena in favourable animals [41]. In our study, the phenomena of hypertension (increase in systolic pressure over 140 mmHg) was not observed in both groups, that is, both rations do not lead to an increase in blood pressure. It is a non-trivial task to determine exactly which mechanism made it possible to lower blood pressure and heart rate in the animals of the experimental group when using the experimental ration. For example, rations with different contents of protein, carbohydrates, and fats can affect the tone of the sympathetic nervous system, leading to significant fluctuations in hemodynamic parameters [42].

An increase in the absolute daily consumption of water is observed in both groups of animals when comparing the volumes of consumption before the beginning and after the end of the experimental period. Absolute water consumption increased by 36.6-70.2% on average.

First of all, such an increase is due to the increase in the body weight of experimental animals and the associated increase in metabolic water needs. Indicators of relative water consumption changed significantly only in the control group of animals at the end of the experimental period and were 24.4% more compared to similar indicators of animals in the experimental group (P<0.05). It should be noted that the increased consumption of water caused by a salt diet, even against the background of preserving the normal filtering function of the kidneys, is an important cause of the development of hypertension. The increased intake of water and salt leads not only to an increase in the volume of the total intracellular and extracellular hydrogen compartments, but also causes an increase in the volume of blood plasma. Hypervolemia, which develops at the same time, is one of the most powerful factors in the development of long-term hypertension.

Searching for optimal feed rations is a complex task that requires assessment of many factors. In a similar work by American authors, it was shown that a faster increase in body weight per energy unit of the ration is not yet sufficient for an unequivocal conclusion about the quality of the proposed ration [43]. In the future, for a more detailed analysis of the influence of the proposed ration on the vital activity of rats, it is necessary to conduct additional studies on other age groups, as well as to expand the number of studied physiological, biochemical and functional indicators.

4 Conclusion

The use of different feed rations when feeding laboratory animals in vivarium conditions can significantly affect their physiological indicators and critically change the results of experimental work. Our proposed new ration for feeding laboratory rats, which is based on the use of combined feed PC 120-1, made it possible to accelerate the growth and development of animals (increase in live weight) compared to the standard vivarium ration, which included a combination of natural feeds: grains, fish, root crops.

When using PC 120-1 combined feed, an increase in the number of erythrocytes and the hemoglobin content in the blood of experimental animals was recorded, and the content of total protein and albumin in the blood serum increased. Also, with the same salt content in both rations, animals fed PK 120-1 compound feed had more optimal hemodynamic parameters (blood pressure and heart rate).

Based on the obtained data, it is possible to conclude that, compared to the standard ration, combined feed PC 120-1 is a more accessible source of amino acids and is superior to it in terms of vitamin and mineral composition. Thus, it is necessary to recommend the use of compound feed PC 120-1 as the basis of a balanced ration for laboratory rats.

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