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## Productivity of germinative duck chimaeras and their descendants

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**Abstract.** The relevance of the study is conditioned upon the necessity to explore the possible influence of chimerisation on the productivity of germinative duck chimaeras and their descendants. To obtain duck chimaeras, the method described by Aige-Gil & Simkiss and Tagirov was applied. Shanma duck embryos were used as recipients and Shaoxin duck embryos homozygous for the plumage colour gene (wild type) were used as donors. To evaluate the egg production of germinative chimaeras of ducks, the analysis of experimental animals and their control counterparts was performed. Analysis of the age of sexual maturation (laying the first egg) indicates that the chimaeras matured later. While in the control group the average age of puberty was 139±9 days, in the chimaera group it was 148±13 days. Thus, it can be concluded that in this

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experiment chimaeras matured later than control animals, which may be related to the effect of busulfan during the sterilisation of recipient embryos. The average live weight of ducks in the control group was lower, and the group itself was more united. Thus, in control ducks, the weight was  $1422.40 \pm 57.00$  g, and in chimaeras –  $1608.80 \pm 94.76$  g. The advantage of chimaeras over the control group in terms of live weight may be related to the fact that the control group consisted of recipients of the Shanma breed. Egg production of ducks for the entire research period was  $87.5 \pm 0.05\%$  (control) and  $79.5 \pm 0.12\%$  (busulfan). The weight of eggs in ducks of two groups for the entire period was:  $70.62 \pm 0.199$  g (control) and  $71.15 \pm 0.157$  g. Morphometric parameters of eggs of the researched groups of ducks: average values of egg length –  $6.056 \pm 0.0564$  cm (control) and  $6.269 \pm 0.1341$  cm (busulfan); egg width –  $4.520 \pm 0.0053$  cm (control) and  $4.529 \pm 0.004$  cm (busulfan). There were no statistical intergroup differences in the morphometric parameters of the eggs of the research groups. Analysis of the productivity of daughters of germinative duck chimaeras demonstrates that, in general, the chimerisation of parents did not affect the productivity of their daughters. The analysis of the productivity of the group of daughters obtained from chimeric animals demonstrates that, by most indicators, this group occupies an intermediate place between the groups whose breeds served as donors and recipients. The method author of the research uses to obtain chimaeras is of practical value for the conservation of genetic resources

**Keywords:** germinative chimaera, Shaoxing duck, shanma, busulfan, egg productivity of ducks

## Introduction

Due to its high reproductive potential, short intergeneration interval, and embryonic development outside the mother's body, poultry provides unique opportunities for its use in fundamental and applied biological research (Mozdziak & Petite, 2004; Kagami, 2016). The methods of cloning and transgenesis have become a routine tool for designing animal models of development (Kathleen *et al.*, 2010), diseases (Ogilvie *et al.*, 2017), bioreactors and producers of valuable biologically active drugs (Petitte & Mozdziak, 2002; Pavlou & Reichert, 2004), highly productive aquaculture objects (Devlin *et al.*, 2009). However, using the conventional technique for microinjection of foreign DNA into the pronucleus of a fertilised egg, which is well-developed for many mammalian species

(Gordon & Ruddle, 1981), encounters difficulties when involved with birds (Perry, 1988; Love *et al.*, 1994). The development of a transgenic bird is complicated by the structure of its opaque egg cell with a large yolk and the unique reproductive system of this class. Direct microinjection of DNA into the oocyte, which is frequently used in mammals, is practically impossible for birds since fertilisation occurs in the infundibulum of the reproductive tract and can be polyspermic (Mozdziak & Petite, 2004). Therefore, manipulation of the zygote proved difficult to use in the development of a transgenic bird (Love, 1994). Over the past decades, some alternative strategies have been developed to obtain transgenic poultry by using chimeric animals established by the transfer of blastodermal cells.

Primordial germ cells are successfully used to establish transgenic birds (Ginsburg & Eyal-Giladi, 1987) and as a tool for preserving the genetic resources of local breeds (Kagami *et al.*, 1997; Kino *et al.*, 1997; Yi-Chen Chen *et al.*, 2019). However, currently, the efficiency of transgenic poultry in many cases remains very low, and the technique of using ducks to establish transgenic birds is practically not developed (Sztań *et al.*, 2012).

### **Analysis of Recent Researches and Publications**

Nowadays, the duck (*Anas platyrhynchos* Linnaeus, 1758) is an understudied scientific (breeding) object in comparison with the species *Gallus gallus domesticus*, *Coturnix coturnix* but one of the most economically promising poultry species. A duck can secrete a lot of protein in the oviduct and can regularly produce eggs over a 20-24-hour cycle, which is a very appealing means for the synthesis of therapeutic proteins since the sterile content of eggs is protected by a hard eggshell. Busulfan is used to suppress cell proliferation. Injection of busulfan into the subgerminal cavity is one of the methods that increase the number of donor cells during the development of chimaeras (Aige-Gil & Simkiss, 1991; Tagirov, 2010).

However, the methods for developing germinative duck chimaeras encounter difficulties associated with the structure of the eggshell in waterfowl; the consequences of chimerisation and its potential influence on the productivity of chimaera offspring remain poorly understood (Sawicka *et al.*, 2011). Transgenic animals are almost not inferior to their non-transgenic counterparts (Korol *et al.*, 2019). The effect of the reproductive season on the sperm productivity of germinative drake chimaeras was

previously explored (Doroshenko *et al.*, 2018). For the analysis of survival, Korol *et al.* (2021) used embryos obtained using various methods of introducing the DNA.

To evaluate the egg productivity of daughters from germinative chimaeras (males), a study was performed on three groups of ducks with different origins. Analysis of the productivity of daughters from germinative duck chimaeras demonstrated that, in general, the chimerisation of their parents did not affect the performance of daughters. An analysis of the productivity (egg production, pieces, length, width, egg weight, and shape index) in a group of daughters obtained from chimeric animals indicates that, according to most indicators, this group occupies an intermediate position between the groups whose breeds served as donors and recipients. The method author of the research used to obtain chimaeras can be successfully used on ducks to preserve genetic resources. Preservation of frozen germ cells of rare bird species and native bird breeds with the prospect of their reproduction using germinal chimaeras will reduce the risks of a decrease in the genetic diversity of birds (Doroshenko *et al.*, 2021).

Thus, the productivity of daughters from drake chimaeras was explored. However, the egg productivity of female chimaeras remains understudied. This work is devoted to this issue.

### **Materials and Methods of Research**

All experiments with animals were performed according to the provisions of the “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (1986).

The objects of research were ducks (*Anas platyrhynchos*) of Shanma (Shan partridge duck,

Shan Ma duck) and Shaoxing breeds kept at the Zhuji Guowei Poultry Development Co, Ltd (China). The study was conducted in the poultry genetics laboratory of the Zhejiang Academy of Agricultural Sciences at a duck farm of Zhejiang Generation Biological Science and Technology Co., Ltd. (Zhejiang Province, China).

To obtain duck chimaeras was used a method such as the production of somatic and embryonic chimaeras in chickens by transferring early blastodermal cells (Petitte *et al*, 1990; Tagirov, 2010) with changes in time according to the embryonic development of the duck. Sterilisation of duck embryos was done with busulfan (Aige-Gil & Simkiss, 1991; Tagirov, 2010). To identify the offspring of chimeric donors, the microsatellite analysis of the parents was used (Kostenko *et al.*, 2017).

*Isolation of blastodermal cells.* Blastodiscs were isolated from freshly hatched fertilised eggs using a filter paper ring (Lucas & Jamroz, 1961). The obtained embryos were washed twice from the yolk in a phosphate-buffered saline (PBS) solution (170 mM NaCl; 3.4 mM KCl, 4 mM Na<sub>2</sub>HPO<sub>4</sub>; 1.8 mM KH<sub>2</sub>PO<sub>4</sub>; pH 7.2). Then, 10-12 embryos were transferred into 1 ml of PBS containing 0.25% trypsin and 0.04% ethylenediamine-tetraacetate (EDTA), and incubated for 10 minutes at 37°C, then pipetted with a Pasteur pipette and centrifuged for 10 s at 1500 rpm/min. The pellet was resuspended in 1 ml of RPMI 1640 nutrient medium containing 10% fetal calf serum. The cell suspension was concentrated by centrifugation for 10 s at 1500 rpm, followed by the removal of 0.7 ml of the supernatant, and then the cells were resuspended again in the medium that remained.

*Obtaining duck chimaeras.* Shanma duck embryos were used as recipients, and Shaoxing duck embryos, homozygous for the plumage

colour gene allele (wild type), were used as donors. Donor cells were injected into the subgerminal cavity of recipients with a micropipette (outer diameter 50–70 µm) through a round opening (window) with a diameter of 0.7 cm in the eggshell. Each embryo was injected with 3–4 µl of the suspension, which contained 600–1000 donor cells. The opening in the egg was covered with a piece of thin plastic wrap, which was glued to the shell with protein and then sealed on top with a larger adhesive tape. Busulfan (SigmaAldrich, United States) was used as a chemical agent that suppresses the division of primary germ cells in recipient embryos.

*Preparation of recipient eggs.* An opening in the eggshell (window) of the recipients (Shanma breed) was made between the blunt and sharp ends of the eggs. It reduced the distance between the injector and the embryo needle. The eggs from recipients were incubated for 8–10 hours at a temperature of 38°C.

*Preparation of busulfan solution.* Busulfan was dissolved immediately before use in 10% dimethyl sulfoxide (DMSO), diluted with 3–5 µl of RPMI 1640 nutrient medium. The concentrations used were 300 ng/egg, 150 ng/egg, and 75 ng/egg.

*Busulfan treatment.* After incubation of recipient eggs for 8 hours, the windows were opened in them. Busulfan was injected into the subgerminal cavity of the embryo with a micropipette (1.5–3 µl of liquid). After busulfan injection, the empty cavity was filled with nutrient medium (RPMI-1640) supplemented with antibiotics (ampicillin, streptomycin), the opening was closed with plastic wrap and adhesive tape. The eggs were incubated for 24 hours at a reduced temperature (+32°C) to prolong the duration of busulfan action on the primary germ cells.

Experimental and control animals were kept in individual cages in the same room with constant access to water and food. The egg productivity of 5 experimental animals, which were obtained as a result of their treatment in the embryonic period with busulfan (chimera group), and 10 control animals was explored. A total of 1617 eggs were examined in 142 days in the period from December 13, 2016, to May 3, 2017.

The body weight was determined individually with an accuracy of 10 g for all ducks aged from 41 to 61 weeks.

Average egg weight and size were measured every day. The egg length (L) and width (W) were measured with an accuracy of 0.1 mm with a vernier caliper.

Eggs were weighed on a JM-A 20001 electronic balance with an accuracy of 0.1 g. The egg shape index (SI) was calculated using the formula:

$$SI = W/L \times 100. \quad (1)$$

The obtained data were statistically processed on a computer by a spreadsheet processor "MS Excel 2010" using descriptive statistics and the F-test for two samples for deviation procedures (Zhelyazkov & Tsvetanova, 2002).

## Results of the Research and their Discussion

As a result of the experiments, animal chimaeras ( $F_0$ ) were obtained. For the first time, to obtain blastodermal chimaeras of ducks, busulfan (1,4-butanediol dimethanesulfonate) was used as an agent that suppresses the development of

primary germ cells, an alkylating agent whose mechanism of action is based on cross-linking of DNA strands, as a result of which the replication process is disrupted. A method was designed for developing germinative duck chimaeras using busulfan injections. It has been demonstrated that duck embryos are more sensitive to busulfan than hens embryos. Injection of busulfan at a concentration of 300 ng/egg results in the mortality of 95.0-96.3% of duck embryos. More than 50% of embryos died in the first 2-3 days after the beginning of incubation. Head and neck disorders were observed in 1.2% of embryos. When using busulfan at a concentration of 150 ng/egg, a mortality rate of 33.3-75.3% was observed. A decrease in a concentration up to 75 ng/egg led to 18.75-38.5% embryonic mortality.

The assessment of duck chimerism by means of the analysis of microsatellite loci and analysis of the phenotype indicates that the efficiency of obtaining germinative duck chimaeras was 65-77.8%.

Analysis of the age of puberty (laying of the first egg) indicates that the chimaeras matured later. If the average age of puberty in the control group was  $139.5 \pm 9.67$  days, then in the group of chimaeras –  $148.2 \pm 13.13$  days. Thus, the author of the research can attest that in this experiment, the chimaeras matured later than the control animals, which may be due to the effect of busulfan in the sterilisation of recipient embryos. After the onset of puberty in this generation, the author analysed the live weight in two groups of ducks aged from 41 to 61 weeks.

**Table 1.** Average indicators in the control and experimental groups of ducks

Rate	Control group		Busulfan group	
	M±m	Cv±mCv	M±m	Cv±mCv
Egg production index (142 days), %	87.5±4.53**	16.4±0.090	79.5±11.8	32.8±0.181
Live weight of ducks, g	1422.40±57.00	12.7±0.079	1608.08±94.76	13.2±0.114
Puberty age, days	139.5±9.67	21.8±0.104	148.2±13.13	19.8±0.140
Egg weight, g	70.6±0.198***	9.20±0.006	71.4±0.157	5.07±0.071
Egg length, cm	6.05±0.056	3.65±0.042	6.26±0.134	4.93±0.070
Egg width, cm	4.52±0.053	3.89±0.044	4.53±0.041	2.09±0.045
Egg shape index, %	75.7±0.3	0.7 0.018	75.2±0.3	0.8±0.028

**Note:** Statistical significance at \*  $p<0.05$ ; \*\*  $p<0.01$ ; \*\*\*  $p<0.001$

The average live weight of ducks in the control group was lower and the group itself was more consolidated. Thus, the control ducks weighed 1422.40±57.00 g and chimaeras – 1608.08±94.76 g. The predominance of chimaeras over the control group in terms of live weight may be explained by the fact that the control group consisted of recipients of the Shanma breed. This breed is characterised by egg productivity and is lighter than the Shaoxing breed (embryos of this breed served as donors). Thus, donor cells could be affected by the weight gain of chimaeras. The author's previous study demonstrated that the average live weight of daughters from Shanma drakes was 1554.20±23.54 g, in the group of daughters from Shaoxing drakes – 1505.47±17.06 g, and a group of daughters from germinative chimaeras – 1535.69±17.34 g (Doroshenko *et al.*, 2021).

Thus, the data of the average live weight of ducks obtained as a result of biotechnological procedures associated with using busulfan, correspond to similar indicators of both the control group and the offspring of male germinative chimaeras.

The egg production index in ducks for the entire study period was 87.5±4.53% (control) and 79.5±11.8% (busulfan).

In previous studies, the mean values of egg production per month in the investigated ducks were 27.52±0.84 % in the group of daughters from the Shanma breed drake, 27.47±0.61% in the group of daughters from Shaoxing breed drake and 27.73±0.53 eggs in the group of daughters from germinative chimaeras (Doroshenko *et al.*, 2021). It corresponded to approximately 91.56-92.4%.

Thus, the sterilisation of recipient embryos could have an impact on egg production. One of the experimental duck egg production index was only 34.92%. The reproductive ability of this chimeric duck was impaired, 50.43% of the eggs after artificial insemination were unfertilised. Therewith, the percentage of fertilised eggs in the initial population was 87.5±3.032 – 92.5±2.414%, depending on the age of the ducks (Chepiha *et al.*, 2017).

The egg weight in ducks of two groups for the entire period was 70.6±0.198 g (control) and 71.4±0.157 g ( $p<0.001$ ).

In general, it can be noted that the average weight of eggs in experimental ducks is normal, as according to the standard in Shaoxing ducks it should be 62–68 g and becomes relatively stable (69–73 g) at the end of egg-laying.

Thus, according to the results of our previous analysis of physical and morphological parameters in Shaoxing ducks, it was reliably established that the average weight of eggs with a green shell is greater than white eggs (71.43±0.208 g and 68.52±0.415 g;  $p<0.01$ ) (Chepiha *et al.*, 2017).

The egg weight is one of the main indicators affecting their quality. Notably, according to this indicator, the egg weight (71.21 g) in the group of ducks of the Shanma breed unambiguously prevailed in comparison with other groups of ducks. But the eggs of chimeric animals were significantly larger (69.94 g) than the eggs of Shaoxing ducks (69.12 g). The range of egg weights for various duck breeds is 60–90 g (Gorski *et al.*, 1998; Adamski, 2005; Rahman, 2010; Xia *et al.*, 2019). For example, the maximum egg weight in the Longyan breed ducks was 65.2 g in the period from 23 to 57 weeks of age and 66.9 g in the period from 41 to 57 weeks of age (Huang & Lin, 2011).

The egg morphometric parameters of the studied duck groups: the average values of egg length were 6.056±0.0564 cm (control) and 6.269±0.1341cm (busulfan); egg breadth – 4.520±0.0053 cm (control) and 4.529±0.004 cm (busulfan). There were no statistical intergroup differences in the morphometric parameters of the eggs of the studied groups. Results similar to the previous ones concerning the egg production of daughters from chimaera drake were obtained.

Thus, the average values of egg length were in the range of 5.98±0.022 cm, 6±0.02 cm,

and 6.06±0.02 cm according to the experimental groups. In addition, a similar feature was observed for the egg width – 4.55±0.01 cm, 4.8±0.01 cm, 4.49±0.01 cm. Analysis of the productivity of daughters from germinative chimaeras of ducks demonstrated that, in general, the chimerisation of their parents did not affect the performance of daughters. An analysis of the productivity of a group of daughters obtained from chimeric animals demonstrates that, according to most indicators, this group occupies an intermediate position between the groups whose breeds served as donors and recipients (Doroshenko *et al.*, 2021).

The egg index of the two studied groups (control – 0.758 and busulfan – 0.748) did not have statistically significant differences.

The index of egg in the 1st group showed slightly higher values compared to the values in the 2nd and 3rd groups, but the difference in values was not statistically confirmed (Doroshenko *et al.*, 2021).

In the previous studies on the productivity of Shaoxing and Shanma ducks, the association of duck productivity with age (Chepiga *et al.*, 2017), egg colour (Chepiga *et al.*, 2017) and microsatellite loci (Chepiga *et al.*, 2018) were presented.

Hypothetically, the procedure for obtaining chimeric offspring cannot affect the productive qualities of their offspring. It is known that reproductive chimaeras can have reduced fertility and be sterile (Doroshenko *et al.*, 2017). However, the descendants of chimaeras are not connected with the process of chimerisation of their parents. The descendants of donors have the properties of donors and the descendants of recipients – recipients, respectively, since the chimerisation procedure does not affect hereditary information. Using busulfan as an agent

that inhibits the proliferation of recipient cells can cause a mutagenic effect, but teratogenic effect and high embryonic mortality were observed. Possibly, the chimerisation procedure affects the survival of primary germ cells and, thus, cell selection occurs at the early stages of development. The data obtained may indicate the necessity of further investigation of the effect of chimerisation procedures on the first generation of chimaeras and their descendants.

Notably, the populations investigated by authors are not pure breeding lines but polymorphic at the loci of quantitative traits, which could affect the results of the studies.

### Conclusions

The method used to obtain chimaeras can be successfully applied in ducks to preserve genetic resources. Analysis of the age of puberty (laying of the first egg) indicates that the chimaeras matured later. If in the control group the average age of puberty was 139.5±9.67 days, then in the group of chimaeras – 148.2±13.13 days. Thus, the author of the research can attest that in this experiment, the chimaeras matured later than the control animals, which may be due to the effect of busulfan in the sterilisation of recipient embryos. The average live weight

of ducks in the control group was lower and the group itself was more consolidated. Thus, the control ducks weighed 1422.40±57.00 g and the chimaeras 1608.80±94.76 g. The predominance of chimaeras over the control group in terms of live weight may be explained by the fact that the control group consisted of recipients of the Shanma breed. The egg production of ducks for the entire study period was 87.5±0.05% (control) and 79.5±0.12% (busulfan). The egg weight in ducks of two groups for the entire period was 70.62±0.199 g (control) and 71.15±0.157 g ( $p<0.001$ ). The egg morphometric parameters of the studied duck groups: the average values of egg length were 6.056 ± 0.0564 cm (control) and 6.269±0.1341 cm (busulfan); egg width – 4.520±0.0053 cm (control) and 4.529±0.004 cm (busulfan). There were no statistical intergroup differences in the morphometric parameters of the eggs of the studied groups. Results similar to the previous ones concerning the egg production of daughters from chimaera drake were obtained.

Preservation of frozen germ cells of rare bird species and native bird breeds with the prospect of their reproduction using germinal chimaeras will reduce the risks of a decrease in the genetic diversity of birds.

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## Продуктивність гермінативних химер качок та їхніх нащадків

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**Анотація.** Актуальність дослідження зумовлена необхідністю вивчення можливого впливу химеризації на продуктивність гермінативних химер качок та їхніх нащадків. Для отримання химер качок застосували метод, описаний Aige-Gil & Simkiss та Тагіровим. Як реципієнтів використовували ембріони качок Шанма, а донорів – ембріони качок Шаосінь, гомозиготні за алелем гена кольору оперення (дикий тип). Для того, щоб оцінити яєчну продуктивність гермінативних химер качок був проведений аналіз експериментальних тварин та їх контрольних аналогів. Аналіз віку статевого дозрівання (відкладання першого яйця) свідчить про те, що химери дозріли пізніше. Якщо в контрольній групі середній вік статевого дозрівання становив 139±9 діб, то в групі химер – 148±13 діб. Отже, можна засвідчити, що в нашому експерименті химери дозріли пізніше, ніж контрольні тварини, що може бути пов'язано з дією бусульфану при стерилізації ембріонів-реципієнтів. Середня жива маса качок контрольної групи була нижчою, а сама група була більш згуртованою. Так, у контрольних качок вага складала 1422,40±57,00 г, у химер – 1608,80±94,76 г. Перевага химер над контрольною групою за живою масою може бути пов'язана з тим, що контрольну

групу складала реципієнти породи Шанма. Яєчність качок за весь період дослідження становила  $87,5 \pm 0,05\%$  (контроль) і  $79,5 \pm 0,12\%$  (бусульфан). Маса яєць у качок двох груп за весь період становила:  $70,62 \pm 0,199$  г (контроль) і  $71,15 \pm 0,157$  г. Морфометричні показники яєць досліджуваних груп качок: середні значення довжини яйця –  $6,056 \pm 0,0564$  см (контроль) та  $6,269 \pm 0,1341$  см (бусульфан); ширина яєць –  $4,520 \pm 0,0053$  см (контроль) і  $4,529 \pm 0,004$  см (бусульфан). Статистичних міжгрупових відмінностей за морфометричними параметрами яєць досліджуваних груп не було. Аналіз продуктивності дочок гермінативних химер качок свідчить, що загалом химеризація батьків не вплинула на продуктивність їхніх дочок. Аналіз продуктивності групи дочок, отриманих від химерних тварин, свідчить, що за більшістю показників ця група займає проміжне місце між групами, чиї породи слугували донорами та реципієнтами. Метод, який ми використовували для отримання химер, становить практичну цінність для збереження генетичних ресурсів

**Ключові слова:** гермінативна химера, качка шаосінь, шанма, бусульфан, яєчна продуктивність качок



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## Development of lifetime productivity of cows depending on the live weight of heifers of different ages

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**Abstract.** One of the problems of modern dairy farming is the short period of productive use of cows. It is observed both in Ukraine and in most countries of the world with developed dairy cattle breeding. The consequence of a short period of productive use is a decrease in the lifetime productivity of cows. The purpose of this work was to investigate the possibility of influencing the duration of use and lifetime milk yield of cows by selecting heifers by live weight during their growing period. The study analysed the lifetime productivity of 1071 cows of the Ukrainian black-and-white dairy breed, starting from their rearing and up to retirement from the herd. Animals were divided into five groups by live weight at the age of 3, 6, 12 and 15 months using standard deviation ( $\sigma$ ) from the mean. Within the groups, the number of calvings, productive life expectancy, lifetime estrus and estrus for higher lactation and the average period between calvings were determined. It was established that the hope for higher lactation is associated with the weight of heifers at the onset of puberty and sexual maturity. Animals, which at 6 months had a live weight of  $+0.5-1.5 \sigma$  and at 12 months more than  $+1.5 \sigma$  from the average in the herd, were characteristics of the highest milk yield. The group of traits of lifetime productivity (number of calvings, duration of productive use and lifetime milk yield) was positively influenced by the live weight of heifers at the age of 3, 6, 12 and 15 months, which exceeded the average for the herd by 0.5-1.5  $\sigma$ . Cows belonging to these groups exceeded other groups by 0.2-1.4 calving. During the period of use, these cows received 11-32% more milk than the average for the herd. The results of the research expand the understanding of the impact of heifer breeding on the development of lifetime productivity of cows and can be used for the selection of livestock and correction of plans for the cultivation of dairy cattle.

**Keywords:** dairy cattle breeding, milk yield, reproductive capacity, calving, duration of productive use

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Klymkovetskyi, A. (2021). Development of lifetime productivity of cows depending on the live weight of heifers of different ages. *Animal Science and Food Technology*, 12(4), 18-25.

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## Relevance

The duration of use of cows is only 3–4 years (De Vries and Marcondes, 2020; Siatka *et al.*, 2020), while it should recover the costs of their breeding and ensure profitable maintenance through milk production. It is implemented through the development of high productivity and long-term use of cows. It was identified that the vast majority of cows leave the herd before the 600th day of productive use (Macalovich *et al.*, 2018), which significantly limits the lifetime productivity of animals. In the breeds common in Ukraine (Ukrainian Red and Black-and-White dairy and Holstein), cows are used in herds for an average of 2.32–2.50 lactations, and their lifetime milk yield is 14940–18669 kg (Mazur *et al.*, 2018). The reduction of productive longevity of cows is gradually recorded (Milostiviy *et al.*, 2017), so the study of the factors that determine it remains relevant.

### Analysis of Recent Studies and Publications

To increase the lifetime productivity of cows, the possibility of using selection methods for the duration of productive use is being explored. A close correlation was identified between the duration of cow use and lifetime milk yield and milk fat yield (Hdud *et al.*, 2018). Therewith, the influence of hereditary factors on the manifestation of lifetime productivity traits is insignificant (Milostiviy *et al.*, 2017), and the selection is complicated by a long evaluation period and the receipt of final results after the animal's retirement. Therefore, there is a necessity to apply other approaches to improve the lifetime productivity of cows. One such measure is considered to be the intensification of growing young stock, which reduces the cost of keeping heifers and increases the productivity of cows (Kruglyak, 2018). The fact

that the development of high lifetime productivity of cows is influenced by the conditions of their use, the origin and management of the dairy herd, and the features of growing heifers is indicated by other researchers (Schuster *et al.*, 2020). There is evidence of an increase in the lifetime productivity of cows when introduced into the herd at the age of 22–26 months (Sawa *et al.*, 2019). In particular, to extend life expectancy and improve other productivity traits, it is recommended to inseminate heifers aged 15–16 months with a live weight of at least 412 kg, provided that at 12 months it is 327–347 kg, or inseminate heifers at 17–18 months with a live weight of 426 kg, provided that at 12 months they weighed 316–344 kg (Levina *et al.*, 2019). In Simmental cows, the highest milk yields and milk fat were observed when their live weight at birth was 33–34 kg, at 6 months of age – 171–190, at 12 months – 291–300, and at 18 months – 401–415 kg (Fedorovych, 2017).

Features of changes in the live weight of heifers during the growing period are significant. It was established that in terms of milk yield, milk fat and protein yield, first-born animals have an advantage over animals with a slow decline in relative growth rate (Polupan & Siriak, 2019). In subsequent lactations, the advantages of such animals in terms of productivity have not been confirmed, but since the milk yield for the first lactation is a significant proportion of lifetime productivity, this pattern should be considered. In addition, it was established that the lifetime milk yield of cows decreases by 15–37% if short-term growth delays occur during heifer rearing (Klimkovetskyi *et al.*, 2020)

To improve milk yields during life, work is performed to establish benchmarks for heifer growth. They include such traits as optimal age and live weight, condition, and development of

body tissues during the stages of growing and beginning of productive use of animals. An important role is devoted to the development of the udder. There are still concerns that the development of the mammary gland is disturbed when the increase in live weight exceeds a specific threshold, which adversely affects milk yield. It has been demonstrated (Van Amburgh *et al.*, 2019) that mammary gland development during the prepubertal growth phase does not decrease due to high energy intake, the overall growth of the mammary parenchyma depended on the attainment of puberty, and the udder, like most reproductive organs, grows in proportion to body size and not in proportion to nutrient intake. Therewith, achieving reasonable targets for the live weight of heifers is essential. To increase milk production in terms of dry matter, it was confirmed (Martín *et al.*, 2020) the importance of heifers reaching the target live weight at 12 and 15 months of age, while a limited lag behind the target at 6 and 9 months of age had little effect on cow performance.

Thus, the literature data confirm the possibility of influencing the lifetime productivity of cows by targeted breeding of heifers. For this

purpose, it is advisable to justify the target indicators of their growth. It is advisable to determine the optimal criteria within individual breeds, and apply them in the conditions of the level of productivity of cows, on which the research was conducted.

The purpose of the research – determine the live weight of heifers of different ages, which is associated with the development of high lifetime productivity of cows.

### Material and Methods of Research

The productivity of animals of the Ukrainian black-and-white dairy breed of Shevchenkivsky PAE of the Kyiv-Svyatoshinsky district of the Kyiv region was analysed. The results of the lifetime use of 1071 cows for the period from 1992 to 2014 were explored. The average productivity of cows was  $4565 \pm 46.2$  kg for 305 days of lactation, life expectancy  $5.9 \pm 0.30$  years, including productive use  $3.1 \pm 0.27$  years.

The live weight at birth at the age of 3, 6, 12 and 15 months was explored in animals. According to these indicators using the standard deviation from the mean ( $X \pm \sigma$ ), the animals were divided into 5 groups (Table 1).

**Table 1.** Criteria for dividing animals into groups by live weight of heifers

Group	Selection criteria	Range in live weight (kg) at age			
		3 months	6 months	12 months	15 months
1	$< X - 1,5\sigma$	$\leq 57$	$\leq 93$	$\leq 165$	$\leq 203$
2	$X - 0,5...1,5\sigma$	57-71	94-117	166-203	204-247
3	$X \pm 0,5\sigma$	72-86	118-142	204-242	248-292
4	$X + 0,5...1,5\sigma$	87-100	143-166	243-280	293-336
5	$> X + 1,5\sigma$	$\geq 101$	$\geq 167$	$\geq 281$	$\geq 337$

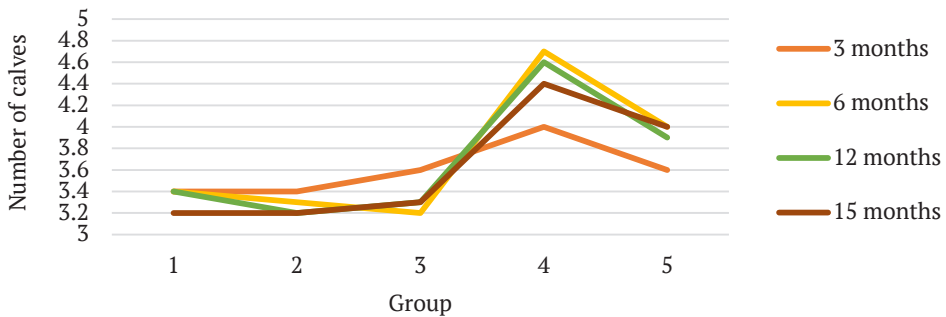
Within each of the groups, the results of lifetime use of cows were explored, in particular, the number of calvings, productive life expectancy, lifetime milk yield and milk yield for higher lactation and the average period between calvings.

The influence of the live weight of heifers on the lifetime productivity of cows was determined by graphical analysis. The significance of the difference between the groups was determined using Student's t-test ( $p < 0.05, 0.01, 0.001$ ).

## Research Results and their Discussion

The analysis demonstrated that there is a regularity between the growth of heifers and the number of calves obtained from cows. A greater number

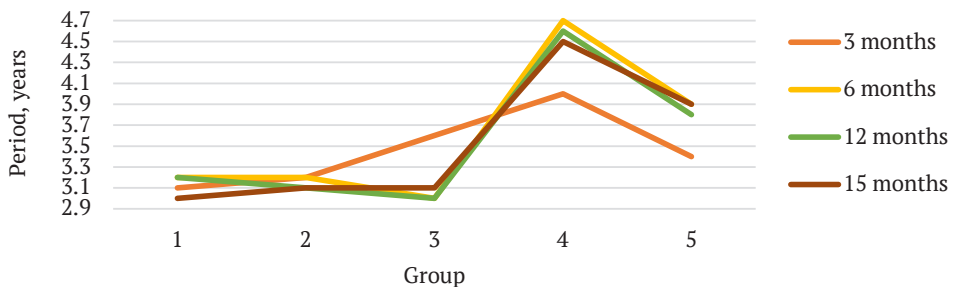
of calvings during life was obtained from animals that had a higher than average live weight during the analysed age periods. Most calves were obtained from livestock assigned at the age of 3, 6, 12 and 15 months to the fourth group (Fig. 1).



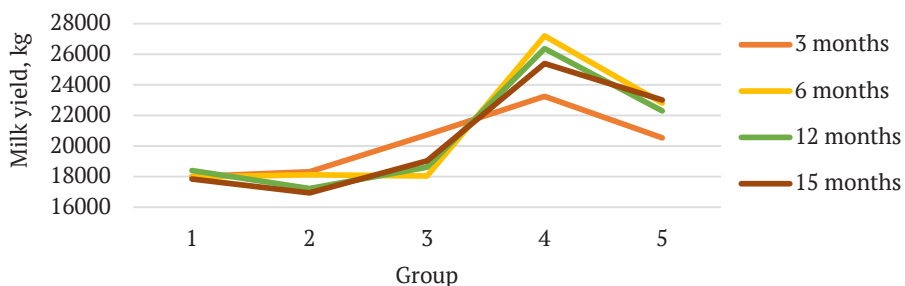
**Figure 1.** Effect of live weight of heifers of different ages on lifetime number of calvings

In particular, animals included in the fourth group by live weight at the age of 3 months exceeded others by 0.2-0.6 calving; at the age of 6 months by 0.4-1.5 ( $p < 0.05 \dots p < 0.001$ ); at 12

months by 0.7-1.4 ( $p < 0.05 \dots p < 0.001$ ) and at 15 months by 0.4-1.2 calving. A similar result was for the duration of productive use (Fig. 2) and lifetime milk yield (Fig. 3).



**Figure 2.** Influence of live weight of heifers of different ages on the duration of productive use of cows

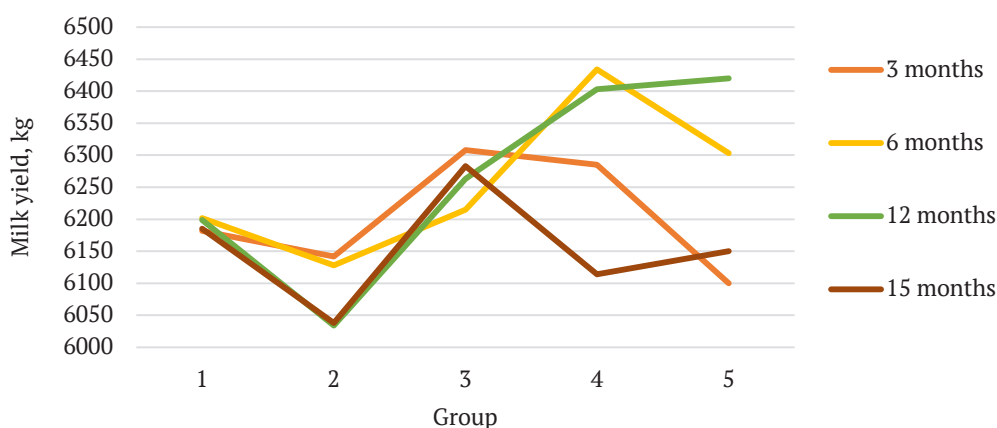


**Figure 3.** Effect of live weight of heifers of different ages on lifetime milk yield of cows

The graphs demonstrate that the low live weight of heifers, at the level of the herd average ( $\pm 0.5 \sigma$ ) and below, reduces the duration of productive use of cows. But the lifetime productivity of cows decreased when heifers reached the highest live weight in all controlled age periods (indicators of the fifth group). Thus, it can be argued that the live weight of heifers that exceeds the average in the herd by  $1.5 \sigma$  is not desirable. The study identified the optimal parameters for the live weight of heifers, which allows for obtaining the highest lifetime productivity of cows. They correspond to the criteria of the fourth group at the age of 3, 6, 12 and 15 months. Animals assigned to this group not only outperformed cows of other groups, their productivity was higher than the average for the herd. The advantage in lifetime milk yield of animals of the fourth group by live weight at the age of 3 months was 11%. In terms of live weight at the age of 6 months, the difference with the herd average reached 32% ( $p < 0.001$ ). Thus, in the populations of the Ukrainian black-

and-white dairy breed, to ensure maximum lifetime productivity, the live weight of heifers at the age of 3, 6, 12, and 15 months should be within the range of  $+0.5...+1.5\sigma$  from the average herd. In the conditions of the investigated herd, the optimal for maximum lifetime productivity was live weight at the age of 3 months 87-100 kg, at the age of 6 months – 14-166, at 12 months – 243-280 and at 15 months – 293-336 kg.

One of the criteria for assessing the milk production of cows is the hope for higher lactation. Features of the development of this trait of cows, depending on the live weight of heifers, differ from the trends established by the duration of use and lifetime milk yield. A relationship was identified between milk yield for 305 days of higher lactation and the live weight of heifers at the age of 3 and 15 months (Fig. 4). Cow productivity was highest when the live weight of heifers of this age corresponded to the criteria of the third group. But no significant difference between the groups was established.

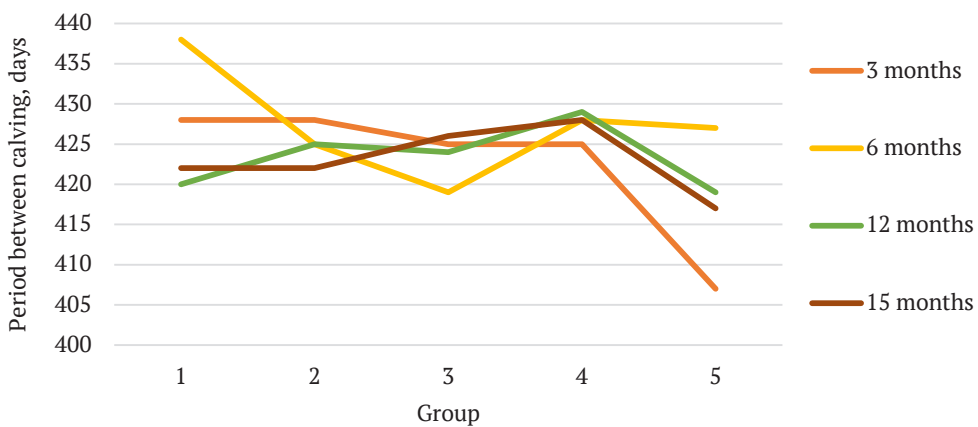


**Figure 4.** Influence of live weight of heifers of different ages on milk yield during 305 days of higher lactation

It was established that the increase in the live weight of heifers at the age of 6 and 12 months, which coincides with the onset of puberty and the onset of sexual maturity, has a positive effect on the increase in milk yield during higher lactation. Optimal at the age of 6 months to obtain the highest milk yield was live weight in the range of 143-166 kg (group 4), and at 12 months - more than 280 kg (group 5). Thus, for the development of high productivity, it is

of great importance to achieve sufficient development in live weight at the age of 12 months, which partially coincides with the conclusions (Martín *et al.*, 2020) made in the analysis of cow productivity in New Zealand.

Indicators of reproductive capacity, which in cows with two or more calvings were estimated by the duration of the period between calvings, depending on the live weight of heifers did not change significantly (Fig. 5).



**Figure 5.** Effect of live weight of heifers of different ages on the average period between calving

There was a tendency to shorten the period between calving in animals of group 5 by live weight at the age of 3 months and prolongation in animals of the first group by live weight at 6 months. It may be evidence of the influence of growth rate or general level of nutrition of heifers of this age on the development of the ability of cows to reproduce effectively, but the statistical analysis did not confirm a significant difference between the individual groups.

### Conclusions and Perspectives

By selecting heifers during the growing period, the lifetime productivity of cows can be

influenced. The longest life expectancy, productive use and lifetime milk yield are characterised by animals that at the age of 3, 6, 12 and 15 months have a live weight of  $0.5 \dots 1.5 \sigma$  more than the average for the herd, thus, these parameters can be considered optimal.

The milk yield for 305 days of the highest lactation was higher in animals that at the age of 6 months had a live weight of  $0.5 \dots 1.5 \sigma$  higher than the average for the herd, and at 12 months, reached its highest indicators. The influence of the live weight of heifers at the age of 3, 6, 12 and 15 months on the duration of the period between calving was not confirmed.

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## Формування довічної продуктивності корів залежно від живої маси телиць різного віку

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**Анотація.** Однією з проблем сучасного молочного скотарства є короткий період продуктивного використання корів. Це спостерігають не лише в Україні, а і в більшості країн світу з розвиненим молочним скотарством. Наслідком короткого періоду продуктивного використання стає зниження довічної продуктивності корів. Метою цієї роботи було вивчити можливість вплинути на тривалість використання і довічний надій корів шляхом добору телиць за живою масою в період їх вирощування. В дослідженні було проаналізовано довічну продуктивність 1071 корови української чорно-рябої молочної породи, починаючи з їх вирощування і до вибуття зі стада. Тварин за живою масою у віці 3, 6, 12 і 15 місяців розподілили на п'ять груп з використанням стандартизованого відхилення ( $\sigma$ ) від середньої величини. В межах груп визначали кількість отелень, тривалість продуктивного життя, довічний надій і надій за вищу лактацію та середній період між отеленнями. Було встановлено, що надій за вищу лактацію пов'язаний із масою телиць у період початку статевого дозрівання і настання статевої зрілості. Тварини, які в 6 місяців мали живу масу  $+0,5-1,5 \sigma$  і в 12 більше ніж  $+1,5 \sigma$  від середньої в стаді, характеризувались найбільшим надоем. На групу ознак довічної продуктивності (кількість отелень, тривалість продуктивного використання і довічний надій) позитивно вплинула жива маса телиць у віці 3, 6, 12 і 15 місяців, яка перевищувала середню по стаду на  $0,5-1,5 \sigma$ . Корови віднесені до цих груп переважали інші групи на  $0,2-1,4$  отелення. За період використання, від цих корів отримали на  $11-32 \%$  молока більше, ніж в середньому по стаду. Результати досліджень розширюють розуміння впливу вирощування телиць на формування довічної продуктивності корів та можуть бути використані для добору поголів'я і корекції планів вирощування великої рогатої худоби молочних порід

**Ключові слова:** молочне скотарство, надій, відтворювальна здатність, отелення, тривалість продуктивного використання



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## **Growth and survival of *Clarias catfish* (*Clarias gariepinus* B., 1822) at different stages of cultivation with the addition to the fodder of “Chiktonik”**

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**Abstract.** Stressful situations decrease the growth rate and survival rate of fish, thus, the search for ways to reduce their adverse impact is relevant. The purpose of the research – to evaluate in aquaculture conditions the effect of different concentrations of vitamin-amino acid complex “Chiktonik” on the growth and survival of larvae and fry of African clarius catfish (*Clarias gariepinus* B., 1822) after stressful situations. The stressful situation for the fish arose from significant fluctuations in the content of ammonia, nitrites and nitrates in the water environment of the closed recirculation aqua system during the period of start-up of the biological filter, until the equilibrium was established. A series of experiments were performed, during which it was established that the addition of the drug at the rate of 1 ml per 1 kg of feed accelerates the growth of fish in experimental variants compared to the control. Experimental use of high doses of the drug (5, 15, 30 and 45 ml/kg of fodder) initially inhibited the growth of fish body weight, but 10-30 days after the experiment, the growth rate of the experimental material was equal to that of the control group of fish and even exceeded the control values in the future. The positive effect of the vitamin-amino acid complex “Chiktonik” on the survival of young clarius catfish at the stage of completion of the larval period of life and in the early stages of the fry period was established. In the experiment with older fish, which were fully developed fry, such an effect of the drug was not observed: the survival rate of fish was at the same level both in the experiment and in the control. Therewith, it was established that the fry reacted worse to higher doses of the drug compared to the grown larvae. The growth rate of fry after using high doses of the drug did not equal that of fish from the control group within a month after the experiment, unlike younger fish. In general,

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the expediency and safety of the practical use of the drug “Chiktonik” for young clarius catfish as a fodder additive at a dose of 1 ml/kg of fish fodder have been proved

**Keywords:** vitamin-amino acid complex, the establishment of biological balance, feeding, stress, aquaculture

## Relevance

At the current level of technical and technological support of aquaculture, the conditions for growing fish in the vast majority of fish farming enterprises cannot be considered optimal. Various stress factors in the form of significant fluctuations in hydrochemical, temperature and oxygen regimes, and the impact of various infectious and nutritional diseases cause growth retardation and fish mortality (Bahareva & Grozesku, 2000).

A big problem for industrial-type fish farms is the purification of used water from nitrogenous compounds ( $\text{NH}_{3/4}$ ,  $\text{NO}_2$  and  $\text{NO}_3$ ), which enter the fish farming system in the process of decomposition of organic matter of fodder and waste products of cultivation objects. To remove nitrogenous compounds from the water, a biofilter is used, in which, under the influence of bacteria, these compounds are converted into substances less toxic to fish. In case of malfunctions or during the start-up phase, before the biological equilibrium is established, when the biofilter is not yet able to effectively remove nitrogenous compounds, the latter accumulate in the water and reach concentrations that are lethal to fish (Bregnballe, 2015; Sharylo *et al.*, 2019). In English literature, the period of establishing biological equilibrium is called the “new tank syndrome” (Alderton, 2019; Ebeling & Timmons, 2010).

Fish poisoning by nitrogenous compounds causes damage to the nervous system and

muscular apparatus. Outwardly, this is manifested in the form of convulsions. In addition, the gill apparatus is affected, the liver and spleen abnormally increase in size, and the haemoglobin content in the blood decreases (Potrohov *et al.*, 2006; Krasnyuk, 2009). In the absence of timely reaction of technologists, fish can die within a few days or even hours (Tilak *et al.*, 2002; Kofonov, 2017)

With a timely response to the problem, the fish can be saved, but it will still get poisoned, and if its body is not supported at the rehabilitation stage, the death of fish, for this reason, may continue.

The relevance of this scientific research is conditioned upon the necessity to increase the survival rate and maintain the rate of mass accumulation in fish after stressful situations, in particular, by using the vitamin-amino acid preparation “Chiktonik” at different stages of the technological process.

## Analysis of Recent Studies and Publications

According to the results of the analysis of foreign and domestic sources of scientific and technical information, it was established that using vitamin-amino acid complexes in aquaculture has great prospects.

During embryogenesis and the transition to external nutrition, fish go through stages called “critical periods” (Martseniuk &

Martseniuk, 2021). During such periods, the highest fish mortality is observed, and it is connected with the fact that the organism enters into new ecological relations with the environment. To prevent the death of fish in the embryonic period, scientists (Lyubomirova *et al.*, 2021) recommend keeping eggs in a solution of vitamins, which positively affects the process of embryogenesis and increases the yield of larvae from eggs. Such larvae are characterised by increased viability and growth rate.

Another use of the complex of vitamins and amino acids is to eliminate the effects of poisoning in fish, long-term fasting, strengthen immunity and increase resistance to bacterial diseases. Scientists (Lyubomirova *et al.*, 2021; Eleev *et al.*, 2019; Osipova *et al.*, 2005; Metallov *et al.*, 2013) believe that the positive effect of using such substances can be both improved health and increased survival and body weight gain of fish.

Chiktonik is a feed additive for animals that contains a balanced amount of vitamins and amino acids, including essential ones. Using the drug is intended to compensate for the deficiency of biologically active substances in the body of animals, regulate metabolism, and promote nonspecific resistance to adverse environmental factors. In the case of unbalanced feeding and stress, during the period of intensive growth and high productivity, using this additive in the composition of fodder increases the safety of livestock, especially young animals, and increases productivity and duration of use of animals.

Chiktonik has been used in agricultural animal husbandry for more than 10 years. This drug was developed initially for poultry farming, but later it was used in other livestock sectors (Gorchakova, 2013; Adullina, 2014), and more

recent research on the effects of Chiktonik on fish has started (Kuznecova & Mosyagina, 2015).

It should be noted that the improper use of vitamin preparations may have adverse effects, namely hypervitaminosis (Ksenofontova, 2019).

The purpose of the study – to evaluate the effect of the drug “Chiktonik” at different concentrations on the growth and survival of juvenile catfish (*Clarias gariepinus* B., 1822).

## Materials and Methods of Research

The material for the research was larvae and fry of clarius catfish. Research methods – generally accepted in fish farming (Martseniuk & Martseniuk, 2020).

To estimate the growth rate, the total index of masonry accumulation in the group of fish in the experiment and the percentage of this index to that in the control were used. The survival of fish in each group was determined by counting the number of juveniles and calculating the percentage of survival. Data collection was performed during control catches, with a frequency of once every 10-15 days. All fish were counted and weighed in groups.

The research was conducted in 4 stages, which were distinguished by various experimental conditions and various concentrations of the drug: 1, 5, 15, 30 and 45 ml/kg of fish fodder. The first, second and third stages of the experiments were conducted in the aquarium laboratory of the Department of Aquaculture of the Center for Aquatic Bioresources and Aquaculture of NUBIP of Ukraine. The fourth stage of research was conducted in the production conditions of a private fish farm for the cultivation of clarius catfish, located in the village of Yushky, Obukhov district, Kyiv region.

The stress factor for fish, the consequences of which were planned to be overcome using

the drug “Chiktonik”, were increased concentrations in water of  $\text{NH}_{3,4}$  (0.5-1 mg N/l),  $\text{NO}_2$  (0.25-0.5 mg N/l) and  $\text{NO}_3$  (30-60 mg N/l). Fish were planted in the growing tanks of a closed aquaculture system until the beginning of the period of biological equilibrium in the water. During the first 10 days, when the fish were feeding the preparation with the addition of artificial fodder, an increase in the content of nitrogen compounds in the water was observed. Part of the ammonia and nitrites were removed using a biofilter, part – by replacing water in the volume of 10% daily. Later, after the completion of the biofilter start-up period, the main hydrochemical parameters were stabilised.

For the first three stages of research, 6 autonomous mini-fish farms with closed water supply were installed. Each unit included a 100-litre glass fish tank (aquarium) and a water regeneration unit (mechanical and biological filters). For water circulation in the system, a pump “MinJang NS F801” with a capacity of 1200 l/h was used. Porous foam sponges connected to the water pump served as mechanical filters. The mechanical filter was cleaned manually as it became dirty. The biological filter was filled with highly porous filler “Separax” produced by “JBL Micromec” as a substrate for the development of the bacterial film.

The water temperature in the UZV was maintained in the optimal range for the cultivation of *clarius* catfish (27-28°C), using thermostats “Resun Sunlike 200”.

The concentration of nitrogenous compounds in aquarium water was determined using express tests TM “Ptero”.

The fourth stage of the experiment was performed in fish ponds with a working volume of 1 m<sup>3</sup>. Biological water treatment in the system was performed using the classic plastic filler Aqua for the biofilter. The fish were reared in a closed-type aquaculture system in a private fish farm.

To prepare the fodder with Chiktonik, the latter was taken from the bottle with a syringe with a needle in the required volume. The solution of the drug for feeding was prepared with the addition of a small amount of distilled water to evenly distribute the solution over the entire volume of fodder and, therewith, minimally moisten the daily portion of fodder. The required amount of solution was evenly applied to the surface of the fodder with a syringe.

For feeding juvenile *clarius* catfish, fodder of the trademark “Aller Aqua” was used with the size of grains or pellets from 0.1 mm to 6 mm, depending on the age of the fish. Fish were fed 5 times during daylight hours. The daily fodder rate was 7% of the body weight of 15- and 30-day-old juveniles and 3% for 65-day-old fry. Fodder with the addition of the drug was given to fish once a day, mainly in the morning.

The dosage of the drug “Chiktonik”, age groups and the amount of experimental material by variants in the context of the stages of the experiment are presented in Table 1:

**Table 1.** Scheme of the experiment to assess the effect of the drug “Chiktonik” on young *clarius* catfish

Stage no.	Variant	Dose of the drug, ml/kg of fodder	Aquatic system No.	Characteristics of the experimental material of fish			Stage duration, days
				age group	average weight, g	quantity, pcs.	
1	Control		1	15-day grown larva	0.08	56	20
			2			56	

Table 1. Continued

Stage no.	Variant	Dose of the drug, ml/kg of fodder	Aquatic system No.	Characteristics of the experimental material of fish			Stage duration, days
				age group	average weight, g	quantity, pcs.	
1	Experiment	1	3	15-day grown larva	0.08	56	20
			4			56	
			5			56	
			6			56	
2	Control	-	1	30-day old fry	0.39	82	35
			2			82	
	Experiment 1	15	3			82	
			4			82	
	Experiment 2	45	5			82	
			6			82	
3	Experiment 1	15	1	65-day old fry	32.08	6	53
			2			6	
	Experiment 2	30	3			6	
			4			6	
Experiment 3	45	5	6				
		6	6				
4	Experiment	5	pool 1	30-day old fry	0.41	1000	8
	Control	-	pool 2			1000	

As the table demonstrates, the research at the first two stages was conducted with repeated variants, from 2 to 4. In the third and fourth stages, the study was conducted without repeated variants.

The duration of the experiment by stages depended mainly on the ability of the water regeneration unit to maintain the biological balance in the aquatic system. The experiment was stopped as soon as the system could no longer cope with the organic load, which grew as the weight of the fish increased. The exception was the fourth stage, which lasted only 10 days since the biological filter in the aquaculture system of the enterprise, where the research was conducted, failed after the second control

catch, which resulted in a sharp deterioration of water quality. The fish began to die en masse, prompting the owner of the enterprise to plant the surviving fish in another aquatic system to prevent further losses of biological material.

### Research Results and their Discussion

In the first stage of research, the smallest by age and weight experimental material was used, and the minimum, so-called basic dose of the drug "Chiktonik" in fish feed was applied. Three control catches were conducted during the stage: at the beginning, middle and end of the stage. The results of processing the material of control catches are presented in Table 2.

**Table 2.** Changes in the total mass and number of experimental materials during the 1st stage of the experiment

Variant	Aquatic system No.	Date of control catch		
		01.08.21	10.08.21	20.08.21
Weight of experimental material, g ( $\pm$ average to control, %)				
Control	1	4.25	82.00	296.00
	2	4.25	85.00	304.00
	average	4.25	83.50	300.00
Experiment	3	4.25	94.00	316.00
	4	4.25	87.00	310.00
	5	4.25	92.00	315.00
	6	4.25	82.00	325.00
	average	4.25	88,75 (+6,3)	316,5 (+5,5)
Fish survival, pcs ( $\pm$ experiment average to control, %)				
Control	1	56	49	45
	2	56	51	45
	average	56	50	45
Experiment	3	56	53	51
	4	56	55	52
	5	56	52	50
	6	56	54	51
	average	56	53,5 (+0,9)	51 (+13,3)

As the table demonstrates, the addition of the drug “Chiktonik” to fish fodder at a dose of 1 ml/kg had a positive effect on the growth and survival of juvenile clarius catfish. Thus, in terms of masonry accumulation, the advantage of the experimental variant over the control at the end of the stage was +5.5%, and in terms of survival – +13.3%.

The purpose of the following stages of the experiment was to explore the effect of high doses of vitamin-amino acid complex “Chiktonik” (5, 15, 30 and 45 ml/kg of fodder) on young clarius catfish in the laboratory (2 and 3 stages) and production conditions (4 stage). The results of the second stage of research are presented in Table 3.

**Table 3.** Changes in the total mass and number of experimental materials during the 2nd stage of the experiment

Variant	Aquatic system No.	Date of control catch			
		24.08.21	04.09.21	14.09.21	29.09.21
Weight of experimental material, g ( $\pm$ average to control, %)					
Control	1	32.00	116.00	482.60	930.00

Table 3. Continued

Variant	Aquatic system No.	Date of control catch			
		24.08.21	04.09.21	14.09.21	29.09.21
Control	2	32.00	115.00	529.00	1041.00
	average	32.00	115.50	505.8	985.5
Experiment 1	3	32.00	108.00	531.00	1320.00
	4	32.00	88.00	523.40	1207.00
	average	32.00	98,00 (-15,2)	527,2(+4,2)	1263,5 (+28,2)
Experiment 2	5	32.00	108.00	622.00	1406.00
	6	32.00	108.00	582.00	1352.00
	average	32.00	108,00 (-6,5)	602,0 (+19,0)	1379 (+39,9)
Fish survival, pcs ( $\pm$ experiment average to control, %)					
Control	1	82	72	62	50
	2	82	68	63	50
	average	82	70	62.5	50
Experiment 1	3	82	78	77	76
	4	82	74	70	70
	average	82	76 (+8,6)	73,5 (+17,6)	73 (+46,0)
Experiment 2	5	82	71	70	70
	6	82	65	61	60
	average	82	68 (-2,8)	65,5 (+4,8)	65 (+30,0)

According to the data of Table 3, a significant increase in the content of the drug “Chik-tonik” in fodder resulted in the following effects: ten days after the start of the stage, according to the control catch, in experimental variants 1 and 2 there was a lag behind the control in terms of masonry accumulation by 15.2 and 6.5%, respectively. In variant Experiment 2, the lowest survival rate was recorded compared to the control and variant Experiment 1, in which, on the contrary, this indicator was the highest.

During the second “ten days” fish from experimental variants both reached and overtook fish from the control group in terms of masonry accumulation: Experiment 1 – by

28.2, experiment 2 – by 39.9% more than in the control. In terms of fish survival rate from the beginning of the experiment, the experimental variants demonstrated better results compared to the control: Experiment 1 – by 46.0, experiment 2 – by 30.0% more.

The task of the third stage of the experiments was to test high doses of the drug “Chik-tonik” (15, 30 and 45 ml/kg of fodder) on catfish fry, twice as old as the experimental material of the 2nd stage and almost ten times larger. Due to the limited number of juvenile *clarius* catfish in this age group, the study was conducted without repeating the variants. The results of the third stage of the experiment are presented in Table 4.

**Table 4.** Changes in the total mass and number of experimental materials during the 3rd stage of the experiment

Variant	Aqua system No.	Date of control catch					
		01.10.21	12.10.21	22.10.21	03.11.21	13.11.21	23.11.21
Weight of experimental material, g ( $\pm$ average to control, %)							
Control	1	775	1080	1456	1906	2458	3050
Experiment 1	2	760	1031 (-4,5)	1364 (-6,3)	1863 (-2,3)	2502 (+1,8)	3572 (+17,1)
Experiment 2	3	770	1035 (-4,2)	1383 (-5,0)	1891 (-0,8)	2570 (+4,6)	3467 (+13,6)
Experiment 3	4	775	1040 (-3,7)	1380 (-5,2)	1896 (-0,5)	2050 (-16,5)	2560 (-16,0)
Fish survival, pcs ( $\pm$ experiment average to control, %)							
Control	1	6	6	6	6	6	6
Experiment 1	2	6	6	6	6	6	6
Experiment 2	3	6	6	6	6	6	6
Experiment 3	4	6	6	6	6	6	6

As the table demonstrates, the best result in terms of masonry accumulation was obtained in Experiment 1 (concentration of “Chiktonik – 15 ml/kg of fodder), the worst – in Experiment 3 (concentration of the drug – 45 ml/kg of fodder). The variant Experiment 2 had the second result, and Control – the third. During 34 days from the beginning of the experiment, research variants 1 and 2 lagged behind the control in terms of the rate of body weight accumulation but then reached and overtook the latter, and variant Experiment 3, on the contrary, increased the gap in this indicator. The difference between the results of the third stage and the second one can be explained by the fact that the metabolism of fish decreases with age, as the body’s reaction to overcome the adverse effects of adverse factors, in this case – excessive concentration of vitamins, which could probably result in hypervitaminosis.

The third stage of the experiment demonstrated no advantages for any variant in terms of survival rate, as there was no fish death at all.

The fourth stage of the experiment was performed in the production conditions of an existing enterprise for the cultivation of clarius catfish in a recirculating aquaculture system. In this stage, there were only two options: control and experimental, with a concentration of “Chiktonik” in fodder of 5 ml/kg of fodder. The short duration of the experiment at this stage is explained by force majeure circumstances that arose at the enterprise – the failure of the biological filter a few days after the second control catch, which resulted in mass mortality of fish in the aquatic system pools and termination of the experiment. The results of the fourth stage, according to the indicators of two control catches, are presented in Table 5.

**Table 5.** Changes in the total mass and number of experimental materials during the 4th stage of the experiment

Variant	Pool No.	Date of control catch	
		01.10.21	11.10.21
Weight of experimental material, g ( $\pm$ average to control, %)			
Control	1	775	1080
Experiment	2	760	1031 (-4,5)
Fish survival, pcs ( $\pm$ experiment average to control, %)			
Control	1	1000	965
Experiment	2	1000	890 (-7,8)

As the table demonstrates, the results of the fourth, so-called production, stage of the experiment, in general, repeated the results of the second and third stages conducted in laboratory conditions. In addition, during the first ten days from the beginning of the experiment, fish in the experimental pool, which received fodder with vitamin-mineral additives, lagged behind the rate of body weight gain from fish in the control pool by 4.5%, and the mortality rate of juveniles was 7.8% higher than that in the control. It can be assumed that, in the case of continuation of the experiment, the indicators of fish in the experimental variant after a while would be equal to those in the control and possibly exceed them. To check this assumption, it is advisable to conduct a repeated experiment in production conditions.

### Conclusions and Perspectives

According to the results of the research, it was established:

1. Vitamin-amino acid complex "Chiktonik" has a biologically active effect on larvae and fry of clarius catfish. It was established that at a concentration of 1 ml per 1 kg of fodder the drug

has a positive effect on the growth and survival of clarius catfish under stressful situations.

2. At high concentrations (5, 15, 30 and 45 ml/kg of fodder) "Chiktonik" for some time (in research conditions – from 10 to 30 days from the beginning of the experiment) initially caused a slowdown in the growth of fish in experimental variants compared to fish in the control, which did not receive the drug supplement. Subsequently, fish from the experimental variants reached and overtook fish from the control group in terms of weight gain rate. Therewith, in younger fish, this process was faster: in a 30-day fry – during the next ten days, in older fish – in 20-30 days.

3. The survival rate of fish under the influence of vitamin-amino acid supplementation in the experiment, in general, significantly increased: by 13.3-46.0%, depending on the variant of the experiment.

4. It is considered promising to continue research to clarify the doses of the drug for different age groups of clarius catfish. In addition, it is advisable to evaluate the effect of the drug "Chiktonik" on the fertility of females, the quality of caviar and the offspring of clarius catfish.

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## **Ріст та виживаність кларієвого сома (*Clarias gariepinus* B., 1822) на різних стадіях вирощування з додаванням в корм препарату «Чиктонік»**

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**Анотація.** Стресові ситуації призводять зниження темпу росту та виживаність риб, тому пошук шляхів зменшення їх негативного впливу є актуальним. Мета досліджень – оцінити в умовах аквакультури вплив різних концентрацій вітамінно-амінокислотного комплексу «Чиктонік» на ріст та виживаність личинок і мальків африканського кларієвого сома (*Clarias gariepinus* B., 1822) після стресових ситуацій. Стрессова ситуація для риб виникла внаслідок значних коливань вмісту аміаку, нітритів та нітратів у водному середовищі замкнутої рециркуляційної аквасистеми протягом періоду запуску біологічного фільтра, до встановлення рівноваги. Було проведено серію дослідів, в ході яких встановлено, що додавання препарату з розрахунку 1 мл на 1 кг корму прискорює ріст риб у дослідних варіантах, у порівнянні з контролем. Експериментальне використання високих доз препарату (5, 15, 30 і 45 мл/кг корму) спочатку призвело до гальмування приросту маси тіла риб, але через 10-30 днів після завершення експерименту темп росту дослідного матеріалу зрівнявся з таким у контрольній групі риб і навіть перевищив показники контролю надалі. Встановлено позитивний вплив вітамінно-амінокислотного комплексу «Чиктонік» на виживаність молоді кларієвого сома на етапі завершення личинкового періоду життя і на перших стадіях малькового періоду. В експерименті з рибами старшого віку, які були повністю сформованими мальками, такого ефекту від використання препарату не спостерігалось: виживаність риб була на одному рівні як в досліді, так і в контролі. Одночасно було встановлено, що мальки гірше реагують на підвищені дози препарату, у порівнянні з підросшими личинками. Темп росту мальків після використання високих доз препарату так і не зрівнявся з таким у риб з групи контролю протягом місяця після завершення експерименту на відміну від молодших за віком риб. Загалом доведено доцільність і безпечність практичного використання препарату «Чиктонік» для молоді кларієвого сома як кормової добавки в дозі 1 мл/кг рибного корму

**Ключові слова:** вітамінно-амінокислотний комплекс, встановлення біологічної рівноваги, годівля, стрес, аквакультура



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## Quality of queen bees in different ways of their production

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**Abstract.** Timely replacement of queens is one of the main zootechnical methods for maintaining the vital activity of bee families, and searching for ways to improve their quality is an urgent problem. The purpose of this study was to evaluate the effect of the method of obtaining queen bees of *Apis mellifera sossimai* and *Apis mellifera carpatica* breeds on their reproductive functions and bee family productivity. To conduct research in the conditions of the apiary of honey-pollination area, three groups were established - control and two experimental, with nine bee colonies in each. In the first (control) group, uteruses were artificially removed from the nursery, in the second group - fistula uteri and the third – swarm uteri. The apiary was located at a point of 50×50 meters, bee families were kept in hives. According to the results of the study, it was established that the queen bee *Apis mellifera carpatica* was heavier than the queen bee *Apis mellifera sossimai*. The queen bees from the nursery are the smallest, and the swarm queen bees are slightly heavier than in other groups, although the difference was not statistically significant. Fistula queen bees produce the most eggs and provide the best brood quality. Fistula queen bees have better weight uniformity than swarm bees, which affects the productivity of families. Colonies with swarm queen bees are characterised by different levels of development, which complicates the maintenance of these families. The quality of queens is affected by the breeding method and the location of the queen cell in the honeycomb. The more queens the colony grows, the worse their quality. As it is complicated to establish optimal conditions in the nursery and the number of queen cells is large, this is the reason for the worst quality of queens. The results obtained are of practical importance for choosing a method of rearing queen bees of high quality to establish well-developed bee colonies

**Keywords:** hive, bee family, fistula queen, swarm queen, nursery, bee brood

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## Introduction

Honeybees can only live and work in a bee colony. There is normally only one queen bee in a typical bee colony. It is the colony's only fully matured female and the mother of a young queen, drone, and worker bees. When a bee colony loses its queen bee and is unable to reproduce a new one, it eventually dies. The queen bees have a sting, which they use during fights with other queens and when laying eggs.

The queen bee is much larger than a worker bee and longer than a drone but not as thick as he is. Her wings are longer than those of bees and drones. The movements are slow, although if necessary, she can run very fast. Day and night, she searches for honeycombs prepared by bees and lays eggs in them. She is constantly surrounded by young bees (retinue). They feed the queen bee with milk they produce, lick it and remove or eat its excrement. The egg production by queen bees depends on the strength of the colony. In a strong colony, the queen bee is larger, feeding is better, thus, it produces more eggs.

The queen bee lays an egg in a honeycomb, cleaned of dust, and old cocoons, and licked by young bees. She will not lay an egg in a dirty cell.

In a strong colony, where bees rebuild many cells, the queen bee can lay 2-3 thousand eggs a day. In a weak colony less, about 1-2 thousand eggs. Occasionally, a good queen bee lays 2-3 eggs in a cell if there are few clean cells. In this case, the bees leave only one egg, destroying the excess.

### Analysis of Recent Researches and Publications

The presence of the queen bee in the bee colony of honey bees fully influences their flight activity in collecting bee pollen. In its absence,

the harvesting of pollen and its processing, the extraction of wax and the construction of honeycombs, the cultivation of brood, and the collection of nectar are significantly hindered and then completely stopped. With the appearance of the queen bee, all functions of the colony as an integrated biological system are restored (Niño *et al.*, 2012).

Timely replacement of queen bees every 2 years, and increasing and maintaining the strength of bee colonies are the main zootechnical measures to maintain the optimal vital activity of bees and obtain the maximum amount of production from them (Mishchenko *et al.*, 2020). Most parameters characterising queen bee quality are reflected in the queen's body weight, which is considered a robust and the best indicator of queen quality (Prešern & Smodiš Škerl, 2019). The age of grafted larvae had a statistically significant effect on the queen's weight, body length, thorax width and length, and wing length of the queen (Okuyan & Akyol, 2018).

Reducing the intensity of work or their complete cessation in the absence of the queen bee is an important biological adaptive response of bee colonies, which allows for maintaining the strength and ability of bees to grow large numbers of brood (Rangel *et al.*, 2016; Walsh *et al.*, 2016).

Regarding the age of the queen bee and the harvesting activity of bees, the research demonstrates that the age of the queen bee and the activity of collecting bee pollen are related: the younger the age of the queen bee, the greater the collecting activity, the more bee pollen comes to colonies (Mishchenko *et al.*, 2020). The species composition and weight of the pollen collected by bees are influenced by many factors, primarily

the number and variety of plant pollen in nature, the development of which depends less on climatic conditions than nectar productivity of plants (Urcan *et al.*, 2017; Radev, 2018).

The first question that arises when choosing queens concerns the optimal timing of their use. Some consider that queens in bee colonies should be replaced every year, while others suggest replacing them in the 2nd or even 3rd year. However, notably, according to the natural conditions, in particular, the duration of the active period, the strength of the bee colony, the intensity of egg-laying and breeding characteristics of queens, the terms of their effective use will be different. If the active period of life of the colony and the oviposition of the queen bee is short, the colony is weak and the queen bee lays about 75-100 thousand eggs a year, then its physiological age will come later. On the contrary, during a long active period in strong colonies, the queen bee can lay 150-200 thousand eggs per season. In this case, the queen bee's body deteriorates faster and physiological old age occurs earlier (Mishchenko *et al.*, 2020).

The purpose of the study was to compare the quality of reproductive function in queen bees of *Apis mellifera sossimai* and *Apis mellifera carpatica* breeds with different methods of obtaining them and the impact on the productivity of the bee colony.

## Materials and Methods of Research

The apiary is located on a plot of land measuring 50x50 meters. Beehives with 20 frames are used to keep bee colonies. Bees are kept in the apiary of the Ukrainian field breed. The apiary has a honey pollination area. A winter house, a mobile vehicle, and a suitable chamber for honey pumping are all available at the apiary. Mustard is sown around the apiary every year.

To conduct experimental work in the apiary, three control groups and two experimental groups were established, with nine bee colonies in each group (Table 1). In the first group, the queen bees were artificially raised in the nursery, in the second group – fistulous queen bees, and in the third – swarm queen bees.

**Table 1.** The experimental design

Group	Number of bee colonies
1 – control	9
2 – experimental	9
3 – experimental	9

## Results of the Research and their Discussion

Egg-laying, or queen bee reproduction, begins in March. The activity of the queen bee develops gradually. Initially, she lays several hundred eggs a day. After the overwintering of bees and their flight, the number of laid eggs increases. With the onset of warmth and

natural flow, egg-laying reaches a maximum. In steppe areas, it is the end of June, and in forest areas – the beginning of June. In the second half of summer, the work of the queen bee gradually hinders and, with the onset of colds, stops. If autumn is warm, egg-laying lasts until October. Thus, the queen bee rests only a few months a year.

The queen bee cannot live long without bees. Even under favourable temperature conditions and good feeding, she lives no more than 2-5 days. In a wooden cage with 10 bees, she lives 15-20 days, sometimes a month. In a bee colony, the queen bee lives up to 5-9 years, more than drones and bees. In the first two years, she lays the largest number of eggs, then productivity decreases. With age, she loses her drone semen and lays more and more unfertilised eggs, from which drones are hatched, thus, it is impractical to keep the queen bee in industrial apiaries for more than two years. Annually, it is necessary to change at least half, and preferably 80-100% of all queens.

According to the method of obtaining, queen bees are divided into 4 groups. Bees that feed the queen bee constantly lick it. After licking the queen bee, the bee immediately begins to share the licked queen bee's substance (pheromones) with other bees. The queen bee's substance is secreted by the maxillary glands. When the queen bee cares for herself, it spreads it all over the body. If worker bees receive a sufficient amount of royal jelly, they do not lay queen cells on the eggs and larvae of worker bees.

The queen bee's substance has been learned to be obtained artificially. Bee colonies, in which queens were removed but given the queen bee's substance in the form of drops on paper, continued to function normally.

In an old queen bee, very little of this substance is produced, and the bees begin to rebuild the bowls, and the queen bee lays eggs in them. After 16 days, the young queen bee is born from an egg. After mating with drones, the young queen bee begins to lay eggs. This change in the queen bee is called quiet. With this change, young and old queens live and lay eggs jointly without expressing hostility to each other.

In a colony where a quiet change of the queen bee is planned, the old queen bee secretes about 1/4 of the queen bee's substance produced by the young fertile queen bee. If placed in a weak colony, the bees will not lay queen cells to change the queen bee. Practice demonstrates that if in the summer in a colony preparing for a quiet change, the queen cells are broken, then such a colony often does not lay them and stays to spend the winter with the old queen bee, which usually dies in the winter. The quiet change of the queen bee depends on the breed of bees. Mountain Grey Caucasian honey bees change 40% of their queen bees annually. Carpathian bees frequently change queens.

If the bee colony loses the queen bee, the bees begin to raise the queens from the eggs and young larvae of the worker bees. Such queens are named fistulous. Biologically, they are complete and frequently better than a swarm.

In normal colonies, the quality of the fistulous queen bee depends on the age of the larvae from which the queen bee will be raised. Larvae up to 3 days old give full-fledged queens, and larvae older than 3 days (at least 6 hours) produce transitional forms of queens with varying degrees of development of signs of worker bees (wax mirrors, baskets), with fewer ovaries and a small ovary. Larvae older than 90 hours are not able to develop into queens, they grow only as worker bees. When the colony prepares for swarming, the bees make bowls, in which the queen bee lays eggs. After the appearance of larvae, bees complete the bowls, turning them into queen cells. Queen bees obtained from such queen cells are called a swarm.

Swarm queen cells are laid, as a rule, on the side, bottom, and middle of the cells. They are similar to a ripe acorn or a thimble. Swarm queen bees are not the same. They grow best in

the queen cells, which are located at the top of the cell (frame). Here they get more food, more temperature, and more humidity. In the lower part of the cell, the temperature frequently fluctuates, as warm and cold air passes through the cell. In addition, there are fewer nursing bees at the bottom, thus, they feed the larvae worse, and queen bees are born smaller. Queen bees from such larvae lay fewer eggs, die more often in winter, and are less durable.

Nurseries produce fistulous queens, which are commonly called artificial. For this, firstly, to prepare the queen bee family: to remove the queen bee and open brood and give the larvae of worker bees of one-day age, etc.

The queen bees were removed at the beginning of the main foraging season, and instead, the bees produced fistula queens. Young queen bees were weighed after fertilisation and yielded comparable findings (Table 2, 3).

**Table 2.** Live weight of fertile queen bees depends on the growth environment

Origin	Number of queen bees	Weight, mg
Artificial queen bees from the nursery	9	237.9 ± 7.1
Their daughters were raised in their colonies during the main forage period (fistulous)	9	272.9 ± 5.9
Their granddaughters were raised in their colonies at the end of the main forage period (fistulous)	9	230 ± 9.7

**Table 3.** Live weight of fertile queen bees

Origin	<i>Apis mellifera carpatica</i> , mg	<i>Apis mellifera sossimai</i> , mg
The queen bee from the nursery	277.1 ± 6.0	223.6 ± 2.8
Their daughters (fistulous)	288.5 ± 5.4	238.7 ± 4.3

During the egg-laying period, the queen bees were weighed. The queen bees were not related but belonged to the same Carpathian

breed. According to the data in Table 4, the queen bees from the nursery are smaller than the queen bees from their colonies.

**Table 4.** Oviposition and live weight of queen bees depending on their origin

Origin	The mass of the fertile queen bee at the beginning of oviposition, mg	Live weight during egg-laying, mg	Oviposition, pcs.
Fistulous	247.6 ± 4.0	292.2 ± 5.0	1629 ± 113.0
Swarm	249.7 ± 6.0	300.2 ± 5.8	1206 ± 94.0

In addition to the Carpathian breed, the Ukrainian steppe breed was tested, comparing fistula queens with swarm queens. Queen bees are slightly heavier, as presented in

Table 4, although this difference is not statistically significant. Fistulous queen bees, on the other hand, produce much more eggs. The brooding quality was greater and higher

in colonies with fistulous queen bees than in swarm queen bee colonies. As a result, swarm queen bees are not worse than fistulous. Colonies with fistulous queen bees are more convenient to work with.

The data on the weighting of swarm and fistulous queens at the beginning of egg-laying is summarised in Table 4. At the beginning of oviposition, the amount of swarm and fistulous queen bees are nearly identical. Only the difference in maximum and minimum weights is significant: 85 mg in swarm and 48 mg in fistulous. As a result, fistulous queen bees have better uniformity than swarm, which has an impact on colony productivity. As swarm queen bees can be both good and harmful, their colonies emerge with variable levels of ability, complicating maintenance.

July is the most favourable month for breeding and changing queens. At this time, bee colonies reach maximum strength, the hive is constantly receiving nectar and pollen, there are no sharp temperature fluctuations. All this provides the best conditions for breeding the largest bees and queen bees. It is almost impossible to establish such conditions for the artificial breeding of queens in the nursery. Encouraging feeding (100-200 g of syrup), which is distributed to foster colonies,

does not provide the colony and offspring with proper nutrition.

The quality of queens is influenced by the method of breeding and location of the queen cell in the honeycomb, the presence of fodder base in nature during their cultivation, the strength of the colony, and the number of queen cells in the colony. The more queens the colony grows, the worse their quality.

The young queen bee should fly around. If she does not do this within a month, she loses the ability to mate with drones, and she begins to lay unfertilised eggs, from which only drones are hatched. This queen bee is called a cobweb. A colony with such a queen bee usually dies if a beekeeper does not help it.

During a strong flow, the queen bee cannot lay many eggs because the bees fill the cells with honey. As the flow decreases and the number of free cells increases, she lays more eggs. After the end of the flow season, the queen bee stops laying eggs as the bees feed them worse. In winter, they do not lay eggs.

## Conclusions

1b. During the artificial rearing of queens, the foster colony does not accept eggs for rearing, thus they give her larvae. The younger the larva, the better the queen bee.

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## Якість бджолиних маток за різних способів їх отримання

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**Анотація.** Своєчасна зміна маток є одним з основних зоотехнічних прийомів для підтримання життєдіяльності бджолиних сімей, а пошук шляхів підвищення їх якості є актуальною проблемою. Метою даного дослідження, було оцінити вплив способу отримання бджолиних маток порід *Apis mellifera sossimai* та *Apis mellifera carpatica* на їх відтворювальні функції та продуктивність бджолиної сім'ї. Для проведення досліджень в умовах пасіки медово-запилувального напрямку було сформовано три групи – контрольну та дві дослідні, по дев'ять бджолосімей у кожній. У першій (контрольній) групі були штучно виведені матки із розплідника, у другій групі – свищеві матки та в третій – ройові. Пасіка розміщувалась на точку розміром 50×50 метрів, бджолині сім'ї утримували у вуликах-лежаках. За результатами дослідження було встановлено, що бджолині матки *Apis mellifera carpatica* були важчі за бджолині матки *Apis mellifera sossimai*. Бджолині матки з розплідника найменші, а ройові бджолині матки дещо важчі ніж в інших групах, хоча різниця не була статистично значущою. Свищеві бджолині матки виробляють найбільше яєць і забезпечують кращу якість розплоду. Свищеві бджолині матки мають кращу вирівняність за масою, ніж ройові, що впливає на продуктивність сімей. Колонії з ройовими бджолиними матками характеризуються різними рівнями розвитку, що ускладнює утримання цих сімей. На якість маток впливають спосіб розведення і розташування маточника в стільнику. Чим більше маток вирощує колонія, тим гірша їх якість. Оскільки в розпліднику важко створити оптимальні умови, а кількість маточників велика, це є причиною найгіршої якості маток. Отримані результати мають практичне значення для вибору способу вирощування маток високої якості для створення добре розвинених бджолиних колоній

**Ключові слова:** вулик, бджолина сім'я, свищеві матки, ройові матки, розплідник, бджолиний розплід



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## Changes in haematological parameters in hens under short-term exposure to adverse environmental factors

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**Abstract.** Short-term exposure to adverse factors is a common phenomenon in industrial egg production. An urgent problem is to understand the impact of environmental changes on poultry use. A comparative analysis of changes in haematological parameters in hens under short-term exposure to adverse environmental factors of different natures was performed. For this purpose, a control and 3 experimental groups of hens were established in a modern complex for the production of food eggs. Within 24 hours, the hens of the 2nd group were deprived of fodder, the 3rd group – of light, and the 4th group – were kept in significant overcrowding. The smallest changes in the blood system of hens were observed under the influence of the factor of lack of fodder, namely an increase, within the physiological standard, in the content of leukocytes and erythrocyte sedimentation rate, a decrease in haemoglobin concentration, hematocrit, erythrocytes, platelets, and a violation of the ratio of different forms of leukocytes – an increase in the concentration of heterophils (3.3% > normal) against a decrease in the concentration of monocytes (1.6% < normal), lymphocytes and basophils. Therewith, under the influence of the factor of the absence of light, a higher content of leukocytes in the blood by 10.6%, a lower concentration of haemoglobin by 22.4%, hematocrit – by 4.2%, platelets – by 9.8%, and a higher erythrocyte sedimentation rate by 9.8%, a higher concentration of heterophils by 5.9% and a lower concentration of lymphocytes – by 4.6% were identified compared to the factor of the absence of fodder. The most significant changes in the blood system were noted under the influence of the factor of significant over-consolidation of hens, namely, a higher content of leukocytes in the blood by 17.1 and 5.9%, a lower concentration of haemoglobin by 29.6 and 9.2%, hematocrit – by 5.9 and 1.7%, erythrocytes – by 10.3%, platelets – by 35.8 and 28.8%, and higher erythrocyte sedimentation rate by 4.9%, a higher concentration of heterophils by 11.3 and 5.4 % and lower concentration of monocytes by 0.8 and 0.4%, lymphocytes by 9.4 and 4.8% and eosinophils by 0.7% compared to the factor of lack of fodder and lack of light,

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respectively. Thus, in production conditions, it is necessary to avoid the over-compaction of poultry, as this factor has the greatest adverse effect

**Keywords:** hens, adverse environmental factors, haematological parameters, stress

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## Relevance

Intensive management of the poultry industry includes several technological operations that cause excessive stress on the adaptive systems of the body of hens and the development of stress in them (Scanes, 2016; Zhuchaev *et al.*, 2019). The effect of adverse environmental factors or technological stressors, such as high density of housing, changes in the microclimate of production facilities, housing conditions and diet composition, vaccination, transportation and movement, reduce the level of the immunological reactivity of the poultry body (Reber *et al.*, 2007; Sloan *et al.*, 2010; Hall *et al.*, 2014), which decreases its productivity (Lara & Rostagno, 2013; Stoianovskiy *et al.*, 2018; Sakhatsky *et al.*, 2020). In conditions of intensive production, it is impossible to avoid completely the influence of stressors, however, determining the degree of influence of technological factors depending on their nature on the physiological state of poultry is a prerequisite for the development of new methods of stress prevention in the selection of optimal ways of keeping hens.

## Analysis of Recent Studies and Publications

It is known that during stress in hens, the activity of all body systems is strained, which is designed to protect themselves and adapt to new living conditions (Dhabhar *et al.*, 2012; Kang *et al.*, 2018; Gorelik *et al.*, 2020). A prerequisite for the development of the stress response is the enhancement of the function of the endocrine glands, particularly the hypothalamus-anterior

pituitary-adrenal cortex system (Olubodun *et al.*, 2015). The main role in the development of stress, according to Selje, is performed by the adrenal cortex, which, under the influence of the pituitary gland, increases the secretion of steroid hormones involved in the adaptation process (Selje, 1979). Therefore, it is believed that the main mechanisms in the implementation of the stress state in the body of poultry are the sympathoadrenal and hypothalamic-pituitary-adrenal-corticotrophic systems, i.e. the development of adaptive responses to the effects of various nonspecific environmental factors on the body occurs by a common mechanism: through the hypothalamic-pituitary-adrenal system and the sympathoadrenal system with the participation of catecholamines (Gavreliuk & Chykina, 2017; Infante *et al.*, 2017). Due to biological effects, catecholamines ensure the transition of the body from a state of rest to a state of excitement and allow it to remain in this state for a long time. Therewith, the emergence and course of physiological reactions in the body of poultry under the action of hormones of the medullary layer of the adrenal glands and mediators of the sympathoadrenal system are accompanied by an increase and qualitative change in metabolic processes in immunocompetent tissues (Stoianovskiy *et al.*, 2018), which is reflected in their blood system.

Changes in the blood system in response to stressors such as lymphopenia, eosinopenia and neutrophilia were first described by Hans Selye in 1936 (Selye, 1936). Modern studies

demonstrate that acute stress primarily causes significant changes in the quantitative and qualitative composition of leukocytes in hens (Nicol *et al.*, 2006; Sekeroglu *et al.*, 2011; Nwaigwe *et al.*, 2020). Leukemoid blood reaction in hens has been described under the influence of stressors such as starvation (Najafi *et al.*, 2015), temperature (Prieto & Campo, 2010), light (Huth & Archer, 2015), contamination with microorganisms (Redmond *et al.*, 2011), transportation (Al-Murrani *et al.*, 1997), movement restriction (Bedanova *et al.*, 2007), etc. However, the vast majority of studies are devoted to the effects on the body of hens, usually only one stressor. In turn, the issue of physiological changes in the body of poultry under short-term exposure to stressors of different natures has been understudied.

The purpose of the research was to compare changes in haematological parameters in hens under short-term exposure to adverse environmental factors of different natures.

## Materials and Methods of Research

As the object of research were used egg hens of the industrial flock “Hy-Line W-36”. Experiments with experimental animals were conducted following the rules of the European Convention

for the Protection of Vertebrate Animals (Official Journal of the European Union L276/33, 2010).

In the conditions of a modern complex for the production of food eggs in poultry houses with an area of 2915 m<sup>2</sup>, 3 groups of hens (101 heads in each) were established at the age of 52 weeks, each of which was kept in a separate cage-analogous in area and equipment manufactured by “Big Dutchman” (Germany). The control group was established from 10 representatives of each of the 3 experimental groups before the simulation of acute technological stress. Blood samples were taken from them, from which a control group of 30 heads was established. Subsequently, each group was exposed to short-term adverse environmental factors of different natures (acute stressors). In particular, hens of the 2nd group were deprived of fodder, the 3rd group – of light, and the 4th group – were kept with significant overcrowding (Table 1). Modelling the effects of acute stressors in hens of the 2nd and 3rd groups was achieved by turning off the corresponding systems in the poultry house – the fodder distribution line and lighting. In the 4th group, 60 hens were planted in the cage to ensure significant over-compaction of the livestock.

**Table 1.** Experiment scheme

Characteristic	Group of hens			
	1	2	3	4
Environmental factor	control (before the factor influence)	absence fodder      light		increased density of content
Duration of the factor exposure, hours	24			
Number of cages per floor	1176			
Number of birds per cage	101		161	
Planting density, birds / m <sup>2</sup>	24.9		39.7	
Area coverage, cm <sup>2</sup> /birds	401.4		251.8	
Cage area, cm <sup>2</sup>	40544			
Number of nipples per cage, pcs.	12			
Feeding front, cm	7.2		4.5	
Poultry house area, m <sup>2</sup>	2915			

Exposure to the factor in all groups was 24 hours, after which haematological parameters of hens were determined. For this purpose, 1.0-1.5 ml of blood was taken from the subwing vein of 30 hens of each group into a test tube with EDTA. Haematological parameters of laying hens were determined on a Micros 60 haematological analyser (Horiba Ltd.) in the laboratory "Bald" (certificate No. LB/02/2016).

The obtained digital results were processed by methods of variation statistics. The significance of differences between the mean values was determined by Student's t-test, differences were considered significant at  $p < 0.05$ .

## Research Results

Haematological parameters of hens of all experimental groups at the beginning of the research were within the physiological standards for each parameter (group 1). No significant differences were identified between the groups. According to the results of the research, after 24 hours of exposure to adverse environmental factors, regardless of their nature, the content of haemoglobin, erythrocytes, hematocrit, platelets and erythrocyte sedimentation rate in the blood of hens were within the physiological standard, and the leukocyte content exceeded them (Table 2).

**Table 2.** Haematological parameters of laying hens

Indicator	Group				Reference values, (Jain, 1993)
	1	2	3	4	
Leukocytes, thousand/ $\mu$ l	26.9 $\pm$ 0.46	36.9 $\pm$ 0.61***	40.8 $\pm$ 0.39*****	43.2 $\pm$ 0.37*****	20-40
Haemoglobin, g/dl	11.8 $\pm$ 0.20	9.8 $\pm$ 0.11**	7.6 $\pm$ 0.23*****	6.9 $\pm$ 0.18*****	7-13
Hematocrit, %	32.9 $\pm$ 0.68	31.1 $\pm$ 0.37*	26.9 $\pm$ 0.53*****	25.2 $\pm$ 0.52*****	22-35
Erythrocytes, mln/ $\text{mm}^3$	3.2 $\pm$ 0.08	2.9 $\pm$ 0.06**	2.9 $\pm$ 0.08**	2.6 $\pm$ 0.07*****	2.5-3.5
Platelets, thousand/ $\text{mm}^3$	73.8 $\pm$ 0.82	69.3 $\pm$ 0.47***	62.5 $\pm$ 0.36*****	44.5 $\pm$ 0.42*****	32-100
The erythrocyte sedimentation rate, Mm/hour	5.4 $\pm$ 0.04	6.1 $\pm$ 0.09***	6.1 $\pm$ 0.06***	6.4 $\pm$ 0.03*****	4.0-6.5

**Notes:** \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  – in comparison with the first group;  $^{\circ}p < 0.05$ ,  $^{\circ\circ}p < 0.01$ ,  $^{\circ\circ\circ}p < 0.001$  – in comparison with the second group;  $^{\prime}p < 0.05$ ,  $^{\prime\prime}p < 0.01$ ,  $^{\prime\prime\prime}p < 0.001$  – in comparison with the third group

In particular, there was an increase in the content of leukocytes in the blood of hens under the influence of adverse environmental factors. In hens of the 2nd group, the leukocyte content was higher by 37.2% ( $p < 0.001$ ) compared to the 1st group but was within the physiological standard. Therewith, in hens of group 3, the blood leukocyte content exceeded the physiological standard by 2.0% and was higher by 51.7% ( $p < 0.001$ ) and 10.6% ( $p < 0.001$ ) compared to groups 1 and 2, respectively. In hens of group 4, the leukocyte content exceeded the physiological standard by 8.0% and was higher by 60.6% ( $p < 0.001$ ) compared to group 1 and by 17.1% ( $p < 0.001$ ) and 5.9% ( $p < 0.001$ ) compared to

groups 2 and 3, respectively. Thus, the highest content of leukocytes in the blood of hens was observed under the influence of the factor of significant overcompaction, and then in descending order – in the absence of light and deprivation of fodder. In addition, when hens were deprived of fodder, the content of leukocytes in their blood did not exceed the physiological standard, although it was close to its upper limit.

Haemoglobin content and hematocrit in hens of all groups were within the physiological standard. However, there was a decrease in haemoglobin content under the influence of adverse environmental factors. Thus, in hens of group 2, the haemoglobin content in the

blood was lower by 16.9% ( $p<0.001$ ) compared to group 1. Therewith, haemoglobin content in hens of the 3rd group was lower by 35.6% ( $p<0.001$ ) and 22.4% ( $p<0.001$ ) compared to the 1st and 2nd groups, respectively. Therewith, the haemoglobin content in hens of group 4 was higher by 41.5% ( $p<0.001$ ) compared to group 1 and by 29.6% ( $p<0.001$ ) and 9.2% ( $p<0.05$ ) compared to groups 2 and 3, respectively.

In addition, there was a decrease in hematocrit under the influence of adverse environmental factors. In hens of group 2, hematocrit was lower by 1.8% ( $p<0.001$ ) compared to group 1. Therewith, hematocrit in hens of group 3 was lower by 6.0% ( $p<0.001$ ) and 4.2% ( $p<0.001$ ) compared to groups 1 and 2, respectively. While in hens of group 4 hematocrit was lower by 7.7% ( $p<0.001$ ) compared to group 1 and by 5.9% ( $p<0.001$ ) and 1.7% ( $p<0.001$ ) compared to groups 2 and 3, respectively.

The influence of adverse environmental factors during the keeping of hens was accompanied by a decrease in the concentration of red blood cells in their blood within the physiological standard. Thus, in hens of the 2nd and 3rd groups, the concentration of red blood cells in the blood was the same and, therewith, lower than in the 1st group by 9.4% ( $p<0.01$ ). Therewith, in hens of the 4th group, the erythrocyte concentration was lower by 18.8% ( $p<0.001$ ) compared to the 1st group and by 10.3% ( $p<0.01$ ) compared to the 2nd and 3rd groups, respectively.

The concentration of platelets in the blood of hens decreased under the influence of adverse environmental factors. Thus, in hens of the 2nd group, the platelet content in the blood

was lower by 6.1% ( $p<0.001$ ) compared to the 1st group. In hens of group 3, the platelet content in the blood was lower by 15.3% ( $p<0.001$ ) compared to group 1 and by 9.8% ( $p<0.001$ ) compared to group 2. Whereas in hens of group 4, the platelet concentration was lower by 39.7% ( $p<0.001$ ) compared to group 1 and by 35.8% ( $p<0.001$ ) and 28.8% ( $p<0.001$ ) compared to groups 2 and 3, respectively.

The parameters of erythrocyte sedimentation rate in the blood of hens of all groups, regardless of the presence and nature of the factor of influence, were within the physiological standard. Therewith, there was an increase in the erythrocyte sedimentation rate under the influence of environmental factors. In particular, in hens of the 2nd and 3rd groups, the erythrocyte sedimentation rate was higher by 13.0% ( $p<0.001$ ) compared to the 1st group. Therewith, in hens of the 4th group the erythrocyte sedimentation rate was higher by 18.5% ( $p<0.001$ ) compared to the 1st group and by 4.9% compared to the 2nd ( $p<0.05$ ) and 3rd ( $p<0.01$ ) groups, respectively.

The influence of adverse environmental factors during the keeping of hens was reflected in the ratio of leukocytes in their blood (Table 3), namely accompanied by an increase in the number of heterophile in the blood. In hens of groups 2-4, which were kept in the absence of fodder, light and significant overcompaction, the content of heterophile in the blood exceeded the physiological standard by 3.3%, 9.2% and 6.8%, respectively. In particular, the concentration of heterophile in hens of the 2nd group was higher by 12.3% ( $p<0.001$ ) compared to the 1st group.

**Table 3.** Leukogram of laying hens, %

Indicator	Group				Reference values, (Jain, 1993)
	1	2	3	4	
Monocytes	7.3±0.22	3.4±0.26***	3.0±0.18***	2.6±0.10****	5-10

Table 3. Continued

Indicator	Group				Reference values, (Jain,1993)
	1	2	3	4	
Lymphocytes	64.3±0.48	57.0±0.48***	52.4±0.89*****	47.6±0.33*****	45-70
Eosinophils	4.5±0.34	3.9±0.14	3.4±0.31*	3.2±0.20***	1.5-6.0
Basophils	2.8±0.17	2.3±0.13*	2.0±0.13***	2.0±0.18**	1-3
Heterophiles	21.0±0.53	33.3±0.61***	39.2±0.27*****	44.6±0.28*****	15-30

**Notes:** \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$  – in comparison with the first group; ° $p<0.01$ ; °° $p<0.001$  – in comparison with the second group; ‘ $p<0.05$ , ‘° $p<0.001$  – in comparison with the third group

In hens of group 3, the number of heterophile was higher by 18.2% ( $p<0.001$ ) and 5.9% ( $p<0.001$ ) than in groups 1 and 2, respectively. Thus, the content of heterophile in hens of group 4 was the highest and exceeded the indicators of group 1 by 23.6% ( $p<0.001$ ), group 2 – 11.3% ( $p<0.001$ ) and group 3 – 5.4% ( $p<0.001$ ).

The increase in the level of heterophile occurred against the background of a decrease in the number of other forms of leukocytes. In particular, in hens of the 2nd group the concentration of monocytes did not reach the physiological standard by 1.6% and was lower by 3.9% ( $p<0.001$ ), and in hens of the 3rd group – by 2.0% and was lower by 4.3% ( $p<0.001$ ) compared to the 1st group. Therewith, in hens of the 4th group, the concentration of monocytes did not reach the physiological standard by 2.4% and was lower by 4.7% ( $p<0.001$ ) compared to the 1st group and by 0.8% ( $p<0.01$ ) and 0.4% ( $p<0.05$ ) compared to the 2nd and 3rd groups, respectively.

The number of lymphocytes in the blood of hens decreased under the influence of adverse environmental factors, but within the physiological standard. In particular, in hens of the 2nd group the concentration of lymphocytes was lower by 7.3% ( $p<0.001$ ) compared to the 1st group, and in hens of the 3rd group – by 11.9% ( $p<0.001$ ) and 4.6% ( $p<0.001$ ) compared to the 1st and 2nd groups, respectively. While in hens of group 4, the concentration of lymphocytes was lower by 16.7% ( $p<0.001$ ) compared to group 1 and by 9.4% ( $p<0.001$ ) and 4.8% ( $p<0.001$ ) compared to groups 2 and 3, respectively.

In addition, a decrease in the content of eosinophils and basophils in the blood of hens within the physiological standard was observed under the influence of environmental factors. In particular, the content of eosinophils in hens of the 2nd group was at the same level as in the 1st group. In hens of the 3rd group, the concentration of eosinophils was lower by 1.1% ( $p<0.05$ ) compared to the 1st group and did not differ from the 2nd group, and in hens of the 4th group – by 1.3% ( $p<0.01$ ) and 0.7% ( $p<0.05$ ) compared to the 1st and 2nd groups, respectively, and did not differ from the 3rd group.

The content of basophils in hens of group 2 was lower by 0.5% ( $p<0.05$ ), in hens of groups 3 and 4 – by 0.8% ( $p<0.01$ ) compared to group 1. There were no differences between 2-4 groups, i.e. depending on the nature of the factor of influence, in the content of basophils.

Thus, the short-term effect of adverse environmental factors on the body of hens was accompanied by changes in their blood system, which were reflected in an increase in the content of leukocytes by increasing the number of heterophile and decreasing the level of monocytes and depending on the nature of the factor of influence. An increase in leukocyte levels is a characteristic response of immunocompetent tissues to the action of glucocorticoids and catecholamines, the concentration of which in the blood of hens increases under the influence of various stress factors (Sapolsky, 2000; Jiang *et al.*, 2017). In general, the development of leukocytosis is based on an increase in the content of

heterophile in the blood of hens, which was noted in the research (Table 3). According to several authors (Christopher and Link, 2007; Dhabhar *et al.*, 2012), the increase in leukocyte content due to heterophile occurs due to hypercortisolemia and hypercatecholamanemia caused by stress, which increases their number and mobilisation in the blood. Increasing the pool of circulating heterophile is the result of preparing the body for a protective response in response to possible damage (Kubes, 2018; Liew & Kubes, 2019).

### Conclusions and perspectives

The short-term impact of adverse environmental factors on the body of hens was accompanied by changes in their blood system, which were reflected in an increase in the content of leukocytes, increasing the number of heterophile and decreasing the level of monocytes, and depending on the nature of the factor of influence. The smallest changes in the blood system of hens were observed under the influence of the factor of lack of feed, namely an increase, within the physiological standard, in the content of leukocytes by 37.2% and erythrocyte sedimentation rate – by 13.0%, a decrease in haemoglobin concentration by 16.9%, hematocrit – by 1.8%, erythrocytes – by 9.4%, platelets – by 6.1%, and violation of the ratio

of different forms of leukocytes – increase in the concentration of heterophils by 12.3% (3.3%>normal) against the background of a decrease in the concentration of monocytes – by 3.9% (1.6%<normal), lymphocytes – by 7.3% and basophils – by 0.5%. Therewith, under the influence of the factor of the absence of light, a higher content of leukocytes in the blood by 10.6%, a lower concentration of haemoglobin by 22.4%, hematocrit – by 4.2%, platelets – by 9.8%, and a higher erythrocyte sedimentation rate by 9.8%, a higher concentration of heterophils by 5.9% and a lower concentration of lymphocytes – by 4.6% were identified compared to the factor of the absence of fodder.

The most significant changes in the blood system were noted under the influence of the factor of short-term significant over-consolidation of hens, namely, a higher content of leukocytes in the blood by 17.1 and 5.9%, a lower concentration of haemoglobin by 29.6 and 9.2%, hematocrit - by 5.9 and 1.7%, erythrocytes - by 10.3%, platelets - by 35.8 and 28.8%, and higher erythrocyte sedimentation rate by 4.9 %, a higher concentration of heterophils by 11.3 and 5.4 % and lower concentration of monocytes by 0.8 and 0.4 %, lymphocytes by 9.4 and 4.8 % and eosinophils by 0.7 % compared to the factor of lack of fodder and lack of light, respectively.

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## **Зміни гематологічних параметрів у курей за короткотермінового впливу негативних факторів навколишнього середовища**

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**Анотація.** Короткочасний вплив негативних факторів, поширене явище під час промислового виробництва яєць. Актуальною проблемою є зрозуміти вплив змін зовнішнього середовища на використання птиці. Проведено порівняльний аналіз змін гематологічних параметрів у курей за короткотермінового впливу негативних факторів навколишнього середовища різної природи. Для цього в умовах сучасного комплексу з виробництва харчових яєць сформували контрольну і 3 дослідні групи курей. Впродовж 24 годин кури 2-ї групи були позбавлені корму, 3-ї – світла, а 4-ї – утримувались за значного переущільнення. Найменші зміни в системі крові курей спостерігались за впливу фактора відсутності корму, а саме підвищення, в межах фізіологічної норми, вмісту у крові лейкоцитів та швидкості осідання еритроцитів, зниження концентрації гемоглобіну, гематокриту, еритроцитів, тромбоцитів, а також порушення співвідношення різних форм лейкоцитів – підвищення концентрації гетерофілів (3,3 % > норми) на тлі зниження концентрації моноцитів (1,6 % < норми), лімфоцитів та базофілів. Тоді як за впливу фактора відсутності світла було виявлено вищий вміст у крові лейкоцитів на 10,6 %, нижчу концентрацію гемоглобіну на 22,4 %, гематокриту – на 4,2 %, тромбоцитів – на 9,8 %, а також вищу швидкість осідання еритроцитів на 9,8 %, вищу концентрацію гетерофілів на 5,9 % та нижчу концентрацію лімфоцитів – на 4,6 % у порівнянні з фактором відсутності корму. Найсуттєвіші зміни в системі крові відмічені за впливу фактора значного переущільнення курей, а саме вищий вміст у крові лейкоцитів на 17,1 і 5,9 %, нижчу концентрацію гемоглобіну на 29,6 і 9,2 %, гематокриту – на 5,9 і 1,7 %, еритроцитів – на 10,3 %, тромбоцитів – на 35,8 і 28,8 %, а також вищу швидкість осідання еритроцитів на 4,9 %, вищу концентрацію гетерофілів на 11,3 і 5,4 % та нижчу концентрацію моноцитів – на 0,8 та 0,4 %, лімфоцитів – на 9,4 і 4,8 % та еозинофілів – на 0,7 % у порівнянні з фактором відсутності корму та відсутності світла відповідно. Таким чином у виробничих умовах необхідно уникати переущільнення птиці, оскільки цей фактор має найбільший негативний ефект

**Ключові слова:** кури, негативні фактори навколишнього середовища, гематологічні параметри, стрес



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## Investigation of the effect of sodium humate fodder additive on sterlet in cage farming

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**Abstract.** Humic preparations are used as microfertilisers in crop production and growth stimulants in animal husbandry, in aquaculture there is a positive effect of these compounds on individual objects of cultivation, but their use in fish farming has not yet been understudied. The purpose of the work was to present the results of an experiment to evaluate the effect of fodder additive humates on the growth rate and survival of different age groups of sterlet (*Acipenser ruthenus* L., 1758) and the conversion of fodder by fish in aquaculture. A series of experiments were conducted on feeding sterlet with fodder with the addition of sodium humate. The research was conducted in 2018, 2020 and 2021 in the production conditions of the cage fish farm located at the Kaniv reservoir. The effect of various concentrations of sodium humate fodder additive on yearling and two-year-old sterlet was evaluated. Sodium humate was applied to the fodder for the respective groups of sterlet by spraying its aqueous solution in defined proportions with subsequent drying. It was established that the addition of sodium humate to fish fodder at concentrations of 60, 100, 120 and 200 mg/kg of fodder did not significantly affect the growth rate of sterlet. Thus, the advantage of the experimental variants over the control for this indicator was insignificant, within 1.0-1.6%. No effect of sodium humate on the survival of sterlet juveniles reared in cages was established. Therewith, the conversion of fodder with the addition of humates by juvenile and two-year-old sterlet was better by 6.7-17.4%. According to the experimental results, the best result was obtained in the variant using fodder additive humates at a concentration of 200 mg/kg of fodder. Based on the results of the research, it was established that in aquaculture conditions it is advisable to use sodium humate as a fodder additive for yearlings and two-year-old sterlet, designed to increase fodder conversion

**Keywords:** biologically active substances, fish farming, sturgeon, survival, growth rate, fodder conversion

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## Relevance

Improving the fish fodder formula is one of the most effective ways to increase the profitability of commercial fish farming. The main purpose that the researchers set themselves is to achieve a reduction in fodder costs per unit of marketable products both in quantitative and in value terms.

To improve the productive properties of artificial fodder, additives of biologically active substances are frequently used, which, with a slight increase in the cost of fodder, significantly improve its conversion. Such substances include easily soluble salts of humic acids – humates. These substances have the properties of immunostimulants, probiotics, toxin adsorbers, and digestive and growth stimulants (Horovaya *et al.*, 1995). According to Kovalenko & Polishhuk (2018), the effect of humates on fish, under the conditions of their cultivation in different aquaculture systems, has not yet been understudied, which determines the relevance of conducting appropriate research on various fish farming facilities, in particular on sturgeon.

### Analysis of Recent Studies and Publications

The interest in the search for fodder additives with biologically active properties that can improve the conversion of artificial fodder for farm animals has existed for a long time. Fodder additives of different origins, both made from natural raw materials and synthetic, are widely used (Polishhuk & Bulavkina, 2010). An essential place among the latter is occupied by humates – water-soluble salts of humic and fulvic acids (Popov, 2004).

Humates are mainly used in crop production as growth stimulants and microfertilisers (Luchnyk *et al.*; Semenyuk *et al.*, 2016).

Significant positive experience has been accumulated in using these substances as micro-additives in animal and poultry fodder (Druzhynyna, 2002; Islam, 2005; Bezuhlova & Zynchenko, 2016), including for the treatment and prevention of diseases (Stepchenko & Hryban, 1997; Belyaev *et al.*, 2012; Hrybanova & Karymova, 2015).

Information on using humates in aquaculture is limited and fragmentary. Thus, it is known about the positive effect of fodder supplementation of humic substances on the fish farming performance of channel catfish (Neronin *et al.*, 1990), carp (Abdel-Wahab, El-Refaae & Ammar, 2012) and tilapia (Ahmed El-Ashram & Maaly Mohammed, 2012). There is no information on using humates in the sturgeon fodder.

The first investigation of the effect of humates on the growth rate and survival of sterlet (*Acipenser ruthenus Linnaeus*), a valuable object of commercial sturgeon farming, was conducted in 2017 (Kovalenko & Polishhuk, 2018). It was established that feeding fodder with the addition of humates to three-year-old sterlet, using the cage method of rearing this fish, had a positive effect on fish breeding performance. Thus, the average daily weight gain of fish in the experimental variant with the addition of humates in the amount of 30 mg/kg of fish fodder was 0.695 g/day, and in the control – 0.59 g/day. The survival rate of fish during the experiment in experimental cages was 92.7%, and in control cages – 82.5%. The obtained result of the experiment was the foundation for continuing research in this area.

### The Purpose of the Research

The purpose of the research was to investigate the effect of different concentrations of humate additives in fish fodder on sterlets of different

ages when grown in the production conditions of cage fish farming.

The object of research – the fish-breeding effect of adding sodium humate to fish fodder.

The subject of the research – growth and survival rates of various age groups of sterlet, the efficiency of conversion of fish fodder with the addition of sodium humate.

Objectives of the research:

- to test the effect of feed additive sodium humate at different concentrations on the growth rate and survival of sterlet juveniles, and on the efficiency of fodder use;

- to collect biological material for laboratory tests on the content of heavy metals in fish.

## Materials and Methods of Research

The research was conducted in 2018, 2020 and 2021 at the production facility of the private fish farm of industrial type “Research and Production Agricultural Enterprise” Bester”. The gardens of the enterprise are located in the

waters of the Kaniv reservoir, in the coastal zone near the village of Trypillya, Obukhov district, Kyiv region.

The material for the research was this yearling (in 2018 and 2020) and two-year-old sterlet (2021), for their cultivation in fish cages with intensive feeding with the product feed “INICIO 917” of the Danish company BioMar, one of the world leaders in fodder production in the field of aquaculture.

The source of sodium humate – the drug “Reasil Humic Health” in powder form, produced by the international company UAB “Life Force Baltic” (Lithuania). The content of humic compounds in dry matter – not less than 80%. The product corresponds to the generally accepted requirements for bacteriological indicators and sanitary and hygienic standards for the permissible content of radionuclides and heavy metals (Feed material Reasil® Humic Health, 2021). The research was conducted using different concentrations of sodium humate additive to fodder according to the scheme (Table 1).

**Table 1.** The scheme of research

Year	Age group of fish	Concentration of sodium humate in fish fodder, mg/kg		
		Control	Experiment 1	Experiment 2
2018	Yearlings	0	60	120
2020	Yearlings	0	100	200
2021	Two-year-olds	0	200*	-

**Notes:** \* – one pilot variant was established in 2021

The research was performed with double replication. For each variant of the experiment, 2 specifically made rectangular frame cages with side dimensions of 1x1x1 m and a mesh size of 5 mm were allocated.

The planting density of the experimental material in all orchards was the same and

generally corresponded to the technological requirements (Sudakova *et al.*, 2006).

The preparation of sodium humate in the form of an aqueous solution was applied to the fodder in the required proportion using a household sprayer, after which the fodder was dried before feeding to the fish. To balance the conditions

of the experiment, the fish fodder in the control variant was sprinkled with distilled water.

Fish feeding was performed with the frequency and according to the provisions proposed by BioMar, based on the actual weight of sterlet in the cages, obtained from the data of periodic control catches and the schedule of planned fish growth.

The duration of the research period, excluding landing and fishing days, was: 70 days in 2018 (from July 27 to October 4), 90 days in 2020 (from August 2 to October 30) and 35 days in 2021 (from September 15 to October 20).

Fish growth was monitored during control catches, every 8-10 days. All fish from each cage were inspected, counted and weighed.

Assessment of the growth rate and survival of fish and fodder conversion efficiency was

performed according to the methods generally accepted in fish farming. Thus, the growth rate was determined by the values of average absolute and relative body weight gain and specific growth rate (Shherbyna & Hamihyn, 2006), and survival rate – by the percentage of fish that survived to the end of the experiment among the planted ones (Marcenyuk & Marcenyuk, 2020).

The fodder conversion efficiency was estimated by the feed coefficient, which was defined as the ratio of the amount of consumed fodder to the body weight gain of fish (Marcenyuk & Marcenyuk, 2020).

## Research results

The evaluation indicators of the experiment conducted in 2018 are presented in the table (Table 2).

**Table 2.** Results of research conducted in 2018

Indicators	Experiment variant						
	Control		Experiment 1		Experiment 2		
	cage 1	cage 2	cage 3	cage 4	cage 5	cage 6	
Small fry planted	units	50	50	50	50	50	50
		total 100		total 100		total 100	
	average weight, g/units	13.3	12.3	13.2	12.6	11.8	11.2
		average 12.8		average 12.9		average 11.5	
Caught yearlings	units	48	49	47	49	49	47
		total 97		total 96		total 96	
	average weight, g/units	80.2	56.5	89.4	57.1	63.3	60.6
		average 68.4		average 73.3		average 62.0	
Absolute growth, g/units		66.9	44.2	76.2	44.5	51.5	49.4
		average 55.6		average 60.4		average 50.5	
Relative growth, %		143.10	128.49	148.54	127.69	137.15	137.60
		average 135.79		average 138.11		average 137.37	
Specific growth rate, %		2.04	1.84	2.12	1.82	1.96	1.97
		average 1.94		average 1.97		average 1.96	

Table 2. Continued

Indicators	Experiment variant					
	Control		Experiment 1		Experiment 2	
	cage 1	cage 2	cage 3	cage 4	cage 5	cage 6
Fish survival, %	96.00	98.00	94.00	98.00	98.00	94.00
	average 97.00		average 96.00		average 96.00	
Fodder coefficient	0.99	1.11	0.87	1.12	1.03	1.06
	average 1.04		average 0.97		average 1.04	

As evident from the table, in terms of relative body weight gain, the best result (138.11%) was obtained in experimental variant 1, the average (137.37%) – in experimental variant 2, the worst (135.79%) – in the control group. Experimental variant 1 was the best in terms of absolute body weight gain and specific growth rate (60.4 g/unit and 1.97%, respectively), the control variant had the second result (55.6 g/unit) in terms of growth and the third – in terms of specific growth rate (1.94%). Experimental variant 2 is the second in terms of specific growth rate (1.96%) and the third in terms of absolute growth (50.5 g/unit). In general, the advantage of the experimental variants over the control in terms of growth rate was insignificant and was in the range of 1.2-1.7%.

Thus, there is a close correlation between the starting weight of fry and the absolute weight gain of sterlet yearlings. A significantly smaller difference in the values of relative growth and specific growth rate of fish between experimental variants 1 and 2 than between the control and each of the experimental variants can be perceived as an additional confirmation of the positive effect of sodium humate fodder additive on fish. According to the survival rate of sterlet from fry to juveniles,

there was no significant advantage of any variant of the experiment, as the absolute difference between the control and experimental variants in the number of fish at the end of the experiment was only 1 fish.

The best result of fodder conversion was recorded in experimental variant 1, with an average value of fodder coefficient of 0.97. In the variants, Experiment 2 and Control the same values of this indicator were obtained (1.04).

Based on the analysis of the results of the experiment conducted in 2018, it can be concluded that the addition of sodium humate at the rate of 60 mg per 1 kg of fish fodder had a positive effect on the growth of yearling sterlet and the efficiency of their use of fodder.

In 2020, a repeated experiment was conducted on yearling sterlet to assess the effect of the fodder additive sodium humate on the growth and survival of fish and their absorption of fodder. The number of fish in each variant of the experiment doubled compared to the previous year. In addition, the concentration of sodium humate in the fodder for the experimental variants was increased: in Experiment 1 – up to 100 and in Experiment 2 – up to 200 mg/kg of fodder. The results of the experiment are presented in Table 3.

**Table 3.** Results of research conducted in 2020

Indicators	Experiment variant						
	Control		Experiment 1		Experiment 2		
	cage 1	cage 2	cage 3	cage 4	cage 5	cage 6	
Small fry planted	units	100	100	100	100	100	100
		total 200		total 200		total 200	
	average weight, g/units	15.2	23.3	17.5	24.5	29.4	21.3
		average 19.3		average 21.0		average 25.4	
Caught yearlings	units	99	99	100	100	100	100
		total 198		total 200		total 200	
	average weight, g/units	96.3	123.1	129.2	124.2	155.2	121.4
		average 109.8		average 126.7		average 138.4	
Absolute growth, g/units		81.1	99.8	111.7	99.7	123.8	100.1
		average 90.5		average 105.7		average 112.0	
Relative growth, %		143.48	134.98	152.28	132.76	143.74	140.30
		average 140.24		average 142.52		average 142.02	
Specific growth rate, %		1.59	1.50	1.69	1.48	1.60	1.56
		average 1.55		average 1.59		average 1.58	
Fish survival, %		99.00	99.00	100.00	100.00	100.00	100.00
		average 99.0		average 100.00		average 100.00	
Fodder coefficient		1.39	1.24	0.93	1.50	1.15	1.02
		average 1.32		average 1.22		average 1.09	

As the table demonstrates, in terms of relative body weight gain, experimental variants 1 and 2 demonstrated almost the same result (142.52% and 142.02%, respectively), which was higher than in the control (140.24%). The values of absolute body weight gain were higher in experimental variants 1 and 2, which was largely influenced by the superiority of these variants over the control planting material: 15.2 and 21.5 g, or 16.7 and 23.8%, respectively). However, in terms of relative growth and specific growth rate, which consider the difference in the starting weight of fish, the advantage of

experimental variants over the control was not that evident. Thus, the best results in terms of relative growth and specific growth rate in experimental variants 1 and 2 (142.52 and 1.59% and 142.02 and 1.58%, respectively) were only 1.3 and 1.6% higher than the corresponding figures in the control variant.

The survival of sterlet from fry to yearlings in the 2020 experiment was at a high level: from 99% in the control to 100% in both experimental variants. These indicators were significantly higher than the regulatory values (70-80%), due to the high level of the technological process

at the base fishery. A slight advantage for this indicator in the experimental variants over the control (only 1% or 1 fish per cage) does not give grounds for a conclusion about the presence of a positive effect of sodium humate on the survival of sterlet yearling in the first year of rearing under cage conditions.

The most effective use of artificial fodder by fish in terms of fodder coefficient was recorded in experimental variant 2 (1.09), the second result was observed in experimental variant 1 (1.22), and the third – in the control (1.32). The advantage of experimental variants 1 and 2 over the control for this indicator was 7.6 and 17.4%, respectively.

Thus, according to the results of the experiment conducted in 2020, it can be concluded that the addition of sodium humate in concentrations of 100 and 200 mg per 1 kg of fish fodder had a slight positive effect on the growth

of sterlet yearlings, but contributed to a much better fodder conversion.

The main objective of the experiment conducted in 2021 was to collect experimental material to assess by laboratory methods the protectionist effect of humates on protecting the fish organism from the accumulation of heavy metals in the second year of commercial sterlet rearing, to prevent fish diseases and ensure food safety of fish products. Therefore, and considering the fact that simultaneously research was started to assess the impact of humates on sterlet yearlings during their cultivation in a closed recirculating aquaculture system, the cage experiment was conducted in two variants: experimental (200 mg of sodium humate per 1 kg of fodder) and control. The duration of the experiment was significantly reduced compared to previous years of research. The results of the experiment are presented in Table 4.

**Table 4.** Results of research conducted in 2021

Indicators	Experiment variant				
	Control		Experiment		
	cage 1	cage 2	cage 3	cage 4	
Planted annuals	units	40	40	40	40
		total 80		total 80	
	average weight, g/units	53.7	54.5	53.8	54.0
		average 54.1		average 53.9	
Two-year-olds caught	units	36	32	34	35
		total 68		total 69	
	average weight, g/units	106.3	115.0	109.0	112.2
		average 110.4		average 110.6	
Absolute growth, g/units	52.6	60.5	55.2	58.2	
	average 56.3		average 56.7		
Relative growth, %	65.75	71.39	67.81	70.04	
	average 68.57		average 68.93		

Table 4. Continued

Indicators	Experiment variant			
	Control		Experiment	
	cage 1	cage 2	cage 3	cage 4
Specific growth rate, %	1.46	1.59	1.51	1.56
	average 1.53		average 1.54	
Fish survival, %	90.00	80.00	85.00	87.50
	average 85.00		average 86.26	
Fodder coefficient	1.55	1.71	1.49	1.45
	average 1.63		average 1.47	

As the table demonstrates, the experimental and control variants almost did not vary in terms of body weight gain and growth rate of fish. A slight advantage in these indicators (within 0.5-1.0%) was demonstrated by the experimental variant.

The survival rate of sterlet from yearlings to two-year-olds had no significant differences in the variants of the experiment and, in general, was slightly lower than the technological standard (90%). The latter can be explained by the abnormally intense and long-term development and death of planktonic algae (the so-called “water bloom”) in the Dnipro reservoirs during August-October this year, which caused a significant deterioration in the living conditions of aquatic organisms by reducing the oxygen concentration in the water below the optimum level and a high risk of fish poisoning by decay products of algae (particularly blue-green algae).

The values of the fodder coefficient for the variants of the experiment were significantly higher than those recommended by the fodder manufacturer (1.1-1.2): experiment – 1.43, control – 1.67. Presumably, this was caused by the above-mentioned “water bloom”, which resulted in the deterioration of the quality parameters of the aquatic environment for fish,

in particular – to reduce the concentration of oxygen dissolved in water. But, even under unfavourable growing conditions, the addition of sodium humate to fish fodder had a positive effect: using the fodder by fish in the experimental variant was 10.9% more effective than the control one.

After completion of the experiment, fish material was selected for testing for heavy metals and transferred to a certified laboratory for ecological and toxicological research.

### Conclusions and Perspectives

According to the results of the research, the following was identified:

1. Using humates as a fodder additive is rather common in livestock farming. However, the influence of humates on the growth rate and survival of fish farming objects, in particular sturgeon, has been understudied.

2. The addition of sodium humate to fish fodder at a concentration of 60, 100, 120 and 200 mg/kg of fodder did not significantly affect the growth rate of sterlet yearlings in the cage method of rearing. The advantage of the experimental variants over the control for this indicator was within 1.0-1.6%.

3. There was no significant effect of fodder additive humates on the survival of sterlet

yearlings during their cultivation in experimental cages of a production enterprise with a high level of technological support of the production process.

4. The conversion of fish fodder with the addition of sodium humate was noted to be better by 6.7-17.4% when feeding to yearlings and two-year-old sterlet.

5. According to the results of the experiments, the greatest level of positive impact on the results of sterlet rearing in cages had to

feed fish with the addition of humates at a concentration of 200 mg/kg of fodder.

6. It is considered promising to explore the effect of fodder additives of humates on the growth and survival of valuable fish farming objects under different conditions of keeping, in particular – in closed recirculating aquaculture systems, and to continue the search for the optimal concentration of these additives in fish fodder for different age groups of fish.

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## Дослідження впливу кормової добавки гумату натрію на стерлядь за садкового способу вирощування

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**Анотація.** Гумінові препарати використовують, як мікродобрива у рослинництві і стимулятори росту у тваринництві, в аквакультури також існує позитивна дія цих сполук на окремі об'єкти культивування, але їх застосування за вирощування риби ще недостатньо вивчене. Метою роботи було висвітлити результати експерименту з оцінки впливу кормової добавки гуматів на швидкість росту і виживаність різних вікових груп стерляді (*Acipenser ruthenus* L., 1758) та конверсію корму рибою за утримання в умовах аквакультури. Було проведено серію експериментів з годівлі стерляді кормами з добавкою гумату натрію. Дослідження проходили у 2018, 2020 і 2021 рр. у виробничих умовах садкового рибницького господарства, розташованого на Канівському водосховищі. Було оцінено вплив різних концентрацій кормової добавки гумату натрію на цьоголітків і дволітків стерляді. Гумат натрію наносили на комбікорм для відповідних груп стерляді шляхом розпилювання його водного розчину у визначених пропорціях з подальшим висушуванням. Встановлено, що додавання гумату натрію до рибного комбікорму в концентраціях 60, 100, 120 і 200 мг/кг корму суттєво не вплинуло на швидкість росту стерляді. Так, перевага дослідних варіантів над контрольним за цим показником була незначною, в межах 1,0-1,6 %. Не встановлено впливу гумату натрію на виживаність цьоголітків стерляді, за їх вирощування в садках. Разом із тим, відмічено кращу на 6,7-17,4 % конверсію кормів з добавкою гуматів цьоголітками і дволітками стерляді. За результатами експериментів найкращий результат отримано у варіанті з використанням кормової добавки гуматів у концентрації 200 мг/кг корму. Виходячи з результатів досліджень, встановлено, що в умовах аквакультури доцільно використання гумату натрію, як кормової добавки для цьоголіток і дволіток стерляді, призначеної для підвищення конверсії корму

**Ключові слова:** біологічно активні речовини, рибництво, осетрові риби, виживаність, швидкість росту, конверсія корму



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## Biotechnological features of production and quality assessment of lactose-free yoghurt

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**Abstract.** The production of lactose-free yoghurts as a dietary product for people with milk sugar intolerance requires its extraction, which can affect the sensory and physicochemical properties of the finished product, thus, their evaluation to improve the technology is relevant. The purpose of the work was to explore yoghurts produced by conventional and lactose-free technologies. The object of the study was organic drinking yoghurt with “blueberry” filler (with probiotic) 2.5% and lactose-free organic yoghurt (with probiotic) 2.5%. The experimental samples were determined by a group of tasters organoleptic indicators, and titrated acidity and active acidity, conditional viscosity and structural and mechanical parameters of the product. According to the results of the research, organic drinking yoghurt with blueberry filler tasted sour-milk, without foreign flavours and odours, moderately sweet, with a pronounced taste of “blueberry” filler, consistency – homogeneous, tender, dense, without gas generation, with fresh blueberry particles distributed throughout the yoghurt, colour – with a shading characteristic of blueberries. Organic lactose-free yoghurt had a sour taste, fermented milk, without foreign flavours and odours, and colour – white. According to the results of physicochemical studies of the experimental samples of organic drinking yoghurt with “blueberry” filling and lactose-free organic yoghurt, it was established that the titrated acidity was 80 and 85°T, pH was 4.7 and 4.5, respectively. The mass fraction of carbohydrates in drinking yoghurt with the “blueberry” filling was 9.8 g/100g compared to 4.4 g/100g in lactose-free yoghurt, including sugar, respectively – 5.8 and 0 g/100 g. The energy value and caloric content were higher in the yoghurt sample with “blueberry” filling. The conditional viscosity of the test samples was respectively 1 min. 30 sec. and 59 sec. The degree of syneresis in the samples of organic drinking yoghurt with “blueberry”

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filling and lactose-free organic yoghurt was 55 and 45%, moisture content was 44.07 and 39.49%, respectively. The results obtained are relevant for understanding the changes in the properties of lactose-free yoghurts compared to the conventional type of this fermented milk product

**Keywords:** lactose, lactase, yoghurt, organoleptic evaluation, physicochemical parameters, viscosity, functional products

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## Relevance

Milk and dairy products play a significant role in human nutrition, as they are a source of highly valuable, easily accessible substances that are in balanced proportions and are characterised by high digestibility in the body (Slavov, 2018; Vlasenko, 2012; Kovalchuk, 2020). However, not all people can consume dairy products. A significant part of the world's population suffers from milk sugar intolerance (partial or complete lactose intolerance) and, as a result, cannot consume dairy products in their natural form (Khavkin, 2009; Ipatova, 2013). To provide dietary dairy products to people with such pathology of digestive organs in Ukraine and all over the world, the production of functional products, in particular lactose-free dairy products, has been launched. Thus, nowadays, it is relevant to explore the technological features of production and evaluation of nutritional properties of lactose-free fermented milk products.

## Analysis of Recent Studies and Publications

The theoretical and practical foundations of the production of functional and lactose-free dairy products are presented in the works of many well-known foreign (Dekker, Koenders, 2019; Dekker, 2016; Kárnyáczki; Csanádi, 2017) and domestic scientists (Misnyk, 2007; Khavkin, 2009; Ipatova, 2013). In their works, the working hypothesis of the production of

high-quality lactose-free dairy products is based on the assumption that the extraction of lactose from milk does not significantly affect the organoleptic and physicochemical parameters of fermented milk products. Currently, there is an entire range of technical and technological methods for reducing lactose in milk and dairy products: enzymatic hydrolysis, membrane treatment, obtaining products of an artificial combination of components, etc.

The conventional way to reduce the amount of lactose in dairy products is the fermentation process during the gelation and coagulation of casein (Vlasenko *et al.*, 2016). Thus, during the production of fermented milk products or cheese ripening, lactose is naturally degraded (Mashkin & Parish, 2006). When the starter is added, lactic acid bacteria ferment milk sugar and produce lactic acid, which is necessary to start the coagulation process of casein (Gvozdev *et al.*, 2013). Thus, in all fermented milk products, cheeses are low-lactose, because milk sugar is fermented by bacteria, and as a result – its amount in them is less than in ordinary milk.

Lactose-free dairy products are obtained after membrane filtration of milk and the addition of lactase enzyme to it. Having lost lactose in this way, milk does not change its taste, colour and fully preserves mineral compounds and vitamins. According to the authors (Slavov, 2019; Vlasenko, 2015), lactose-free products

differ from conventional products only in the absence or low content of lactose and have advantages in terms of dietary and functional nutrition. Therewith, it becomes less caloric due to a decrease in carbohydrate concentration by 35-45%, which is lower than in conventional milk (Dekker, Koenders, 2019). And this, in turn, can have an adverse effect on the consumer properties of some types of lactic acid products.

Therefore, a comparative assessment of the organoleptic and physicochemical characteristics of yoghurts produced by conventional and lactose-free technologies is quite relevant and is of both practical and scientific interest.

The purpose of the research – explore in a comparative aspect the biotechnological

features of yoghurts produced by conventional and lactose-free technologies and evaluate their organoleptic and physicochemical properties.

### Material and Methods of Research

The research was conducted in the laboratory of the Department of Processing Technologies and Quality of Livestock Products of Polissya National University, Zhytomyr.

The objects of the study were samples of yoghurts purchased in the branded outlets of LLC “Organic Milk”, Zhytomyr, namely: organic drinking yoghurt with “blueberry” filler (with probiotic), mass fraction of fat 2.5% and lactose-free organic yoghurt (with probiotic) fat content 2.5% (Fig. 1).



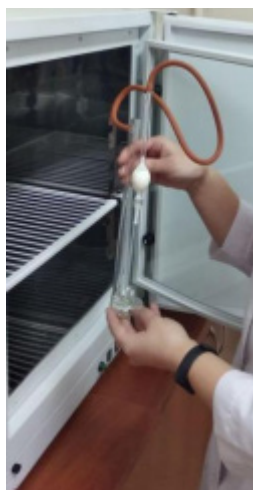
**Figure 1.** Research samples

During sampling, the yoghurts corresponded to the three-day use-by date. After sampling, the samples were stored in a refrigerator at 6°C.

Organoleptic parameters (appearance, structure and consistency, taste and smell, colour) of yoghurt were evaluated according to DSTU 4343:2004 “Yogurts. General technical conditions” and were conducted by a group of five tasters. At the time of the study, all samples had a valid date of use and were coded with a three-digit code. In the experimental samples of yoghurt, physicochemical parameters

were determined: titrated acidity (titrimetric method), and active acidity (potentiometric method using an ionometer). The mass fraction of fat was determined according to GOST 5867-90 by the Gerber acid method. The mass fraction of protein was determined by the formal method.

The conditional viscosity was determined on an Oswald viscometer, and the duration (in seconds) of a continuous flow of the product was measured, which was 1 min. 30 sec. and 59 sec. respectively (Fig. 2).



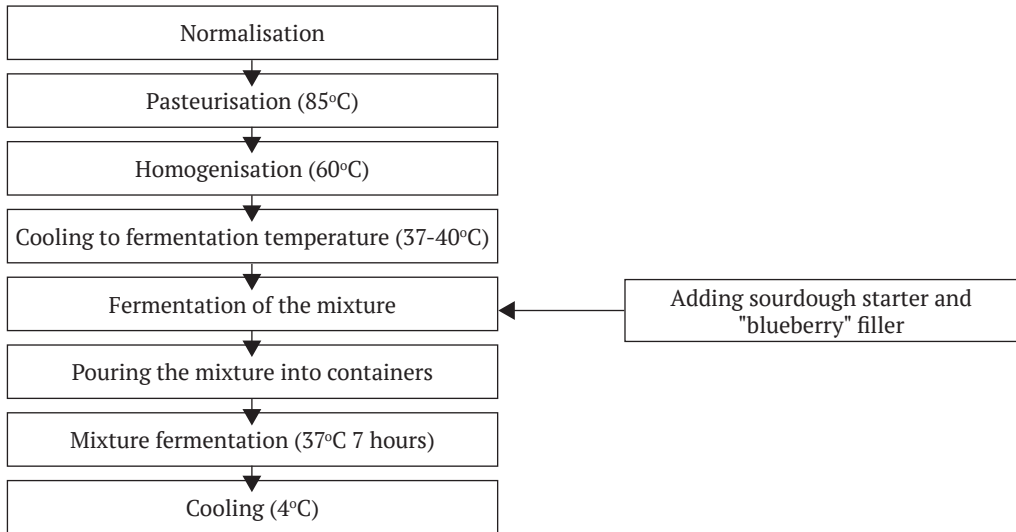
**Figure 2.** Measurement of conditional viscosity on the Oswald viscometer

To evaluate the structural and mechanical properties of the prototypes, the conditional viscosity was determined by the time of outflow of the product with a capacity of 10 cm<sup>3</sup> with an outlet of 5 mm and the degree of syneresis – by the amount of serum produced per 1 hour of free filtration. The moisture content in the experimental samples was determined on a moisture meter. The conditional viscosity was determined on an Oswald viscometer, and the duration (in seconds) of a continuous flow of the product was measured.

All analyses were performed in triplicate. The obtained results were processed biometrically using the built-in statistical functions package of MS Excel.

### Research Results and their Discussion

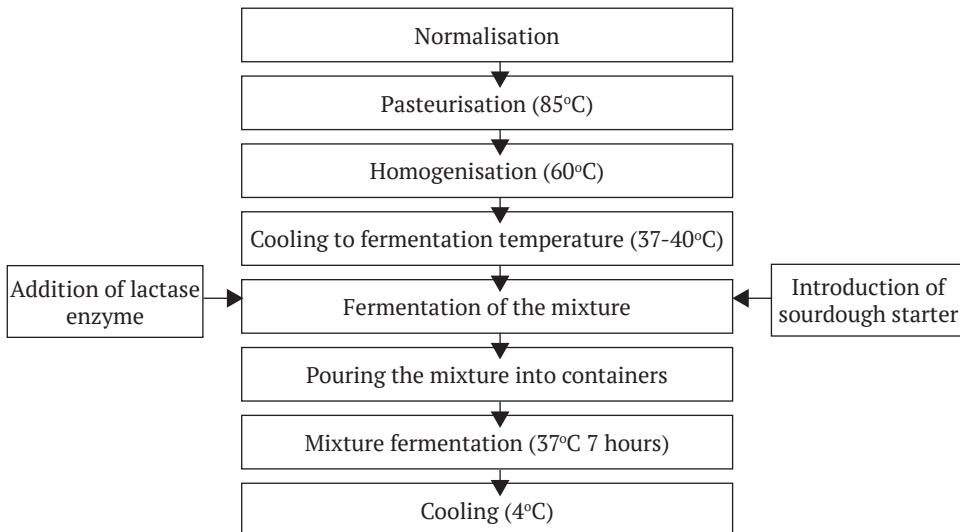
In the conditions of LLC “Organic Milk” organic drinking yoghurt with “blueberry” filling is produced according to the conventional scheme by thermostatic method (Fig. 3).



**Figure 3.** Scheme of technological process of organic drinking yoghurt production with "blueberry" filling

The most common way in the world to reduce the lactose content in milk is to add the enzyme lactase to the product (Slavov *et al.*, 2019; Vlasenko *et al.*, 2018; Skorchenko &

Greek, 2012). The enzyme splits up to 98% of the lactose in milk. In the conditions of LLC "Organic Milk", this scheme of lactose-free yoghurt production is used (Fig. 2).



**Figure 4.** Scheme of the technological process of lactose-free yoghurt production in the conditions of LLC "Organic Milk"

The difference in technological schemes is that lactose-free yoghurt contains the enzyme lactase. Due to the addition of this component to milk, people with lactose intolerance can enjoy dairy products and not feel any discomfort. Considering that lactose intolerance is the absence (lack) of the enzyme lactase, it is simply added to the products. Thus, milk sugar is already split in this product.

The results of the organoleptic evaluation of lactose-free yoghurt are presented in Table 1. According to the main organoleptic indicators, lactose-free yoghurt with 2.5% fat content practically did not vary from organic drinking yoghurt with “blueberry” fillers of similar fat

content. Some differences were observed only in the sweet and sour taste. The lactose-free version of fermented organic milk with probiotics had a less pronounced sweet taste than organic drinking yoghurt and was sourer. It is explained by the fact that products with lactose hydrolysis have a more sour taste due to the lower content of milk sugar (Dekker, 2016). Organic lactose-free yoghurt (with probiotic) with 2.5% fat content had a sour taste, fermented milk, without foreign tastes and odours, and white colour. The consistency of lactose-free yoghurt was homogeneous, tender, and dense, without gas production, and practically did not vary from organic drinking yoghurt.

**Table 1.** Organoleptic evaluation of research samples

Indicator	Characteristic	
	organic drinking yoghurt with “blueberry” filling (with probiotic) 2.5 %	organic lactose-free yoghurt (with probiotic) 2.5 %
Taste and odour	Sour milk, without foreign tastes and odours, moderately sweet, with a pronounced taste of “blueberry” filler	Sour milk, without foreign flavours and odours, sour taste
Consistency	Homogeneous, tender, dense, without gas production, with fresh blueberry particles distributed throughout the yoghurt mass	Homogeneous, delicate, moderately dense, without gas production
Colour	With a shade that is typical for blueberries	White

One of the most significant factors that allow using fermented milk for human consumption is its acidity. In addition, viable bacterial cultures are the foundation for the production of fermented milk, and their activity causes changes in the finished fermented milk beverages (Dekker & Koenders, 2019). In these studies, the effect of lactose hydrolysis on the acidity of fermented milk was hardly noticeable (Table 2). In the samples of this drink, the titrated acidity was slightly higher

(85°T) compared to drinking yoghurt (80°T). A similar picture was observed for active acidity, which was 4.5 in lactose-free yoghurt against 4.7 in drinking yoghurt. Similar conclusions were reached by (Csanádi, 2017), who noted that lactose-free yoghurts had a lower pH and a higher concentration of lactic acid than the control yoghurts using classical technology. The authors explain their findings by the fact that the splitting of lactose into monosaccharides facilitates

the metabolism of bacteria and enhances the fermentation process.

Regarding the indicators of total nutrition, a significant difference between the samples was observed only in the concentration of

carbohydrates. Thus, the mass fraction of carbohydrates in drinking yoghurt with “blueberry” filling was 9.8 g/100 g for 4.4 g/100 g in lactose-free yoghurt, including sugar 5.8 and 0 g/100 g, respectively (Table 2).

**Table 2.** Physicochemical parameters of the research samples

Indicator	Organic drinking classic yoghurt (with probiotic) 2.5%	Organic lactose-free yoghurt (with probiotic) 2.5%
Titrated acidity, °T	80	85
Active acidity	4.7	4.5
Mass fraction of fat, g/100g	2.5	2.5
Mass fraction of protein, g/100g	2.5	2.9
Mass fraction of carbohydrates Including sugar, g/100g	9.8	4.4
	5.8	0
Energy value, kJ/100 g	327	216
Calorie content, kcal/100g	78	55

Energy value and caloric content were slightly higher in the sample of yoghurt with “blueberry” filling.

Evidently, this is connected with the higher mass fraction of carbohydrates and sugar in the composition of this yoghurt.

As for the ingredient composition of the yoghurt samples, the only difference between them was that the first sample contained “Blueberry” filler, and the second – Lactase enzyme. The starter and probiotic consisted of the same lactic acid bacteria (Table 3).

**Table 3.** Composition of yoghurt of research samples

Organic drinking yoghurt with “blueberry” filler (with probiotic) 2.5%	Organic lactose-free yoghurt (with probiotic) 2.5%
Organic cow’s milk normalised	
«Blueberry» filler 8% (organic frozen blueberries, organic sugar, citric acid - acidity regulator, pectin - thickener)	-
organic sugar	-
yoghurt sourdough starter (streptococcus termophilus, lactobacillus delbrueckii subsp bulgaricus)	
probiotic: lactobacillus rhamnosus	
-	lactase enzyme

One of the significant indicators of nutritional evaluation of fermented milk drinks is syneresis – the separation of the liquid phase from the gel. This process can be spontaneous or occur only when the gel is mechanically destroyed by cutting, shaking or freezing. This visible defect can occur during the storage of fermented milk drinks and can affect the consumer properties of the final product (Dekker & Koenders, 2019). This indicator is primarily influenced by the total solids concentration and protein content in fermented milk, which increases the hardness of the gel and the retention capacity of whey in yoghurt. In addition, the type of milk and the type of starter used

can affect the syneresis of fermented beverages (Kárnyáczki & Csanádi, 2017). In these studies (Table 4), it was established that lactose-free yoghurt had a lower syneresis (45%), while the samples of drinking yoghurt with “blueberry” filling (55%). Similar data were observed (Dekker, 2016). The authors attributed this phenomenon to a lower amount of synthesised exopolysaccharides in lactose-free yoghurt at a higher enzyme concentration.

A similar picture was observed for the humidity content in the research samples. In addition, it was lower in lactose-free yoghurt and amounted to 39.49% against 44.07% in drinking yoghurt with “blueberry” filling (Fig. 5).

**Table 4.** Structural and mechanical indicators of research samples

Indicator	Organic drinking yoghurt with “blueberry” filling (with probiotic) 2.5%	Organic lactose-free yoghurt (with probiotic) 2.5%
Conditional viscosity	1 min. 30 sec.	59 sec
Degree of syneresis, %	55	45
Humidity content, %	44.07	39.49



**Figure 5.** Determination of humidity content in research samples

## Conclusions and Perspectives

The conducted studies on biotechnological features and consumer qualities of yoghurts produced by conventional and lactose-free technologies provide grounds to state:

1) LLC “Organic Milk” organic drinking yoghurt with “blueberry” filling is produced from organic milk according to the conventional scheme by the thermostatic method. During the production of lactose-free yoghurt, the addition of lactase enzyme is provided in the technological line;

2) lactose-free version of fermented organic milk in its consumer qualities practically did not differ from the drink made by the classical method and had high consumer quality;

3) some differences in the organoleptic

evaluation and physicochemical parameters were not significant and were caused by the technological feature of the preparation of the fermented milk product;

4) lactose-free yoghurt had a less distinct sweet taste than organic drinking yoghurt and was sourer;

5) the mass fraction of carbohydrates in drinking yoghurt was significantly higher than in lactose-free yoghurt, caused by the addition of “blueberry” filler. In addition, lactose-free yoghurt had no sugar in its composition;

6) in the future, it would be advisable to explore the effect of the lactase enzyme on the viability of fermented milk microflora, which is a source of probiotics in finished products for functional purposes.

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## Біотехнологічні особливості виробництва та оцінка якості безлактозного йогурту

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**Анотація.** Виробництво безлактозних йогуртів, як дієтичного продукту для людей з непереносимістю молочного цукру потребує його вилучення, що може позначитись на сенсорних та фізико-хімічних властивостях готового продукту, тому їх оцінювання для удосконалення технології є актуальним. Метою роботи було дослідження йогуртів, виготовлених за традиційною та безлактозною технологіями. Об'єктом дослідження були йогурт органічний питний з наповнювачем «чорниця» (з пробіотиком) 2,5 % та йогурт безлактозний органічний (з пробіотиком) 2,5 %. У дослідних зразках визначали групою дегустаторів органолептичні показники, а також титровану кислотність і активну кислотність, умовну в'язкість та структурно-механічні показники продукту. За результатами досліджень, йогурт органічний питний з наповнювачем «чорниця» на смак був кисломолочний, без сторонніх присмаків і запахів, у міру солодкий, з вираженим присмаком наповнювача «чорниця», консистенція – однорідна, ніжна, щільна, без газоутворення, з частками чорниці свіжої, які розподілені за всією масою йогурту, колір – з відтінком, характерним для чорниці. Йогурт безлактозний органічний мав кислуватий смак, кисломолочний, без сторонніх присмаків і запахів, колір – білий. За результатами фізико-хімічних досліджень дослідних зразків йогурту органічного питного з наповнювачем «чорниця» та йогурту безлактозного органічного встановлено, що титрована кислотність становила 80 та 85 °Т, рН відповідно – 4,7 та 4,5. Масова частка вуглеводів у йогурті питному з наповнювачем «чорниця» становила 9,8 г/100 г за 4,4 г/100 г в йогурті безлактозному, у тому числі цукру відповідно – 5,8 та 0 г/100 г. Енергетична цінність та калорійність була вищою у зразку йогурту з наповнювачем «чорниця». Умовна в'язкість дослідних зразків становила відповідно 1 хв. 30 сек. та 59 сек. Ступінь синерезису у зразках йогурту органічного питного з наповнювачем «чорниця» та йогурту безлактозного органічного становив 55 та 45 %, вміст води відповідно – 44,07 та 39,49 %. Отримані результати мають значення для розуміння змін у властивостях безлактозних йогуртів у порівнянні з традиційним видом цього кисломолочного продукту.

**Ключові слова:** лактоза, лактаза, йогурт, органолептична оцінка, фізико-хімічні показники, в'язкість, функціональні продукти



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## **Influence of methods of selection of parents by the index of similarity of antigens in blood groups on weight and linear growth of bulls**

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**Abstract.** In solving the problem of qualitative improvement of meat breeds, an important place is occupied by the improvement of methods of practical use of existing methods of selection of parental pairs using histocompatibility antigens, polymorphic proteins and blood group systems. The purpose of the work is to determine the effect of homogeneous and heterogeneous selection of parental pairs by blood group factors on the weight and linear growth of Ukrainian beef bulls. Ukrainian meat breed is bred using four breeds and is characterised by high variability in polymorphic traits. The type of selection of parents was determined by the index of antigenic similarity ( $r_{as}$ ) of antigens of the B blood group system of cattle. To calculate the index of antigenic similarity of parents, the formula of D.A. Zhivotovskiy and A.M. Mashurov was used. The selection was considered homogeneous if the parents'  $r_{as} \geq 0.268$ , and heterogeneous if  $r_{as} \leq 0.267$ . It is proved that bulls that come from parents with higher  $r_{as}$  prevail in the test by average daily gain and have a higher live weight. For  $r_{as}$  in parents over 0.268, animals tend to improve growth rate by 8 months of age. This trend continues after weaning. Bulls sired by parents with  $r_{as}$  up to 0.267 have better average daily gains in the period from 15 to 18 months, which indicates their lower precocity. According to the index of antigenic similarity of parents over 0.268, animals are better in terms of meat forms at the age of 15 and 18 months. At 15 months of age, bulls obtained from homogeneous selection for  $r_{as}$  have smaller height measurements, the better developed front part of the body in terms of chest width and depth, and longer torso and hindquarters. Homogeneous

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selection of parental pairs according to the index of similarity of antigens of the B blood group system improves the weight growth and expressiveness of meat forms in bulls of the Ukrainian beef breed

**Keywords:** selection, antigenic similarity, live weight, average daily gain, measurements, beef cattle breeding

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## Relevance

In the genetic progress of beef cattle, the rationale for the selection of parental pairs is of great significance. With a significant (about 90%) use of natural mating during its breeding, inbreeding is inevitable, which can increase homozygosity for recessive non-functional alleles, which can increase embryonic mortality (Upperman *et al.*, 2019) and reduce animal productivity. Currently, a growing problem in the Ukrainian meat breed is the decline in animal productivity. In females, it is manifested by weight growth, reproductive capacity and milk production. Therewith, the methods of selection of parents in local breeds of beef cattle are not sufficiently justified, which is a problem. Parental pairs are selected in different ways, regardless of the degree of their kinship. Therefore, inbred depression is manifested, particularly in local breeds.

One of the genetic methods that allow increasing the variability of traits in offspring is using antigens of the B blood group system during the selection of parents. They are protein or polysaccharide compounds that result in the development of antibodies. According to I.Y. Gorbatenko, and M.I. Gil (2006), antigens of erythrocytes and some proteins are used to determine the origin of animals, evaluate the gene pool of breeds and account for productivity indicators after the selection of parental pairs. Immunobiological features of blood groups,

based on immunogenetic analysis by the index of antigenic similarity, allow predicting of the results of parental selection by the features of its selection.

Therefore, it is essential to explore the weight and linear growth of beef bulls in the local Ukrainian beef breed, under the influence of the selection of parental pairs that have a different index of similarity of antigens ( $r_{av}$ ) of the B blood group system.

## Analysis of Recent Studies and Publications

One of the methods for assessing the genetic potential of animal productivity currently using the system of B blood groups is still. It combines relative simplicity of implementation on large livestock, with rather high results (Chizhova *et al.*, 2015). Despite the ambiguous results obtained when studying the relationship between blood groups and animal performance traits, research in this area continues, thanks to data obtained on cattle (Bukarov, 1995), sheep (Kopylov, 2019) and pigs (Goncharenko, 2009).

The data of studies (Nazarenko, 1986; Tyutyunnikov, 1995; Ivanova and Rossokha, 1996) indicate the relationship between blood groups on the one hand and some traits of cattle productivity, which have an essential role in cattle breeding, on the other hand, it

was established (Tyutyunnikov, 1995) that the lower the index of antigenic similarity of father and mother by blood group antigens, the higher the fertility of their daughters. After mating of cows and bulls at a low (up to 0.20) coefficient of antigenic similarity, the fertility of cows is higher by 5.7%, the number of walks, respectively ( $p>0.999$ ) is lower by 0.14, and the service period is shorter by 21.6 days (Nazarenko, 1986). The most productive were identified (Ivanova & Rossokha, 1996) pairs “cow-bull” with a low degree (from 0.10 to 0.39) of antigenic similarity.

The opposite feature was obtained (Ugnivenko and Nosevych, 2014a) in females of the Ukrainian beef breed at the initial stages of complex reproductive crossing for reproductive traits. As the degree of antigenic similarity of parents by blood group factors increases, the average milk yield and lifetime milk yield per 1 day of the life of their daughters tend to increase (Uhnivenko and Nosevych, 2014b).

Thus, the purpose of the study was to determine the influence of the selection of parents by the index of antigenic similarity ( $r_{as}$ ) of cattle of local Ukrainian beef breeds on the traits of weight and linear growth of their sons and to substantiate its optimal options for complex reproductive crossing.

## Materials and Methods of Research

The research was performed on bulls in the breeding plant “Volia”, Zolotonosha district, Cherkasy region. Animals up to 6-7 months of age were raised near cows on suckling. After weaning, well-developed bulls were selected for the group and tested for the probability of origin by blood group factors. Until 8 – months of age, they were accustomed to a typical diet and conditions of detention. Intensive rearing was performed from 8- to 18-months of age. The general level of feeding was calculated to obtain an average daily gain of 1000 to 1200 g. During this period, the animals were fed with the fodder of their products according to the rations established by the standards. The mass of fodder eaten by each bull was calculated every ten days (two days in a row) by weighing the given fodder and its residues. Based on the consumed fodder, their energy value (in oat feed units) and costs per 1 kg of live weight gain were determined. Bulls were examined indoors with a tethered keeping system. Each time before giving a new portion, the fodder that remained in the feeder was weighed and determined how much it was consumed by the animals. There was no significant difference in its consumption between the bulls of the groups (Table 1).

**Table 1.** Fodder consumption from 8 to 18 months of age by bulls obtained from a different selection of parental pairs by the index of antigenic similarity ( $r_{as}$ ),  $M\pm m$

Fodder	$r_{as}$ above 0.268 (n=15)		$r_{as}$ up to 0.267 (n=11)	
	fodder unit	%	fodder unit	%
Concentrated	1480±31.5	49.5±0.85	1407±58.5	46.8±0,64
Rough	482±53.7	15.7±1.57	493±52.8	15.9±1.41
Juicy	543±35.9	17.9±1.00	554±60.4	18.1±1.59
Greens	536±41.7	17.4±1.69	568±57.0	19.2±2.09

Table 1. Continued

Fodder	$r_{as}$ above 0.268 (n=15)		$r_{as}$ up to 0.267 (n=11)	
	fodder unit	%	fodder unit	%
Total	3032±77.6	100.0	3017±124.7	100.0
Per 1 kg of growth	8.9±0.44	-	9.3±0.67	-

To calculate the index of antigenic similarity of parents, erythrocyte antigens of bovine blood groups according to the B system were used. It was determined by the formula of Zhivotovsky-Mashurov (1974):

$$r_{as} = \frac{S}{n_1 + n_2 - S}, \quad (1)$$

where  $r_{as}$  – index of antigenic similarity of parents;  $S$  – the number of antigens that coincide in the father and mother;  $n_1$  – the total number of antigens detected in the mother;  $n_2$  – the total number of antigens detected in the father.

To analyse changes in weight and linear growth of bulls, they were grouped by the value of the index of antigenic similarity of parents: Group I –  $r_{as}$  over 0.268 and Group II –  $r_{as}$  up to 0.267. Meat forms in animals were evaluated according to the guidelines (Prakhov *et al.*, 1972). The obtained data were processed using the methods of variation statistics. To

determine the degree of variability of traits, its coefficient (Cv, %) was calculated by the ratio of the standard deviation to the average value for the group.

### Research Results and their Discussion

Animals with an index of antigenic similarity ( $r_{as}$ ) in parents of 0.268 and more tend to deteriorate the growth rate up to 8-months of age. Perhaps it is due to the worse milk productivity of their mothers, which offsets the positive effect of homogeneous selection on the growth rate of offspring in the suckling period. After weaning, it mainly tends to prevail over the indicators of peers from parents with  $r_{as}$  up to 0.267 (Table 2). It contradicts the data (Romanov *et al.*, 1984), according to which the place has a better growth rate in animals obtained from heterogeneous selection for  $r_{as}$ .

**Table 2.** Average daily gain of bulls obtained with different indexes of antigenic similarity ( $r_{as}$ ) of parents

Average daily increase for the period: from-to	$r_{as}$ over 0.268			$r_{as}$ to 0.267		
	n	M±m	Cv, %	n	M±m	Cv, %
0-8	33	872±21.6	14.0	35	906±20.3	13.1
8-12	34	1128±36.4	18.5	35	1075±37.8	20.5
12-15	32	1188±50.2	23.5	32	989±56.1	31.6
8-15	32	1144±33.3	16.2	32	1051±38.5	20.4
15-18	27	903±57.2	32.3	21	932±87.6	42.1
8-18	28	1084±26.0	12.5	21	1032±37.2	16.1

In bulls obtained from parents with  $r_{as}$  up to 0.267, the average daily gains are better in the period from 15 to 18 months (Table 2), which indicates their lower precocity. Due to faster growth in the suckling period, outbred animals

at the age of 8 months tend to increase their live weight. The higher growth rate of bulls from parents with  $r_{as}$  over 0.268 during these periods contributes to an increase in their live weight (Table 3).

**Table 3.** Live weight of bulls obtained with different index of antigenic similarity ( $r_{as}$ ) of parents

Live weight at age: month	$r_{as}$ over 0.268			$r_{as}$ to 0.267		
	<i>n</i>	<i>M</i> ± <i>m</i>	<i>Cv</i> , %	<i>n</i>	<i>M</i> ± <i>m</i>	<i>Cv</i> , %
newborns	35	32.5±0.60	10.8	35	32.5±0.58	10.5
8	35	247±5.4	12.8	35	253±5.1	11.6
12	34	384±7.0	10.5	35	383±6.7	10.2
15	32	490±9.6	10.9	33	472±9.4	11.2
18	28	574±10.3	9.3	21	558±11.5	9.2

The tendency for the advantage in the live weight of animals from heterogeneous selection begins to appear at 15 months of age. Heterogeneous bulls have greater variability in average daily gain compared to homogeneous bulls. It indicates their unequal adaptation to environmental conditions, both during the suckling period and after weaning. The coefficient of variation of live weight obtained from a homogeneous and heterogeneous selection of bulls in all age periods have no difference.

Bulls from homogeneous and heterogeneous selection for  $r_{as}$  maintain a fairly high growth rate until 18 months of age. The offspring of parents with similar antigens grow faster for a longer time. Their average daily live weight gain from 8 to 18 months is higher compared to their peers from the heterogeneous selection. Thus, at the first stages of the establishment of the Ukrainian beef breed, a homogeneous  $r_{as}$  selection of parents improved the average daily weight gain of bulls.

Homogeneous mating increases the tendency of animals to stop growing early and increases feed costs for growth.

The results of this study were compared with those obtained on Aberdeen Angus (Romanov *et al.*, 1984) and Red Steppe (Nazarenko & Voronenko, 1986) breeds. According to L.M. Romanov and colleagues (1984), the group of bulls and heifers born from parents with a high (more than 0.308) index of antigenic similarity are smaller. Growth retardation in young animals was observed in the following age periods. At the age of 12 months, the difference is significant. The difference in body weight between heifers from parents with low and high similarity index at 15 months of age was 13 kg ( $P < 0.999$ ), and at 18 months – 16 kg ( $P > 0.999$ ) in favour of the first ones (Nazarenko & Voronenko, 1986).

The Ukrainian beef breed was established by complex reproductive crossbreeding of Kian (K 3/8), Charolais (Ch 3/8), Simmental (S 1/8),

Grey Ukrainian (*GU* 1/8) cattle (Ugnivenko *et al.*, 2008). Homogeneous selection of animals of four initial breeds with large differences in their pedigrees results in heterosis by average daily gain and live weight after 8 months at the first stages of complex reproductive crossing. Probably, the adverse effects of heterogeneous selection on the index of antigenic similarity regarding the growth of bulls in the initial stages of breeding are levelled by the genetic background of significantly different crossbreeds. The best average daily gains from

birth to 8 months of age are the result of better milk production of cows, which, in combination with the bulls matched to them, had a low index of similarity for blood group factors.

Meat forms of bulls obtained from parents with different  $r_{as}$  are different. Animals with a higher index of antigenic similarity are better in the expression of meat forms at 15 and 18 months (Table 4). Breeding cattle for better-expressed meat forms contributes to obtaining from it a higher growth rate up to 18 months of age and reducing feed costs for growth.

**Table 4.** Expression of meat forms and measurements of bulls obtained with different indexes of antigenic similarity ( $r_{as}$ ) of parents

Feature	$r_{as}$ over 0.268		$r_{as}$ up to 0,267	
	<i>n</i>	<i>M</i> ± <i>m</i>	<i>n</i>	<i>M</i> ± <i>m</i>
Meat forms (points) at age: 15 months	19	54.1±0.93	14	52.8±0.97
Meat forms (points) at age: 18 months	15	55.6±0.79	11	53.8±1.07
Measurements at the age of 15 months, cm.				
Height at the withers	3	126.7±4.32	5	127.2±3.51
Height at the sacrum	3	137.3±4.32	5	138.0±3.89
Breast depth	3	68.7±0.41	5	66.4±1.60
Breast width	3	46.3±1.78	5	46.2±1.19
Width in clusters	3	45.0±1.41	4	46.3±2.02
Oblique length of the body (stick)	3	152.7±5.21	4	145.5±3.35
Oblique rear length	3	52.0±2.55	4	50.5±2.13
Chest girth	3	188.0±3.08	4	190.0±3.24
Heel girth	3	20.8±0.20	4	20.8±0.35

At 15 months of age, bulls with a higher index of antigenic similarity ( $r_{as}$  more than 0.268) have smaller height measurements, the better developed front part of the body in terms of chest width and depth, and longer torso and rear and heel girth. Animals from parents with a lower index of

antigenic similarity ( $r_{as}$  up to 0.267) are relatively taller, with wider clusters and larger chest girth.

### Conclusions and Perspectives

Selection of parents with a high (homogeneous) index of antigenic similarity ( $r_{as}$  more than 0.268)

for erythrocyte antigens of the B blood group system with large differences in their genealogies increases the average daily gain and live weight of sons and improves the expression of meat forms.

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## Вплив способів підбору батьків за індексом подібності антигенів системи В груп крові на ваговий і лінійний ріст бугайців

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**Анотація.** У розв'язанні проблеми якісного поліпшення м'ясних порід важливе місце посідає удосконалення способів практичного використання наявних методів підбору батьківських пар за використання антигенів гістосумісності, поліморфних білків та систем груп крові. Метою роботи є визначення впливу гомогенного і гетерогенного підбору батьківських пар за факторами груп крові на ваговий і лінійний ріст бугайців української м'ясної породи. Українська м'ясна порода виведена з використанням чотирьох порід і характеризується високою мінливістю за поліморфними ознаками. Тип підбору батьків визначали за індексом антигенної подібності ( $r_{as}$ ) антигенів системи В груп крові великої рогатої худоби. Для розрахунку індексу антигенної подібності батьків використали формулу Д. А. Животовського і А. М. Машурова. Гомогенним вважали підбір за  $r_{as}$  батьків  $\geq 0,268$ , а гетерогенним при  $r_{as} \leq 0,267$ . Доведено, що бугайці, які походять від батьків за більшого  $r_{as}$  переважають на випробуванні за середньодобовими приростами і мають більшу живу масу. За  $r_{as}$  у батьків понад 0,268 тварини мають тенденцію до поліпшення швидкості росту до 8-місячного віку. Після відлучення ця тенденція зберігається. У бугайців, отриманих від батьків із  $r_{as}$  до 0,267 середньодобові прирости кращі у період від 15 до 18 місяців, що свідчить про меншу їх скороспілість. За індексу антигенної подібності батьків понад 0,268 тварини кращі за вираженістю м'ясних форм у віці 15 та 18 місяців. У 15 місяців бугайці, отримані від гомогенного підбору, за  $r_{as}$  мають менші висотні проміри, краще розвинену передню частину тулуба за шириною і глибиною грудей, довший тулуб і зад. Гомогенний підбір батьківських пар за індексом подібності антигенів системи В груп крові призводить до поліпшення у бугайців української м'ясної породи вагового росту та вираженості м'ясних форм

**Ключові слова:** підбір, антигенна подібність, жива маса, середньодобовий приріст, проміри, м'ясне скотарство

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