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ON THE PROBLEM OF USING NATURAL BIOLOGICALLY ACTIVE SUBSTANCES TO ACTIVATE ARTEMIA EGGS

Abstract. Currently branchiopod from the genus of Artemia Leach, 1819 became the common starting feed №1 for growing young aquatic organisms. However, there is often reduced hatching of nauplius from the collected eggs and artificial activation is required. As a result of empirical research, it was possible to experimentally detect the activating effect of excretion products – diluted adult urine. The research was carried out in several stages. It was found that the rate of germination growth for different populations ranges from 0, 2 to 0, 6% per day. In the first experiment, cysts with 24% of hatching were placed in a solution of lake water diluted with urine to a concentration of 105 g/l. After two weeks, the outage reached 66. 7 % and exceeded the control 2.55 times. But over time, the activity of the embryos decreased and by the end of April, the hatching reached zero. By using eggs from the 1st experiment with 66. 9% hatching in a fresh solution of «lake water + urine» (105 g / l) on the first day, the hatching exceeded the control by 52. 1% and by 23.7% the data from experiment 1. Later, when 80.8% were hatched, a large number of weakened and dead nauplius was observed.

In the third experiment, spring egg with a hatching rate of 36. 7% was used. Different salinity concentrations (110-140 g/l) were used due to dilution of lake water (233 g/l) with urine and fresh water. When activated in a solution with a salinity of 110 g/l after one month, the discharge exceeded the control by 1. 65 times. At the end of the experiments, an increase in the salt concentration reduced the activating effect with a slight decrease in hatching. The mechanisms of activating action of the components of the proposed liquid folds the physiological effects of steroid hormone metabolites on the formation of the larva in the egg and the destructive physical and chemical effects of a number of substances on the egg shell. For complex activation of the development of the embryo with the destruction of egg's shells, savings are achieved in the plastic and energy substances available in the egg, preserving the nutritional value of nauplius.

Keywords: Artemia, eggs, shell, hatching of nauplius, urina.

Introduction. Currently branchiopod from the genus of Artemia Leach, 1819 became the common starting feed №1 for growing young aquatic organisms. The main problem with using Artemia eggs to get nauplius is a low percentage of hatching; that requires activities for the activation. For a long period and to the present time the development of methods for activating Artemia eggs by the action of various factors and reagents has been underway: light and magnetic field, ultraviolet rays, freezing, mechanical impact when grinding eggs with sand, chemical reagent (components, included in the salt solution, sodas and borax, hydrogen peroxide), organic solvents, (acetone, butane, ethyl alcohol), parapsychical impacts and mums, the literature review is given in [1-3].

The authors of this article, as a result of empirical research, were able to experimentally detect the activating effect of excretion products – dilute urine of an adult. The relevance of the research is that an incubating and activating medium for the cultivation of Artemia eggs has been developed, which is characterized by ease of manufacture, economic and technological feasibility.

Materials and methods. Artemia's eggs were collected for experiments in the autumn in salty reservoirs of the Pavlodar region. The hatching of nauplius was determined by using standard methods [4]. Determination of the size of the hatching was carried out under round-the-clock lighting, at a temperature of 25-28°C for 24 hours. In different variants of the experiments the salt brine was diluted with fresh urine of an adult to a certain concentration of salt. Quantitative data were processed using statistical methods [5].

Research results. At the first stage of research, was experimentally determined the amount of hatching of nauplius from autumn Artemis eggs in local reservoirs with different levels of salinity. During this period, the embryos reach the stage of gastrula and become close to the state of diapause, characterized by a sharp decrease in the intensity of metabolism and cease the forming processes, and have a low percentage of hatching nauplius. In spring, eggs in the reservoir usually have 72-99% hatch rate [6]. To achieve those values of maturation (germination) of eggs it should undergo through the activation process. The ability to hatch increases gradually during the period of egg storage in a humid environment. The rate of growth of the germination index varies for different populations and ranges from 0, 2 to 0, 6% per day. The conducted research has shown that for performing artificial activation of Artemia's eggs diapause, it is necessary to prepare salty environment of a certain concentration, taking into account the natural mineralization of the mother reservoir. The salinity gradients found in experiments that are optimal for hatching nauplius are most likely favorable for the targeted use of various activating reagents [7,8], including the isolation products used by the authors.

The second stage of experimental research consisted of activation of eggs in liquid environment with different proportions of the activating substance (adult urine) and salt lake water.

Experiment 1. Artemia's egg, collected in a lake with a mineralization of 285 g / l in early October, was stored in a humid environment at a temperature of minus 5 to minus 17°C. In mid-December, the outage rate was 24% (22. 4–25. 2). The egg sample was placed in a solution of lake water (natural salinity in the lake – 285 g/l), diluted with urine to a concentration of 105 g/l. Preliminary experiments have shown that this amount of mineralization is optimal for activation of crustacean eggs. During the experiments, the samples were stored at a temperature of + 4° – + 6°C. In the control, during the entire activation period, there was a gradual increase in the hatching rate. However, in General, the final result was very low (40. 4 %) and was almost 1. 7 times less for achievement the product standard (at least 70%)(figure 1).

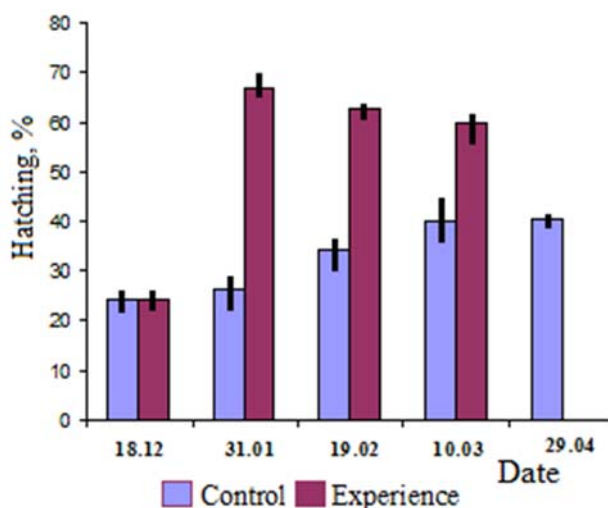


Figure 1–Hatching of Artemianauplius in a controlled and activated environment

The use of fresh urine in the activation solution stimulated the release of Artemianauplius, and in two weeks this indicator reached a value of 66. 7 %, exceeding the control value by 2. 55 times. In the future, the activity of the embryos began to decrease, and by the end of April, the offspring fell to zero.

Experiment 2. After the 1st experiment the hatching of the Artemia's egg reached 66. 9 % (31. 01), part of the experimental eggs were placed in a fresh solution of "lake water + urin" with a concentration of 105g/l. The distribution of the activation has been introduced in three different environments (figure 2).

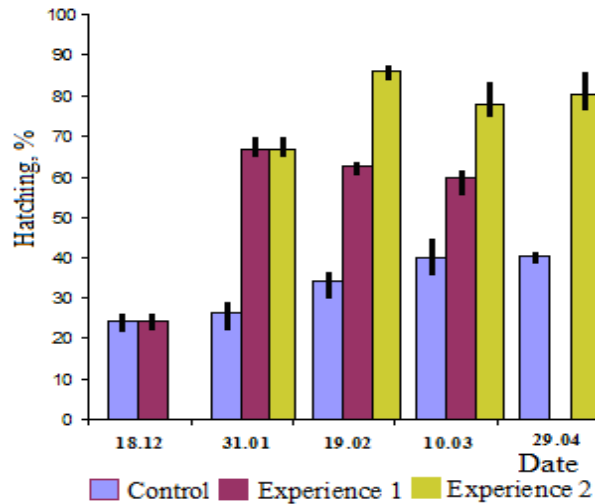


Figure 2 – Hatching of Artemia'snauplius in a controlled and activated environment

The fresh solution immediately stimulated high development of eggs, and after 19 days, hatching increased by 52.1% compared to the control and 23.7% exceeded the data of 1st experiment. In the future, a fairly high level of hatching was maintained, but on 29.04, when 80.8% were hatched, a large number of weakened and dead naupliuses were observed.

Experiment 3. An Artemia's egg collected in the spring (March) in a lake with a mineralization of 233g/l showed a very low percentage of hatching, total 36,7% (27,0 – 46,5).

The egg was laid for activation in various concentrations of salt and urine:

1. Lake water (natural salinity 233 g/l) is diluted with urine to a concentration of 110 g/l.
2. Lake water (233 g/l) is diluted with urine of 140 g/l.
3. Lake water (233 g/l) is diluted with urine to 150 g/l, and then diluted with addition of the fresh water to 140 g/l.
4. Lake water (233 g/l) is diluted with urine to 160 g/l, and then diluted with addition of the fresh water to 140 g/l.
5. Lake water (233 g/l) is diluted with urine to 170 g/l, and then diluted with addition of the fresh water to 140 g/l.

The main monitoring objects were eggs in a salt solution with a concentration of 140 g/l. Figure 3 shows data on the hatching of Artemia'snauplius after 20 and 30 days of activation. Salt concentrations before dilution with fresh water are shown in parentheses. The results of experiments showed that when Artemia's eggs are activated in a solution with a mineralization of 110 g/l, there is a constant growth of hatching, which in one month exceeded the control by 1.65 times. An increase in the salt concentration reduced the activating effect and there was even a slight decrease in hatching at the end of the experiments. Diluting the "lake water + urin" solution with fresh water to the required concentrations reduced the effectiveness of the solution, and at higher dilutions, complete death of the embryos was observed.

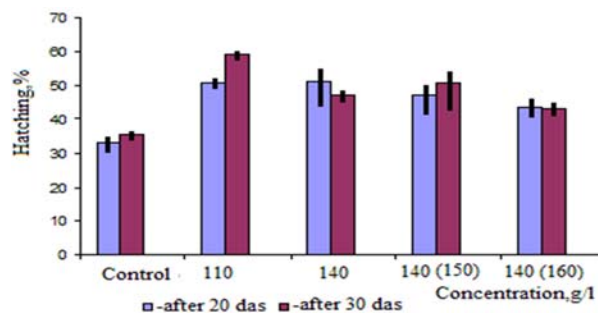


Figure 3 – Disabling Artemia'snauplius in a controlled and activated environment, %

Discussion of research results. The mechanisms of activating effects of the components of the proposed liquid are formed, on the one hand, from the physiological effects of steroid hormone metabolites on the formation of the larva in the egg, on the other – from the destructive physical and chemical effects of a number of substances on the egg shell.

Metabolites of adrenal cortex hormones (mineralocorticoids and glucocorticoids) contained in the urine of people and animals of any age [9] can have an activating effect on the development of the embryo of any animal by enhancing metabolic processes in tissues [10].

Metabolites of sex steroid hormones present in the urine of an adult (while in the female body, along with estrogens, a small amount of androgens is synthesized, and in the male body – estrogens), have an anabolic effect and contribute the formation of tissues and organs of the embryo. The destructive effect of the components of urine on the egg shell, which facilitates the exit of nauplius (and thus increases the viability of larvae, preserving their energy resources and increasing their feed value), is as follows. The egg shell of the arthropods consists of several layers and includes chitin, lipids, and tanned proteins. Urea, also known as carbamide, the content of which in human urine reaches 2%, and in the prepared activating liquid-about 1%, partially pollinating fats due to the alkaline reaction (which means that it converts them to a soluble state), softens tanned proteins, and then destroys their secondary and tertiary structure and leads to partial hydrolysis. Egg's shell passes liquid inside and undergoes further destruction. The properties of urea as a macerating substance are well known in biology and medicine (and find appropriate application where it is necessary to achieve partial or complete maceration of tissues). In practical biochemistry, urea is widely and successfully used to destroy the quaternary structure of proteins and supramolecular protein complexes (including antigen – antibody [11]).

In addition, urea and other organic substances – non-electrolytic, contained in the urine, are antagonists of salt ions. They contribute swelling of egg shells, performing, in fact, the same function as fresh water in the spring, formed when snow and ice melt on salt lakes. Proteins, partially hydrolyzed by urea, also attach water and swell, speeding up the overall process of destruction of the shell.

A certain role in the swelling of the egg shells could play the order of penetration of substances into the shell. Apparently, at first there is a partial destruction of the shell under the action of urea, and afterwards mobile sodium ions penetrate through the concentration gradient, and only then – organic substances available in the urine (urea, a small amount of other metabolic products, steroids). Sodium cations penetrate the hydrate shell and have water-retaining properties. Metabolites of steroid hormones also contribute swelling and further rupture egg's shells due to the fact that, penetrating into the shell, they delay sodium cations [12], which means that they contribute to the accumulation of liquid since monovalent cations have hydrating properties for living tissues [13].

Destruction of egg's shells promotes the penetration of oxygen, which also has a stimulating effect on the development of nauplius.

It is possible that with comprehensive activation in the development of the embryo with the destruction of egg shells, savings are achieved in the plastic and energy substances available in the egg.

First of all, the hatching nauplius does not spend extra energy on breaking the egg shells, which by the time the larva leaves are destroyed or significantly lose their strength. Secondly, the products of hydrolysis of proteins and lipids of the shell may well be assimilated by the embryo for plastic or energy needs. Secondly, the products of hydrolysis of proteins and lipids of the shell may be assimilated by the embryo for plastic or energy needs. Third, urea and other ultimate products of protein metabolism can inhibit excessive catabolic breakdown of proteins on the principle of auto-inhibition (thereby partially preventing the strengthening of protein catabolics caused by corticosteroid metabolites).

Fourth, metabolites of sex hormones (and especially androgens) enhance anabolic processes (and androgens are also present in the female body due to their synthesis by the adrenal glands). Fifth, corticosteroid metabolites contribute to the accumulation of sugars in tissues, thereby forming a stock of easily and quickly oxidizing energy substances [10].

The death of formed embryos in eggs at the attempt of trying to activate them with undiluted adult urine may be associated with several, not excluding each other, reasons.

First, a high concentration of urea could cause a violation of the structure and partial hydrolysis of proteins not only of the shells, but also of the embryo itself, whereas in dilute urine, urea is consumed for hydrolysis and destruction of shell proteins, and its concentration is insufficient to cause fatal changes in the tissues of the embryo.

Secondly, a high concentration of urea and other products of nitrogen metabolism can cause intoxication of the emerging and already formed embryo.

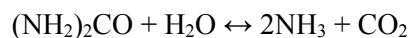
Third, urea, along with other products of nitrogen metabolism, contained in the urine, can suppress (and in high concentrations – irreversibly) the vital activity of any kind of living organisms on the principle of auto-inhibition [11].

Fourth, it is also possible that corticosteroids and their metabolites contained in the urine accelerate catabolic processes, thereby depriving the emerging, but not yet released nauplius protein and inhibiting its anabolic processes. Which means that such an embryo may not even have enough opportunities to exit the egg shells and start self-feeding; in addition, due to excessive catabolics of proteins, it may not be fully formed and is doomed to death.

Fifth, when using pure urine as an activating agent, without adding salt brine, the macerating effect of urea in the absence of salts is stronger and destroys the developing embryo.

It is somewhat more difficult to explain the facts of death of the formed larvae in cases when distilled water was added to the mixture of urine and lake salt brine – while maintaining the same concentration of salts in the prepared activating liquid. The following assumptions can be advanced.

In a dilute solution, urea is more susceptible to decomposition (hydrolysis) with the formation of a toxic product – ammonia, as well as carbon dioxide, which inhibits the life of the larva.



According to the law of mass activity (and the following law of Ostwald dilution), when a weak electrolyte (or a salt of a weak acid or a weak base, or other hydrolysable product) is diluted with water, both dissociation and hydrolysis processes are enhanced.

$$K = \alpha^2 C / (1 - \alpha),$$

where, C is the concentration of the substance, K is the constant, and α is the degree of decomposition of the substance (dissociation or hydrolysis).

Since the degree of dissociation α is very small and close to zero, it can be ignored in the denominator, and then:

$$K = \alpha^2 C \text{ or } C = K / \alpha^2,$$

in other words, there is an inverse relationship between the concentration and the degree of dissociation or hydrolysis [12].

When activating *Artemia*'s eggs with such a dilute urea, steroid hormones have a stimulating effect on the embryo, but the formed nauplius dies from the action of ammonia and carbon dioxide, which are released and penetrate into the shells as the eggs are kept in such an activating solution. This assumption is also supported by the facts noted in experiment 1: the long-term content of eggs in a brine + urine solution without replacing the activating liquid led to significant death of nauplius, which could be associated with the gradual decomposition of urea into ammonia and carbon dioxide. At the same time, replacing the activating solution with a fresh portion of it increased the yield of nauplius and did not reduce the viability of the larvae.

Thus, the use of the natural product of human and animal excretion in the proposed concentrations and in compliance with the specified terms of egg activation significantly increases the percentage of nauplius yield without reducing their viability and without accumulation of toxic or foreign substances for living organisms in the base feed facility.

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АРТЕМИЯ ЖҰМЫРТҚАЛАРЫН БЕЛСЕНДІРУ ҮШІН ТАБИҒИ БИОЛОГИЯЛЫҚ БЕЛСЕНДІ ЗАТТАРДЫ ПАЙДАЛАНУ МӘСЕЛЕСІ БОЙЫНША

Аннотация. Қазіргі уақытта *Artemia Leach* тұқымының желбезекаяқтылар отрядының шаянтәрізділері 1819 жас гидробионттарды жасанды өсіру барысында бастапқы жем болып саналады. Дегенмен, жұмыртқалардан жиналған науплиус шығымының төмендегені байқалады және жасанды белсендіруді талап етеді. Эмпирикалық зерттеулер нәтижесінде тәжірибелі жолмен бөлу өнімдерін белсендіретін әрекет – ересек адамның сұйылтылған несесі екені анықталды. Зерттеулер бірнеше кезеңдерде жүргізілді.

Зерттеудің бірінші кезеңінде тәжірибелік жолмен эмбриондар диапауза жағдайына жақын болған кезеңде және табиғи белсендіруден кейінгі көктемгі кезеңде артемийдің күзгі жұмыртқаларынан науплиустардың шығу мөлшері анықталды. Әртүрлі популяцияларда орташа өсу қарқыны тәулігіне 0, 2-ден 0, 6%-ға дейін ауытқиды.

Эксперименталды зерттеулердің екінші кезеңі белсенді заттың (ересек адамның несесі) және тұзды көл суы қосылған сұйықтық ортада жұмыртқаны белсендіруден тұрады.

Бірінші тәжірибеде 24% шығымы бар цистадағы көл суының ерітіндісі 105 г/л концентрациясы бар несеппен араластырылды. Ерітіндіні активтендіруде жаңа несепті пайдалану *Artemia* науплиустар шығымын ынталандырды және екі аптадан кейін бұл көрсеткіш 66,7% -ға жетті, бұл бақылау көлемінен 2,55 есе артық. Алдағы уақытта эмбриондар белсенділігі төмендеп, сәуір айының соңында нөлге дейін жетті.

Екінші тәжірибеде бірінші тәжірибеден алынған жұмыртқа қолданылды, яғни сол жұмыртқаның шығымы 66,9 % (31. 01) және «көл суы + несеп» (105 г/л) жаңа ерітіндісіне орналастырылды. 19 күннен кейін, бақылаумен салыстырғанда, науплиус шығымы 52,1%-ға өсті және бірінші тәжірибеден 23,7%-ға артты. Алдағы уақытта жоғары деңгей сақталған, бірақ 29. 04. 80,8%-дағы шығу деңгейі кезінде көптеген әлсіреген және өлі науплиус саны байқалды.

Үшінші тәжірибеде 233 г/л минералданған және 36,7% өскен көлден алынған көктемгі жұмыртқа қолданылды. Көл суын (233 г/л) несеп және тұщы сумен араластыру есебінен тұздың түрлі концентрациясы (110-140 г/л) пайдаланылды. Активтендірудің ұзақтығы 20 және 30 күнге созылды. Ерітіндіні 110 г/л минералдап, белсендірілген кезде науплиус шығымы өсті және бір айдан кейін бақылаудан 1,65 есе асып түсті. Тәжірибе соңында тұз концентрациясының артуы науплиус шығымын төмендетіп, белсендіруші әсерді баяулатты. Ерітіндіні тұщы сумен араластыру ерітіндінің әсер ету тиімділігін төмендетті, ал жоғары араласқанда эмбриондардың толық жойылғаны байқалды.

Ұсынылатын сұйықтық компоненттерінің белсендіретін әсер ету механизмдері стероидты гормон метаболиттерінің жұмыртқада личинканың қалыптасуына физиологиялық ықпалы әрі жұмыртқа қабығына бірқатар заттардың деструктивті физикалық-химиялық әсері бар. Бүйрек үсті без қабығы гормондарының (минералокортикоид және глюкокортикоид), сондай-ақ адам мен жануар несесіндегі жыныс гормондарының метаболиттері тіндегі алмасу үдерісін күшейтетіндіктен, кез келген жануар эмбрионының дамуына белсенді әсер етуі мүмкін. Несеп компоненттерінің жұмыртқа қабығына деструктивті әсері, науплиус шығымын жеңілдетеді әрі липидтерді ішінара шаю, ақуыз құрылымы мен гидролиздің бұзылуы, ұлпаны ажырату әсері бар. Карбамид және басқа да органикалық заттар – несептегі электролитті емес тұз иондарының антагонисі болып есептеледі және жұмыртқа қабығының ісінуіне ықпал етеді.

Несеп нәрмен ішінара гидролизденген ақуыздар, сондай-ақ су, қабық деструкциясының жалпы үдерісін жылдамдатады. Жұмыртқа қабығы ісінгенде заттардың қабық ішіне ену тәртібі маңызды рөл атқарады. Алдымен несепте нәр әсерінен қабықтың ішінара деструкциясы пайда болады, содан кейін концентрация градиенті бойынша ішке жылжымалы натрий иондары кіреді, содан кейін несептегі органикалық заттар (карбамид, басқа аз мөлшердегі алмасу өнімдері, стероидтар) енеді. Натрий катиондары гидрат қабығына еніп, су өткізбейтін қасиеттерге ие болады. Стероидты гормон метаболиттері қабық ішіне еніп, натрий катиондарын (минералокортикоидтар) ұстап қалады, демек, сұйықтықтың жиналуына ықпал етеді, себебі бір валентті катиондар тірі тінге гидрат жасайтын қасиеттерге ие. Жұмыртқа қабығының деструкциясы оттегінің ішіне енуіне ықпал етеді, ол да науплиус дамуын ынталандырады.

Жұмыртқа қабығының деструкциясы бар эмбрионның дамуын осындай кешенді белсендіруде науплиустардың қоректік құндылығын сақтай отырып, жұмыртқаның пластикалық және энергетикалық субстанцияларын үнемдейді.

Түйін сөздер: артемия, жұмыртқа, қабық, науплиустардың шығуы, несеп.

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К ПРОБЛЕМЕ ИСПОЛЬЗОВАНИЯ ЕСТЕСТВЕННЫХ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ ДЛЯ АКТИВАЦИИ ЯИЦ АРТЕМИИ

Аннотация. В настоящее время жаброногий рачок рода *Artemia* Leach, 1819 является хорошим стартовым кормом при искусственном выращивании молоди гидробионтов. Однако часто наблюдается пониженный выклев науплиусов из собранных яиц и требуется проведение искусственной активации. В результате эмпирических исследований удалось опытным путем обнаружить активирующее действие продуктов выделения – разбавленной урины взрослого человека. Исследования были проведены в несколько этапов.

На первом этапе исследований опытным путем определили величину выклева науплиусов из осенних яиц артемий в период, когда эмбрионы становятся близки к состоянию диапаузы и в весенний период после естественной активации. Установлено, что в среднем темп прироста всхожести для разных популяций колеблется от 0, 2 до 0, 6% в сутки.

Второй этап экспериментальных исследований состоял в активации яиц в жидких средах с различной долей активирующего вещества (урины взрослого человека) и соленой озерной воды.

В первом опыте цисты с выклевом 24% поместили в раствор озерной воды разбавленной уриной до концентрации 105 г/л. Использование свежей урины в растворе активации стимулировало выклев науплиусов артемий, и уже через две недели этот показатель достиг величины 66,7 %, превышая величину контроля в 2,55 раза. В дальнейшем активность эмбрионов начала снижаться, и к концу апреля выклев упал до нуля.

Во втором опыте использовалось яйцо из опыта 1 с выклевом 66,9 % (31.01), помещенное в свежий раствор «озерная вода + урина» (105 г/л). Через 19 дней выклев повысился на 52,1 % по сравнению с контролем и на 23,7 % превысил данные опыта 1. В дальнейшем сохранялся высокий уровень выклева, но 29.04. при выклевом 80,8 % наблюдалось большое число ослабленных и мертвых науплиусов.

В третьем опыте использовалось весеннее яйцо из озера с минерализацией 233 г/л и всхожестью 36,7%. Были использованы различные концентрации солености (110-140 г/л) за счет разбавления озерной воды (233 г/л) уриной и пресной водой. Продолжительность активации составляла 20 и 30 суток. При активации в растворе с минерализацией 110 г/л шел рост выклева и через один месяц превысил контроль в 1,65 раза. Увеличение концентрации соли снизило активирующий эффект с некоторым снижением выклева в конце опытов. Разбавление раствора пресной водой снизило эффективность действия раствора, а при более высоких разбавлениях наблюдалась полная гибель эмбрионов.

Механизмы активирующего действия компонентов предлагаемой жидкости складываются из физиологических влияний метаболитов стероидных гормонов на формирование личинки в яйце и деструктивных физико-химических воздействий ряда веществ на оболочку яйца. Метаболиты гормонов коры надпочечников (минералокортикоидов и глюкокортикоидов), а также половых гормонов, содержащиеся в моче людей и животных, могут оказать активирующее воздействие на развитие эмбриона любого животного за счет усиления обменных процессов в тканях. Деструктивное влияние компонентов урины на оболочку яиц, облегчающее выход науплиусов, заключается в частичном омылении липидов, нарушении структуры и гидролизе белков, мацерирующем действии. Карбамид и другие органические вещества-неэлектролиты, содержащиеся в моче, являются антагонистами ионов солей и способствуют набуханию яйцевых оболочек. Белки, частично гидролизованные мочевиной, также присоединяют воду и набухают, ускоряя общий процесс деструкции оболочки. Определенную роль в набухании яйцевых оболочек мог сыграть порядок проникновения веществ внутрь оболочки. По-видимому, сначала происходит частичная деструкция оболочки под действием мочевины, затем внутрь по градиенту концентрации проникают подвижные ионы натрия, и лишь затем – органические вещества, имеющиеся в моче (карбамид, небольшое количество других продуктов обмена, стероиды). Катионы натрия проникают в гидратной оболочке и обладают водоудерживающими свойствами. Метаболиты стероидных гормонов также способствуют набуханию и дальнейшему разрыву яйцевых оболочек за счет того, что, проникая внутрь оболочки, они задерживают катионы натрия (минералокортикоиды), а значит, способствуют накоплению жидкости, поскольку одновалентные катионы обладают для живых тканей гидратирующими свойствами. Деструкция оболочек яйца способствует проникновению внутрь кислорода, который также оказывает стимулирующее воздействие на развитие науплиуса. При такой комплексной активации развития зародыша с деструкцией яйцевых оболочек достигается экономия имеющихся в яйце пластических и энергетических субстанций, сохраняя питательную ценность науплиусов.

Ключевые слова: артемия, яйца, оболочка, выклев науплиусов, урина.

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