The research presents the results of experimental studies of the influence of various physicochemical factors (phonophoresis, electrophoresis, electrospray, diffusion, etc.) on the rate of transport of biologically active substances through the protective layer of hatching eggs shell of crossbred Haysex Brown. The aim of the study was to compare the effectiveness of the use of physicochemical methods of transportation of biologically active substances through the shell of hatching eggs of chickens.

For the experiment, three batches of eggs were formed, which were obtained from layer hens Haysex Brown, 144 pieces in each experimental group. The kinetic parameters of BAS transport through bioceramic protective barriers of eggs were calculated based on the determination of BAS concentration on the surface and inside the egg by mass spectrometric method (mass spectrometer with ionization of $^{252}$Cf fission fragments "MSBH", (Ltd "SELMI", Sumy, Ukraine). The degree of permeability of bioceramic layers of the shell relative to the model gas mixture which is identical to the atmospheric air was studied by the method of V. Breslavets et other and the mass spectrometric method (gas mass spectrometer "MX 7304A"). Electron microscopic studies were performed on a scanning electron microscope REMMA-102; Visilog 6.11 (Noesis, Belgium) was used to process the obtained digital images to determine the number of shell microdefects per unit area of the digital image.

It has been experimentally proved that to increase the efficiency of transport of biologically active substances (BAS) through the bioceramic layers of the shell of hatching eggs of Loman Brown hens, it is advisable to use ultrasonic treatment (phonophoresis, sonophoresis), substances - enhancers, including plant terpenes (L-menthol), DMSO and cyclodextrin. Phonophoretic treatment of hatching eggs increases the hatchability of eggs by 7.6%. At the same time, the gas permeability of the bioceramic layer increases significantly (by $0.43 \times 10^{-4} \text{ m}^2/\text{m}^2 \cdot \text{s}$).

Key words: biotechnology, technology, egg incubation, disinfectants, biologically active substances.

DOI: https://doi.org/10.32845/bsnau.lvt.2020.4.15

One of the newest promising approaches to the urgent problem of increasing the hatchability of hatching eggs and the quality of young poultry is the regulation of metabolism of avian embryos during incubation using various physicochemical factors, including biologically active substances (BAS) of synthetic and natural origin [1,3,8,14,17]. Despite the fact that, as shown by numerous studies, as a BAS can be used a variety of organic and inorganic substances, and still remain unresolved certain aspects of non-destructive transport of these BAS through the bioceramic protective barrier of eggshell [2,7,10,21].

The problem of delivery of BAS to the area embryo development is achieved by fundamentally different methods [5,6,9,25]. One of them is the introduction of BAS and vaccines in the middle of the hatching egg ("in ovo") using a precision needle microdose (technology Embrex Inc., USA). However, this technology involves a local violation of the integrity of the bioc-
eralic layer [4,11]. Also known are technologies for regulating the metabolism of avian embryos that use passive and active transport of BAS through the bioceramic layer. The main driving factor of the first is the free diffusion of substances that are part of the BAS, through the calcite layer of the shell [12,15,18,19].

Factors of active transport include accelerated diffusion and transfer by means of "transporter molecules" and so-called "enhancers" - substances that enhance the transfer processes [13, 24, 26]. Peculiarities of both approaches to transshell transfer are combined in the technology of "artificial cuticle" for hatching eggs [16]. We note that due to the complex, multicomponent and heterogeneous structure of bioceramic and glycoprotein protective layers of the shell and supra- and subshell membranes, detailed studies of the mechanisms and kinetic parameters of transport of organic substances that are part of BAS, different molecular weight, shape and charge 20,22,23,27.

The aim of the study was a comparative study of the efficiency of transport of biologically active substances through the shell of hatching eggs of Haysex Brown chickens.

**Materials and methods of research.** The work used hatching eggs (15-20 weeks of egg-laying), obtained from a high sex brown bird, which was kept in accordance with the established norms of keeping and feeding. The kinetic parameters of BAS transport through bioceramic protective barriers of eggs were calculated based on the determination of BAS concentration on the surface and inside the egg by mass spectrometric method (mass spectrometer with ionization by 252 Ce fragments "MSBH", (Ltd "SEMB", Sumy, Ukraine). Reagents: creatine phosphate disodium salt, 5% (Sigma, USA); adenosine monophosphate (AMP) (Sigma, USA); glutamine (Glu) (Serva, Germany); cysteine (Cis) (Reanal, Hungary), dimethyl sulfoxide (DMSO); cyclodextrin, (Sigma, USA)*. The composition of the model aqueous solution of BAS (MR-BAS) for experiments to determine the efficiency of transport of BAS in the middle of the egg: (AMP (0.1%) + Glu (0.1%) + Cis (0.1%) + creatine phosphate disodium salt (0.1%), Concentrations of substances "enhancers": DMSO (0.05.5%), α- cyclodextrin (0.5-3.0%), L-menthol (0.5-2.5%), quaternary ammonium compounds (QAC) - Cid-20 (CID-Line, Belgium) (0.5-2.0%). The study of the efficiency of transporting BAS in the middle of the egg (n = 144) was performed as follows:

Control (Intensity of BAS diffusion under conditions of equal temperatures outside and inside the egg): 50 μl of MR-BAS was applied to the lateral surface of the egg (thermostat; humidity 95-98%; 18±0.20C; 12 hours);

Group 1 (Free diffusion of BAS under conditions of elevated temperature inside the egg): egg kept in a thermostat for 2 hours at a humidity level of 95-98%; 25±0.2C, immersed for 15 minutes in a solution of MR-BAS 15±0.10°C (water thermostat);

Group 2 (Substance "enhancer" α- cyclodextrin): 50 μl of MR-BAS + α-cyclodextrin (0.5%) was applied to the lateral surface of the egg (thermostat; humidity 95-98%; 180±2°C; 12 hours);

Group 3 "Substance" enhancer "from the group of terpenes (L-menthol): on the side surface of the egg was applied 50 μl of MR-BAS + L-menthol (0.5%) (thermostat; humidity 95-98%; 180±2°C; 12 hours);

Group 4 (DMSO enhancer substance): on the side surface of the egg was applied 50 μl of MR-BAS + DMSO (0.05%) (thermostat; humidity 95-98%; 18±2°C; 12 hours);

Group 5 *"Substance" enhancer "from the QAC group (CID-20): on the side surface of the egg was applied 50 μl of MR-BAS + CID-20 (0.5%) (thermostat; humidity 95-98%; 18±2°C; 12 hours);

Group 6 (Hydraulic shock): a solution of MR-BAS was applied to the side surface of the egg with a diameter of 0.3 mm in a pulsed mode (0.5 s) under a pressure of 2.5 atm for 2 minutes;

Group 7 (Electrophoresis): on the side surfaces of the egg from above and below were placed foam washers with a diameter of 1 mm, saturated with a solution of MR-BAS (1 ml), Platinum electrodes were connected to the washers (voltage 3.5 V; current direct; 10 min.; thermostat 180±2°C;

Group 8 (Electrospray "electrospray") (diameter of droplets of aerosol of solution MR-BAS 200 nm - 1 μm; voltage + 7 kV;

Group 9 (Phonophoresis; sonophoresis; ultrasonic treatment)); 180±0.2°, 22 kHz for 2-15 s).

The degree of permeability of bioceramic layers of the shell relative to the model gas mixture which is identical to the atmospheric air was studied by the method of V. Breslavets et al. [3] and mass spectrometric method (gas mass spectrometer "MX 7304A"). Electron microscopic studies were performed on a scanning electron microscope REMMA-102, when processing the obtained digital images to determine the number of microdecks of the shell per unit area of the digital image (Y; number of channels,%; table), used the program Visilog 6.11 (Noeas, Belgium). Experimental results (repetition not less than n = 5) were processed statistically using the package Statistica 5.1.

**Results of research and discussion.** The table shows the results of a series of experiments comparing the degree of efficiency of methods of transporting BAS through the biocrystalline layer of the shell. The control was taken as the amount of BAS received by conventional diffusion for 12 hours at a humidity level of 95-98% and a temperature of 180±0.2°C; in the middle of the egg at equal temperatures outside and inside the egg. At the end of the experiment, a drop of BAS on the outer surface of the shell was dried, then the egg was carefully retrieved from the protein and yolk, dried again, then selected 0.5 cm2 shell with a layer of BAS applied to the outer surface, broken into 2 equal parts, fixed on the disk surface to keep the sample of the device "IASB" (one part of the outer surface, the other inner). Thus, the analysis of the organic component of the outer and inner surfaces of the shell allowed a comparison in a certain approximation of the number of BAS received during incubation in the middle of the egg. Thus, in the case of normal diffusion in the middle of the egg received 0.03 ± 0.009% of the amount of BAS contained on the surface.

The table shows that the most promising factors for non-destructive transfer of BAS through the biocrystalline layer are ultrasonic treatment (phonophoresis, sonophoresis), plant terpenes (L-menthol), which are already used in biotechnology as enhancers of BAS transport through natural protective structures (leather, bioceramics, etc.), DMSO and CD.

In particular, phonophoretic treatment of hatching eggs of Lohmann Brown crosses increases the hatchability from the initial value of 80.4% to 88.0% (+7.6). This significantly increases the gas permeability (up to 1.91 from the initial value of 1.48. 10⁻⁴ m3/m² s) (+ 0.43) under conditions of transfer to...
the middle of the egg 79.2% of the BAS of the total number of eggs applied to the surface (Table 1).

### Table 1

**Comparison of the efficiency of different physicochemical methods of transportation of biologically active substances (BAS) in the middle of hatching eggs (cross Lohmann brown)**

| Groups | Kind of transportation | The content of BAS in the middle of the egg, % of control | Y, % | Gas permeability $10^{-4} \text{ m}^2/\text{m}^2/\text{sec}$ | Eggs hatchability, % |
|--------|-----------------------|--------------------------------------------------------|------|-----------------------------------------------|---------------------|
| C      | Control (intensity of diffusion of BAS under conditions of equal temperatures outside and in the middle of the egg) | 0.03±0.009 | 65.2±1.84 | 1.48±0.052 | 80.4±2.42 |
| 1      | Free diffusion of BAS under conditions of elevated temperature in the middle of the egg | 2.5±0.11** | 65.0±2.33 | 1.48±0.091 | 81.1±3.10 |
| 2      | Cyclodextrins (CD) | 13.4±0.09** | 65.6±0.71 | 1.56±0.173* | 85.6±0.71** |
| 3      | L-menthol | 58.2±0.27** | 65.0±1.54 | 1.49±0.056 | 86.0±0.94** |
| 4      | Dimethyl sulfoxide (DMSO) | 65.1±0.14** | 66.0±2.03 | 1.48±0.183 | 86.0±0.22** |
| 5      | Quaternary ammonium compounds (QAC) CID-20 | 1.8±0.31** | 65.6±2.40 | 1.44±0.114 | 82.8±1.61 |
| 6      | Hydraulic shock | 22.1±0.05** | 81.1±3.33** | 1.51±0.273 | 81.7±0.40 |
| 7      | Electrophoresis | 56.4±0.23** | 78.0±3.44** | 1.71±0.363** | 86.0±2.33* |
| 8      | Electrospray | 35.3±0.16** | 69.4±1.73* | 1.56±0.150* | 82.8±2.03 |
| 9      | Phonophoresis | 79.2±0.08** | 74.0±2.11** | 1.91±0.091** | 88.0±1.10** |

**Notes:** * P <0.05; ** P <0.01.

It is noteworthy that there is no unambiguous correlation between the level of morphological "ordering" of bioceramic layers of the shell, which in our work is expressed by Y (the number of microdefects in the shell per unit area of digital image of the shell), and the level of gas permeability of the bioceramic layer. Thus, the number of microdefects of the bioceramic layer per unit area of the shell in comparison with the control significantly increases (+ 15.9%) under the influence of "hydraulic shock", as well as with the use of phonophoretic techniques (+ 8.8% relative to control). However, the corresponding increase in the number of BAS received in the middle of the egg is + 22.07% and 79.17% compared to the control. These results provide grounds for assuming the existence of different mechanisms of transfer of organic compounds through the membrane systems of living tissues, in the case of solid-phase structures is not effective enough. In this aspect, the electrospray technique proved to be more effective - as can be seen from the table, the number of microdefects of the bioceramic layer per unit area of the shell does not differ significantly from the control. However, the efficiency of trans shell transfer exceeds the corresponding figure inherent in the technique of "hydraulic shock" by 58.1%.

According to our data, very promising are the classic substances - "enhancers" - DMSO and plant terpenes (L-menthol). These substances significantly increase the rate of trans shell shell transfer (+ 65.07% and 58.17%, respectively) and at the same time have little effect on the degree of ordering of heterogeneous layers of bioceramics (table). DMSO and L-menthol also have a positive effect on the egg hatchability, although they are not able to increase the gas permeability of the bioceramic layer.

**Conclusions.** It has been experimentally proved that to increase the efficiency of transportation of biologically active substances (BAS) through the bioceramic layers of the shell of hatching eggs of Loman Brown chickens, it is advisable to use ultrasonic treatment (phonophoresis, sonophoresis), enhancers, including plant terpenes (L-menthol), DMSO and cyclodextrin.

Phonophoretic treatment of hatching eggs increases the hatchability of eggs by 7.6%. At the same time, the gas permeability of the bioceramic layer increases significantly (by 0.43. 10-4 m3 / m 2·s).

**Acknowledgment.** The work has been performed under the financial support of the Ministry of Education and Science of Ukraine (state registration number 0119U100551).

---

**References:**

1. Bessarabov, B. F., 2006. Inkubatsiya yaiets s osnovami embriologii selskohozyaystvennoy ptitsy [Incubation of eggs with the basics of poultry embryology]. M : Kolos. pp 264.
2. Breslavets, V. O., 2006. Doslidzhennia hazo- ta volohopronyknosti shkaralupy yaiets kurei riznykh porid tori viku [Investigation of gas and moisture permeability of eggshells of chickens of different breeds and ages]. Ptakhivnytsto : mizhvid. nauk. zv. IP UAAN, issue 58, pp. 355-360.
3. Breslavets, V. O., 2001. Inkubatsiia yaiets silskohospodarskoi ptytsi : metodychniy posibnyk [Incubation of poultry eggs: a guide]. Kh: IEKVM., pp. 92.
4. Dobrenko, A., 2010. Obrabotka yaiets v magnitnom pole. Ptitsevodstvo. Nr 4. S. 21 - 22.
5. Ivanov, V. O., 2004 Vplyv biologichno aktyvnykh rechovyn, vvedenykh khimichnym sposobom v yaytsa, na vyvodimist' yaiets' miyanykh i yayechnykh ptakhiv. Suchasne ptakhivnytsto. issue 4, pp. 2 - 3.
6. Krilov, N. K., 2001. Elektrofizicheskiye metody vozdeystviya v tekhnologii inkubatsii yaiets Izv. Nats. Akad. Nauki i
7. Medvedev, A., 2001. Bezopasnye sredstva dla ya dezinfektii. [Safe disinfectants]. Pitsevodstvo, issue 4, pp. 37-41.
8. Prokudina, N. A., 2008. Inkubatsiya yait selskokhozyaystvennoy ptitsy [Incubation of poultry eggs]. H.: «NTMT», pp. 386.
9. Sakhtatskiy, I., 2005. Dezinfectinski zasoby dla ptakhivnytstva: porivilna efektyvnist [Disinfectants for poultry: comparative effectiveness]. Veterynarna medytsyna Ukrainy, issue 1, pp. 40-43.
10. Stehni, B. T., 2005. Porivilna otsinka preparativ dlia peredinkubatsiini obrobky yait [Comparative evaluation of drugs for pre-incubation treatment of eggs]. Mzhzarnodnyi tematychnyi naukovyi zbiry. Kharkiv. T. 2, issue 85, pp. 1022-1025.
11. Iakubchak, O. M., 2006. Chym krashche obrobky? Porivilna otsinka suchasnykh I tradysytnyk dezinfektysnih zasobiv, shcho vykorystovuvutisja v haluzi ptakhivnytstva [What is more beautiful to grind? Ratio assessment of the occasional and traditional disinfectious diseases, how to get sick in the bird poultry]. Suchasne ptakhivnytstvo, issue 6, pp. 14-15.
12. Yakymenko, I. L., 2003. Rehulyatorna diya monokhromatychnoho vydymoho svita neteplovoyi int [Control of ultraviolet light for the development of feathers]. Avtoref. dys. ... doktora biol. nauk: 03.00.02; Kyyiv nats. un-t. - K., p. 34.

Wei Xiao, Junbo Xu, Xiaoyan Liu, Qiaoling Hub and Jianguo Huang, 2013. Antibacterial hybrid materials fabricated by nanocoating of microfibril bundles of cellulose substance with titaania/chitosan/silver nanopartic le composite films. J. Mater. Chem. B, issue 1, pp. 3477 -3485.

13. D’Alba, L., Jones, D. N., Badawy, H. T., Eliasson, C. M. and Shawkey, M. D., 2014. Antimicrobial properties of a nanostructured eggshell from a compost egg [Comparative ecology]. Journal of Hazardous Materials, issue 240, pp. 37-50. https://doi.org/10.1016/j.jhazmat.2013.04.030.
14. D’Alba, L., Maia, R., Hauber, M. E. and Shawkey, M. D., 2016 Evolution of avian eggshell structure in relation to nesting ecology. Proc. R. Soc. Lond. B. V. 283: 20160687 doi: 10.1098/rspb.2016.0687.
15. Gang, Xiao., 2015. Synthesis of core-shell bio a finny chitosan-TiO2 composite and its environmental applications. Journal of Hazardous Materials, issue 283, pp. 888-896.
16. Su, Hyun Kim, Hong, Kyoon, No and Witoon, Prinyawiwatkul. 2007. Effect of Molecular weight, type of chitosan, and chitosan solution pH on the shelf-life and quality of coated eggs. Journal of food science. Vol. 72, issue 1, pp. 44-48.
17. Bainer, B. M., Mcdade, K., Burchmore, R., 2013 Enhancing the egg’s natural defence against bacterial penetration by increasing cuticle deposition. Animal Genetics. V. 44, issue 6, pp. 661-668. doi: 10.1111/age.12071.
18. Liu, Z., Sun, X., Cai, C., He, W. and Linhardt, R. J., 2016 Characteristics of glycocalyx layers on chicken eggshells and the influence of disaccharide composition on eggshell properties. Journal of food science. Vol. 72, issue 1, pp. 2879–2888. doi: 10.1111/j.1365-2621.2016.03110.x.
19. Maria, P., Montero Garcia, M., Carmen, G. M. and Gustavo, V., 2017. Edible films and coatings: fundamentals and applications. CRC Press, Taylor & Francis Group. Issue 24, pp. 598.
20. Maureen, B., Yves, N., Filip, V., 2011. Immersenf food science, technology and nutrition improving the safety and quality of eggs and egg products: Volume 2 Egg safety and nutritional quality. Woodhead Publishing. Issue 38, pp. 448.
21. Yuceer, M., Caner, C., 2014 Antimicrobial lysozyme-chitosan coatings affect unctional properties and shelf life of chicken eggs during storage. J. Sci. Food Agric, issue 94, pp.153–162. doi: 10.1002/jsfa.6322.
22. Yu, Shao, Changsheng, Cao, Shiliang, Chen, Xiao, He, Xiaofang, Li and Danzhen, Li., 2015. Investigation of nitrogen doped and carbon species decorated TiO2with enhanced visible light photocatalytic activity by using chitosan. Applied Catalyst B: Environmental, issue 177, pp. 344–351.

Список використаної літератури:

1. Бессарабов Б. Ф. Інкubaція яєць із основами эмбriологии сельскохозяйственной птицы. М: Колос. 2006. С. 264.
2. Бреславець В. О. Дослідження газо- та вологопроникності шкапули яєць курей різних порід та віку. Птаховинство. Міжвід. темат. наук. зб. ІП УААН. 2006. № 58. С. 355-360.
3. Бреславець В. О. Інкubaція яєць сельськогосподарської птиці : методичний посібник. Х.: ІЕІКВМ. 2001. С. 92.
4. Добрехо А. Обработка яєць в магнітном полі. Птицеводство. 2010. № 4. С. 21 – 22.
5. Іванов В. О. Вплив біологічно активних речовин, введенних хімічним способом в яйце, на виводимість яєць маємих і яєць пасічних. Сучасне птаховінство. 2004. № 4. С. 2 - 3.
6. Кириллов Н.К. Електрофізичні методи впливу в технології інкубації яєць. Ізв. Нац. Акад. Наук і
ефективності використання фізико-хімічних методів транспортування біологічно активних речовин через шкаралупу інкубаційних яєць курей. Для проведення досліду було сформовано три партії яєць, котрі були отримані від курей-несучок Хайсекс браун, по 144 штук в кожній експериментальній групі. Кінетичні параметри транспортування БАР через біокерамічні захисні бар’єри яєць вираховували виходячи з визначення концентрації БАР на поверхні і всередині яйця мас-спектрометричним методом (мас-спектрометр з іонізацією уламками поділу $^{252}\text{Cf}$ "МСБХ", (BAT "SELMI", Суми, Україна). Ступінь проникності біокерамічних шарів шкаралупи щодо модельної газової суміші яка є ідентичною атмосферному повітря, вивчали методом В. О. Бреславця та ін. та мас-спектрометричним методом (газовий мас-спектрометр "МХ7304А". Електронно-мікроскопічні дослідження проводили на скануючому електронному мікроскопі РЕММА-102; при обробці отриманих цифрових зображень для визначення кількості мікродефектів шкаралупи на одиницю площі цифрового зображення, використовували програму Visilog 6.11 (Noesis, Бельгія). Експериментально доведено, що для підвищення ефективності транспортування біологічно активних речовин (БАР) через біокерамічні шари шкаралупи інкубаційних яєць курей кросу Ломан браун доцільно використовувати ультразвукову обробку (фонофорез, сонофорез), речовини "енхансери", зокрема рослинні терпени (L-ментол), ДМСО і ІД. Фонофітретична обробка інкубаційних яєць підвищує показник виводимості яєць на 7,6%. При цьому значно зростає газопроникність біокерамічного шару (на $0.43 \cdot 10^{-4} \text{м}/\text{м}^2 \cdot \text{с}$).

**Ключові слова:** біотехнологія, технологія, інкубація яєць, дезінфектанти, біологічно активні речовини

Дата надходження до редакції: 08.12.2020 р.