

## THE IMPACT OF POTENTIAL FEED ADDITIVE NANOCOMPOSITE (AG, CU, FE AND MN DIOXIDE) ON EGGS' QUALITY PARAMETERS OF LAYING HENS COMPARED WITH METAL SALTS

Orobchenko O. L., Roman'ko M. Ye., Kutsan O. T.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: toxi-lab@ukr.net

**Summary.** The purpose is to study veterinary-sanitary characteristics of laying hens' egg quality under the conditions of influence of nanocomposite (Ag, Cu, Fe and Mn dioxide) (NcMe) and metal salts in the conditions of chronic toxicological experiment. Methods. The research was conducted on 72 laying hens cross Hayseks Brown (4 groups were formed: one control and three experimental n=18). Chickens from control group in addition were administered saline with feed; experimental chickens were given feed additives every day for 30 days: group I — a mixture solution of metal salts at a dose of 0.3 mg/kg, group II — nanocomposite metals in its biotic dose (0.3 mg/kg body weight) and group III — NcMe in toxic dose (4.0 mg/kg body weight), after cessation of additives administration the poultry was observed for 15 days. During the experiment there was conducted eggs' record from experimental poultry, and their veterinary-sanitary examination was carried out. Results. The data on the impact NcMe and metal salts on productivity of laying hens and veterinary-sanitary characteristics of eggs' quality was obtained. Conclusions. Introduction of NcMe with feed affect quantity and quality characteristics of laying hens productivity, predominating the effect of salts of the metals, that is characterized by increased levels of egg laying during the experiment in poultry from the third group in average by 36.1% and egg weight in the second experimental group — 24.7% ( $P \leq 0.05$ ) and changes in pH level of egg white and yolk, but within the normative values (in accordance with DSTU 5028:2008 'Hen's eggs for human consumption. Specifications').

**Keywords:** laying hens, nanocomposite metals, metal salts, dose, veterinary and sanitary examination, eggs, productivity

**Introduction.** Industrial poultry farming is the most dynamic and knowledge-based industry that makes a significant contribution to the country's Food Program as a major manufacturer of high quality animal protein (eggs and meat). Productivity of poultry and usefulness of products of this industry depend largely on the balance of the diet, the presence of minerals, enzymes and other biologically active compounds in it (Egorov et al., 2011; Laptev et al., 2012). Currently, nanotechnology is recognized as the major driving force of science in the twenty-first century. They begin to be used in livestock, particularly in poultry farming. The literature data shows the superiority of metals in the form of nanoparticles before their salts, metal nanoparticles can easily penetrate all organs and tissues and in biotic doses stimulate metabolism (Nesterov et al., 2014). The purpose of our work was to study veterinary-sanitary characteristics of laying hens' egg quality under the conditions of the influence of nanocomposite (Ag, Cu, Fe and Mn dioxide) and metal salts in the conditions of chronic toxicological experiment.

**Materials and methods.** The experiment was performed at the Department of Toxicology, Safety and Quality of Agricultural Products, NSC 'IECVM' on 72 laying hens cross Hayseks Brown weighing 1.5–1.8 kg, aged 365 days (4 groups were formed: one control and three experimental, 18 chickens in each).

Experimental composite mixture — nanocomposite metals (NcMe) — was composed of silver nanoparticles ( $31.5 \pm 0.9$  nm), iron nanoparticles ( $100.0 \pm 10.0$  nm), copper nanoparticles ( $70.0 \pm 5.0$  nm) and dioxide manganese nanoparticles ( $50.0 \pm 3.0$  nm) in aliquots relation with the final concentration  $100.0 \mu\text{g}/\text{cm}^3$  for each metal, similar to the concentration of the metals in ionic (macro disperse) form in a solution of mixture of salts —  $\text{AgNO}_3$ ,  $\text{CuSO}_4 \times 5\text{H}_2\text{O}$ ,  $\text{MnSO}_4 \times 5\text{H}_2\text{O}$  and  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$  respectively.

After holding experimental chickens from all groups on a standard diet for 15 days (equalization period), chickens of control group in addition were given additives to feed daily for 30 days, group I — a mixture solution of metal salts in doses of 0.3 mg/kg, group II — NcMe in biotic dose (0.3 mg/kg body weight) and group III — NcMe in toxic dose (4.0 mg/kg body weight), after cessation of supplementation the birds have been observed for 15 days. Biotic and toxic doses were established in accordance with previous studies (Kutsan, Roman'ko and Orobchenko, 2012; Orobchenko, Roman'ko and Kutsan, 2014) when studying acute and chronic toxicity NcMe on laboratory animals. During the experiment there was conducted clinical observation and collection of eggs from experimental birds to determine the level of productivity and quality of product by physical-chemical parameters.

Veterinary-sanitary examination of eggs was conducted under the rules (Ukraine. Chief Inspector of Veterinary Medicine of Ukraine, 2001), we were guided by the requirements of DSTU 5028:2008 ‘Hen’s eggs for human consumption. Specifications’ (DSSU, 2009). Statistical analysis of the results of research was carried out with the help of applied package Microsoft Excel 2003 (for Windows XP).

**Results and discussion.** According to the results of veterinary-sanitary examination of eggs from laying hens of control and research groups there was found that their quality meets the requirements of the current DSTU 5028:2008 ‘Hen’s eggs for human consumption. Specifications’ (DSSU, 2009) for the duration of the study: egg shell was intact, strong, without damage, smooth; the yolk was bright yellow, evenly colored, elastic texture, shape maintained; the white was pure and transparent, viscous, with no signs of damage; peculiar smell for fresh eggs.

Birds from experimental group III (NcMe 4.0 mg/kg body weight) were the most productive — from these birds during the experiment there were obtained 200 eggs, which exceeded the number of eggs received from poultry of control group in average of 36.1% ( $P < 0.05$ ). Slightly lower productivity was recorded in chickens of experimental group II (biotic NcMe dose, 0.3 mg/kg body weight) — 159 eggs, that meaningfully exceeded the number of eggs ( $n = 147$ ) received from the control birds, in average of 8.2% ( $P < 0.05$ ). The lowest productivity ( $n = 138$ ) was recorded in chickens from the group I which received a solution of metal salts at a dose of 0.3 mg/kg body weight, that was close to the level of the control group.

In accordance with the requirements of DSTU 5028:2008 ‘Hen’s eggs for human consumption. Specifications’ (DSSU, 2009), eggs are divided into categories according to weight (Table 1).

**Table 1** – Classification characteristic of chicken eggs for food in compliance with DSTU 5028:2008 ‘Hen’s eggs for human consumption. Specifications’ (DSSU, 2009)

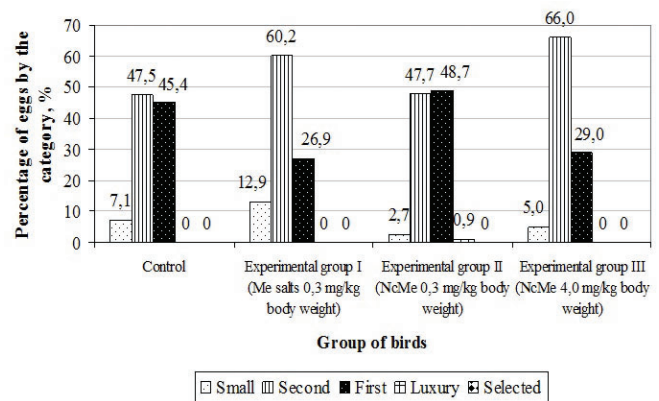
Category	The weight of one egg, g
Selected or XL	73.0 and more
Luxury or L	From 63.0 to 72.9
First or M	From 53.0 to 62.9
Second or S	From 45 to 52.9
Small	From 35 to 44.9

During 15 days of administration of the preparations the highest percentage of small eggs was found in the experimental group, where chickens received solution of metal salts 0.3 mg/kg, and the smallest — in the research group II (biotic dose of NcMe). The percentage

of the second category of eggs was higher in the third experimental group; and in control, I and II groups it was almost on the same level.

The percentage of eggs weight from 53.0 to 62.9 g (first category) was the highest in the second experimental group, while for chickens from the third experimental group (NcMe 4.0 mg/kg body weight) there was recorded the minimum rate. Luxury eggs were found only in the second experimental group, where the chickens received biotic dose of NcMe.

After 30 days there was observed a similar picture, with the exception of second category eggs percent increase in the experimental group I. Also it was interesting from a practical point of view that the percentage of eggs of the first category was higher than the second category in the second experimental group (Fig. 1).

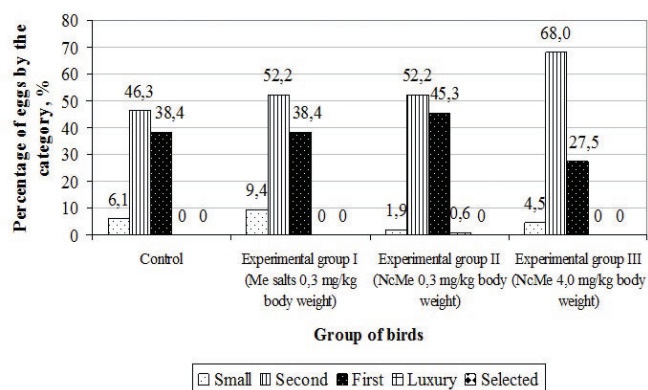


**Figure 1.** Percentage of eggs from experimental hens by the category on the 30<sup>th</sup> day of experiment

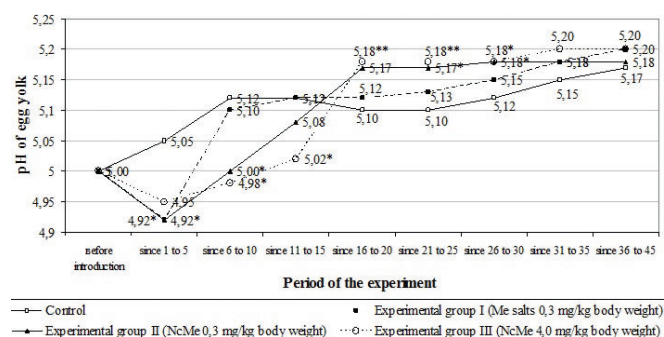
On the 7<sup>th</sup> day after the cessation of additives introduction there was determined unchangeable dynamic by the percentage of small eggs, increase the percentage of eggs of the second category in group II, and increase of eggs of the first category in control group.

And on the 15<sup>th</sup> day after the cessation of additives introduction there was recorded stabilization of eggs by the categories, except the third experimental group, where a higher percentage of eggs the second category maintained (Fig. 2).

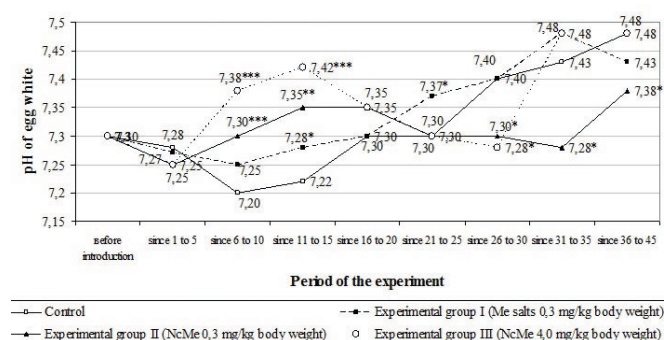
Another indicator by which egg quality is regulated is yolk and white pH level (data is presented in Fig. 3–4). From the first to the 5<sup>th</sup> day of the experiment the pH level of egg yolk from hens of the first and second research groups was significantly lower than the control, 3.0% (Fig. 3), while in the third experimental group it had only a downward trend. In this term of study for pH of egg white from hens of experimental groups there were not served probable deviations from control (Fig. 4).



**Figure 2.** Percentage of eggs from experimental hens by the category in 15<sup>th</sup> days after cessation of additives introduction



**Figure 3.** Dynamics of pH level of egg yolks from experimental laying hens ( $M \pm m$ ,  $n=4$ ), \* —  $P < 0.05$ ; \*\* —  $P < 0.01$  — relative to control



**Figure 4.** Dynamics of pH level of egg white from experimental laying hens ( $M \pm m$ ,  $n=4$ ), \* —  $P < 0.05$ ; \*\* —  $P < 0.01$ ; \*\*\* —  $P < 0.001$  — relative to control

Since the 6<sup>th</sup> to the 10<sup>th</sup> day of the experiment likely decrease in pH level of egg yolks of hens, which received NcMe was recorded: in group II — by 2.3%, and in group III — by 2.7%, while in the experimental group I this figure did not differ from control.

pH level of egg white tended to increase in the first experimental group, and in the second and third groups was higher than the control ( $P < 0.001$ ) by 1.4 and

2.5% respectively. Since the 11<sup>th</sup> to the 15<sup>th</sup> day of the experiment the pH level of egg yolks from hens of the first experimental group did not differ from control, in the second experimental group there was observed a tendency to decrease, and in the third group there was probable decrease in pH level of 2.0%. In this term of the study pH of egg white in all experimental groups significantly exceeded control: in the group I — by 0.8%, in the group II — by 1.8% and in the group III — by 2.8%.

Since the 16<sup>th</sup> to the 20<sup>th</sup> day there was observed excess of control pH level of egg yolk in all research groups, and in groups I and II there was a tendency, in group III — significant increase by 1.6%, while the pH of the white had not probable deviations from control.

Since the 21<sup>st</sup> to the 25<sup>th</sup> day of the experiment there was noted the excess of control pH level of yolk in all research groups, and in group I there was a tendency. In groups II and III there was significant increase by 1.4 and 1.6% respectively. The pH of the white did not differ from control in the second and third experimental groups, and in the first group it was higher than the control by 1.0% ( $P < 0.05$ ).

A similar dynamics in the pH level of egg yolks has been observed since the 26<sup>th</sup> to the 30<sup>th</sup> day of the experiment, while the pH level of egg white in this period of the study did not differ from control in experimental group I, and in the second and third groups was significantly lower than the control by 1.4 and 1.6% respectively.

Since the 31<sup>st</sup> day and to the end of the experiment there was observed a tendency to increase the pH level of eggs yolks in all experimental groups. While the pH level of egg white since the 31<sup>st</sup> to the 35<sup>th</sup> day tended to increase in the first and third groups, and in the second group it was significantly lower than the control by 2.0%, and in the last period of the study pH level of white from hens in the experimental group III did not differ from control, in the second group there was a decrease by 1.3% ( $P < 0.05$ ), and in the experimental group I there was a tendency to decrease.

Thus, the data indicates that metal nanoparticles affect the quantity and quality characteristics of laying hens' productivity, and they have an advantage over macro size forms of such metals (metal salts), as evidenced by the increase of egg-laying qualities (III experimental group) and egg weight (II experimental group) during the period of introduction of additions, and also it causes changes in pH level of egg yolk and white, but within the norms regulated by the requirements of DSTU 5028:2008 'Hen's eggs for human consumption. Specifications' (DSSU, 2009).

**Conclusions.** Introduction with feed of metal nanocomposite (Ag, Cu, Fe and Mn dioxide) affects

the quantity and quality of productivity of laying hens, predominating the effect of salts of the metals, that is characterized by increased levels of egg laying during the experiment in poultry from the third group in average of 36.1% and egg weight in the second experimental group — 24.7% ( $P \leq 0.05$ ).

Introduction of metals as additives in macro and nanoscale form causes changes in the pH level of egg white and yolk, but within the norms regulated by the requirements of DSTU 5028:2008 'Hen's eggs for human consumption. Specifications' (DSSU, 2009)).

### References

- DSSU [State Committee for Technical Regulation and Consumer Policy] (2009) DSTU 5028:2008. *Hen's eggs for human consumption. Specifications [Yaitsia kuriachi kharchovi. Tekhnichni umovy]*. Kyiv: Derzhspozhyvstandart Ukrainy.
- Egorov, I., Andrianova, E., Prisyazhnaya, L. Blazhinkas, D. and Buteykyk, G. (2011) 'Application vilzim Multi-enzyme preparation vilzim pri vyrashchivani tsipliat-broylerov', *Ptitsevodstvo*, 8, pp. 21–23. Available at: <http://elibrary.ru/download/10660226.pdf>. [in Russian].
- Kutsan, A. T., Roman'ko, M. Ye. and Orobchenko, A. L. (2012) Evaluation of safety and toxicity metal nanoparticles as veterinary nanonutritsevtika prototypes, to identify systematic biomarkers in experiments in vitro and in vivo [Otsenka bezopasnosti i toksichnosti nanochastits metallov, kak prototipov veterinarnogo nanonutritsevtika, po opredeleniyu sistemnykh biomarkerov v eksperimentakh in vitro i in vivo], In: *Materiály VIII mezinárodní vědecko-praktická konference «Moderní vymoženosti vědy–2012». Díl 22. Biologické vědy. Zvěrolékařství. Praha: Publishing House «Education and Science»*, pp. 84–87. Available at: [http://www.rusnauka.com/4\\_SND\\_2012/Veterenaria/1\\_100160.doc.htm](http://www.rusnauka.com/4_SND_2012/Veterenaria/1_100160.doc.htm). [in Russian].
- Laptev, G. Y., Novikov, N. I., Nikonov, I. N. and Egorov, I. A. (2012) 'Application of Tsellobakterin-T — a biological product based on producer of bacterial cellulases, in poultry' [Primenenie Tsellobakterina-T — biopreparata na osnove produtsenta bakterial'nykh tsellyulaz v ptitsevodstve]. In: Polyakov, V. A. and Rimareva, L. V. (eds.). *Perspektivnye fermentnye preparaty i biotekhnologicheskie protsessy v tekhnologiyakh produktov pitaniya i kormov*. Moskva: VNIIPBT, pp. 397–401. [in Russian].
- Nesterov, D. V., Sipaylova, O. Yu., Sizova, E. A. and Sheyda, E. V. (2014) 'Comparative assessment of different methods of introduction of copper nanoparticles for exchange of toxic elements in muscle tissue broiler chickens' [Srvnitel'naya otsenka vliyaniya razlichnykh sposobov vvedeniya nanochastits medi na obmen toksichnykh elementov v myshechnoy tkani tsipliat-broylerov], *Aktual'nye problemy transportnoy meditsiny*, 3(37), pp. 146–150. Available at: <http://elibrary.ru/download/22153298.pdf>. [in Russian].
- Orobchenko, A. L., Roman'ko, M. Ye. and Kutsan, A. T. (2014) 'Toxicological evaluation nanocomposite of metals (Ag, Cu, Fe and Mn dioxide) in the level of biochemical markers in the blood of rats in chronic experiment' [Toksikologicheskaya otsenka nanokompozita metallov (Ag, Cu, Fe i dviuokis' Mn) po urovnyu biokhimicheskikh markerov krovi krys v usloviyakh khronicheskogo eksperimenta], *Veterinariya, zootekhnika i biotekhnologiya*, 3, pp. 21–29. Available at: <http://elibrary.ru/download/90944192.pdf>. [in Russian].
- Ukraine. Chief Inspector of Veterinary Medicine of Ukraine (2001) *Rules of veterinary and sanitary expertise of poultry eggs [Pravyla veterynarno-sanitarnoi ekspertyzy yaiets sviiskoi ptytsi]*, approved by the Chief Inspector of Veterinary Medicine of Ukraine (decree № 70, 07.09.2001), registered by the Ministry of Justice of Ukraine (№ 850/6041, 27.09.2001). Available at: <http://zakon.rada.gov.ua/laws/show/z0850-01>. [in Ukrainian].