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Prospects of betulin application in broiler farming

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SUMMARY

It is an urgent task today to seek and implement nature-like technologies in broiler production and obtain biologically complete and safe poultry products, thus refusing from antibiotic use in feed. Feed additives based on natural components can be an effective tool for implementation of preventive and therapeutic veterinary measures. The paper presents research study results of application of betulin-based phytobiotics in broiler farming. The tests were carried out on Ross-308 cross-breed broiler chickens within the production cycle at one of the poultry farms of the Sverdlovsk Oblast. Birds of the experimental group received compound feed supplemented with dry betulin at 2.5 mg/kg of live weight from day 21 to day 35 of growing. The introduction of betulin-based feed additive into the diet contributed to increase in live weight gain and 7.6% pectoralis muscle output as compared with the control group. It was established that the phytobiotic consumption resulted in reduced deposition of subcutaneous and abdominal fat, higher biological value of meat by increasing the ash content, improved technological properties of meat due to increasing water-holding capacity of muscle fiber and intensity of formation and maturation of muscle fiber. Histological studies of pancreatic tissue samples from broiler chickens showed increase in the mass of islets of Langerhans and insulin-producing cell complexes. The pancreas was activated due to effects of the betulin-based feed additive. The results obtained indicate that the use of betulin in broiler production is a promising trend.

Key words: broiler chicken, feed additive, betulin, phytobiotics, live weight, muscle fibre, fat, pancreas, islets of Langerhans.

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Перспективы применения бетулина в бройлерном птицеводстве

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РЕЗЮМЕ

Поиск и внедрение природоподобных технологий при выращивании цыплят-бройлеров и получении биологически полноценной и безопасной продукции птицеводства в условиях отказа от кормовых антибиотиков на сегодняшний день является актуальной задачей. При проведении профилактических и терапевтических ветеринарных мероприятий эффективным средством могут быть кормовые добавки на основе природных компонентов. В работе представлены результаты научного исследования по применению фитобiotика на основе бетулина в бройлерном птицеводстве. Испытания проведены в условиях технологического цикла на одной из птицефабрик Свердловской области на цыплятах-бройлерах кросса «Росс-308».

Птице в опытной группе бетулин в сухом виде вводили в комбикорм с 21-го по 35-й день выращивания из расчета 2,5 мг/кг живой массы. Введение в рацион кормовой добавки на основе бетулина способствовало повышению прироста живой массы и выхода грудных мышц на 7,6% по отношению к контрольной группе. Установлено, что потребление цыплятами фитобиотика приводило к снижению отложения подкожного и абдоминального жира, повышало биологическую полноценность мяса за счет увеличения содержания зольных элементов, улучшало технологические свойства мяса за счет повышения влагоудерживающей способности мышечного волокна, а также за счет интенсивности формирования и созревания мышечного волокна. При проведении гистологических исследований образцов тканей поджелудочной железы цыплят-бройлеров выявлено увеличение площади островков Лангерганса, инсулин-продуцирующих клеточных комплексов. Под воздействием кормовой добавки на основе бетулина происходила активация работы поджелудочной железы. Полученные результаты свидетельствуют о том, что применение бетулина в бройлерном производстве является перспективным направлением.

Ключевые слова: цыплята-бройлеры, кормовая добавка, бетулин, фитобиотик, живая масса, мышечное волокно, жир, поджелудочная железа, островки Лангерганса.

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INTRODUCTION

The trend of implementing nature-like technologies in broiler poultry farming is determined by the European Union requirements and is related to the production of biologically complete and safe products. By 2025 Russian poultry establishments will have to completely refuse from feed antibiotics in broiler chicken production, therefore, the search for and introduction of alternative biological additives into production seem to be extremely urgent today [1, 2]. Phytobiotics based on natural components and used for preventive and therapeutic veterinary measures fit well into the technological cycle and demonstrate pronounced biological and economic effects [3–6]. The betulin-based phytobiotic is currently one of the promising modern developments.

Betulin is derived from birch bark (from Latin *Bétula*). It belongs to triterpene alcohols and has a high physiological activity. The substance has antiseptic, wound-healing and anti-inflammatory properties, as well as anti-ulcer, choleric and hepatoprotective activity. It can be used for prevention of liver diseases, which often occur in meat-producing poultry and are associated with alimentary factors [7]. The hypolipidemic and hypocholesterolemic activity of betulin is effective for normalizing lipid metabolism [8]. It also has antibacterial effect on some streptococcus bacteria [9, 10].

Betulin has been used in medicine for a long time, yet its unique properties have been reviewed in veterinary medicine and agriculture just recently. This phytobiotic has aroused increased interest of both scientists and practitioners; the possibility of its use in the technological cycle is being looked into, and its positive zootechnical, as well as physiological and biochemical effects on chicken are being studied. Betulin enhances the vaccination efficacy

in chicks, which is economically beneficial for a poultry establishment [3, 9].

Betulin used in poultry improves glycemic indicators. It was proved to reduce sera lipid concentration, which is shown in a statistically significant decrease in the concentration of total cholesterol and triglycerides, and is also accompanied by an improvement in the liver function parameters (a pronounced decrease in the activity of alanine aminotransferase and aspartate aminotransferase). At the same time the antioxidant and anti-inflammatory effect of betulin was established (indicators of lipid peroxidation, catalase activity, superoxide dismutase, concentration of anti-inflammatory cytokines). The normalization of the ratio of immunoregulatory subpopulations of lymphocytes (CD4+ and CB8+), a decrease in the blood concentration of inflammatory cytokines (IL-6, IL-8, IL-12, IL-18, TNFα, IFNγ) were shown. Due to its antiseptic, wound-healing and anti-inflammatory properties betulin can be used for wound aseptic care and treatment of gastrointestinal inflammation (antiulcer activity) [3, 8].

The aim of this paper is to study effects of the betulin-based feed additive on growth performance and biological parameters of Ross-308 cross-breed broilers.

MATERIALS AND METHODS

Animals. Two groups of 80 Ross-308 cross broiler roosters in each group were formed to conduct research in production conditions using the analogy principle. Chickens of the experimental group received feed supplemented with dry betulin at 2.5 mg per kg of body weight at days 21–35 of growing, the second group was control.

All experiments were carried out in poultry in accordance with the requirements of Directive 2010/63/EU of

Table
Broiler chicken growth parameters before and after the experiment (n = 160)

Таблица
Технологические показатели выращивания цыплят-бройлеров до и после опыта (n = 160)

| Parameter | Control Group | Experimental Group |
|--------------------------|------------------|--------------------|
| Body weight at day 21, g | 520.00 ± 18.56 | 522.80 ± 26.31 |
| Body weight at day 35, g | 1 924.80 ± 44.93 | 1 942.80 ± 57.70 |
| Body weight gain, g | 1 404.80 | 1 420.00 |
| Homogeneity, % | 85 | 90 |
| Livability, % | 100 | 100 |

the European Parliament and the Council on the protection of animals used for scientific purposes of September 22, 2010.

Histological examination. The tissue samples were taken for histological analysis from five broiler chickens selected by random sampling in each group (in accordance with the recommendations of the All-Russian Scientific Research and Technology Institute for Poultry, 2010) and were subjected to control slaughter. Histological samples of pectoral muscles and pancreas were fixed in 10% neutral formalin solution. The general study was conducted and structural changes were examined in paraffin sections, using hematoxylin and eosin staining according to the generally accepted method. Histological findings were recorded using Leica DM2500 light microscope with a Leica camera.

Biochemical blood tests were carried out using Chem Well 2910 Combi automatic biochemical analyzer (Awareness Technology Inc., USA) and standard Vital Diagnostics SPb (Russia) and Diasys (Germany) test kits.

The digital data were processed using standard statistical methods of Microsoft Excel 2007 and Statistica 6.0 software. The reliability was calculated using the Student's t-test.

RESULTS AND DISCUSSION

Ross-308 cross hybrid was breed by specialists of the Aviagen company (U.K.) and is the result of a complex cross-breeding scheme of five breeds in four generations. This is a fast growing broiler with efficient feed conversion and high meat performance. Increased body weight indicated a positive effect on body of broiler chickens when various additives were introduced into the diet during the feeding period. Moreover, it is important to take into account whether the increase occurred due to muscle growth or fat deposition. Therefore, the birds were weighed at the beginning and at the end of the experiment. The results are presented in the Table.

The data presented in the Table indicate an insignificant increase in body weight of the birds in the experimental group. However, it should be noted that the anatomical cutting of carcasses revealed 36% less subcutaneous and abdominal fat deposition on the internal organs and mesentery of the intestine in broilers of the experimental group, and the performance of the pectoral and leg muscles thereof was 7.6% higher in relation to the con-

trol group. This confirms the hypolipidemic properties of betulin.

The flock livability indicators were at 100% in both groups. The homogeneity of the flock shows the uniformity of broiler body weight gain during the rearing period, it was 5% higher in the experimental group as compared with the control, indicating a positive effect of betulin on the growth and development parameters of broilers.

The histological examination was conducted for a more detailed study of muscle fiber characteristics. The correlation of meat quantity and quality with the size of muscle fibers was established. A higher number of muscle fibers indicated greater and better meat quality, as well as lower fat content. The muscle fiber thickness decreases with an increase in the mass of individual muscles due to the appearance of thinner new ones. The structure of the muscle tissue and interstitial connective tissue of chickens in the experimental groups (Fig. 1 and 4), as compared with the control ones, was represented by a compact muscle tissue, with nearly completed maturation process and fine

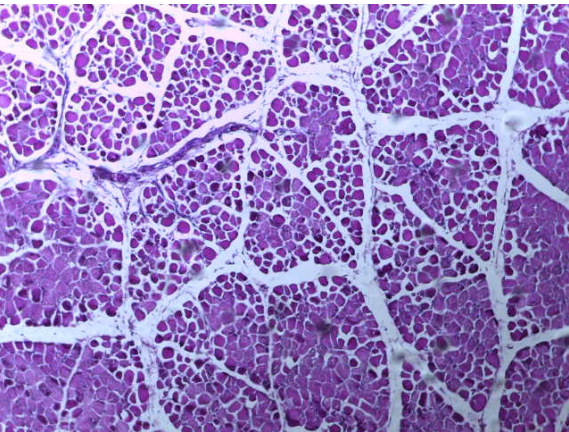


Fig. 1. The structure of femoral muscle fiber bundles in birds of the experimental group. Hematoxylin and eosin staining (100x magnification)

Рис. 1. Структура пучков мышечных волокон бедренной группы мышц птиц опытной группы. Окраска гематоксилином и эозином (увеличение ×100)

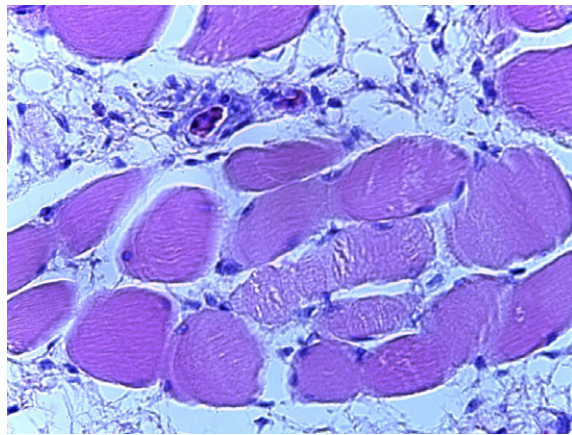


Fig. 2. Immature muscle fibers in the bundle (control group). Hematoxylin and eosin staining (400× magnification)

Рис. 2. Незрелые мышечные волокна в пучке (контрольная группа). Окраска гематоксилином и эозином (увеличение ×400)

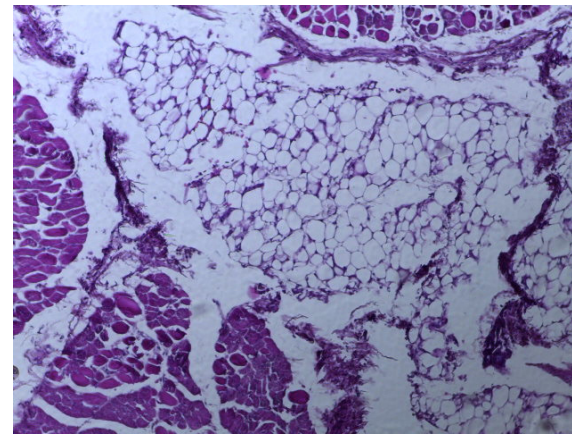


Fig. 3. Muscle fiber bundles with a fat cell layer (control group). Hematoxylin and eosin staining (200× magnification)

Рис. 3. Пучки мышечных волокон с прослойкой жировых клеток (контрольная группа). Окраска гематоксилином и эозином (увеличение ×200)

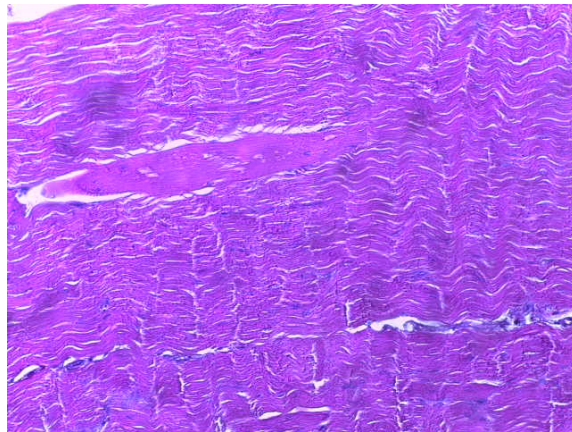


Fig. 4. The breast muscle of the birds in the experimental group. The striated pattern is clearly expressed indicating the maturity of this muscle fiber. Hematoxylin and eosin staining (200× magnification)

Рис. 4. Грудная мышца цыплят опытной группы. Поперечнополосатая исчерченность четко выражена, что свидетельствует о созревании данного мышечного волокна. Окраска гематоксилином и эозином (увеличение ×200)

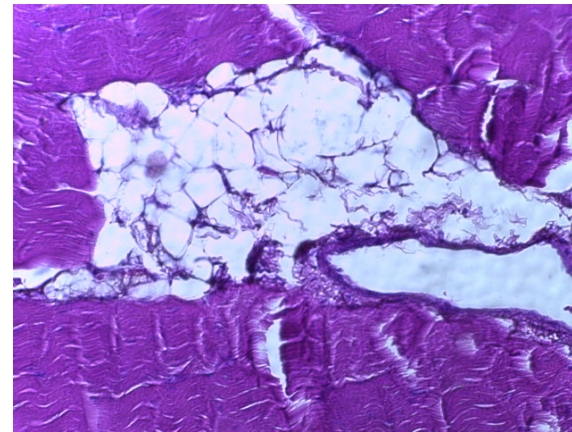


Fig. 5. Perivascular deposition of large fat droplets in the muscles of control broilers. Hematoxylin and eosin staining (200× magnification)

Рис. 5. Периваскулярное отложение крупных капель жира в мышцах контрольных бройлеров. Окраска гематоксилином и эозином (увеличение ×200)

droplets of fat located perivascularly. Larger blood vessels appear inside the muscle, which contributes to better tissue nutrition.

In the samples obtained from birds of the control group, the process of formation and maturation of muscle tissue in both the thoracic and femoral muscle groups had a tendency towards incomplete maturation (Fig. 2) with formation of coarse adipose connective tissue. Adipose tissue, which is located along the intermediate connective tissue, contains a significant amount of fat. Adipocytes are larger, in some areas they are located tightly to each other, forming a continuous layer. Fatty layers with connective tissue sometimes penetrate between small muscle bundles (Fig. 3 and 5).

It should be noted that introduction of betulin-based phytobiotics into the diet promoted the formation of maturer muscle fiber. This kind of meat is the safest for humans and has good nutritional properties.

Betulin had a positive effect on the water-holding capacity of the pectoral muscle fibers, which is an important technological parameter for cooling, freezing or storage processes, as well as for manufacture of sausages and smoked products from broiler meat. The loss of meat juice during heat treatment causes dehydration of tissues, decrease in juiciness, deterioration in the consistency, structure and taste of the finished product. Studies have shown that betulin increased the water-holding capacity of breast muscles in broiler chickens by 5.1% as compared with the control group.

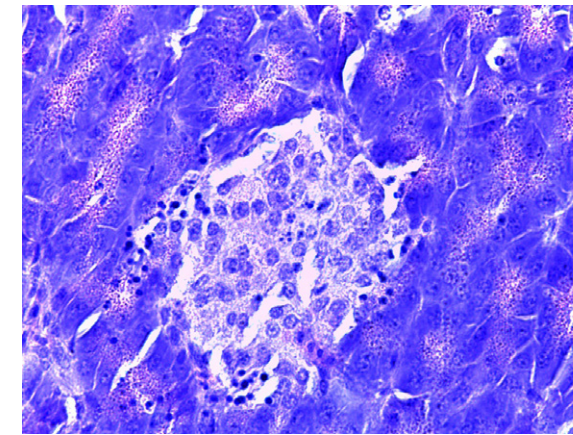


Fig. 6. Pancreas of chickens in the control group. The islets of Langerhans are clearly defined, beta cells are in active secretion. Hematoxylin and eosin staining (400× magnification)

Рис. 6. Поджелудочная железа цыплят контрольной группы. Островки Лангерганса четко очерчены, бета-клетки в состоянии активной секреции. Окраска гематоксилином и эозином (увеличение ×400)

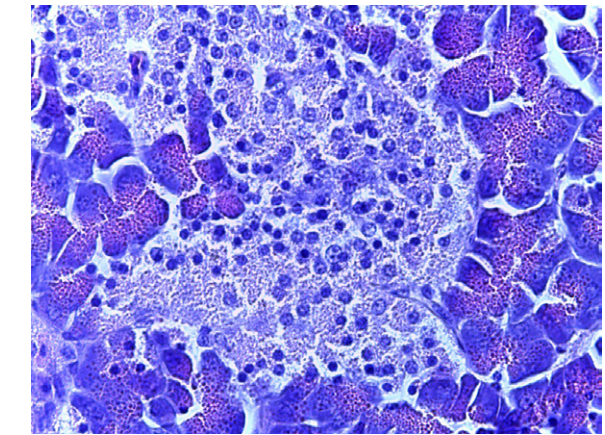


Fig. 7. Pancreas of chickens in the experimental group. The size of the islets of Langerhans has sharply increased. Hematoxylin and eosin staining (400× magnification)

Рис. 7. Поджелудочная железа цыплят опытной группы. Островки Лангерганса резко увеличены в размерах. Окраска гематоксилином и эозином (увеличение ×400)

Additional tests of muscle tissue chemical composition showed increased accumulation of ash elements in the muscle fiber of chickens by 41.9% in the experimental group (1.08 ± 0.16 in the control group, 1.86 ± 0.25 in the experimental group), which also indicates increase in biological value of meat from broiler chicken that received betulin.

Biochemical blood tests showed significant changes in the concentration of uric acid in the control ($450.07 \pm 35.97 \mu\text{mol/L}$) and experimental ($230.17 \pm 26.78 \mu\text{mol/L}$) groups (at $p \leq 0.01$). This is due to blocking the breakdown of purine bases in broilers. The processes of protein synthesis and the formation of muscle fiber proceeded more intensively in chickens in the experimental group.

It was found that betulin had impact on the development and condition of internal organs. Histological studies of the broiler chicken pancreas in the control group showed that the islets of Langerhans were clearly delineated, the beta cells were in active secretion (Fig. 6).

The pancreas of broilers in the experimental group that received the betulin-based feed additive secreted normally, but the islets of Langerhans sharply increased in size (Fig. 7). Active insulin production was recorded. Thus, the increased area of islets of Langerhans (insulin-producing cells) was revealed in the pancreas of broiler chickens. The pancreatic function was activated due to betulin effect.

CONCLUSION

The study showed that the betulin-based feed additive decreased the deposition of subcutaneous and abdominal fat with a slight difference in body weight of broiler chickens; increased the meat biological value due to raised content of ash elements; improved the meat technological properties by increasing the water-holding capacity of muscle fibers; promoted the accumulation of proteins and minerals in meat and the intensity of the formation and maturation of muscle fibers. The increased area of islets of Langerhans (insulin-producing cells) was

recorded in the pancreas of birds indicating the pancreatic activation.

The betulin-based feed additive fits perfectly into the broiler production technology and is one of the promising areas for implementation of environmentally friendly technologies in poultry farming.

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Canine parvovirus enteritis: epidemic situation analysis and perspectives

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SUMMARY

Parvovirus enteritis is one of canine dangerous diseases which poses a particular concern for practitioners and dog owners around the world. Parvovirus type 2 (CPV-2) can affect dogs at any age, but puppies between 6 weeks and 6 months old are most susceptible to infection. One of the main biological properties of parvovirus is its continuous genetic evolution, which led to the replacement of the original virus type by new antigenic variants – CPV-2a, CPV-2b and CPV-2c. According to the literature data, all three variants of the virus are currently circulating in the domestic dog population worldwide. The paper presents analysis of the epidemic situation and seasonal occurrence of canine parvovirus enteritis in certain regions of the Russian Federation in 2017–2019. It was shown that parvovirus enteritis was ranked first among the registered infectious diseases of dogs and accounted for 37% during the study period. It has been established that the disease is registered all year round, but the frequency of disease cases depends on the season. Canine parvovirus infection mainly occurs in spring, late autumn and early winter, which is probably associated with changes in daily temperature during these periods and decreased animal resistance. Despite extensive vaccination, the main reason for the wide spread of the virus is either interference with maternal antibodies in vaccinated puppies or low level of immune protection in adult dogs. It has been concluded that it is necessary to monitor the parvovirus circulation and spread in order to study the genetic and antigenic properties of newly identified isolates for the timely update of vaccine strains used for development of specific means of prevention.

Key words: parvovirus (CPV), canine parvovirus enteritis, canine diseases, epidemic situation.

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Парвовирусный энтерит собак: анализ эпизоотической ситуации и перспективы

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РЕЗЮМЕ

Парвовирусный энтерит является одним из опасных заболеваний собак и вызывает определенную обеспокоенность у практикующих врачей и владельцев собак по всему миру. Парвовирус 2-го типа (CPV-2) может поражать собак в любом возрасте, но наиболее подвержены риску заражения щенки в возрасте от 6 недель до 6 месяцев. Одним из основных биологических свойств парвовируса является его непрерывная генетическая эволюция, которая привела к тому, что исходный тип вируса был заменен новыми антигенными вариантами – CPV-2a, CPV-2b и CPV-2c. Согласно литературным данным, в настоящее время все три варианта вируса циркулируют в популяции домашних собак по всему миру. В работе представлен анализ эпизоотической ситуации и сезонности заболеваемости собак парвовирусным энтеритом в 2017–2019 гг. в отдельных округах Российской Федерации. Показано, что среди регистрируемых инфекционных болезней собак парвовирусный энтерит находится на первом месте, его доля за исследуемый период составила 37%. Установлено, что заболевание фиксируется круглогодично, но частота случаев зависит от сезона. Заражение собак парвовирусом в основном происходит весной, поздней осенью и ранней зимой, что, вероятно, связано с перепадами суточной температуры в эти периоды и снижением резистентности организма животных. Несмотря на обширную вакцинацию, основной причиной широкого распространения вируса является либо вмешательство материнских антител у вакцинированных щенков, либо низкая эффективность иммунной защиты у взрослых собак. Сделан вывод о необходимости проведения мониторинга циркуляции и распространения парвовируса с целью изучения генетических и антигенных свойств вновь выявляемых изолятов для своевременного обновления вакцинных штаммов, используемых при создании средств специфической профилактики.