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Morphological and immunological profile of piggles blood under the influence of feed additive “Globigen Jump Start” and adverse microclimate parameters

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KO: Project administration; Conceptualization; Methodology; Investigation; Formal analysis; Writing — original draft, review & editing.

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The article presents the results of a study of the influence of adverse microclimate parameters on the morphological and immunological blood parameters of piglets in the neonatal period of development, and also evaluates the effectiveness of the use of the feed additive “Globigen Jump Start” to correct the identified violations. The study was conducted under production conditions with elevated air temperature and above-normal indoor ammonia concentration. It was established that deviations of microclimate parameters from reference values negatively affect the functional state of the piglets’ organism, which is manifested by changes in hematological and immunological blood parameters. The use of a feed additive containing egg immunoglobulins IgY, β-glucans and mannan-oligosaccharides increased the level of erythrocytes, hemoglobin and hematocrit ($P<0.05$–0.01), and also contributed to the normalization of the ratio of individual forms of blood leukocytes, in particular, an increase in the proportion of lymphocytes and a decrease in the number of neutrophils. The results obtained indicate a pronounced immunomodulatory and adaptogenic effect of the feed additive “Globigen Jump Start” and the feasibility of its use to increase the adaptive capacity and resistance of the piglet organism in the critical period of early postnatal development under the influence of environmental stress factors.

Key words: piglets, neonatal period, microclimate, heat stress, immunity, hematological indicators, feed additive, IgY

Introduction

Intensification of livestock farming in combination with global climate change is accompanied by an increase in the level of technological and environmental stresses, which negatively affect the physiological state, metabolic homeostasis and immune reactivity of farm animals. An increase in ambient temperature, deterioration of microclimate parameters in livestock premises and accumulation of harmful gases lead to disruption of heat exchange, water-salt balance and activation of stress-releasing mechanisms, especially in young animals in the early periods of ontogenesis [1, 5, 7].

Piglets are especially vulnerable to the effects of adverse environmental factors in the neonatal period, when the body’s adaptive mechanisms are not yet fully formed, and the immune system largely depends on passively acquired protective factors. Under such conditions, even minor deviations of microclimate parameters from optimal values can cause the development of immunodeficiency states, reduced resistance, increased morbidity and mortality of young animals, which has significant economic consequences for the pig industry [6, 13, 16].

An important role in the formation of the body’s adaptive response to stress factors is played by mechanisms of nonspecific resistance and the immune response regulation system. In particular, nitric oxide and related NO-dependent processes are considered as a component of the stress-limiting system, which ensures short- and long-term adaptation of the body to adverse environmental conditions [17]. At the same time, the effectiveness of the immune response in young piglets is largely determined by the level of passive immunity obtained with colostrum and the functional state of the cellular and humoral components of the immune system.

Modern pig farming technologies, in particular early weaning of piglets from sows, often do not take into account the biological features of the development of the immune system, which leads to increased sensitivity of animals to the effects of stress and pathogenic factors in the post-weaning period [9, 13]. In this regard, a relevant direction of scientific research is the search and implementation of effective immunotropic agents capable of increasing the adaptive potential and resistance of the piglet organism under conditions of negative environmental influences. In this context, feed additives containing immunoglobulins, β-glucans and mannan-oligosaccharides, which provide passive immune protection, stimulate nonspecific resistance and support the functional state of the digestive and immune systems, are of significant scientific and practical interest. One of such additives is “Globigen Jump Start”, which includes egg immunoglobulins IgY and inactivated feed yeast [13, 2, 8, 14–15].

Taking into considerstion the above, the aim of the research was to determine the impact of adverse microclimate parameters on the morphological and immunological blood parameters of piglets in the neonatal period of development, as well as to assess the effectiveness of using the feed additive “Globigen Jump Start” to increase the adaptive capacity and immune resistance of the animal organism under the influence of environmental stress factors.

Materials and Methods

The study of the influence of microclimate parameters on the body of piglets in the neonatal period of their development was carried out in production conditions, in particular in one of the farms of Rivne region. The ventilation system is supply and exhaust, which is carried out by opening windows and doors, exhaust shafts located in the ceiling above the passage, as well as by using exhaust fans that are turned on manually.

Table 1. Indoor microclimate parameters

Indicators	Day 5	Day 19	Day 33	Acceptable norm
Outside air temperature, °C	30	33	36	—
Indoor air temperature, °C	32	34	36	20–22
Air speed, m/s	2,8	3,0	3,0	1–3
Carbon dioxide CO2, ppm	1310	1670	2346	3000
Ammonia NH_3, mg/m^3	32	67	71	0–20
Hydrogen sulfide H_2S, mg/m^3	0	0	0	0–5

Two groups of farrowing sows of the Large White breed were formed (control and experimental groups of 5 animals in each group). The sows of the control group were fed standard compound feed. The sows of the experimental group, starting from the 5th day before the expected farrowing and until the 28th day of lactation, in addition to standard compound feed, received the feed additive “Globigen Jump Start” in the amount of 0.5 kg/t of finished feed. Piglets born from sows of the control and experimental groups, starting from 5 days of age, received prestarter compound feed, balanced in terms of essential nutrients, according to existing standards. At the same time, piglets of the experimental groups, starting from 5 days of age, were fed with the feed additive “Globigen Jump Start” in the amount of 1 kg/t of feed along with the standard compound feed. Piglets were weaned from sows at 28 days of age.

The feed prebiotic additive “Globigen Jump Start” (*EW Nutrition GmbH*, Germany) contains 26.25 % egg powder (source of IgY, an analogue of IgG antibodies in mammals) and inactivated dry feed yeast up to 100 %, containing beta-glucans and mannan-oligosaccharides (MOS) [14].

During the study period, the clinical condition, safety and growth of animals were monitored. During the experiment, the main microclimate parameters were monitored using a *Dozor S-M-2* gas analyzer: temperature, air velocity, concentration of carbon dioxide, ammonia and hydrogen sulfide.

The material for laboratory studies was blood taken from piglets on days 5, 19 and 33. In heparin-stabilized blood, the total number of erythrocytes (RBC), leukocytes (WBC) and platelets (PLT), hemoglobin concentration (HGB), hematocrit (HCT), mean erythrocyte volume (MCV), mean hemoglobin content in erythrocytes (MCH) were determined on the *MYTHIC 18 Vet* hematology analyzer. The ratio of individual forms of leukocytes (blood leukogram) was calculated using visual microscopy, according to the methods described by us in the reference book [18].

Results and Discussion

Analysis of the research results showed that the microclimate parameters significantly differed from the recommended reference values (table 1). Thus, the air temperature (°C) in the room for suckling piglets at 5, 19 and 33 days of age was 10; 12 and 14 °C higher compared to the recommended maximum temperature values; the ammonia concentration (mg/m3) was 1.6; 3.3 and 3.5 times higher, respectively. The discrepancy between the microclimate parameters during manual control of the supply and exhaust ventilation system in the room and the reference values led to a significant negative impact on the activity of the studied systems of the piglets’ organism (table 2), which is also evidenced by the results of our previous studies [13, 19].

The use of the feed additive "Globigen Jump Start" in feeding piglets during the critical period of neonatal development under the negative influence of unfavorable microclimate parameters caused positive changes in morphological, biochemical and immunological blood parameters (tables 2 and 3). Thus, from the data presented in table 2, we see that the number of erythrocytes in the blood of piglets of the experimental group at 19 and 33 days of age was 24.4 ($P<0.05$) and 34.6 % ($P<0.01$) higher than in animals of the control group. Similar changes, only less pronounced, were established when determining the concentration of hemoglobin and the content of hemoglobin in erythrocytes. At the same time, the significantly higher content of hemoglobin in erythrocytes in all periods of the studies attracts attention. The higher hemoglobin content in erythrocytes in the blood of piglets at 5 days of age is probably due to the effect of the studied supplement, which was consumed by the farrowing sows. At the same time, in the blood of piglets of the experimental group at 33 days of age, an increase of 14.7 % in the hematocrit value ($P<0.05$) was recorded. These data indicate the activating effect of the components of the studied feed supplement on the oxygen-transport function of the blood.

High temperature and accumulation of harmful gases in a room with poor ventilation significantly affected the ratio of individual forms of leukocytes in the blood of piglets (table 3). When exposed to stress and irritation by harmful gases, such as ammonia, an increase in the number of neutrophils in the blood is observed. This is because neutrophils are the first cells to respond to inflammatory processes and tissue damage. Simultaneously with the increase in the number of neutrophils, a decrease in the proportion of lymphocytes was detected, as a sign of suppression of adaptive immunity. From the results of the studies presented in table 3, we see that the use of the feed additive "Globigen Jump Start" to sows and piglets born from them has a regulatory effect on the ratio of their individual forms, which helps animals better adapt to stressful conditions. This is indicated by a lower number of segmented neutrophils in the blood of piglets of the experimental group compared to the control group on the 19th day of life ($P<0.05$) and a higher proportion of lymphocytes ($P<0.05$) throughout the entire research period. It is likely that the components of "Globigen Jump Start" stimulate the adaptive immune response and support the activity of lymphocytes. Analysis of the percentage of other types of leukocytes showed that the number of eosinophils in the blood of piglets of the experimental group on the 33rd day of life was half ($P<0.05$) less than in the control group. High concentrations of harmful gases can irritate the mucous membranes and respiratory tract, causing allergic reactions, which leads to an increase in the level of eosinophils.

The decrease in the level of eosinophils in the blood of suckling piglets in the experimental group is apparently due to the presence of beta-glucans and MOS in the feed additive, which help maintain healthy microflora and reduce allergic reactions to harmful gases.

Table 2. Hematological blood parameters of piglets (M±m, n=5)

Indicators	Group	Periods of the experiment		
		Day 5	Day 19	Day 33
Erythrocytes (RBC), th/l	C	3.88±0.59	4.76±0.42	5.11±0.40
	E	4.93±0.39	5.92±0.26*	6.88±0.34**
Hemoglobin (HGB), g/l	C	84.0±5.15	96.8±4.68	98.4±4.20
	E	94.8±6.11	111.4±4.17*	115.2±4.07*
Leukocytes (WBC), g/l	C	8.76±0.98	8.38±0.57	13.5±1.18
	E	9.82±0.99	10.28±0.72	12.08±0.77
Hematocrit (HCT),%	C	35.5±3.18	36.6±1.84	38.1±1.76
	E	37.9±1.37	40.0±2.02	43.7±1.53*
Mean erythrocyte volume (MCV), fL	C	72.1±2.29	73.1±1.38	74.7±1.78
	E	73.6±1.71	75.6±1.66	77.7±1.46
Mean hemoglobin content in erythrocytes (MCH), pg	C	18.0±0.59	18.8±0.62	19.1±0.78
	E	20.9±0.69*	21.2±0.74*	21.7±0.69*

Note. In this and the following tables: * — $P<0.05$, ** — $P<0.01$, *** — $P<0.001$ — significant difference in animals of the experimental group compared to the control group.

Table 3. Leukogram of piglet blood, % (M±m; n=5)

Indicators	Group	Periods of the experiment		
		Day 5	Day 19	Day 33
Basophils	C	0.6±0.24	0.4±0.4	0.2±0.2
	E	0.8±0.20	0.8±0.2	0.8±0.37
Eosinophils	C	3.0±0.71	4.2±0.86	5.4±0.93
	E	2.2±0.49	3.0±0.45	2.6±0.68*
Young	C	0.4±0.24	0.6±0.40	0.8±0.37
	E	0.6±0.24	0.6±0.24	0.4±0.24
Rod-shaped neutrophils	C	3.6±0.68	2.4±0.51	2.2±0.58
	E	2.6±0.60	2.6±0.68	2.0±0.55
Segmented neutrophils	C	37.6±1.72	39.0±1.52	38.4±1.50
	E	34.2±1.43	33.5±1.54*	33.0±2.00
Lymphocytes	C	52.0±1.34	50.0±1.87	49.8±1.88
	E	56.6±1.08*	56.2±1.83*	57.2±1.66*
Monocytes	C	2.8±0.73	3.4±0.93	3.2±0.58
	E	3.0±0.71	3.8±0.66	4.0±0.71

In general, the results of the conducted studies indicate that the effect of elevated air temperature and above-normal indoor ammonia concentrations causes the development of a stress reaction in the body of piglets in the neonatal period, which is accompanied by a violation of the oxygen-transport function of the blood and changes in immune reactivity. A decrease in erythrocyte indices and the formation of a neutrophil-lymphocyte imbalance can be considered as a consequence of hypoxia and activation of nonspecific inflammatory mechanisms.

The use of a feed additive containing IgY, β-glucans and mannan-oligosaccharides causes activation of erythropoiesis and an increase in the functional capacity of erythrocytes in the body of piglets, which is manifested by an increase in the number of erythrocytes, hemoglobin concentration and hematocrit value. Such changes are adaptive in nature and contribute to an increase in the efficiency of oxygen transport under conditions of heat stress.

Normalization of the leukocyte formula of the blood, in particular an increase in the proportion of lymphocytes and a decrease in the number of segmented neutrophils and eosinophils, indicates a decrease in the intensity of the nonspecific inflammatory response and maintenance of the adaptive link of immunity. The detected effects are probably due to the complex immunomodulatory effect of the components of the supplement, which combines the mechanisms of passive immune protection and stimulation of the nonspecific immune response. Thus, egg yolk immunoglobulins (IgY), as well as biologically active components of dry yeast, in particular β-glucans and MOS, are characterized by a pronounced immunomodulatory and metabolic-regulatory effect, which indirectly affects the oxygen-transport function of the blood and the ratio of individual forms of leukocytes [2].

As is known, Ig Y provides passive immunity, which is especially important for animals in the first days of life, when their own immune system is still developing [2, 8]. Immunoglobulins Y, entering the body, are able to specifically bind antigens of pathogenic microorganisms and their toxins in the intestinal lumen, reducing the level of antigenic and endotoxic load. This leads to a decrease in the systemic inflammatory response, normalization of the production of pro-inflammatory cytokines (IL-1β, TNF-α) and a decrease in oxidative stress. As a result, favorable conditions are created for stabilizing erythropoiesis, preserving the structural and functional integrity of erythrocyte membranes and increasing the affinity of hemoglobin for oxygen, which has a positive effect on the oxygen-transport function of the blood [10].

Dried yeast enriched with β-glucans interacts with innate immune receptors (Dectin-1, CR3, TLR-2/6) on the surface of macrophages, neutrophils and dendritic cells. Activation of these receptors contributes to a moderate stimulation of phagocytosis, increased antioxidant defense and optimization of cytokine balance. Reduction of excessive inflammatory response and oxidative damage to erythrocytes may contribute to an increase in their functional activity, in particular, improvement of gas exchange and tissue oxygenation [3–4, 12].

Mannan-oligosaccharides exert their effect mainly through the sorption of pathogenic bacteria that have mannose-specific fimbrial adhesins, as well as through the modification of the composition of the intestinal microbiota. Improving the intestinal barrier function reduces the translocation of bacterial endotoxins into the systemic circulation, which indirectly reduces the burden on the immune system and hematopoietic organs. Under such conditions, the need for compensatory leukocytosis decreases, the ratio of neutrophils and lymphocytes stabilizes, and the proportion of immunocompetent cells of the adaptive link increases [4].

Thus, the complex action of IgY, β-glucans and MOS promotes the redistribution of leukocyte forms in the direction of reducing the relative number of stress-associated neutrophils and increasing the proportion of lymphocytes, which may indicate a decrease in the level of chronic inflammation and increased immune resistance of the body. Simultaneous normalization of immune homeostasis and reduction of oxidative stress create the prerequisites for improving the oxygen-transport function of the blood by stabilizing hemoglobin, hematocrit and functional fullness of erythrocytes.

Thus, the components of the feed additive, namely: Ig Y, beta-glucans and MOS help reduce the impact of pathogens and support the immune function of the piglets' organism under the influence of adverse climatic factors. At the same time, the hematopoietic function of the bone marrow is activated, which leads to an increase in the number of erythrocytes and an increase in the level of other hematological indicators, as an adaptive reaction that helps the body compensate for hypoxia caused by insufficient oxygen levels due to poor ventilation.

It was established that keeping piglets in the neonatal period under conditions of elevated air temperature and ammonia concentration is accompanied by the formation of a stress state, which negatively affects the oxygen-transport and immune function of the blood. This is manifested by a decrease in erythrocyte indices, an imbalance in the leukocyte formula and a shift in the immune response towards nonspecific inflammation.

The use of the feed additive "Globigen Jump Start", containing egg immunoglobulins IgY, β-glucans and mannan-oligosaccharides, causes an activating effect on the oxygen-transport function of the blood of piglets, as evidenced by a significant increase in the number of erythrocytes, hemoglobin concentration, hemoglobin content in erythrocytes and hematocrit value, especially at 19 and 33 days of age ($P<0.05–0.01$). These changes are adaptive in nature and are aimed at compensating for hypoxia caused by heat stress and impaired gas exchange indoors.

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Морфологічний та імунологічний профіль крові поросят за впливу кормової добавки «Глобіген Джамп Старт» та несприятливих параметрів мікроклімату

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У статті представлено результати дослідження впливу несприятливих параметрів мікроклімату на морфологічні та імунологічні показники крові поросят у неонатальному періоді розвитку, а також оцінено ефективність використання кормової добавки «Глобіген Джамп Старт» для корекції виявлених порушень. Дослідження проводили в умовах виробництва з підвищеною температурою повітря та концентрацією аміаку в приміщенні вище норми. Встановлено, що відхилення параметрів мікроклімату від референтних значень негативно впливають на функціональний стан організму поросят, що проявляється змінами гематологічних та імунологічних показників крові. Використання кормової добавки, яка містить яєчні імуноглобуліни IgY, β-глюкани та маннан-олігосахариди, підвищило рівень еритроцитів, гемоглобіну та гематокриту ($P<0{,}05$–$0{,}01$), а також сприяло нормалізації співвідношення окремих форм лейкоцитів крові, зокрема, збільшенню частки лімфоцитів та зменшенню кількості нейтрофілів. Отримані результати свідчать про виражений імуномодулюючий та адаптогенний ефект кормової добавки «Глобіген Джамп Старт» та доцільність її використання для підвищення адаптивних можливостей та стійкості організму поросят у критичний період раннього постнатального розвитку під впливом стресових факторів навколишнього середовища.

Ключові слова: поросята, неонатальний період, мікроклімат, тепловий стрес, імунітет, гематологічні показники, кормова добавка, IgY

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