Physiological state and reproductive qualities of sows when using probiotic preparations A₂ and Immunoflor

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Abstract. The paper provides research and practice rationale for feasibility of using the complex probiotic preparations A₂ and Immunoflor in the pig production and growing technology with the aim of improving reproductive qualities of sows through increasing nonspecific resistance of the organism. The probiotic preparations were given with the feed twice, at the beginning of pregnancy and 14 days before farrow, A₂ in the amount of 1.62 g per animal in Experimental Group 1 and “Immunoflor” in the amount of 0.05 g per animal in Experimental Group 2. It was established that pig production from sows of both Experimental Groups 1 and 2 was 7.2 and 10.3 % higher, comparing that in the Control Group. At that, the number of healthy and viable pigs obtained from sows of the given experimental groups was reliably 8.5 and 12.5 % greater than in the Control Group (P<0.01). The new complex probiotic agents A₂ and Immunoflor, having significant antagonistic activity against putrefactive bacteria, decreased the mortalitility of pigs 1.4 and 2.1 times, respectively, increased litter size by 11.4 and 3.6 % and the milkability of sows by 4.2 kg and 4.7 kg.

1. Introduction
In conditions of industrial livestock management technologies animals experience pressing of ecological and technological factors and, as a result, their protection and adaptation reactions of the organism’s functional systems in response to external stimuli change [1]. The majority of these stimuli are stress-inducing for the organism, and they lead to disruptions, among others, in the activity of the immune system of the organism [2]. In its turn, this caused disruptions in the physiological condition of animals, diseases of various etiology and mortality, which is explained, first of all, by a decrease of nonspecific resistance of the organism and its immunobiological response, especially in critical ontogenesis periods – during the last third of sows’ pregnancy and in the period of neonatality of pigs [3]. The influence of unfavorable factors leads to birth of animals with immunodeficiency states, and they are more prone to diseases, develop and grow poorly [4].

In lieu of the above said, according to many Russian and foreign scientists, today it is important to move safely from the conventional existing triad: unhealthy animal → diagnosis → treatment, to a
new concept: animal population → hygienic surroundings → prevention, i.e. from industrial technologies to an ecology-adaptive system [5]. This system is directed at satisfaction, first of all, of physiological needs of the organism and only then at satisfaction of technological needs by creating comfortable hygienic conditions for “dam → fetus → newborn animal” biosystem functioning [6].

Biologisation of the modern swine breeding field, along with methods of genome analysis, DNA testing and marker-based selection, suggests strict adherence with hygienic conditions of feeding, housing and care of animals, provision of a high level of nonspecific protective forces of the organism directed at fulfillment of the genetically predetermined adaptive, productive and reproductive potential of the organism and improvement of economic traits [7]. In the view of the above said, obtaining of biologically complete organic products is a relevant problem of modern biological science and practice [8].

Therefore, in this aspect the use of complex probiotic preparations is both of scientific interest and of practical meaning [9-11]. Probiotic preparations can be used at all stages of production of swine breeding products [12]. They are used for various goals such as productivity improvement, morbidity rate reduction, increasing of productive and reproductive qualities of swine and environmental pollution reduction [13].

The aim of the present work is to study the influence of the probiotic preparations A2 and Immunoflor on sows’ physiological condition and reproductive qualities.

2. Materials and methods

The zoohygienic conditions of housing and feeding of sows of the experimental groups were identical. Russian probiotic preparations A2 and Immunoflor were used to activate nonspecific resistance of the organism of farrow sows and improve their productive qualities. The animals of Experimental Group 1 were given, together with their feed, the probiotic preparation A2, twice, at the beginning of pregnancy and 14 days before farrow, in the amount of 1.62 g per animal, while the animals of Experimental Group 2 were given Immunoflor, twice at the same times, in the amount of 0.05 g per animal, in accordance with the directions for use.

A2 is a probiotic preparation intended for maintaining and restoring of the normal microflora of the gastrointestinal tract of agricultural animals, improving the natural resistance of the organism, preventing development of mycotoxicoses and dysbacterioses, restoring the immunity following vaccination, improving feed conversion, stress reduction, normalizing the microbial balance in the digestive tract following antibiotic therapy, stimulating growth, increasing safety and productivity, growing healthy young stock. It was created on the basis of new bacterial strains that have exceptional characteristics of the antagonist activity to enteropathogens of Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Salmonella typhi, high resistance to tetracycline, streptomycin and other antibacterial agents as well as xylanase, amylase and protease activity. A2 preparation contains Bacillus licheniformis, BKM B-2713D strain – not less than 2×10⁹ CFU/g and Bacillus subtilis, BKM B-2711D strain – not less than 2×10⁹ CFU/g as well as lactose and bran as the excipient. There are no genetically modified organisms in it. The preparation was developed by LLC “Nova” (Moscow, Russia).

Immunoflor is a probiotic preparation intended for balancing of diets and their enrichment with the aim of maintaining and restoring positive microflora of the gastrointestinal tract as well as for improving the immunity, stimulating growth and development of agricultural animals and birds. The composition of the probiotic includes: lyophilically dried biomass of Bifidobacterium globosum, Enterococcus faecium, Bacillus subtilis, Bacillus licheniformis, Saccharomyces cerevisiae bacteria with the total concentration of 1×10⁹ CFU/g (table 1). The preparation was developed by LLC “PK KrosFarm”, Mytishchi (Moscow Region, Russia).
Table 1. Composition of Immunoflor probiotic preparation per 1g of preparation.

| Main components                | Supplemental components                  |
|--------------------------------|------------------------------------------|
| *Bacillus subtilis* 1×10^9 CFU/g | Lactose – 10%                            |
| *Bacillus licheniformis* 1×10^9 CFU/g |                                           |
| *Bifidobacterium globosum* 1×10^9 CFU/g | Chitosan – 0.5%                          |
| *Enterococcus faecium* 1×10^9 CFU/g   |                                           |
| *Saccharomyces cerevisiae* 1×10^9 CFU/g | Excipient (maltodextrin) up to 100%      |
| CFU – colony-forming unit        |                                           |

The basic air basin parameters in houses for farrow and lactating sows were determined monthly during 3 days in a row: in the middle of the swine houses and in diagonal end corners. In the sow houses the ambient temperature, relative humidity and illumination intensity were measured using the “TKA-PKM” instrument, Model 42 (manufactured by LLC “TKA Scientific Instruments”, St. Petersburg, Russia), the air velocity was measured with the “TKA-PKM” thermoanemometer, Model 50 (manufactured by LLC “TKA Scientific Instruments”, St. Petersburg, Russia), CO₂, NH₃ and H₂S concentrations in air were measured with the UG-2 gas analyzer (manufactured by LLC “Promecopribor”, St. Petersburg, Russia), the microbial and dust contamination was measured with the use of the Yu.A. Krotov apparatus (manufactured by LLC “NIKI MLT-Povolzhye”, Penza, Russia).

The light factor (LF) was determined with the ratio of the total area of all windows to the same of the sow house floor, and the daylight factor (DLF) was determined with the ratio of illumination inside the sow house to the outside illumination, and the factors were expressed in percent:

$$DLF = \frac{O_i}{O_o} \times 100,$$

where $O_i$ is illumination in the sow house, $lx$; $O_o$ is outside illumination (with diffuse sky light), $lx$.

The sows’ body temperature was measured with a medical thermometer, the pulse rate was registered over the femoral artery by palpation, the number of breaths per minute – by counting the respiratory sounds in the lungs during breathing in and out using a phonendoscope by the auscultation method.

The red blood cell count, hemoglobin concentration and leukocyte count were determined with the use of the PCE 90 Vet automatic veterinary hematologic analyzer (Erma Inc, Japan). The analyzer state, graph plotting and measuring are displayed on a large LCD screen. The hematologic analyzer is controlled by means of an integrated keyboard. The PCE 90 Vet automatic veterinary hematoletic analyzer automatically takes the blood sample, dilutes, mixes, lyses, supplies and flushes it.

Then the phagocytic activity of white blood cells was determined with the use of day-old *Staphylococcus aureus* agar culture, blood plasma lysozyme activity – with *Micrococcus lysodeikticus*, blood serum bactericidal activity – with *Escherichia coli*, by means of the FEK-56M photoelectric colorimeter (Zagorsky optiko-mekhanicheskyy zavod (Zagorsky Plant of Optical Mechanics, Russia).

The digital material of the experiments was processed by the method of variation statistics on the validity of the difference in the compared indicators (P < 0.05-0.001) using a personal computer in the Microsoft Excel program.

3. Results of the study

Table 2 provides zoohygienic microclimate parameters in sow houses for farrow and lactating sows.

The main microclimate parameters in houses for farrow and lactating sows met the zoohygienic standards and had the following values: T – 17.1 ± 0.21 and 19.0 ± 0.19 ºC, R – 72.5 ± 1.11 and 70.0 ± 1.17 %, v – 0.27 ± 0.02 and 0.18 ± 0.03 m/s, microbial contamination – 49.7 ± 2.13 and 44.2 ± 1.99 ths/m³, NH₃ – 14.3 ± 0.73 and 12.1 ± 0.71 mg/m³, H₂S – 7.1 ± 0.47 and 6.6 ± 0.38 mg/m³, CO₂ – 0.15 ± 0.01 and 0.17 ± 0.01 %, CO – not detected, solid aerosols – 3.4 ± 0.27 and 2.7 ± 0.32 mg/m³.
With geometric regulation of illumination in sections of farrow sows and lactating sows LF made up 1:15, and with lighting one – DLF made up 0.85 ± 0.01 and 1.0 ± 0.01 %, respectively.

**Table 2. Air environment parameters in animal houses.**

| No. | Parameter                              | House for farrow sows | House for lactating sows |
|-----|----------------------------------------|------------------------|--------------------------|
| 1   | Ambient temperature, °C                | 17.1 ± 0.21            | 19.0 ± 0.19              |
| 2   | Humidity, %                            | 72.5 ± 1.11            | 70.0 ± 1.17              |
| 3   | Air velocity, m/s                      | 0.27 ± 0.02            | 0.18 ± 0.03              |
| 4   | Microbial contamination, thousand/m³   | 49.7 ± 2.13            | 44.2 ± 1.99              |
| 5   | NH₃, mg/m³                              | 14.3 ± 0.73            | 12.1 ± 0.71              |
| 6   | H₂S, mg/m³                             | 7.1 ± 0.47             | 6.6 ± 0.38               |
| 7   | CO₂, mg/m³                             | 0.15 ± 0.01            | 0.17 ± 0.01              |
| 8   | CO, mg/m³                              | -                      | -                        |
| 9   | Content of aerosols, mg/m³             | 3.4 ± 0.27             | 2.7 ± 0.326              |
| 10  | Light factor                           | 1 : 15                 | 1 : 15                   |
| 11  | DLF, %                                 | 0.85 ± 0.01            | 1.0 ± 0.01               |

The indices of the sows’ clinical physiological condition are given in table 3.

The data of table 3 demonstrate that the use of A₂ and Immunoflor probiotic preparations together with feed twice, at the beginning of pregnancy and two weeks before farrow, for the sows of Experimental Group 1 in the amount of 1.62 g per animal and the sows of Experimental Group 2 in the amount of 0.05 g per animal, did not influence the indices of the clinical physiological state of the animals.

**Table 3. Sows’ clinical physiological indices.**

| No. | Groups of animals | Observation periods, days | Body temperature, °C | Pulse rate, fluctuations/min | Breathing, movements/min |
|-----|-------------------|---------------------------|----------------------|-----------------------------|--------------------------|
| 1   | Control Group     | 30 – 25                   | 39.1 ± 0.24          | 71 ± 1.65                   | 16 ± 0.67                |
|     |                   | 15 – 10                   | 39.2 ± 0.13          | 70 ± 1.17                   | 15 ± 0.67                |
|     |                   | 3 – 5                     | 39.3 ± 0.15          | 69 ± 1.64                   | 17 ± 0.93                |
| 2   | Experimental Group 1* | 30 – 25                 | 38.8 ± 0.15          | 71 ± 1.72                   | 15 ± 0.74                |
|     |                   | 15 – 10                   | 39.3 ± 0.10          | 72 ± 1.22                   | 16 ± 0.88                |
|     |                   | 3 – 5                     | 39.2 ± 0.11          | 70 ± 1.45                   | 17 ± 0.74                |
| 3   | Experimental Group 2** | 30 – 25                   | 39.2 ± 0.18          | 72 ± 0.99                   | 16 ± 0.41                |
|     |                   | 15 – 10                   | 39.1 ± 0.22          | 71 ± 1.58                   | 15 ± 0.83                |
|     |                   | 3 – 5                     | 39.0 ± 0.18          | 70 ± 1.16                   | 15 ± 0.76                |

* Time of A₂ introduction: at the beginning of pregnancy and 14 days before farrow.
** Time of Immunoflor introduction: at the beginning of pregnancy and 2 weeks before farrow.

Sows’ reproduction indices are given in table 4.

Under the influence of the probiotic preparations, the number of viable pigs obtained from farrow sows in Experimental Groups 1 and 2 was 7.2 and 10.3 % higher than the one in the Control Group, but the difference revealed turned out to be unreliable (P>0.05). It should be noted that with the use the probiotic preparations A₂ and Immunoflor the number of stillbirths in Experimental Group 1 and 2 was 1.4 and 2.1 times less than in the Control Group. The probiotic preparations tried by us for the
first time increased the milkability of sows by 4.2 and 4.7 kg and litter heaviness – by 11.4 and 3.6 %, respectively. These data are given in table 4.

Thus, the probiotic preparations A2 and Immunoflor enabled more complete fulfillment of the bioresource potential of sows’ reproductive qualities by normalizing metabolism in their organisms.

Table 4. Indicators of reproductive qualities of sows.

| Index                  | Control Group | Experimental Group 1 | Experimental Group 2 |
|------------------------|---------------|----------------------|----------------------|
| Number of sows         | 10            | 10                   | 10                   |
| Pigs obtained,         |               |                      |                      |
| total                  | 98            | 107                  | 110                  |
| per 1 sow              | 9.8 ± 0.24    | 10.7 ± 0.41          | 11.0 ± 0.32          |
| Including,             |               |                      |                      |
| viable pigs            | 9.6 ± 0.17    | 10.4 ± 0.31*         | 10.8 ± 0.22*         |
| stillbirths            | 0.4 ± 0.27    | 0.3 ± 0.21           | 0.2 ± 0.12           |
| Litter heaviness, kg   | 1.05 ± 0.07   | 1.17 ± 0.10          | 1.09 ± 0.08          |
| Milkability, kg        | 47.8 ± 1.51   | 52.4 ± 1.21*         | 52.9 ± 1.02*         |

* P<0.05.

The main hematologic indices of the sows are reflected in table 5.

With immunocorrection it was established that hemopoiesis improved in the sows of the experimental groups. For example, the red blood cell count of the sows of Experimental Groups 1 and 2 3-5 days after farrow turned out to be 7.9 and 11.6 % (P<0.05) higher, the hemoglobin level – 5.8 and 9.2 % higher and the white blood cell count – 4.8 and 3.3 % higher, respectively, than in the Control Group.

Table 5. Hematologic indices of sows.

| Group of animals       | Index                      |                      |                      |
|------------------------|---------------------------|----------------------|----------------------|
|                        | red cells, ×10¹²/l        | hemoglobin, g/l      | leukocytes, ×10⁹/l   |
| Control Group          | 5.39±0.17                 | 97±2.05              | 11.2±0.22            |
| Experimental Group 1   | 5.81±0.25                 | 105±1.25*            | 11.7±0.28            |
| Experimental Group 2   | 6.01±0.21*                | 108±1.84*            | 11.6±0.35            |

* P<0.05.

The phagocytic activity of leukocytes and the lysozyme activity of blood plasma of Experimental Groups 1 and 2 sows were higher than in the Control Group throughout the duration of the scientific experiment. For instance, 3-5 days after farrow the phagocytic activity of leukocytes of the given experimental groups turned out to be 8.5 and 9.7 % higher, while the lysozyme activity of blood plasma – 3.2 and 5.8 % higher than the ones in the Control Group.

The dynamics of cellular and humoral factors of nonspecific resistance of the sows’ organisms is visualized in figures 1-4.

It was established that the sows’ blood serum bactericidal activity index in all studied groups increased by the end of pregnancy and reached its peak during the research period 15-10 days before farrow: 52.8 ± 1.52 % (Control Group), 58.8 ± 1.28 (Experimental Group 1) and 59.3 ± 1.24 % (Experimental Group 2). That is, the sows of Experimental Groups 1 and 2 exceeded their herdmates in the Control Group by the given index of the humoral component of the organism’s nonspecific
resistance by 6.1 and 6.6 % (P<0.05), respectively. The results of these studies make it possible to conclude that with the use of the probiotic preparations the blood serum bactericidal activity increased reliably with respect to the Control Group.

**Figure 1.** Phagocytic activity of leukocytes in sows.

**Figure 2.** Phagocytic index in sows.

**Figure 3.** Lysozyme activity of blood plasma in sows.

**Figure 4.** Bactericidal activity of pig blood serum.

The results of these immunobiological studies show that the use of the probiotic preparations А2 and Immunoflor for the sows of the experimental groups increases their reproductive potential by activating the cellular and humoral factors of nonspecific resistance of the organism, with the more apparent effect of the probiotic preparation Immunoflor. It was established that the number of pigs
obtained from the sows of Experimental Groups 1 and 2 was 7.2 and 10.3 % higher than in the Control Group, but this difference was insignificant (P>0.05). It should be noted that with the use of the probiotic preparations A2 and Immunoflor the number of stillbirths in Experimental Groups 1 and 2 was 1.4 and 2.1 times less than in the Control Group. The probiotic preparations A2 and Immunoflor tried by us for the first time increased the litter heaviness by 11.4 and 3.6 % and the milkability of the sows by 4.2 kg and 4.7 kg, respectively.

Thus, by normalizing the metabolic processes in farrow sows, the probiotic preparations A2 and Immunoflor enabled greater fulfillment of the bioresource potential of reproductive qualities of their organisms.

4. Conclusion
Prescription of the probiotic preparations A2 and Immunoflor for farrow sows in the amount of 1.62 g and 0.05 g per 1 animal, respectively, twice, at the beginning of pregnancy and 14 days before farrow, through normalizing metabolism and activating nonspecific resistance of the organism, has positive influence on the fetal development, increasing the number of healthy pigs in the litter by 7.2 and 10.3 %, the litter heaviness – by 11.4 and 3.6 %, and improves the sows’ milkability by 9.3 and 10.3 % (P<0.05).

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