Changes in white blood cell pattern in piglets, as a consequence of administration of aged garlic extract (AGE) or allicin to sows during pregnancy and lactation, were presented. The experiment was performed on piglets born by sows of Large Polish White breed. Animals were kept under standard rearing conditions with free access to fresh water and well balanced diet. The piglets obtained from 18 sows were divided into three groups: one control and two experimental ones. Within those groups there were formed 7 age-differentiated subgroups (each subgroup n = 6): non-suckling newborn piglets (0 d) and at the age of 1, 3, 7, 14, 28 and 35 d. Starting from the 91st d of pregnancy up to the piglets’ weaning on 28th d of their life, the sows were daily administered per os AGE at the dosage of 10 ml/100 kg b.w. or allicin at the dosage of 1.6 mg/100 kg b.w., whereas the controls received 10 ml/100 kg b.w. of vehiculum. The blood samples were collected from the piglets on days 0, 1, 3, 7, 14, 28 and 35 of their life and analysed immediately. The results showed in most cases a significant increase in the white blood cell counts in different periods of piglets’ life. This indicates an improvement in animal performance following treatment with the investigated garlic preparations.

Key words: sows, piglets, garlic, allicin, leukocytes.

Pig breeders continuously search for various methods to improve production and the state of animal health. In their aspirations they often meet various difficulties in which the most common are diseases of pigs. Depending on age, pigs are affected with diseases of different origin. Many diseases of pigs cause not only animal death but also contribute to production losses (inhibition of weight gain, increase in bad feed conversion, elongation of intensive feeding period or progressive cachexia) (19) and large financial expenses (13). Diseases of respiratory system belong to frequent illnesses among pigs and are caused by various bacterial (9, 13, 19, 28), and viral pathogens and mixed infections (13). These diseases are essential considering their appearing in groups of different age (suckling piglets, fatteners) (13). Such diseases as post-weaning diarrhoea and oedema disease, salmonellosis, streptococcal infection, swine dysentery, Aujeszky’s disease or balantidiosis, deserve a special attention because of high mortality in the population of pigs (40).

In relation to that, breeders of pigs make an effort to prevent diseases by prophylactic vaccinations and improvement of breeding conditions (32). In order to improve animal health and productive results, they use various growth stimulators (antibiotic and non-antibiotic) in nutrition of pigs. Antibiotic growth promoters are generally employed as feed supplements for pigs because they afford large productive advantages (improvement of weight gain and state of health, increase in feed consumption, stabilization of alimentary canal microflora, inhibition of pathogenic microorganism extension) and little financial expenses (25).

Considering the prohibition of the use of growth antibiotic stimulators in nutrition of animals from the beginning 2006, breeders of pigs are forced to look for alternatives among non-antibiotic feed supplements. Each of them possesses various properties which are credited to antibiotic growth promoters. For example, non-antibiotic feed supplements such as herbs, acidifying factors (organic acids), minerals (zinc oxide or a combination of zinc oxide with copper sulphate) take a part in the stabilization of alimentary canal microflora (5, 25, 29), others, such as enzymes, proteins or amino acids improve feed conversion (25). Herbs, particularly garlic, draw breeders’ attention due to many prophylactic and therapeutic properties.

Garlic extracts or allicin (a main organic allyl sulphide component of garlic) (26) possess wide spectrum of antimicrobial effects: antibacterial against both Gram-positive (Actinomyces, Bacillus, Clostridium, Enterococcus, Mycobacterium, Staphylococcus, Streptococcus) and Gram-negative (Actinobacillus, Escherichia, Fusobacterium, Leptotrichia, Prevotella, Porphyromonas, Salmonella, Shigella) bacteria (3, 6, 10, 16, 38), antiviral against human cytomegalovirus, influenza B virus, herpes simplex virus types 1 and 2, parainfluenza virus type 3, vaccina virus, vesicular stomatitis virus and human rhinovirus type 2 (37),
antifungal against various species of fungi from the genera: *Aspergillus, Cryptococcus, Candida, Trichophyton, Epidermophyton, Microsporum, Torulopsis* (1, 41) and antiparasitic against *Entamoeba, Giardia, Leishmania, Leptomonas* and *Crithidia* (1, 2, 22).

Moreover, garlic may prevent many diseases considering suppression of LDL oxidation (20), reducing serum cholesterol and triglycerides (4, 36), increasing HDL-cholesterol and fibrinolytic activity (4), inhibiting tumour cell growth, increasing natural killer and other killer cell activities (17), reducing serum glucose level (36) and others.

Little is known about the influence of garlic administration to pregnant and lactating sows on changes of leukocyte pattern in the blood of piglets. It inclined us to undertake these studies in order to broaden the knowledge on garlic preparations.

**Material and Methods**

**Experimental design and sampling procedure.** The experimental procedures used throughout this study are in a compliance with the guidelines for the “Care and Use of Animals” as published by the American Journal of Physiology and were approved by the Local Ethic Committee on Animal Experimentation of the Agricultural University of Lublin, Poland.

The experiment was performed on piglets born by sows of Large Polish White breed. The animals were kept under standard rearing conditions with free access to fresh water and well balanced diet. The piglets obtained from 18 sows were divided into one control and two experimental groups. All the investigated groups of piglets were divided additionally into 7 age-differentiated subgroups (each subgroup n = 6): nonsuckling newborn piglets (0 d) and at the age of 1, 3, 7, 14, 28 and 35 d. Starting from the 91st d of pregnancy up to piglet weaning on the 28th d of their life, the sows were daily treated per os, during morning feeding, with aged garlic extract (AGE group; n = 6) at the dosage of 10 ml/100 kg b.w. or allicin (ALL group; n = 6) at the dosage of 1.6 mg/100 kg b.w., whereas controls (C group; n = 6) received 10 ml/100 kg b.w. of vehicleulm. Piglets obtained from AGE, allicin or vehicleulm-treated sows were assigned to AGE, ALL or C group, respectively. Each age-differentiated subgroup comprised 6 piglets. AGE was prepared as a result of 12-month hydroalcoholic maceration of sliced garlic, according to instructions presented by Staba (22), whereas the allicin was administered in the form of commercial capsular preparation (Alliomega, Herbapol Lublin S. A., Poland).

The blood samples were collected from the vena cava cranialis of piglets at the age of 0, 1, 3, 7, 14, 28 and 35 d to heparinized tubes and analyzed immediately. Haematological analyses were performed with the use of automatic haematological analyser MS9 (MELET SCHLOESING Laboratories, France).

**Statistical analysis.** Statistical analyses were performed using STATISTICA 6.0 software. All the data were presented as means ± SEM. The Student’s t-test was used to determine statistical significance of differences between AGE or ALL groups versus the control. The level of statistic significance was set at P ≤ 0.05 for all comparisons.

**Results**

The results showed the increased number of white blood cells (WBCs) in AGE group from day 3 to 35 and in ALL group on days 3, 7, 28 and 35 of piglets’ life when compared to controls. Significantly higher values in both experimental groups were observed on days 28 and 35 (P < 0.001) and additionally in ALL group on day 7 (P < 0.01) and in AGE group on day 14 of life (P < 0.05). However, the number of WBCs in both experimental groups was lower on days 0 and 1 and additionally in ALL group on day 14 of life when compared to controls. Significantly lower values were observed in AGE groups on days 0 and 1 of life (Table 1).

The number of granulocytes in the blood of both experimental groups was increased on day 35 and additionally in AGE group on day 14 and in ALL group on day 7 of piglets’ life when compared to controls. Significantly higher values were observed in AGE as well as in ALL group on day 35 of life (P < 0.001). However, the number of granulocytes in both experimental groups was decreased on days 0, 1, 3 and 28, and also in AGE group on day 7 and in ALL group on day 14 of life when compared to controls. Significantly lower values were observed in AGE groups on days 1 and 28 and in ALL groups on days 0 and 28 of life (Table 1).

The number of monocytes in the blood of both experimental groups was increased on days 3, 7 and 28 and additionally in AGE groups on days 14 and 35 and in ALL group on day 0 of piglets’ life when compared to controls. Significantly higher values were observed in AGE group on days 28 (P < 0.001) and 35 (P < 0.01) and in ALL group on days 3 and 7 of life (P < 0.01). However, the number of monocytes in both experimental groups was decreased on day 1 and also in AGE group on day 0 and in ALL group on days 14 and 35 of life when compared to controls. Significantly lower values were observed in AGE group on day 14 of life (Table 1).

The number of lymphocytes in the blood of both experimental groups was increased on days 3, 7, 14, 28 and 35 of猪漏’s life when compared to controls. Significant higher values were observed in AGE group on days 28 and 35 (P < 0.001) and additionally in ALL group on days 3, 7, 14, 28 and 35 of piglets’ life when compared to controls. Significant lower values were observed in both experimental groups on day 1 and additionally in AGE group on day 0 of life when compared to controls. There were no statistically significant lower values in AGE as well as ALL group of piglets (Table 1).
Table 1

The mean number of WBCs, granulocytes, monocytes and lymphocytes in piglets at 0, 1, 3, 7, 14 and 35 d of life from sows treated with vehiculum (C group), AGE (AGE group) or allicin (ALL group) during pregnancy and lactation.

<table>
<thead>
<tr>
<th>Day of life</th>
<th>Group</th>
<th>n</th>
<th>WBCs (10^9/l)</th>
<th>Granulocytes (10^9/l)</th>
<th>Monocytes (10^9/l)</th>
<th>Lymphocytes (10^9/l)</th>
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<tr>
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<td>1.9</td>
<td>1.68</td>
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<td>1.08</td>
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<td>1.88</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
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<td>7.09</td>
<td>1.48</td>
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<td>3.31 ***</td>
<td>1.26</td>
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</table>

* P < 0.05; ** P < 0.01; *** P < 0.001, versus group C.

Discussion

Besides properties summarized in introduction, garlic favourably affects microcirculation: decreases systolic and diastolic blood pressure (11), plasma viscosity (18), preserves the structure and function of erythrocytes (23), enhances cytotoxicity of peripheral mononuclear cells (26), inhibits platelet aggregation, adherence, adhesion (31) and thromboxane formation (4) or production of proinflammatory cytokines such as IL-1α, IL-6, and IL-8, tumour necrosis factor-α, and interferon-γ (12) and enhances production of anti-inflammatory cytokine - IL-2 by peripheral blood cells (21). Leukocytic system is associated with a microcirculation and has not been investigated in the relation to supplementation of garlic preparations.

White blood cells are responsible for both non-specific and specific defence of a host. Non-specific defence of the organism plays an important role as the first line protection against pathogens which are not familiar to the organism and possesses two kinds of defence mechanisms: cellular and humoral. Polynuclear blood cells (granulocytes) and some mononuclear blood cells (monocytes and macrophages) take part in the process of phagocytosis (cellular mechanisms of non-specific defence). Other mononuclear blood cells, lymphocytes, play an important role in both cellular (T lymphocytes) and humoral (B lymphocytes) mechanisms of specific defence of a host. Lymphocytes of T class protect a host from intracellular pathogens such as Mycobacterium, Listeria, Brucella or Salmonella and the greater part of viruses and fungi. Moreover, B lymphocytes are responsible for the formation of antibodies, principally immunoglobulin of M or G classes (IgM, IgG), which are in the greatest concentration in blood as a consequence of germ penetration to a host. Humoral mechanisms of specific defence play a role in the action against extracellular pathogens, toxigenic bacteria such as Pasteurella and some viruses (39).

The results showed the increased number of WBCs from 3 to 35 d of piglet life in AGE and ALL groups. This parameter was considerably augmented on days 7, 14, 28 and 35 in AGE and/or ALL groups (above 2-fold in ALL and almost 3-fold in AGE groups on day 35 of piglets’ life) when compared to control. Similarly, we observed the increased number of granulocytes on days 7, 14 and 35 of life, monocytes on days 3, 7, 14, 28 and 35 and lymphocytes from day 3 to 35 of piglets’ life as a consequence of administration of AGE or allicin to pregnant and lactating sows. These changes are very important considering the increase in the number of leukocytes, especially on day 28 of life when the piglets were weaned. In this period, breeders of pigs change a manner of piglets’ feeding. It is very critical for them considering a diminution of piglets’ immunity and insufficient development of the alimentary system with inefficient secretion of digestive enzymes and gastric juices. Mechanisms responsible for an increase in blood leukocyte pattern after administration of garlic are not known. The increased number of WBCs may result from the ability of some garlic extracts to inhibit neutrophil
migration (14, 15). There are no data about an influence of garlic on migration of other leukocytes. On the other hand, some studies reported that various garlic components stimulated or decreased peripheral blood cells proliferation (7, 21, 27). It is interesting whether the observed blood leukocytes are immature or mature. The increased number of immature WBCs could result from the stimulation of proliferation, whereas that of mature ones – from inhibiting leukocyte migration after administration of garlic.

Moreover, our previous studies on AGE and allicin performed on sows and piglets showed the increased activity in both lysozyme and ceruloplasmin (Cp) or the enhanced level of both gammaglobulins (IgG) defence mechanisms (35) and increased activity in both lysozyme and ceruloplasmin (Cp) or the enhanced level of both gammaglobulins (IgG) and total protein content (8, 35) in different periods of sows' and piglets' life, including day 28. Our results suggest that the immune system of sows and piglets is a very important target for biologically active components of garlic. These positive effects on defence mechanisms of the investigated animals perhaps contributed to the improvement of body weight gain and systematic development of piglets reported in other our studies (33, 34).

In conclusion, our results show that administration of garlic preparations such as AGE or Alliominax causes the activation of both non-specific (granulocytes, monocytes, lysozyme, Cp) and specific (lymphocytes, IgG) defence mechanisms (35) and increases favourably growth rate and systemic development of the investigated piglets (33, 34). Our previous and present studies confirmed that garlic preparations can be used as very attractive alternative for antibiotic growth promoters in pig nutrition, especially what concerns their impact on animal performance.

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