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Changes in hematological and immunological indices in piglets transferred from the rearing unit to the fattening unit

ANNA CHMIELOWIEC-KORZENIOWSKA, LESZEK TYMCZYNA, BEATA TRAWIŃSKA, MAREK BABICZ*, MAGDALENA PYRZ, PAWEŁ RÓŻAŃSKI, MARCIN PASTWA*

Department of Animal Hygiene and Environment, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
*Department of Pig Breeding and Production Technology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

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Chmielowiec-Korzeniowska A., Tymczyna L., Trawińska B., Babicz M., Pyrz M., Różański P., Pastwa M.

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Summary

The aim of the present study was to determine the levels of selected hematological and immune indices in growers transported from the rearing unit to the fattening facility. Blood samples were analyzed for the white blood cell count and the leucogram profile. IgA, IgM, and IgG immunoglobulin contents as well as lysozyme concentration were determined in serum. The hematological evaluation indicated a significant impact of the transfer to the fattening unit on the WBC picture. In fatteners aged 11 weeks, an elevated level of neutrophils with a concomitant decline in lymphocyte numbers was observed. A higher concentration of dust as well as chemical and microbial contaminants determined in the air of the fattening unit also affected immunoglobulin concentration and lysozyme content.

Keywords: pig, immunoglobulin, lysozyme

The last decade of swine breeding and animal trade has been marked by organizational and technological changes, the emergence of new mutations of microbes in their habitats, as well as the persistent long-term stress of animals under breeding conditions. These factors may impair the efficiency of the animal immune system and thereby facilitate a more intensive proliferation of conditional pathogens. The available data provide evidence that swine respiratory disorders caused by microorganisms or/and chemicals detected in the air of pig houses are the most common diseases associated with substantial economic losses. The disease incidence rate is also influenced by excessive stocking density in small pig buildings housing a large number of animals. Importantly, frequent moving and regrouping of animals, as well as suboptimal animal management conditions, cause them to develop an adaptive stress, which is an immunosuppressive factor leading to an increased incidence rate of diseases (15). The studies of Frank et al (6) confirm that pig herd management that does not meet the recommendations predisposes animals to bacterial infections, especially to respiratory diseases. On the other had, a low level of immune activation in animals, achieved by providing them with optimal management conditions with low counts of pathogens and potential antigens that stimulate defense mechanisms, as well as by reducing stress factors, not only affects their overall body condition, but also ensures a high production efficiency (2, 3, 11). A critical stage in pig production is the transfer of animals to the fattening facility. It is associated with intense stress produced by changes in the feeding system and management conditions as well as by the development of a new herd hierarchy.

The purpose of the study was to determine the levels of selected hematological and immune indices during the transfer of growers from the rearing unit to the fattening unit.

Material and methods

The research was conducted on a private pig farm stocked on average with 105 LU. A closed-cycle production system was applied in which the premises were either fully stocked or empty. The animals were kept in three separate pig houses: a building with two stalls for boars, another with farrowing and rearing units, and the fattening unit. The rearing unit was divided into 8 pens with an average stock of 20 growers/pen

and 0.3 m^2 floor area/individual with concrete grid flooring. In the fattening unit, the animals were housed in the deep litter system with litters removed after each production cycle. Each pen consisted of a laying area covered with straw and a feeding table. Throughout the research period, the average stock was 100 animals per chamber, which resulted in the mean pen area of 0.8 m^2/unit.

The animals were provided with permanent veterinary service, including standard prophylactic measures. Piglets aged 3 and 13 days were administered a ferric preparation according to a prophylaxis program, and at 5 days of age they had the canine tooth clipped and underwent castration. At 2 weeks of age, the animals were immunized against *M. hyopneumoniae* and disinfested. All the pigs were free from stress susceptibility related to the RYR1CC genotype, as demonstrated by the PCR-RLFP technique.

According to the management policy of the farm, the piglets were weaned at an average weight of 7.0 kg at 28-days-of-age. The animals remained at the rearing facility until 10-weeks-of-age, and then, having reached 25-30 kg body weight, they were transferred to the fattening facility.

The assessment of the effect of transfer from the rearing unit to the fattening unit on the growers' health was based on hematological and immune examinations. For this purpose, 30 healthy growers with no clinical manifestations of any disease (1: 1 sex ratio) were selected. At 9- and 11-weeks-of-age; i.e., one week before the transfer (at the rearing unit) and one week after the transfer (at the fattening unit), blood samples for analyses were collected from the selected animals. To minimize stress, blood was always sampled at the same time of the day in a separate room, and the whole procedure (from the immobilization of the animal) took no more than 22 s. The blood (4.9 ml per sample) was collected from the jugular vein into test tubes with K2 EDTA (Profilab Sc. Warsaw, Poland) and into S-Monovette tubes (Sarstedt AG&Co., Numbrecht, Germany). A total of 60 blood samples were taken from the 30 pigs.

The hematological evaluation of the WBC count, lymphocytes (Lym), monocytes (Mono), neutrophilic granulocytes (Neu), eosinophils (Eos), and basophils (Baso) was performed with a hematological analyzer Melet Schloesing Laboratories (Osny, France). The serum was analyzed by the Single Radial ImmunoDiffusion VMRD procedure to determine the levels of immunoglobulin complexes, such as the IgA, IgM, IgG classes. Lysozyme concentration was determined by the lyso-plate method.

Microclimatic conditions were measured at each of the research series. Temperature, moisture, air movement, cooling, and light were measured at 3 points (P1-P3) in both the rearing unit and the fattening units, at a height of 30 cm, twice at 4 h intervals. This method reduced abrupt jumps in the investigated parameters, especially temperature, and provided a more complete picture of microclimatic conditions in the two units.

The microclimate in the fattening unit was determined by standard zootechnical methods; air temperature and moisture were measured with a thermohygrometer (RT811E, Technik, Warsaw, Poland), and air movement with an anemometer (model A-1200M1, OBRAiUP, Łódź, Poland).

At the same time, the air in the rearing and fattening units was analyzed for the concentrations of ammonia, carbon dioxide, volatile organics (VOCs), and total dust as well as the total counts of bacteria, actinomycetes, and fungi. The concentrations of ammonia, VOCs, and CO_2 were measured with a QRAE detector (PGM-2000, RAE systems, San Jose, USA). Air dust concentration was determined by a gravimetric method, and air samples were collected with an individual aspirator (model 224-PCEX8, SKC, Dorset, England).

The evaluation of microbial air contamination was carried out in compliance with the Polish standard PN-EN 13098:2007 by the aspiration method. Total numbers of bacteria and microscopic fungi were established by the plate dilution method; i.e. serial dilutions were plated onto suitable stable media: trypticase soy agar (TSA) enriched with 5% sheep blood for the total bacterial count, maltose extract agar (MEA) for the total microscopic fungi numbers, and "Agar for Actinomycetes" with supplemental nystatin for the total actinomycetes count. The air samples plated on TSA medium underwent a 7-day incubation period at 30°C (for 1 day), 22°C (3 d), and 4°C (3 d), The samples on MEA medium were incubated at 30°C (4 d) and 25°C (3 d), and those on "Agar for Actinomycetes" for 7 d at 25°C.

The obtained results were analyzed statistically and characterized by the number of samples (n), arithmetic mean (M), and standard deviation (SD). The findings concerning the microclimate in the rearing and fattening units, as well as health parameters of the pigs under investigation, were compared by the Ducan test. All the analyses were performed using SAS v.9 statistical software.

Results and discussion

Throughout the research period, thermal conditions in the rearing and fattening units were consistent with the requirements (Tab. 1). The mean air temperature in the rearing unit was higher by 5°C than in the fattening unit ($P < 0.01$). Compared with the fattening unit, the rearing facility had a significantly lower total dust concentration in the air ($P < 0.05$). Among the chemical contaminants

Tab. 1. Microclimate conditions in the pig buildings

Parameter	Rearing unit		Fattening unit		*P*-value
	M	SD	M	SD	
Temperature and moisture conditions					
Temperature, °C	23.7	1.6	18.7	0.4	0.000
Moisture,%	76.2	6.2	83.0	3.6	0.078
Air movement, m/s	0.1	0.1	0.1	0.0	0.455
Physical and chemical contaminants					
Dust rate, mg/m^3	1.7	2.4	6.1	1.1	0.025
CO_2,%	0.2	0.0	0.1	0.1	0.083
Ammonia, ppm	4.5	2.0	18.8	8.1	0.036
VOCs, ppm	0.2	0.1	0.5	0.2	0.135
Microbial contaminants					
Total bacterial count, × 10^5 cfu/m^3	4.52	1.38	342.7	472.9	0.039
Total fungal count, × 10^5 cfu/m^3	0.05	0.01	1518.1	5024.2	0.340
Total actinomycetes count, × 10^5 cfu/m^3	0.05	0.03	19.9	29.7	0.051

determined, only the ammonia content proved significantly higher in the air of the fattening unit than it was in the rearing unit ($P < 0.05$). Some significant differences were also found in microbial contaminant rates. The total bacterial count in the fattening unit was higher by as many as two orders of magnitude than that in the rearing unit.

The statistical analysis of the obtained hematological results revealed significant differences in the leukocyte count and the percentages of lymphocytes and neutrophils (Tab. 2). In the blood of fatteners transferred to the fattening facility, the lymphocyte numbers were higher by 2.7 10^9/l in gilts and by 3.4 10^9/l in barrows than the corresponding values in the rearing unit ($P < 0.01$). At 11-weeks-of-age a rise in neutrophil concentration with a concurrent decline in the lymphocyte level was noted.

Among the immunoglobulins measured, only the concentrations of immunoglobulin M differed statistically (Tab. 3). The IgM level in the blood of fatteners aged 11 weeks was lower than in the blood of animals aged 9 weeks (on average by 0.6 g/l in gilts and by 0.5 g/l in barrows). At the same time, an increase was observed in the blood lysozyme content. The lysozyme concentration at 11-weeks-of-age was higher by 0.3 mg/l in gilts ($P > 0.05$) and by 0.4 mg/l in barrows ($P < 0.05$) than the concentration measured at 9-weeks-of-age: i.e. before the transfer to the fattening unit.

Tab. 2. Number of white blood cells and leucogram profile in the pigs' blood

Indicator	Sex	n	Rearing unit		n	Fattening unit		*P*-value
			M	SD		M	SD	
WBC, × 10^9/l	gilts	30	16.7	3.8	30	19.4	5.0	0.024
	barrows	30	19.6	5.1	30	23.0	7.5	0.056
Lym,%	gilts	30	57.5	10.1	30	49.7	10.5	0.005
	barrows	30	56.0	7.4	30	46.4	10.7	0.000
Mono,%	gilts	30	4.6	1.4	30	4.6	0.9	0.985
	barrows	30	4.6	1.0	30	4.6	0.8	0.719
Neu,%	gilts	30	34.3	9.9	30	40.2	10.4	0.028
	barrows	30	36.3	7.3	30	43.7	11.9	0.009
Eos,%	gilts	30	3.3	2.1	30	5.0	6.0	0.128
	barrows	30	2.7	1.4	30	4.9	6.5	0.095
Baso,%	gilts	30	0.3	0.2	30	0.4	0.2	0.559
	barrows	30	0.5	0.2	30	0.5	0.3	0.958

Tab. 3. Concentrations of immunoglobulins and lysozyme in the pigs' blood serum

Indicator	Sex	n	Rearing unit		n	Fattening unit		*P*-value
			M	SD		M	SD	
IgA, g/l	gilts	25	0.4	0.1	30	0.4	0.2	0.117
	barrows	24	0.4	0.0	29	0.4	0.1	0.101
IgG, g/l	gilts	25	3.8	3.2	25	4.8	2.3	0.202
	barrows	24	4.3	3.4	30	5.2	2.9	0.344
IgM, g/l	gilts	25	1.4	1.0	29	0.8	0.3	0.011
	barrows	24	1.4	1.0	24	0.9	0.4	0.032
Lysozyme, mg/l	gilts	25	1.6	0.5	30	1.9	0.8	0.486
	barrows	24	1.4	0.2	29	1.8	0.5	0.045

The present investigations have shown that the transfer of growers from the rearing unit to the fattening facility may constitute a serious stressor for the animals. It was associated not only with social stress, related to the regrouping of the animals, but also with adaptation to new and, what is more, unfavorable maintenance conditions. The evaluation of the gaseous composition of the air in the fattening unit showed that contaminant levels, especially those of ammonia, gaseous organic pollutants (VOCs), and total dust, were higher than in the rearing unit, which may have affected the animals' health status. The average ammonia concentration in the fattening unit was higher than in the rearing unit by as much as 14.3 ppm, whereas the mean total dust level in the fattening unit was twice as high as the zoohygienic standard. Toxicological studies have revealed that an ammonia concentration above 7.5 ppm is associated with respiratory disorders and increased susceptibility to infections (4). The combination of ammonia and dust is considered particularly hazardous. Pedersen et al. (13) indicate a significant relationship between high concentrations of dust and ammonia and the incidence of atrophic rhinitis and mycoplasma pneumonitis. Hamilton et al. (8) report that an ammonia concentration above 10 ppm, especially if combined with a high air dust rate, promotes the atrophy of the nasal concha caused by *Pasteurella multocida*.

In addition to chemical contaminants, the environment in the pig building was influenced by biological factors, whose negative effects on animal health may have been mutually reinforced. The total bacterial count in the air of the fattening unit amounted to 342.7×10^5 cfu/m^3, which was 75 times higher than in the rearing unit ($P < 0.05$). Differences in the total fungal content were even more marked ($P > 0.05$). The mean fungal concentration in the air of the fattening unit was higher by as many as four orders of magnitude than that in the rearing facility. These values were higher than those determined by Donham (5), who states that the total microbial count in the air of a pig facility should not exceed 10^5 cfu/m^3. In this case they were several times higher than the zoohygienic norm for the fattening unit (8.0×10^4 cfu/m^3) determined according to the animal health criterion. These results are consistent with studies by Chang et al. (2), which demonstrate that the level of microbial contamination of the air in farm buildings

depends not only on the season and maintenance system, but also on the function of the building. The authors found greater total bacteria numbers in air samples from fattening units and lower in those from farrowing and rearing facilities. Greater microbial pollution was noted in the air of premises contaminated with grass and straw dust, dominated by microbes originating from crops. Hence in the fattening unit a major source of bioaerosol was the litter bedding.

Interactions between different levels of welfare and the systemic biological balance are subject to varying environmental conditions and can be observed throughout the lifetime of animals. The defense mechanisms stimulated by the presence of numerous pathogens trigger a series of reactions making up the inflammatory response. Sensitive indicators of the ongoing inflammation or immune reaction to environmental stress factors are the total counts of white blood cells, macrophages, neutrophils, and lymphocytes (1), which has been confirmed by the present study. One week after transfer to the fattening unit, the blood of fatteners showed an increased leukocyte count. WBC only slightly exceeded the reference values presented by Winnicka (16), but remained within the range given by Odinka et al. (12). The elevated leukocyte count observed in the pigs' blood may have been indicative of an inflammatory response in these animals (9). It may also have been caused by the transfer-related stress or by the reaction to contaminants in the air of the fattening unit. The higher pollution in the air of the fattening facility may have acted as an additional stressor for pigs. This may explain the changes in each WBC indicator observed in fatteners aged 11 weeks; i.e., one week after their introduction to the fattening unit. Compared with the results obtained at 9-weeks-of-age – during the week preceding the transfer – there was a significant rise in neutrophil numbers with a concomitant fall in the lymphocyte content in the animals' blood. Kleinbeck and McGlone (11) confirm that the environment has a significant impact on the white blood cell picture. The authors found a higher WBC count in the blood of piglets kept in facilities with higher air contamination, which is confirmed by von Borell et al. in their studies on growers (1). Von Borell et al. found that WBC, including lymphocytes and monocytes, in growers' blood increased when the indoor air ammonia concentration reached 35 ppm, i.e. 26.2 mg/m^3. Jarvis and Miller (10) report that the inhalation exposure to mycotic glucans also exerts a significant impact on the markers of inflammatory response, including macrophage, eosinophil, and neutrophil contents.

A critical protective role in fighting pathogens and in the removal of their metabolites (endotoxins, glucans) is attributed to immunoglobulins and lysozyme. As Odinka et al. (12) observed, lysozyme activity increases during acute immune response and decreases in chronic inflammatory states. Investigations by Swammy et al. (14) showed that stress caused in pigs by pen changes, isolation or immobilization is associated with a decrease in the IgG fraction and an increase in acute-phase protein (APP) and lysozyme activity. The present study has shown that animal transfer to the fattening unit was accompanied by a lower concentration of immunoglobulin M and a concurrent increase in the lysozyme content. It is difficult, however, to offer a straightforward interpretation of these findings, given numerous discrepancies in the literature (7, 13, 14). The observed changes may have been related to the stimulation of the immune system by known antigens (bioaerosol) also present in the rearing unit. IgM is the first antibody formed when white blood cells are initially exposed to an antigen. When exposed to the antigen for the second time, the pig builds very high levels of antibodies, mostly in the class of IgG.

In conclusion, the present results indicate that all procedures involved in the transport and regrouping of animals constitute serious stressors which have negative effects on their health.

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Corresponding author: dr hab. Anna Chmielowiec-Korzeniowska, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland; e-mail: anna.korzeniowska@up.lublin.pl