DYNAMICS OF VARIATIONS OF ACUTE PHASE PROTEINS IN PIGS FED BIOIMMUNO AND/OR IMMUNISED WITH RESPISURE ONE VACCINE AGAINST MYCOPLASMAL PNEUMONIA

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Abstract

The purpose of the study was to determine the influence of the Bioimmuno administration in feed and/or immunisation with the ‘Respisure’ One vaccine against mycoplasmal pneumonia of swine on the development of non-specific immune response expressed by the level of acute phase proteins (APP). The study was performed on 28 piglets at the age of 4 weeks, divided into four equal groups. The biopreparations were administered according to the following pattern: group I - Bioimmuno (1 kg/50 kg of feedstuff) for 48 h and then vaccination with Respisure One (2 ml/animal i.m.), group II – Bioimmuno (1 kg/50 kg of feedstuff) for 48 h, group III - Respisure One (2 ml/animal i.m.), and group C (control) – PBS (2 ml/animal i.m.). Serum concentration of APP: C-reactive protein, haptoglobin, and ceruloplasmin were determined on days 0, 3, 7, 14, and 21 after immunomodulation and/or immunisation. The statistically significantly lower (P<0.05) levels of all determined APP in the experimental groups in comparison with the control group were demonstrated. This proves the protective value of the biopreparations applied in specific patterns, through their beneficial influence on the decrease in consequences of the respiratory system infections in pigs.

Key words: swine, Bioimmuno, Respisure One, acute phase proteins.

Non-specific immune response in porcine respiratory system plays a very important role in the protection against infectious agents, which penetrate together with the inhaled air. One of the elements of this response, aiming at restricting the inflammation, removing the damaging or infectious agent, and restoring homeostasis, is the so called acute phase response (APR). In its course, there are variations in the concentrations of a number of serum proteins, qualified because of that reason to the so called acute phase proteins (APP) (2, 13, 25, 30, 35, 41). The biosynthesis of these proteins in mammals takes place mainly in hepatocytes, although some of them can also be produced in smaller quantities by activated lymphocytes, monocytes, endothelial cells, and fibroblasts. The most significant signals inducing the transcription factors of APP genes are proinflammatory cytokines IL-1, IL-6, and TNF-α, produced by various types of the immune system cells, as well as epithelial cells and fibroblasts, in response to factors such as: viruses, bacteria, fungi, substances released by dead tissues, and some other factors (12, 26, 27, 34). In pigs, C-reactive protein (CRP), haptoglobin (Hp), and pig major acute phase protein (MAP) belong to major acute phase proteins (13).

Due to their high reactivity and the easiness of determination, APP can successfully be applied in the detection of inflammation in the respiratory system (1, 5, 7, 9, 15, 25, 29, 42).

One of the most frequent bacterial respiratory system disease, recorded in all countries with intense pig breeding, including Poland, is mycoplasmal pneumonia of swine (MPS) (17, 28). The economic losses associated with the MPS result most of all from a decrease in body weight gain, greater use of feedstuffs, as well as an increase in incidence and percentage of secondary infections, which increase the costs of treatment (31, 38). In order to limit the losses caused by MPS, specific immunophrophylaxis is nowadays commonly used. However, it should be remembered that such actions only reduce the inflammatory condition in the lungs and improve the fattening production results, but they do not protect against the settlement of Mycoplasma hypneumoniae (Mhp) organisms in the respiratory system, and they do not set the herd free from the disease. Good effects of eliminating MPS are gained with the simultaneous improvement of environmental conditions of pig breeding. Nowadays, there are many biopreparations available on the market demonstrating the effectiveness in the prevention of clinical signs of the disease (16, 24, 40). In spite of
satisfying effects, the studies are continuously conducted to increase the vaccination effectiveness, which will allow to reduce pulmonary lesions nearly to zero, will not allow for the colonisation of the respiratory system by \textit{Mhp}, and will lead to set the herd free from this pathogen \cite{4, 6}. The alternative or the action supporting vaccinations may be the application of immunomodulators, which intensely activate the cellular and humoral defence reaction. Within the scope of immune mechanisms, an increase in the metabolic and phagocytic activity of neutrophils and monocytes/macrophages, as well as an increase in T and B lymphocyte activity, are stated. It has been indicated that immunomodulation enhances also the production of specific antibodies by plasmatic cells and strengthens the synthesis of interferons, lysozyme, and other substances conditioning humoral response. Anti-infectious immunity stimulated in such a manner allows to maintain a high level of wholesomeness of pig herds and to reduce significantly the losses caused by infectious diseases of the respiratory system \cite{3, 19-22, 37}.

The purpose of the study was to determine the influence of the Bioimmuno administration in feed and/or immunisation with the Respisure One vaccine against MPS in pigs on the development of non-specific immune response expressed by the level of APP.

\section*{Material and Methods}

\textbf{Animals.} The research was carried out in a small pig farm, in which the basic herd consisted of 16 sows, and the production of fatteners from their own pig population, as well as piglets from the purchase, amounted to about 600 animals annually. The weaning population, as well as piglets from the purchase, and immunomodulated (group II) groups. On the 7th d of the experiment, a statistically significant (\(P<0.05\)) increase in the CRP level was observed in the control group (group C) in comparison with the immunomodulated and immunised (group I), and immunomodulated (group II) groups. On the 7th d there were statistically significant differences between control and all experimental groups receiving biopreparations, although in groups I and III, a decrease in the CRP levels was already noticed. Statistically

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Group} & \textbf{Biopreparation} & \textbf{Vaccination} & \textbf{Result} \\
\hline
I & Bioimmuno & Respisure One & Decrease in CRP levels \\
II & Bioimmuno & Respisure One & No difference in CRP levels \\
III & Respisure One & Respisure One & Decrease in CRP levels \\
\hline
\end{tabular}
\caption{Comparison of CRP levels in experimental groups.}
\end{table}

\textbf{Biopreparations.} In the experiment, the following biopreparations were used: inactivated vaccine against MPS - Respisure One (Pfizer Inc. Animal Health Group) – each dose of the vaccine (2 ml) contained NL 1042 \textit{Mhp} 4.5 – 5.2 log_{10} RP strain and Amphigen oil adjuvant, the Bioimmuno preparation (IFI Olsztyn, Poland) containing 40 g of methisoprinol + 960 g of \textit{Saccharomyces cerevisiae} to be given with feedstuff.

The biopreparations were administered according to the following pattern: group I - Bioimmuno with feedstuff at amount of 1 kg/50 kg of feed for 48 h and then immunisation with Respisure One (2 ml/animal i.m.); group II - Bioimmuno with feedstuff at amount of 1 kg/50 kg of feed for 48 h; group III - Respisure One immunisation (2 ml/animal i.m.), and group C (control) - PBS at a dose of 2 ml/animal i.m.

In the immunological assays, for the determination of the non-specific immunity indices, a sample of 6-ml volume of blood from the anterior vena cava was collected from each pig into the tube without anticoagulant, in order to obtain the serum. After coagulation, the blood sample was centrifuged for 10 min at 1,620 x g.

The blood samples were collected on days 0, 3, 7, 14, and 21 after immunomodulation and/or immunisation. Serum levels of APP: C-reactive protein (CRP), haptoglobin (Hp) and ceruloplasmin (Cp) were determined.

The level of CRP was determined by means of the turbidimetric method in the Cobas Emira analyser (Roche Diagnostics), using the suspension of latex particles coated with human antibodies against CRP C-reactive protein (cod 31921, BioSystems S.A. Costa Brava 3,0, Spain). Comments from the article by Martinez-Subiela \textit{et al.} \cite{23} were taken into consideration while interpreting the results.

The determination of the Hp level was performed by means of the turbidimetric method in the Hitachi 912 analyser (Roche Diagnostics), using the Tiina-quant haptoglobin reagent (Cat. No. 11557629, Roche Diagnostics), containing human antibodies against Hp. The interpretation of the results was carried out on the basis of the article by Wiedmeyer \textit{et al.} \cite{43}.

The Cp level was determined by means of the spectrophotometric method according to Rice \textit{et al.} \cite{32}, in modification by Siwicki \textit{et al.} \cite{33}.

The received results were drawn up statistically with the application of the analysis of variance test for the comparison of a number of means (NIR test) at \(P<0.05\) and the determination of standard deviations.

\section*{Results}

The dynamics of variations in the CRP levels in porcine serum after the Bioimmuno administration and/or immunisation with Respisure One was presented in Table 1.

On the 3rd d of the experiment, a statistically significant (\(P<0.05\)) increase in the CRP level was observed in the control group (group C) in comparison with the immunomodulated and immunised (group I), and immunomodulated (group II) groups. On the 7th d there were statistically significant differences between control and all experimental groups receiving biopreparations, although in groups I and III, a decrease in the CRP levels was already noticed. Statistically
significant differences were also found on the 14th d of the experiment in piglets between groups I, II, and III (immunised) and group C, as well as a statistically significantly higher CRP level in group II compared with groups I and III. On the 21st d of the experiment, the CRP level in the pigs immunised with Respisure One and/or stimulated with Bioimmuno was still statistically significantly lower in groups I and III in comparison with groups II and C.

The development of the Hp levels after the Bioimmuno administration and/or immunisation with Respisure One was presented in Table 2.

### Table 1

<table>
<thead>
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<td>I (B+R)</td>
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<td>III (R)</td>
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<td>26.98</td>
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<tr>
<td>C</td>
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Explanations as in Table 1.

### Table 2

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<tr>
<td>II (B)</td>
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<tr>
<td>III (R)</td>
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<tr>
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<td>X</td>
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Explanations as in Table 1.

### Table 3

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<tr>
<td>II (B)</td>
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<td>III (R)</td>
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<tr>
<td>C</td>
<td>X</td>
</tr>
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<td></td>
<td>157.40</td>
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</table>

Explanations as in Table 1.
The Hp level on the 7th d after administration of the biopreparation was statistically significantly (P<0.05) higher in group C in comparison with groups I and II, and statistically significantly higher in group III compared with group II. Statistically significant differences were also found on the 14th d of the experiment in group C in comparison with group I. At the final stage of the experiment, the Hp level in the serum of pigs after the administration of the vaccine and/or immunomodulator in groups I and III was statistically significantly lower in comparison with group C.

The development of the Cp levels after the Bioimmuno administration and/or immunisation with Respisure One was presented in Table 3.

The Cp determinations from as early as the 3rd d after administration of the biopreparation indicated a statistically significantly lower level in all experimental groups in comparison with the control group. On the 7th d of the experiment this level was still significantly lower in groups I and II compared with group C, whereas on the 14th and 21st d in group I in comparison with groups II and C.

Discussion

Proteins of a major significance in the reaction to an infectious factor in pigs were determined in our studies. The administration of the immunomodulator resulted in the lack of an acute phase reaction in the form of the increase in the CRP level in groups I and II on the 3rd d of the experiment, as well as on the 7th and 14th d in all experimental groups receiving biopreparations in comparison with group C. On the 21st d of the experiment, the CRP level in porcine serum after the Bioimmuno administration and/or immunisation with Respisure One was still statistically significantly lower in groups I and III in comparison with groups II and C. The results obtained in groups I and III may be a proof of the protective effect of the immunomodulator and/or vaccine in groups I and III compared with group C. The Hp concentration in pigs increases in the respiratory system diseases, in the course of diarrhoeas, in tail and ear injuries resulting from biting, as well as in retention conditions (36). The usefulness of measuring the Hp level was also demonstrated as an evaluation indicator of weight gain in pigs, particularly important in the early period of the production cycle (8). Cp in pigs is considered to be a more characteristic APP than in other animal species (39). The Cp level in the serum is repeatedly increased in various pathological conditions, i.e. in case of cholestatic jaundice, dyshepatia, neoplastic diseases, as well as in the course of joint infections and inflammations (15). Our studies constitute another confirmation of the already known facts that immunomodulators, e.g. lysozyme dimer, stimulate the phagocytes activity, the IFN-γ production and have a modulating influence on the synthesis of TNF, IL-2, and IL-6 (10). The values of the presented parameters correlated with the CRP level and they were also found in the course of inflammatory processes. In our studies, the acute phase response was indicated in the non-vaccinated groups, which, as it was demonstrated in the studies conducted by Heegaard et al. (9), Knura-Deszcz et al. (11), Magnusson et al. (18), and Petersen et al. (29), is a result of an accompanying infection, which next should be identified in the studies to follow. APR, occurring during an infection, stress or in the presence of another damaging factor, can play the role of a marker of inflammatory reactions, which may frequently remain imperceptible in the clinical study. The lowering of the APP levels indicates, however, the reduction of the inflammatory reaction in the course of pathological processes in the respiratory airways (1, 7, 9).

The results of our studies demonstrated the maintenance of statistically significantly lower (P<0.05) levels of all determined APP in the experimental groups of piglets in comparison with the control group, which proves the protective value of the biopreparations applied in specific patterns, through their beneficial influence on the decrease, in consequences, of the respiratory system infections in pigs.

References


