EFFECT OF THE COUPLED ADMINISTRATION
OF LYDIUM-KLP OR METHISOPRINOL AND RESPISURE
ON THE HEALTH STATUS AND PREVENTION
OF MYCOPLASMAL PNEUMONIA
OF SWINE IN LARGE-SCALE COMMERCIAL BREEDING

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Abstract

The study was aimed on determining the clinical protective value of Lydium-KLP and Methisoprinol in the prevention of respiratory system diseases, as well as their effect on the health status of pigs in large-scale commercial breeding and on the improvement of in the effectiveness of specific immunoprophylaxis of mycoplasmal pneumonia of in swine (MPS). Piglets at the age of 7 d were divided into eight groups of 25-30 piglets each. On days 7 and 21 of life they were immunised with Respisure vaccine (group I) or administrated Lydium-KLP or Methisoprinol (groups IV and VII). In other groups, Lydium-KLP or Methisoprinol were given simultaneously (groups II and V) or 48 h before immunisation with Respisure (groups III and VI). Control piglets were administrated PBS (group C). Clinical observations as well as post-slaughter and animal husbandry analyses indicated that the simultaneous administration of Lydium-KLP or Methisoprinol with Respisure gives better results in comparison to immunisation-only with Respisure. The statistically-significant differences were found in the mean percentage of meatiness between groups receiving Methisoprinol with or 48 h before Respisure and control group, as well in the point evaluation of lesions in the lungs between groups receiving Lydium-KLP with Respisure and Methisoprinol 48 h before Respisure and control group. The study demonstrated the beneficial effect of coupled administration of Lydium-KLP or Methisoprinol and Respisure on the improvement of health status and the productive performance of pigs, as well as on the effectiveness of specific immunoprophylaxis of MPS in large-scale commercial breeding. The most beneficial variants of coupled administration were the simultaneous application of Lydium-KLP and Respisure, and Methisoprinol application 48 h before immunisation with Respisure.

Key words: swine, Mycoplasma hyopneumoniae, mycoplasmoses, Lydium-KLP, Methisoprinol, Respisure, prophylaxis.

Diseases of the respiratory system, including mycoplasmal pneumonia of swine (MPS), occur often in fattening farms and evoke considerable economic losses in the production of pork livestock. Mycoplasma hyopneumoniae (Mhp) antibodies, an aetiological factor of MPS, have been detected in ca. 80% of farms reporting respiratory disorders in swine, whereas typical after-slaughter lesions in the lungs have been reported in different countries in 30%-80% of pigs (17). In Poland, out of 935 blood samples analysed, 23.1% yielded a positive reaction to Mhp antigen, whereas mycoplasmas were isolated from 61% of the lungs with pneumonia-induced lesions (9). Losses linked to MPS result most of all from diminished body weight gains, poorer feed utilisation and in consequence the elongation of the fattening period. It is estimated that Mhp infection decelerates swine growth rate by 2.8%-44% and reduces body weight gains by 14%-20% and feed conversion efficiency by 16%-30% (27, 29). Secondary infections that often accompany Mhp infections increase treatment costs and necessitate the introduction of more complex prophylactic programmes.

The specific immunoprophylaxis of MPS is commonly applied in order to reduce losses resulting from Mhp infections. It is worth emphasising the fact that these actions only restrict the inflammatory state in the lungs and improve fattening performance, but do not protect against the colonisation of the respiratory system by these microorganisms, nor relieve the herd of the disease. Good effects of MPS eradication may only be achieved through the simultaneous improvement in the environmental conditions of pig breeding. Nowadays, a number of biopreparations are available on the market, which are effective in the prevention of the clinical
symptoms of the disease. Most often, inactivated vaccines are prepared on the basis of whole Mhp cells or some of their fragments only (cellular membranes). In the activation of immunocompetent cells, key significance is ascribed to adjuvants used as carriers of an antigen in the vaccines (16). The action of environmental and stress factors has an unfavourable effect on the non-specific defence mechanisms and on the specific immunity of animals. For these reasons, there has been an interest in immunomodulators that strongly activate cellular and humoral defence mechanisms, which enable the acquiring of anti-infectious immunity and allows the maintenance of a high health status in herds of pigs as well as considerably reducing losses that result from infectious diseases of the respiratory system (3, 19).

A number of substances stimulating the immune system have been applied in veterinary medicine, but non-specific immunomodulators with a multi-directional mechanism of action still have the predominant role, and these include, e.g., bacteria of the genus Propionibacterium, levamisole, lysozyme dimer, glucans and isoprinosine (2, 6, 7, 18, 19).

The study was aimed at determining the clinical protective value of Lydium-KLP and Methisoprinol in the prevention of respiratory system diseases, as well as their effect on the health status of pigs in large-scale commercial breeding and on the improvement of the effectiveness of specific immunoprophylaxis of MPS.

Material and Methods

Farm. The study was conducted in a pig industrial fattening farm on 216 piglets of both sexes, with a similar number of boars and gilts, with an initial body weight (b. w.) of ca 2-3 kg (7th d of life), divided into eight groups of three farrows each with 25 – 30 animals per group. The basic stock of the experiment involved 2,200 sows. The annual production of fatteners accounted for ca. 36,000 animals. Piglets were weaned on day 28 of their life. The farm has been implementing the specific prophylaxis of MPS (Respisure) for 5 years. Its facilities include: farrowing houses with 50 farrowing pens and pig fattening buildings with boxes for 10–15 pigs, depending on their body weight, assuring identical feeding and watering conditions. Farm buildings are well equipped, having mechanical ventilation system with manual regulation, which ensures good environmental conditions.

Vaccine. Inactivated vaccine against MPS – Respisure (Pfizer Inc. Animal Health Group) – containing chemically-inactivated whole cells of Mhp and oil adjuvant Amphigen.

Immunomodulators. Lydium-KLP (Nika Health Products Ltd, USA) – an active substance: lysozyme dimer (N-acetylmuramide glycano-hydrolase), a protein substance of natural origin with an enzymatic effect, at a concentration of 0.2 mg/mL; Methisoprinol (Polfa, Poland) – an active substance: isoprinosine; a synthetic compound composed of one molecule of inosine and three molecules of 4-/acetylamino-/benzate-1/-dimethyl amine-2-propanol, at a concentration of 0.1 mg/mL.

Immunisation schedule. The piglets received the preparations intramuscularly into one or both sides of the neck on days 7 and 21 of their life, according to the following group variants:

- **Group I** - Respisure in a dose of 2 ml/piglet,
- **Group II** - Respisure in a dose of 2 ml/piglet with simultaneous administration of Lydium-KLP in a dose of 20 µg/kg b.w.,
- **Group III** – Lydium-KLP in a dose of 20 µg/kg b.w. 48 h before immunisation with Respisure in a dose of 2 ml/piglet,
- **Group IV** – Lydium-KLP in a dose of 20 µg/kg b.w.,
- **Group V** - Respisure in a dose of 2 ml/piglet with simultaneous administration of Methisoprinol in a dose of 20 mg/kg b.w.,
- **Group VI** – Methisoprinol in a dose of 20 mg/kg b.w. 48 h before immunisation with Respisure in a dose of 2 ml/piglet,
- **Group VII** - Methisoprinol in a dose of 20 mg/kg b.w.,
- **Group C** – (control) - PBS at a dose of 2 ml/piglet.

The pigs used in the experiment had not been previously immunised against MPS or been immunostimulated.

Clinical observations covered the monitoring of local and constitutional side-effects, the evaluation of the incidence and character of symptoms manifested by the respiratory system and other disorders of health status, as well as the determination of the number and percentage of medical interventions and death cases.

Animal husbandry examinations involved the determination of the mean duration of fattening period, post-slaughter examination of the mean post-slaughter mass of carcasses, mean percentage of meatiness, and an index of quantitative lesions in the lungs with the point method according to Goodwin and Whittlestone (5).

The results obtained were calculated statistically by means of the analysis of variance for the comparison of multiple mean values (NIR test) at P<0.05 and determination of standard deviations.

Results

No local side-effects were observed in any pig in any experimental group at the injection site of Respisure vaccine or Lydium-KLP or Methisoprinol immunomodulators. No constitutional side-effects were observed after the administration of the vaccine and/or immunomodulators, and the clinical condition of the animals after administration of the biopreparations did not diverge from reference in respect of the control animals.

The results of the clinical observations of the pigs administered with Lydium-KLP or Methisoprinol and/or immunised with a Respisure are shown in Table 1.
Table 1
Results of the clinical observations of pigs administered with Lydium-KLP or Methisoprinol and/or immunised with a Respisure

<table>
<thead>
<tr>
<th>Group*</th>
<th>Number of animals</th>
<th>Number/Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (R)</td>
<td>29</td>
<td>3/10.34</td>
</tr>
<tr>
<td>II(R+L)</td>
<td>26</td>
<td>2/7.69</td>
</tr>
<tr>
<td>III(L-R)</td>
<td>26</td>
<td>3/11.54</td>
</tr>
<tr>
<td>IV(L)</td>
<td>30</td>
<td>5/16.66</td>
</tr>
<tr>
<td>V(R+M)</td>
<td>28</td>
<td>2/7.14</td>
</tr>
<tr>
<td>VI(M-R)</td>
<td>25</td>
<td>2/8.0</td>
</tr>
<tr>
<td>VII(M)</td>
<td>27</td>
<td>4/14.81</td>
</tr>
<tr>
<td>C</td>
<td>25</td>
<td>5/20.0</td>
</tr>
</tbody>
</table>

* Explanations: R - Respisure, L – Lydium-KLP, M - Methisoprinol, C – Control

Table 2
Results of animal husbandry and post-slaughter analyses of the pigs treated with Lydium-KLP or Methisoprinol and/or immunised with a Respisure

<table>
<thead>
<tr>
<th>Group*</th>
<th>Mean fattening period (d)</th>
<th>Mean post-slaughter mass of carcasses (kg)</th>
<th>Mean percentage of meatiness</th>
<th>Point evaluation of lesions in lungs according to Goodwin and Whittlestone</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (R)</td>
<td>185</td>
<td>75.91</td>
<td>52.4</td>
<td>38</td>
</tr>
<tr>
<td>II(R+L)</td>
<td>184</td>
<td>75.65</td>
<td>52.9</td>
<td>32a</td>
</tr>
<tr>
<td>III(L-R)</td>
<td>189</td>
<td>77.23</td>
<td>53.3</td>
<td>40</td>
</tr>
<tr>
<td>IV(L)</td>
<td>199</td>
<td>73.53</td>
<td>52.2</td>
<td>73</td>
</tr>
<tr>
<td>V(R+M)</td>
<td>185</td>
<td>76.98</td>
<td>54.6b</td>
<td>40</td>
</tr>
<tr>
<td>VI(M-R)</td>
<td>185</td>
<td>76.15</td>
<td>54.1a</td>
<td>37a</td>
</tr>
<tr>
<td>VII(M)</td>
<td>196</td>
<td>73.76</td>
<td>52.7</td>
<td>71</td>
</tr>
<tr>
<td>C</td>
<td>201</td>
<td>73.73</td>
<td>51.6b</td>
<td>90b</td>
</tr>
</tbody>
</table>

* Explanations as in Table 1; a, b – P<0.05

The pigs in groups IV, VII, and C, administered only with the immunomodulators or placebo, showed a higher percentage of infections, with symptoms of the respiratory system as compared to the other experimental groups in which the piglets were immunised with the Respisure vaccine alone or in a combination with the immunomodulators. In turn, health disorders not linked with the respiratory system, manifested most of all by diarrhoea, most frequently occurred in groups I, VII, and C. Medical interventions linked with disorders of health condition referred more often to groups VII and C, whereas a lower percentage of interventions was noted in the other groups, especially in groups III, V, and VI treated with a combination of Respisure and Lydium-KLP or Methisoprinol. These groups were additionally characterised by the lowest mortality rate over the experimental period, as compared to groups I, IV, and C in which that rate was reported to be the highest. All changes between groups and parameters presented in Table 1 were not statistically significant.

The results of animal husbandry and post-slaughter analyses referring to the mean duration of fattening period, mean post-slaughter mass of carcasses, and meat yield as well as a macroscopic score evaluation of lesions in the lungs, are presented in Table 2.

Analyses conducted in the study demonstrated that the mean fattening period in groups I, II, III, V, and VI, administered with Lydium-KLP or Methisoprinol and/or Respisure, was considerably shorter (by 7–15 d) than the other groups receiving immunomodulators alone. The longest fattening period (201 d) was noted in the control group (C).

In the post-slaughter examination, the highest mean carcass mass was observed in groups III, V, and VI, which was higher by 1.58–3.70 kg as compared to the other experimental groups. The lowest values of post-slaughter mass were demonstrated for carcasses obtained from groups IV, VII, and C.

Groups III, V, and VI, receiving a combination of the immunomodulators with the vaccine, were additionally characterised by the highest meatiness of carcasses (53.3%–54.6%). A slightly lower percentage
of meatiness was observed in groups I, II, IV, and VII (52.2%–52.9%), and the lowest one – in group C (51.6%). Statistically significant differences (P<0.05) were observed only between groups V and VI receiving Methisoprinol with or 48 h before Respisure and control group.

The point evaluation of macroscopic changes in the lungs demonstrated the highest total number of points (71–90) and the highest percentage of piglets with a lesion index above the null (90.91%) in groups IV, VII, and C that were not immunised against MPS. The lowest number of lesions typical of Mhp, infections, i.e. 32–40 points and 45.45%–63.64% of animals with a lesion index above the null, respectively, was observed in groups I, II, III, V and VI, in which the piglets were administered the immunomodulators and/or immunised with Respisure. In that case statistically-significant differences (P<0.05) were found between group II with coupled administration of Lydium-KLP and Respisure, group VI, which received Methisoprinol 48 h before Respisure and the control group. In turn, no macroscopic lesions in the lungs (index = 0) were observed in over half the animals from groups I, II, III, and V.

Discussion

Studies conducted in pigs from large-scale commercial breeding have confirmed that Lydium-KLP and Methisoprinol immunomodulators as well as Respisure vaccine applied alone or in combination do not induce unfavourable local or generalised side-effects. In a similar study addressing the evaluation of the harmlessness and effectiveness of specific immunophylaxis in the eradication of MPS, Tarasiuk et al. (30) did not observe any side-effects linked with the administration of Respisure vaccine in any of the 419 immunised pigs. Markowska-Daniel et al. (21) did not note any changes in the clinical status and observed the maintenance of the internal body temperature within physiological norms in pigs upon the administration of Suivac A vaccine and/or Propionibacterium avidum (PA) KP-40 immunomodulator. In a similar study with the use of the Respisure vaccine and Inmodulen containing an extract of inactivated cells of Propionibacterium granulosum and detoxicated lipopolysaccharides isolated from E. coli, Markowska-Daniel and Glapiak (19) also did not observe any side-effects after applying these preparations.

In the reported study, clinical observations demonstrated that the administration of Lydium-KLP or Methisoprinol immunomodulators and/or immunisation with Respisure vaccine caused a reduction in the occurrence of disease symptoms manifested by the respiratory system. Similar investigations, with a positive effect of immunomodulators applied on the immunonpotentisation of vaccines, were undertaken by Markowska-Daniel et al. (21), who used PA KP-40 for the stimulation of the immune system of pigs immunised with selected viral antigens. They demonstrated the beneficial effect of the coupled administration of a Suivac A vaccine and PA KP-40. Double immunisation of piglets with a Suivac A vaccine at 4-week intervals, with simultaneous intravenous administration of PA KP-40, yielded better protection against challenge induced 8 weeks after re-vaccination. Having been infected, the immunised and immunomodulated pigs displayed a lower intensity of clinical symptoms, a lack of deaths, the lowest body weight loss, the shortest period of growth inhibition, and the highest dynamics of proliferation of post-vaccinal antibodies in comparison to control groups. In a similar study, these authors also demonstrated the positive effect of intraperitoneal administration of PA KP-40 on the course of a control infection with a classical swine fever virus as compared to the non-immunised animals. Megid et al. (24) showed the positive impact on the coupled immunisation against rabies and immunomodulation with Propionibacterium acnes on the survivability and distribution of rabies virus in tissues of mice after a control infection. The usability of the concept adopted in this study is confirmed by results of another study carried out by Markowska-Daniel and Glapiak (19), who, when evaluating the effect of the Inmodulen on the effectiveness of immunisation against Mhp, confirmed the positive effects of applying immunomodulators in the specific immunophylaxis of MPS.

Clinical observations as well as post-slaughter and animal husbandry analyses conducted in the reported study showed that the best results were achieved in the groups of immunised and immunomodulated pigs and slightly worse in only immunised animals, but these results were not statistically significantly higher as compared to only immunomodulated and control groups. The differences referred to the percentage of animals with disorders of the respiratory system, the number of medical interventions and cases of deaths due to respiratory and other disorders, the extent of pathological lesions in the lungs, body weight gains, and duration of fattening period. The efficacy of the specific immunophylaxis or that of immunomodulation has been confirmed many times in the prophylaxis and therapy of swine diseases, including those of the respiratory and other systems, and on pathological conditions. The efficacy of the application of the Inmodulen in the strengthening of the specific immunophylaxis of porcine respiratory and reproductive syndrome (PRRS) was demonstrated by Pejsak et al. (25). The coupling of the specific and non-specific immunophylaxis diminished the mortality rate of piglets in the period from weaning till transfer to a fattening sector and positively affected the dynamics of body weight gains and the shortening of the fattening period.

The concept of coupled administration of vaccines and immunomodulators is, so to speak, a consequence of previous studies on the evaluation of the effectiveness of specific immunophylaxis or immunomodulation in the prevention and therapy of respiratory system diseases and other disorders of health status. Clinical investigations have explicitly confirmed the efficacy of the application of the Respisure vaccine (3, 8, 15, 31). A study by Tarasiuk et al. (30)
demonstrated that the specific immunoprophylaxis of MPS with the use of the vaccine effected a nearly 3-fold decrease in the number of cases with symptoms manifested by the respiratory system, as well as in the culling index and mortality rate in pigs. Other studies have also proven the effectiveness of the treatment of respiratory system diseases in swine by means of Lydium-KLP (10). Simultaneously, the current study demonstrated that diarrhoea occurred less frequently in the piglets from groups receiving the immunomodulators. This is consistent with the results reported by Markowska-Daniel et al. (22, 23) as well as Markowska-Daniel and Pejsak (20) in studies into the effect of the coupled administration of iron and isoprinosine on the health status of piglets and their haematological and immunological indices, as well as with findings of Pejsak et al. (26) from research on the usability of Lydium-KLP in the therapy of selected bacterial diseases in pigs under field conditions.

Stipkovits et al. (28) compared the effectiveness of antibiotic therapy and the coupled application of antibiotics and immunomodulation with the use of the Inmodulen in the course of enzootic pneumonia in pigs. They speculated that the stimulation of the immune system increases the capability of antibiotics to kill mycoplasmas, which reduce pathological lesions in the lungs and improve the production performance of livestock. The improved health status of animals from the experimental groups receiving the immunomodulators – Lydium-KLP or Methisoprinol (demonstrated in the reported study) was also generally manifested in smaller losses linked with a lower percentage of deaths in these groups, except group III.

The stimulation of the developing immune system is of special significance in the case of young animals (11). The activation of cellular immunity mechanisms through the application of stimulants in piglets in the early period of their life was demonstrated by Bartnicka and Kondracki (1). The enhanced proliferation of T lymphocytes and increased activity of blood phagocytes was observed as soon as in the first week of life of the piglets, and was maintained for a period of 6 weeks. These authors claimed that the prophylactic application of preparations stimulating the immune system as soon as possible after the birth, coupled with appropriate animal husbandry conditions and feeding, may considerably contribute to the improved health status of the piglets in the period of their particular susceptibility to infections. The improvement in the health status of the piglets may as well be reached through the application of immunomodulators in pregnant sows. Krakowski et al. (12) demonstrated the positive effect of non-specific immunostimulation with isoprinosine, TFX, and HMB of pregnant sows 4–6 weeks before farrowing on an increase in IgG level, total protein, and the activity of lysozyme incolostrum. Nevertheless, some caution should be exercised when applying methods of immunomodulation of the immune system. An example may be a study by Kyriakis et al. (13), who demonstrated that the non-specific stimulation of the immune system by means of immunisation (Respisure) or immunomodulation (Baypamun) was likely to increase the replication of PCV-2 and intensify the clinical symptoms of post-weaning multisystemic wasting syndrome (PMWS) in pig herds.

The values of post-slaughter carcass mass and meatiness percentage noted in the reported study, were higher in the groups receiving the vaccine and the combination of the vaccine and the immunomodulators, which is consistent with the results of an earlier study by Dohoo and Montgomery (4) in vaccinations against MPS. These results were, however, not confirmed in a study by LeGrand and Kobisch (14), who did not find any differences in the post-slaughter evaluation of meat yield between the group immunised against MPS and the control group.

The occurrence of macroscopic lesions in the lungs is highly pathognomonic for Mhp infections and it is very reliable and useful in the diagnosis of inflammatory lesions induced by that microorganism. The current study demonstrated a reduction in the extent of inflammatory lesions in the lungs of piglets from groups I, II, III, V, and VI (administered Respisure and a combination of Respisure and an immunomodulator) that were shown to positively affect the inhibition of the development of pathological lesions, which was also confirmed in studies by other authors (3, 14, 19).

Tarasiuk et al. (30) also demonstrated a considerable reduction in inflammatory lesions in the lungs, evaluated in post-slaughter examinations, as well as an increase in body weight gains by 28 g/d in the piglets immunised with the Respisure in the 1st and 3rd week of life, and by 18.1 g/d in the piglets immunised in the 4th and 7th week of life. The evaluation of the macroscopic lesions in the lungs according to Goodwin and Whittlestone (5) allowed proper analysis of the lesions induced by Mhp, excluding general changes induced by other factors.

The analysis of the results obtained in the animal husbandry evaluation indicated that the simultaneous administration of Lydium-KLP or Methisoprinol with Respisure gives better results in comparison to the immunisation only with Respisure. The statistically-significant differences were found in the mean percentage of meatiness between groups receiving Methisoprinol with or 48 h before Respisure and the control group, as well as in the point evaluation of lesions in the lungs between groups receiving Lydium-KLP with Respisure and Methisoprinol 48 h before Respisure and control group. Thus, the administration of Methisoprinol or Lydium-KLP increases the effectiveness of vaccination against MPS, whereas the application of immunomodulators alone without Respisure yields worse results and does not assure adequate protection against Mhp infections.

In summary, the study demonstrated the beneficial effect of the coupled administration of Lydium-KLP or Methisoprinol and Respisure on the improvement in health status and production performance of pigs, as well as on the effectiveness of specific immunoprophylaxis of MPS in large-scale commercial breeding. The most beneficial variants of coupled administration were simultaneous application of Lydium-KLP and Respisure and Methisoprinol application 48 h before immunisation with Respisure.
References


