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EVALUATION OF ANTIBODY FORMATION, DAILY WEIGHT GAIN AND MEAT QUALITY AFTER VACCINATION OF PIGLETS AGAINST *MYCOPLASMA HYOPNEUMONIAE*

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A *Mycoplasma hyopneumoniae* vaccine (Respisure, Pfizer AH) was tested for its effects on antibody formation, daily weight gain (DWG) in different growing periods, lung lesions and quality of meat (chemical composition, physicochemical properties and fatty acid composition). Two groups of conventional piglets were used for the investigation. One group of 11 females and 11 males was vaccinated intramuscularly at the age of 1 and 3 weeks. The other group of 22 piglets was left nonvaccinated as control. The results showed that antibodies against *M. hyopneumoniae* in the vaccinated group had been formed 14 days after the second vaccination and remained present till the end of the study at 147 days of age. In the nonvaccinated group, seroconversion started at 49 days of age and by the end of the study 10 out of 22 pigs had become seropositive. Vaccinated pigs achieved significantly higher daily weight gain (+30 g) and finishing body weight (+6.04 kg) than the nonvaccinated animals. In addition, the vaccinated pigs showed lesions involving 3.27% of the lung surface in average, while in the nonvaccinated pigs 9.04% of the lung surface was affected. Investigation of meat quality showed that the longissimus dorsi muscle of vaccinated pigs contained significantly lower percentage of fat (–0.63%) and its tryptophan/hydroxyproline ratio was significantly lower (–23.57) in comparison with the control animals. In addition, some other parameters also showed a favourable tendency, e.g. lean meat percentage was 0.91% higher, the protein content of the longissimus dorsi muscle was 0.35% higher, its water-binding capacity was also higher by 0.78%, its monounsaturated fatty acid concentration was 2.97% lower, while its polyunsaturated fatty acid content was 1.65% higher in the vaccinated pigs than in the nonvaccinated animals.

Key words: Enzootic pneumonia, pig, antibodies, vaccine, meat quality

Studies show that up to 93% of swine herds worldwide are affected by mycoplasmal pneumonia, which is thus one of the most prevalent and costly

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swine diseases (Ross, 1992). Even at low levels of infection, this chronic respiratory disease represents significant costs because of reduced feed efficiency, lower daily weight gains, lack of uniformity in pig size, repeated antibiotic treatments and decreased carcass price (Clark, 1996). In cases of mixed infection with other respiratory pathogens, *Mycoplasma hyopneumoniae* produces more severe pneumonia than after a single infection (Ross, 1992). Although different eradication programmes are available for *M. hyopneumoniae*, the risk of reinfection of mycoplasma-free herds is very high. Vaccination is an important strategy for controlling mycoplasmal pneumonia (Ross et al., 1984; Djordjevic et al., 1997; Thacker et al., 1998). Field trials demonstrated that *M. hyopneumoniae* vaccines, based on adjuvanted whole cells, had beneficial effects in several herds clinically affected by enzootic pneumonia (Charlier et al., 1994; Dohoo and Montgomery, 1996). In addition to being efficacious and safe, the vaccine should also be cost effective (Walker, 1992). Passive immunity against *M. hyopneumoniae* has also been demonstrated to have an influence on vaccination. The maternally derived antibody level and its persistence in piglets are highly variable. Morris et al. (1994) showed that the median half life of *M. hyopneumoniae* antibodies in piglets born to seropositive sows was 16 days.

At the present time, meat quality is characterised by describing meat value, organoleptic, sanitary-hygienic and technological properties (Jukna and Jukna, 2000). The properties of pig meat including lean meat percentage, physicochemical properties and chemical composition depend on the breed, sex and body weight of the animals and many other factors (Wood et al., 1994). Only little information is available on the effect of vaccination with *M. hyopneumoniae* vaccine on the physical properties or eating quality of meat. The flesh score showed significantly better results for the vaccinated groups compared with the control groups; however, backfat thickness and the quantity of valuable meat parts were not significantly different between the vaccinated and control groups (Radeloff and Heinritzi, 1998).

In this study, we examined the effect of vaccinating piglets against *M. hyopneumoniae* infection on body weight and daily weight gain, on the development of lung lesions characteristic of mycoplasmal pneumonia and on the appearance of specific anti-*M. hyopneumoniae* antibodies, as well as on various carcass and meat quality parameters (thickness of backfat and longissimus dorsi muscle, lean meat percentage as well as the fat, protein, tryptophan and hydroxyproline content and water-binding capacity of the longissimus dorsi muscle and the concentration of some saturated, mono- and polyunsaturated fatty acids in that muscle).

Materials and methods

Forty-four crossbred 7-day-old piglets (22 females and 22 males) from a Lithuanian conventional farm were randomly divided into two groups. The original herd was infected with *M. hyopneumoniae.* Eleven females and 11 males were vaccinated against *M. hyopneumoniae* with the commercially available vaccine Respisure (Pfizer AH, USA) injected in a dose of 2 ml behind the ear, as recommended by the manufacturer. The first dose was administered during the first week of life. After 14 days a second dose was administered. The control group (11 males and 11 females) was not vaccinated. The vaccinated and control animals were housed separately during and after weaning. During the fattening period they were kept in the same air space but were separated by a door. At 28 days of age the piglets were transferred to the post-weaning unit. Pigs were weighed individually when moved between buildings and subsequent to slaughter. Prevention measures (castration, iron injection, needle teeth clipping, tail docking) and other management practices were identical for both groups.

Blood samples were taken from both groups of piglets at the time of the first vaccination and at 21, 35, 49, 63, 91, 119 and 147 days of age. The antibody response of all piglets was examined by blocking ELISA (Dako, Denmark) (Feld et al., 1992). The serum samples were diluted 1:10 and incubated for 1.5 hours in micro wells precoated with *M. hyopneumoniae* antigen. Then, without emptying the wells, peroxidase-conjugated mouse monoclonal antibody to *M. hyopneumoniae*, directed against a specific epitope on 74 kDa protein, was added. After incubation for 15 min the micro wells were washed and chromogenic substrate (1.2 phenylenediamine dihydrochloride) was added. A golden brown colour developed in all wells, and after 10 min the reaction was stopped by the addition of sulphuric acid. The higher the antibody concentration in the pig serum sample, the less monoclonal antibody conjugate was bound to the well and the lower was the intensity of colour detected in the well. The absorbance (OD) in each micro well was read at 490 nm, and the absorbance values of sample wells were compared with that of a buffer control well. Those sera were considered positive in which the OD value was less than 50% of that of the buffer control well.

Live body weight was measured at 7, 28, 107 and 185 days of age. Daily weight gain (DWG) was calculated in each group as the difference between mean body weight at the start and at the end of finishing period divided by the number of fattening days of a group. The obtained data of vaccinated and control groups were compared during the weaning, growing and finishing periods.

Backfat thickness, muscle thickness and lean meat content were measured by ultrasound PIGLOG 105 equipment (SFK Technology, Denmark) before slaughtering. The measurement was performed at two predetermined anatomical sites: fat 1 between the 3rd and 4th lumbar vertebrae (7 cm from the midline), fat 2 and muscle thickness between the 3rd and 4th rib (10 cm) and 7 cm from the

midline. The lungs of vaccinated and nonvaccinated pigs were examined at slaughter by the same person. Lung lesions were scored in percent (Goodwin et al., 1968). The extent of lung lesions was recorded onto a lung diagram and surface areas showing pneumonia for each lobe were given a score from 1 to 5. Total score by percent is 55. This consists of the following: left apical lobe 10%, right apical lobe 10%, left cardiac lobe 10%, right cardiac lobe 10%, cranial edge of left diaphragmatic lobe 5%, cranial edge of right diaphragmatic lobe 5% and intermediate lobe 5%.

Percentage of reduction in lung lesion scores relative to the control group was calculated for the vaccinated group by the following formula: mean of control group minus mean of vaccinated group, divided by the mean of the control group, then multiplied by 100.

Lungs with gross lesions were selected for microbiological investigations. All mycoplasma cultivation procedures were performed according to the methods used in the Mycoplasma Section at the Danish Veterinary Laboratory, Copenhagen (Friis, 1974; Friis, 1975). In brief: for mycoplasma isolation, lung tissues were homogenised in a grinder using 5 ml of Friis medium. Lung suspensions were inoculated in 10–100,000-fold dilutions in Friis medium. Inoculation was carried out at +35–37 °C in a roller drum. Cultures with acid shift were subcultured 3–5 times and inoculated on Friis agar. Isolated strains of mycoplasma were identified by the disc growth inhibition test (DGI), using antisera against the type 'J' strain of *M. hyopneumoniae* and strain Ms 42 of *Mycoplasma flocculare*.

At the end of the fattening period, five pigs were randomly selected from each group for slaughter. The left half carcass was dissected as described in Methods for the Control of Pig Fattening and Slaughtering (1978). Samples of the longissimus dorsi muscle (*m. longissimus dorsi*) were analysed in duplicate for dry matter content. Crude protein was examined by a block digestion method, crude ash and ether extract were determined according to standard methods described in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1990*a*). Other parameters were examined as follows: tryptophan: using *p*-dimethylamino-benzaldehyde, according to the method described by Miller (1967); hydroxyproline: as described in the Methodological Guidelines (1978); meat pH: 24 h after slaughter; colour intensity and water binding capacity: by the method of Grau and Hamm as described by Gumeniuk and Tcherkasskaya (1977), cooking losses of meat: by the method of Schilling (1966).

The fatty acid composition of the backfat was determined according to the AOAC (1990*b*). Extraction of lipids for fatty acid analysis was done with chloroform/methanol (2:1, v/v) as described by Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared using the procedure of Christopherson and Glass (1969). The FAME were separated and quantified using a gas chromatograph (Chrom 5) equipped with a flame ionisation detector and integrator (CI-100) (Pustavoy, 1978). A glass column of 2.5 m × 3 mm containing 0.160–0.200 mm

chromaton N-AW-HMDS with 15% diethylene glycol succinate was used. The carrier gas was nitrogen at a flow rate of 100 ml/min. The detector was supplied with dried air at 400 ml/min and hydrogen at 40 ml/min. The detector and injector were maintained at 250 °C and the column was maintained at 180 °C. Peaks were identified by comparison with the retention times of the standard FAME (Sigma Chemical Co.). The data were analysed statistically. The arithmetic average values (X), standard deviation (SD) and coefficients of variation (CV) were calculated for all data. The significance of differences between the average values was determined according to Snedecor and Cochran (1989). $P \leq 0.05$ was an indicator of significance.

Results

Before vaccination, antibodies against *M. hyopneumoniae* were detected in 12 out of the 44 pigs (27%). These maternal antibodies disappeared from all animals after 21 days. Antibodies against *M. hyopneumoniae* in the vaccinated group had been formed by day 14 after the second vaccination. Antibodies could be detected in all vaccinated pigs until the 147th day. In the control group seroconversion started on day 49 (5 sera out of 22). By the end of the study on day 147, 10 out of 22 piglets were positive (Table 1).

Table 1

Presence of *Mycoplasma hyopneumoniae* antibodies in experimental pigs from day 7 to 147 of age

Groups	Result	Day 7	Day 21	Day 35	Day 49	Day 63	Day 91	Day 119	Day 147
Vaccinated	Positive	3	–	22	22	22	22	22	22
	Negative	19	22	–	–	–	–	–	–
Control	Positive	9	–	–	5	7	7	9	10
	Negative	13	22	22	17	15	15	13	12

The DWG increased by 30 grams (g) in the vaccinated group compared to that of the control group. The finishing body weight of the vaccinated animals was significantly ($P < 0.05$) higher (6.04 kg) than that of the nonvaccinated pigs (Table 2).

A significantly lower proportion (3.27%) of lung surface was affected in the vaccinated animals than in the nonvaccinated pigs. *M. hyopneumoniae* was not isolated from lung samples collected from vaccinated pigs but it was cultured from 6 out of 9 lungs in the nonvaccinated group. In vaccinated animals measurements of fat 1 and fat 2 were less by 1.53 and 0.5 mm, respectively, muscle thickness was greater by 1.6 mm and the percentage of lean meat was higher by 0.91 than in the control animals (Table 3).

Table 2

Effect of vaccination with Respisure on some production parameters of pigs

Parameters	Control	Vaccinated	Difference compared with control
Daily weight gain (g/day)	560	590	30*
Weight (kg) after weaning (at 28 days)	7.47	7.70	+0.15
Weight (kg) before fattening	46.86	48.26	+1.4***
Finishing weight (kg)	105.45	111.49	+6.04***
Percentage of lung lesions (according to Goodwin et al., 1968)	90.5	3.27	5.78***

*P < 0.05; ***P < 0.001

Table 3

Effect of vaccination of pigs with Respisure on meat quality

Parameters	Control	Vaccinated	Difference compared with control
Fat 1 (mm)	16.22	15.09	–1.53
Fat 2 (mm)	16.8	16.38	–0.5
Muscle thickness (mm)	53.9	55.5	+1.5
Lean meat (%)	54.78	55.68	+0.91
Dry matter (%)	25.29	24.96	–0.33
Fat (%)	1.67	1.04	–0.63
Protein (%)	22.48	22.87	+0.39
Ash (%)	1.03	1.02	–0.01
Tryptophan (mg %) (T9)	382.0	358.48	23.57**
Hydroxyproline (mg %) (O)	50.52	47.46	–3.06
Water-binding capacity (%)	54.75	55.53	+0.78
Lauric acid, $C_{12:0}$	0.07	0.06	–0.01
Myristic acid, $C_{14:0}$	0.94	0.93	–0.01
Palmitic acid, $C_{16:0}$	27.69	28.85	+1.16
Stearic acid, $C_{18:0}$	10.66	9.70	–0.96*
Saturated fatty acids, total	39.35	39.52	+0.17
Palmitoleic acid, $C_{10:1}$	4.29	4.64	+0.35
Oleic acid, $C_{18:1}$	51.22	48.88	–2.32
Monounsaturated fatty acids, total	55.48	53.52	–1.96
Linoleic acid, $C_{18:2}$	4.38	6.08	+1.7
Linolenic acid, $C_{18:3}$	0.52	0.41	–0.09
Arachidonic acid, $C_{20:4}$	0.34	0.39	+0.05
Polyunsaturated fatty acids, total	5.17	6.82	+1.65

*P < 0.05; ** P < 0.01

Concerning the chemical composition of meat, no difference was found in tryptophan/hydroxyproline (T/O) ratio between the control and the vaccinated group. At the same time, water-binding capacity was higher by 0.78% in the vaccinated group than in the control. Fatty acid composition of the longissimus dorsi muscle showed no significant difference in monounsaturated fatty acid content; however, the very important polyunsaturated acid content was 1.65% higher in the vaccinated group (Table 3).

Discussion

Our data show that the *M. hyopneumoniae* vaccine used in this study (Respisure, Pfizer AH) induced protection against mycoplasmal pneumonia in pigs, which is in agreement with existing data of the literature (Diekman et al., 1999; Maes et al., 1999; Thacker et al., 2000). Antibodies against *M. hyopneumoniae* were detected by a blocking ELISA (Feld et al., 1992). Maternal antibodies were detected till 21 days of age in both groups. Similar results were reported by Morris et al. (1994). In the vaccinated group antibodies were detected 14 days after the second vaccination and were present in 100% of pigs at day 147. This may indicate the long duration of immunity. In the nonvaccinated pigs the *M. hyopneumoniae* antibodies due to natural infection started to appear on day 49 and the rate of seropositivity increased from 22.2% to only 45.5% by the end of trial. This may explain the successful isolation of *M. hyopneumoniae* from the lungs of nonvaccinated pigs. The DWG, one of the most important biological parameters, showed significant difference (30 g) in favour of the vaccinated pig group. The weight gain was lower than that (40 g) observed in field trials with Stellamune Mycoplasma by some authors (Charlier et al., 1994). In other studies the daily weight gain of vaccinated animals increased by almost 60 g in comparison with that of nonvaccinated animals (Schatzmann et al., 1996). Percentage reduction of lung lesions in the vaccinated group reached 64% and only 3.25% of the lung surface was affected. Ross et al. (1992) described much more extensive lung lesions (30 to 80%) in slaughter pigs. On the basis of the present study we can state that vaccination did not exert a negative effect on meat quality. On the contrary, some parameters (water-binding capacity, concentration of polyunsaturated fatty acids, lean meat %) were even better in the vaccinated group. However, much more data should be generated in this respect to prove the positive effect of vaccination on meat quality.

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