Chemical evaluation of the quality of meat originating from pigs vaccinated with experimental bivalent vaccine against colidiarrhoea and colienterotoxemia

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Summary

In intensive pig production, large and stubborn problems are losses among suckling, especially in weaned piglets caused by infection of enterotoxigenic Escherichia coli (ETEC), usually F4ac+ and / or F18ac+ strains. The research within the project 053-0532265-2255 was established satisfactory imunoprophylactic effect of experimental oral F4ac+ and / or F18ac+ non-ETEC vaccine against colidiarrhoea and colienterotoxemia in weaned piglets. However, in the m. longissimus dorsi orally vaccinated pigs with a bivalent vaccine (containing two strains of E. coli / F4ac+ no ETEC strain P407 and not P4ac+ no ETEC strain P407 and not P4ac+ no experimental oral pigs in relation to the chemical composition of m. longissimus dorsi nonvaccinated pigs.

Key words: pigs, experimental non-ETEC vaccine, meat quality, m. longissimus dorsi

Introduction

In intensive pig production, large and stubborn problems are losses among suckling, especially weaned piglets caused by infection of enterotoxigenic Escherichia coli (ETEC), usually F4ac+ and / or F18ac+ strains. Stressful conditions of intensive farming, which occur in the first few days (usually between 3rd and 7th day of life) at farrowing or immediately after weaning (usually between 21st and 28th day of life), representing a trigger for the invasion immune immature and incompetent young piglets with ETEC strains, which rapidly multiply and colonisated the mucosa of the small intestine, producing up to 10 times more bacterial cells than total cells of the body of the host. Through F4ac and/or F18ac fimbrial adhesins, they bind to specific receptors on enterocytes and secreted enterotoxins, which cause damage to the intestinal epithelium, disorders of absorption, and secretory function of the mucous membrane, resulting with diarrhea, dehydration and acidosis, as in the case of colidiarrhoea, or induce local edema, systemic toxemia and inflammatory lesions in the brain, as in the case of enterotoxaemia of weaned piglets, with a consequent loss of body weight and/or death. Despite the central role of ETEC strains in pathogenesis of colidiarrhoea/colienterotoxemia in weaned pigs, except hereditary factors (recessive homozygotes have receptors for adhesins fimbriatus), predisponing factors are also immune factors (adult pigs acquired immune competence between 7th and 9th weeks of life), because of functional immaturity of systemic and local (gut) immunity produced tolerogenic rather than protective immune responses to intraluminal antigens (Sorce: project 053-0532265-2255). On the basis of literature data it is known that in order to monitoring and prevention of colienterotoxemia and colibacillosis in weaned piglets commonly used vaccines containing monovalent F4+ or F18 + non - ETEC strains, while Tielse et al. (2008) considered that orall administration of bivalent vaccine containing F4+ and F18+ live E. coli strains achieve satisfactory protection of postweaning colibacillosis and colienterotoxemia. Although today there is no effective vaccine against colidiarrhoea and colienterotoxemia, it is known that experimental bivalent F4ac+ and F18ac+ non-ETEC vaccine (VAK, strain 2407, and 2143), administered orally in weaned piglets on the day of weaning (10x1010 CFU/60 mL TSB) is effective in the prevention and control of natural intestinal infections, especially caused by pathogenic strains of E. coli. Therefore, the aim of this study was to evaluate the chemical quality of meat originating from pigs vaccinated with experimental bivalent vaccine (containing two strains of E. coli / F4ac+ non ETEC strain 2407 and F18ac+ non ETEC strain 2143) to prevent colidiarrhoea and colienterotoxemia.

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Material and methods

The research was conducted within the Ministry of Science, Education and Sports project No.: 053-0532265-2255. The study was carried out in 42 days on pig farm in the eastern part of the Republic of Croatia. A total of 40 crossbred pigs (LandracexYorkshirexPietrain), equal number of females and castrates, with an average body weight of 6 kg and good health status were weaned at the age of 26 days. They were divided into two experimental groups (A, C), each containig 20 animals. To piglets from group C (control group) 0 day of the experiment was applied orally 60 mL trypticase soy broth (TSB). To piglets from group A 0 day of the experiment was orally applied bivalent vaccine (containing two strains of E. coli / F4ac+ non ETEC strain 2407 and F18ac+ non ETEC strain 2143) in a dose of 10 x 1010 in 60 mL trypticase soy broth. At the end of the experiment, 42nd day, the animals were detained slaughtering process. From both experimental groups were sampled muscles in the amount of 100 g (m. longissimus dorsi) in order to test the effect of the vaccine on the chemical quality of meat. Experimental animals were treated according to the Croatian legislation on animal protection and in accordance with the recommendations of the European Community (86/609/EEC). At the Department of Hygiene and Technology of Foodstuffs, Faculty of Veterinary Medicine, University of Zagreb shares of water (method ISO 1442 standard), fat (method ISO 1443 standard), protein (method ISO 937 standard) and ash (method ISO 936 standard) were determined. Statistical analyses of experimental data in content of fat and protein in meat of control (untreated) group of animals (C group) and treated group (A group) of piglets, were provided using the t-test for dependent samples.

Results and Discussion

Since previous studies of numerous research groups were not fully defined the safty and effective immunoprophylaxis against colidiarrhoea and colienterotoxemia, it is necessary for further research of administration of attenuated oral vaccine, to the known phenomenon of oral tolerance, as well as the paradoxical physiological role of the mucous of the intestine to absorb intraluminal contents (nutrients/nutraceutic) and to simultaneously discriminate between innocuous antigens from food and harmful antigens of enteric pathogens and their products. During many years of research within the project 053-0532265-2255, was found satisfactory imunoprophylactic effect of experimental oral F4ac+ and / or F18ac+ non - ETEC vaccine against colidiarrhoea and colinterotoxemia of weaned pigs or confirmed thesis of the immunogenic and protective ability of vaccine strains based on production, immune and health indicators (Kovšca et al., 2008; 2009; 2010; Popović i sur., 2008.; Valpotić et al., 2010). According to abovementioned, our research has focused on evaluating effects of bivalent non-ETEC vaccine on the chemical quality of meat originating from vaccinated pigs.

Table 1. Descriptive statistics for the parameters of chemical content of 100 g m. longissimus dorsi of non-vaccinated (C group) and vaccinated (A group) animals.

Variable	Mean(%)	St. Dev.	Var.	SEM
C- m. longissimus dorsi voda	72.29	0.398968	0.159176	0.106629
C- m. longissimus dorsi pepeo	1.28	0.219890	0.048352	0.058768
C- m. longissimus dorsi mast	2.14	0.388644	0.151044	0.103869
C- m. longissimus dorsi bjelančevine	24.19	0.310706	0.096538	0.083040
A- m. longissimus dorsi voda	71.41	0.392232	0.153846	0.104828
A- m. longissimus dorsi pepeo	1.03	0.329585	0.108626	0.088085
A- m. longissimus dorsi mast	2.20	0.360403	0.129890	0.096322
A- m. longissimus dorsi bjelančevine	23.88	0.429413	0.184396	0.114765

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This is supported by research results of Siugzdaite et al. (2003) on the impact of Mycoplasma pneumoniae vaccine (Respisure Pfizer AH) to changed quality of the meat of vaccinated pigs. Namely, in the meat of animals vaccinated by Mycoplasma hyopneumoniae vaccine (Respisu-

impact on the content of the basic ingredients (especially fats) in pig's muscles (Table 3). Results are shown in Table 1 are not fully in concordance with the results of Večkovec (2012), which determined the amount of protein from 16 to 19.4 % and the fat content from 12.9 to

Table 2. Chemical content of muscle tissue (%) (modified from Petričević et al., 2001).

	Indicator	Mean (%)
Muscle tissue	water	72.63-73.01
	proteins	23.53-23.62
	fat	2.25-2.69
	ash	17.80-19.74

re Pfizer AH) was significantly reduced the amount of fat (-0.63%) and the tryptophan/hydroxyproline (-23.57%) compared to non-vaccinated animals. In addition, in the m. longissimus dorsi vaccinated animals compared to non-vaccinated animals was observed for the 0,35% higher amount of protein, 0.78% higher value of the water

22.5 % by the chemical analysis of muscle and fat tissue of pigs in same experiment. These results are expected due to the different anatomical position of the samples in the experiment. All results indicate a need for further research of the effect of orally applied bivalent vaccine (containing two strains of E. coli/ F4ac + no ETEC strain

Table 3. The content of the basic chemical ingredients in m. longissimus dorsi (Rahelić (1984.. cit. Kralik. 2007).

Pig breed	Ingredients (%)					
	Water	Proteins	Fat	Ash	Connective tissue	UP (ppm)
Black slavonian pig	71.43	21.28	5.93	1.20	0.28	14.34
Large white Yorkshire	74.17	22.09	1.69	1.25	0.38	17.62
Swedish Landrace	73.52	22.49	1.99	1.18	0.37	18.62

binding capacity, 1.65% higher amount of polysaturated fatty acid, and 2.97% lower amount of monosaturated fatty acids (Siugzdaite et al., 2003). Results of our research, in Table 1 and on the basis of Table 2, it is shown that the water content, ash, fat and protein in the bulk sample of m. longissimus dorsi originating from non-vaccinated animals (Group C) in accordance with the results of Petricevic al. (2001; Table 2). Furthermore, in a sample of m. longissimus dorsi vaccinated animals (group A) were observed statistically insignificant changes due to water, ash, fat and protein content (probability of error greater than 0.05) compared to the unvaccinated group of animals (Group C). Rahelić (1984, cited Kralik, 2007) states that breed and level of refinement can have a significant

2407 and not F18ac + ETEC strain 2143) on the chemical composition of pig meat.

Furthermore, although statistically insignificant changed, the chemical composition of m. longissimus dorsi of pigs vaccinated with bivalent vaccine (containing two strains of E. coli/ F4ac + no ETEC strain 2407 and not F18ac + ETEC strain 2143), independently of its established beneficial effects on health, immune and production indicators are justification for further exploration of alternative strategies for the control and prevention of infections of animals for consumption in intensive farming (VIP project: No. 2012-11-17), in relation to specific prophylaxis (Mršić et al., 2013; Špoljarić et al., 2013; Špiranec et al., 2013).

Conclusion

Based on multi-year evaluation of our model for the prevention and control of intestinal infections of weaned pigs, with the current support of Ministry of Science, Education and Sports, we have determined relevant scientific approach to the health problems in intensive pig farming.

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References

Kovšca Janjatović, A., G. Lacković, F. Božić, M. Popović, I. Valpotić (2008): Levamisole synergizes proliferation of intestinal IgA(+) cells in weaned pigs immunized with vaccine candidate F4ac(+) nonenterotoxigenic *Escherichia coli* strain. Journal of Veterinary Pharmacology and Therapeutics. 31, 328-333.

Kovšca Janjatović, A., G. Lacković, F. Božić, D. Špoljarić, M. Popović, H. Valpotić, N. Vijtiuk, Ž. Pavičić, I. Valpotić (2009):. Histomorphometric characteristics of immune cells in small intestine of pigs perorally immunized with F18ac+ nonenterotoxigenic *E. coli* vaccinal strain. European journal of histochemistry. 53, 189-198.

Kovšca Janjatović, A., G. Lacković, F. Božić, D. Kezić, M. Popović, H. Valpotić, I. Harapin, Ž. Pavičić, B. Njari, I. Valpotić (2010): Histomorphometric evaluation of intestinale cellular immune responses in pigs immunized with live oral F4ac+ non-enterotoxigenic vaccine *E. coli* against postweaning colibacillosis. European journal of histochemistry. 54, 18-24.

Kralik, G. (2007.): Porijeklo, pasmine i proizvodnost svinja. Poglavlje u sveučilišnom udžbeniku Svinjogojstvo – biološki i zootehnički proncipi (autori: Kralik G., Kušec, G., Kralik, D., Margeta, V.), Grafika d.o.o., Osijek.

Mršić, G., B. Njari, S. Srečec, M.J. Petek, Ž. Cvrtila Fleck, M. Živković, K. Špiranec, D. Špoljarić, D. Mihelić, L. Kozačinski, M. Popović (2013): Kemijska ocjena kakvoće pilećeg mesa podrijetlom od tovnih pilića hranjenih uz dodatak pripravka plemenite pečurke *Agaricus bisporus*. Meso: prvi hrvatski časopis o mesu. 15, 300-306.

Popović, M., H. Valpotić, M. Kardum Paro, Ž. Pavičić, N. Vijtiuk, I. Popović, R. Resanović, I. Valpotić (2008): Qualitative and quantitative parameters of swine cell immunity. Acta veterinaria (Beograd). 58, 149-158.

Petričević, A., G. Kralik, D. Gutzmirtl, T. Crnjac, V. Margeta (2001.): Utjecaj hranidbe na kakvoću svinjskih polovica u obiteljskim gospodarstvima. Poljoprivreda 7, 1, 47-51.

Siugzdaite J., K. Garlaite, D. Urbsiene (2003.): Evaluation of antibody formation, daily weight gain and meat quality after vaccination of piglets against Mycoplasma hyopneumoniae. Acta Vet Hung. 51 (3): 273-81.

Špiranec, K., D. Špoljarić, G. Mršić, I. Špoljarić, S. Srčec, Ž. Cvrtila Fleck, D. Mihelić, L. Kozačinski, M. Popović (2013): Kemijska ocjena kakvoće pilećeg mesa podrijetlom od tovnih pilića hranjenih uz dodatak zeolit klinoptilolita. Meso: prvi hrvatski časopis o mesu. 15, 464-466.

Špoljarić, D., G. Mršić, M. J. Petek, I. Špoljarić, S. Srčec, Ž. Cvrtila Fleck, K. Špiranec, Katarina; D. Mihelić, L. Kozačinski, M. Popović (2013). Kakvoća pilećeg mesa podrijetlom od tovnih pilića hranjenih uz dodatak prirodnog propolisa. Meso: prvi hrvatski časopis o mesu. 15, 380-383.

Tiels P., F. Verdonck, A. Coddens, B. Goddeeris, E. Cox (2008.): The extretion of F18+ *E. Coli* is reduced after oral immunisation of pigs with a FedF and F4 fimbriae conjugate. Vaccine, 2154-2163.

Valpotić, H., A. Kovšca Janjatovića, G. Lacković, F. Božić, V. Dobranić, I. Valpotić, M. Popović (2010). Increased number of intestinal villous M cells in levamisole - pretreated weaned pigs experimentally infected with F4ac+ enterotoxigenic *Escherichia coli* strain. European journal of histochemistry. 54, 88-91.

Večkovec, A. (2012.): Učinci zeolit klinoptiolita i pokusnog dvovaljanog cjepiva protiv kolidijareje i kolienterotoksemije na proizvodnost i imunosni odgovor odbijene prasadi. Disertacija. Veterinarski fakultet Sveučilišta u Zagrebu. Str. 180.

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