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Field application of egg yolk immunoglobulin as the feed additive in prophylaxis of diseases in weaned piglets*)

TADEUSZ STEFANIAK, ANNA RZĄSA, PAULINA JAWOR, ARTUR ZYZAK, WIKTOR NIEMCZUK*, MARIAN KUCZAJ**, MAREK POPŁAWSKI***, JACEK BORKOWSKI****

Department of Immunology, Pathophysiology and Veterinary Preventive Medicine, ul. Norwida 31, 50-375 Wrocław
*Department of Epizootiology and clinic of Bird and Exptic animals, Pl. Grunwaldzki 45, 50-366 Wrocław
**Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, l. Chełmońskiego 38C, 51-630 Wrocław
***POL-LEAN Sp. z o. o., Ferma Trzody Chlewnej, 55-090 Łosice
****Department of Physiology and Biochemistry, University School of Physical Education in Wroclaw, al. l. J. Paderewskiego 35, 51-612 Wrocław

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Stefaniak T., Rząsa A., Jawor P., Zyzak A., Niemczuk W., Kuczaj M., Popławski M., Borkowski J.

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Summary

The influence of yolk immunoglobulin (IgY) as the feed additive for piglets during first two and three weeks after weaning on the selected production and health parameters was evaluated in field conditions. Piglets were fed 0.5 g IgY/kg feed (n = 87), 1 g IgY/kg (n = 180) and 2 g IgY/kg (n = 384). Control group was 847 piglets fed the same feed without IgY. IgY preparation was obtained by air drying the purified yolk gammaglobulin from non-immunized hens. IgY concentration was estimated using radial immunodiffusion and antibody activity against selected enterotoxigenic bacteria using ELISA. It was found that it minimized the losses caused by alimentary tract pathology, but not influenced respiratory tract infections. Higher weight gain was observed in the experimental groups. The addition of 2 g IgY/kg of the feed may be recommended in field conditions in the postweaning period.

Keywords: IgY, weaned piglets, diarrhea

Stress associated with weaning, changes in social relations, as well as change of feeding (including lack of sow milk) are important factors that influence the piglets' susceptibility to infection (23). Those factors contribute to the gastrointestinal tract disturbances (9). Diarrhoea is the most common problem in piglets before 2-3 weeks of life and in postweaning period (8, 11, 14). Passive immunization through oral administration of antibodies derived from serum, colostrum or chicken egg yolk may be effective method to counteract diarrheal diseases in piglets (23). The protective effect of immunoglobulin preparations is usually dose dependent (1, 21). Apart from spray dried swine plasma (12, 13), large interest is associated with egg yolk immunoglobulin (IgY), because of its large scale production possibilities exceeds significantly other sources of immunoglobulins (4, 6, 24, 25). IgY has been found to be relatively resistant to proteases what increases its biological activity within gastrointestinal tract. It is also easy to obtain specific antibody. Hens immunized with vaccines assigned for different species produced and transferred specific antibody to yolk (22). The IgY biological function within the piglets' intestine is similar to that of bovine colostrum immunoglobulins (23).

IgY preparations are produced as feed additives in Germany, Czech Republic, South Korea, Japan, but not in Poland (3, 7, 18). High antibody activity of IgY preparation from non-immunized hens was detected against *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Campylobacter jejuni* and they suppressed the colonization of laying hens' intestine (5).

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Additive 1-2 g IgY from non-immunized hens/kg to the broiler chicken feed reduced gastrointestinal tract *Salmonella* Enteritidis carrier after experimental challenge (26). Addition of IgY from non-immunized hens to the piglets' feed mixture allowed to obtain higher daily gains (15).

The aim of the study was to investigate the effect of IgY feed additive on the gastrointestinal and respiratory tract disturbances, as well as rearing results in weaned piglets in field conditions.

Material and methods

Production of IgY preparation. Eggs were harvested at laying hens farm (Lohman Brown line). Yolk egg immunoglobulin preparation used in experiments I-III was produced on laboratory scale according to patent No 191137 [WUP 03/06, Sposób otrzymywania preparatu gamma globuliny z żółtka jaja kurzego (The method of egg yolk gammaglobulin production)]. After salt precipitation and desalting by diafiltration the preparation was spray dried on the experimental drying column. In experiment IV spray dried egg yolk plasma was utilized. Egg yolk plasma was obtained by sedimentation in acidic conditions, it was spray dried as described above. The presence of *Salmonella* sp. and *Staphylococcus aureus* in produced series of IgY preparations was excluded by bacteriological culture.

Samples (250 mg) from 74 preparations series (69 egg yolk gammaglobulin preparations and 5 egg yolk plasma preparations) were dissolved gently in 4 ml of PBS, and dialyzed overnight against PBS. Total protein concentration was measured by biuret method, and established on 10 g/l. IgY content was measured by radial immunodiffusion in self modification of Mancini method (25). Preparations of known IgY concentration were diluted to obtain 0.1 g IgY/l in PBS containing 0.05% Tween 20 (PBS-T) and antibody activity against whole cell strains of: *Eschericha coli* O157, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Klebsiella pneumoniae* was estimated using non-competitive, indirect ELISA (24). As positive control pooled hen serum and as negative control bovine serum albumin were used.

Respective series of IgY preparations were pooled to obtain mean antibody activity and IgY concentration and next obtained preparation was used as feed additive in the experiments according to doses presented in Table 1. In trials I-III to the commercial feed used at farms additive of IgY preparations were added using mechanical stirrer at farms. Spray dried egg yolk plasma used in trial IV was added during feed production process.

Tab. 1. Size of experimental groups and doses of IgY added to the fodder

Trial	Experimental groups			
	0.5 g IgY	1 g IgY	2 g IgY	Control
I /farm „L"	15	15	15	15
II/farm „L"	72	70	70	138
III/farm DS.	nt*	95	86	47
IV/farm „Ł"	nt*	nt*	213	647
Total	87	180	384	847

Explanation: *nt – not tested

Experiments with animals. Four field trials were carried out on weaned cross-breed piglets polish landrace × polish large white (pbz × wbp) on the 3 commercial farms. The number of experimental animals and the levels of IgY additives to the feed are shown in table 1.

Trial I and II was carried out in modernized pig farm type Bisprol 12000. Piglets were weaned at 24th day of life and moved to nursing sector to pens with plastic slatted floor for 15 heads. The nursing building was divided into 10 rooms, every for 4 pens.

Trial III was carried out in old type pig farm, without separate nursing sector. Piglets were weaned on the 42nd day of life and moved into pens for 22 heads on average. Three straw bedded pens were placed close to farrowing pens, and 7 were in fattening sector with slatted floor.

Trial IV was carried out in modernized pig farm type Bisprol 6000. Piglets were weaned on the 28th day of life and moved to nursing buildings for 200 heads. Every nursing room consisted of 10 pens with plastic grilled floor.

In trials I-III piglets obtained feed containing IgY during first two weeks and in the trial IV for first 3 weeks after weaning.

All piglets were weighed at weaning and after 30 days. Illnesses including type and intensity of pathology were counted, as well as mortality cases. In the trial IV additionally feed conversion (FCR) was evaluated.

Statistical analysis. Comparisons of results were made by the analysis of variance procedures at non-orthogonal scheme using the Statistica 9.1 statistical package (StatSoft Inc., Tulsa, OK). Post-hoc analyses were determined by the Duncan test (trial II and III) and NIR test (trial IV) at 0.05 and 0.01 p-values. Statistical significance were marked with different small ($p \leq 0.05$) and capital ($p \leq 0.01$) letters. The results were presented as mean and standard deviation.

The study design was approved by the Second Local Ethics Commission for Experiments on Animals in Wrocław (no. 69/2007, 58/2011).

Results and discussion

Estimation of IgY content and antibody activity in egg yolk immunoglobulin preparations from non-immunized hens. In collected series of IgY preparations (Tab. 2) there was found high concentration of IgY (14-68% of total protein) and antibody activity against strains isolated from diarrhoeic piglets. The antibody activity against whole cells of investigated strains: *Eschericha coli* 0157, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Klebsiella pneumoniae* was compared to pooled samples of hens' serum used as the positive control. Mean IgY concentration in spray dried plasma preparations was lower compared to study Rząsa et al. (17). Salt precipitation allowed to obtain the higher IgY content (exceeding 40%), however there was high diversity in IgY content among series. It indicates that the drying conditions (time, temperature) varied as the consequence of different volume of dried IgY solutions in successive series.

Tab. 2. Characterization of IgY preparations (E.c. – *Escherichia coli* strain O157, S.t. – *Salmonella* Typhimurium, S.e. – *Salmonella* Enteritidis, K.p. – *Klebsiella pneumoniae*)

	IgY content (%)	ELISA reaction intensity (0.1 g IgY/dm^3)			
		E.c.	S.t.	S.e.	K.p.
Mean from 69 purified IgY preparations The mean IgY from 5 yolk plasma preparations	41.55 ± 17.12 18.2 ± 6.9	0.290 ± 0.120	0.345 ± 0.123	0.320 ± 0.144	0.215 ± 0.160
Mean of pooled hen serum (positive control)		1.050 ± 0.132	1.232 ± 0.150	1.113 ± 0.126	0.840 ± 0.087
Rated evaluation of activity		100%	100%	100%	100%
Activity of spray dried preparations as compared to positive control		27.62%	28.00%	28.75%	25.59%

There was observed decrease of antibody activity of spray-dried IgY (measured as an absorbance) to 25.59--28.75% (against *Klebsiella pneumoniae* and *Salmonella* Enteritidis strains respectively) compared to positive control (Tab. 2). It was expected that high temperature used at spray drying may inactivate part of IgY antibodies. Results from the former study made by our group showed that lyophilization had no negative effect on antibody activity (not published). However because of high costs this method is not suitable for large scale production of feed additives for livestock animals.

Tab. 3. Daily gains of piglets (trial I)

Group	Group daily gain		Piglets daily gain (g)	Mean total body weight gain (kg)
	(kg)	% (as 100% daily gain of control group)		
0.5 g IgY	119.85 ± 81.54	113.84	266 ± 54	7.99 ± 0.65
1 g IgY	118.50 ± 79.23	112.56	263 ± 49	7.90 ± 0.71
2 g IgY	119.70 ± 82.34	113.70	266 ± 56	7.98 ± 0.66
Control	105.28 ± 83.21	100	251 ± 58	7.52 ± 0.58

Experiments with animals.

Trial I. There was no signs of pathology in experimental groups. Only one control piglet showed diarrhoea symptoms and died and there was isolated *E. coli* from its intestine. Mean daily gains for whole populations were 261 g (Tab. 3.) and this result may be assumed as satisfactory as compared to others (14). The lowest daily gain were observed in control piglets (251 g), the slightly higher were noted in groups obtained 0.5 and 2 g additive of IgY/kg.

Trial II. In the experiment repeated in the same farm, according to the same scheme but on higher number of animals (350 heads) no gastrointestinal tract pathology was found. Respiratory tract infections occurred in 5% of control piglets and 2.8% of piglets fed 2 g IgY/kg (differences statistically not confirmed). At the same time 5.8% of control piglets died, whereas only 2.8% of piglets fed 1 g IgY/kg. There was no loses in remaining groups (0.5 g and 2 g IgY/kg) and this result appeared significantly important at $p \leq 0.05$ compared to mentioned earlier two groups. It seems that IgY additive reduced health disturbances (morbidity and mortality) in experimental groups compared to control one, similarly to results observed by Yokoyama at al. (27), but due general low incidence of disturbances in our experimental groups it is difficult to confirm positive effect of increasing dose.

Trial III. Table 4 and 5 shows selected parameters describing health status and causes of death in weaned piglets reared in old fashion farm. The lowest rate of diarrhoea occurred in piglets fed 2 g IgY/kg and this result compared to remaining groups was statistically different ($p \leq 0.01$). The same tendency was observed in accidents of severe diarrhea.

Tab. 4. Morbidity of weaned piglets (trial III)

Morbidity		Groups		
		1 g IgY	2 g IgY	control
Diarrhoea:				
1-2 days (mild)	% min-max	22.11 17.65-29.41	24.41 19.35-33.33	25.53 16.67-31.03
≥ 3days (severe)	% min-max	9.47A 5.88-14.71	2.33Bb 0-6.67	6.38a 0-10.34
Total	% min-max	31.58A 23.53-44.12	26.74B 19.35-40	31.91A 16.67-41.38
Respiratory tract infections:				
1-2 days (mild)	% min-max	6.32 2.94-8.82	10.47 6.45-13.33	8.51 5.56-10.34
≥ 3days (severe)	% min-max	6.32 2.94-8.82	4.65 0-6.67	4.26 3.45-5.56
Total	% min-max	11.57 11.11-11.76	15.12 12-20	12.77 11.11-13.79
Total morbidity:				
	% min-max	43.16 35.29-55.88	41.86 32-60	44.68 27.78-55.17

Tab. 5. Loses of piglets due to mortality and cachexia (%) (trial III)

Group		Mortality caused by			Cachectic piglets
		diarrhoea	respiratory tract infections	total	
1 g IgY	% min-max	7.37 5.88-8.82	5.26 2.94-8.82	12.63 8.82-17.65	17.02 11.11-20.69
2 g IgY	% min-max	4.65 3.23-6.67	4.65 3.23-6.67	9.30 6.45-13.33	13.95 12.00-16.67
Control	% min-max	4.26 3.45-5.56	4.26 3.45-5.56	8.51 6.90-11.11	16.84 11.11-26.47

This result was statistically different to observed in 1 g IgY/kg group at $p \leq 0.01$ and at $p \leq 0.05$ compared to control group. The diarrhoea incidents frequency was similar in control and piglets fed 1 g IgY/kg, but severe cases occurred at higher rate in experimental (9.47%), than in control (6.38%) group. The protective effect of IgY seems to be dose dependent. Similar tendency was observed in Chernysheva et al. (2) study where prevalence of diarrhea and mortality was the smallest in group fed higher dose of IgY (3.2 g per 1 kg of feed) and slightly higher in group obtained lower dose of IgY (0.32 g/1 kg feed) than in control group.

Respiratory tract infections occurred the lowest frequency in piglets fed 1 g IgY/kg, the highest was observed in piglets fed 2 g IgY/kg (Tab. 4). Severe course of respiratory tract infections occurred in the lowest frequency in control animals and in group fed 2 g IgY/kg, and the highest frequency was observed in a group fed 1 g IgY/kg, but this diversity wasn't confirmed statistically (Tab. 4).

Morbidity rate (Tab. 4) was only slightly lower in experimental groups comparing to control one. Very high diversity of morbidity within groups was probably caused by fluctuation of the air temperature outside of the building, and as the consequence fluctuation of humidity and temperature within the piggery.

Mortality caused by diarrhoea and respiratory tract infections (Tab. 5) was the lowest in control piglets (8.51%), slightly higher in piglets fed 2 g IgY/kg (9.3%) and the highest in piglets fed 1 g IgY/kg (12.63%). In contrast, the percentage of cachectic piglets (Tab. 5) was the lowest in group fed 2 g IgY/kg (13.95%) whereas in control piglets and animals fed 1 g IgY/kg was similar (16.84 and 17.02% respectively). Higher dose of IgY (2 g IgY/kg) could protected piglets from morbidity and cachexy, but not prevented mortality of sick animals. Probably higher dose of IgY (similarly to that used by Chernyseva et al. (2) could be sufficient to decrease piglets' mortality.

The highest daily gain (328 g) was observed in piglets fed 2 g IgY/kg, it was distinctly higher than in piglets fed 1 g IgY/kg (279 g) and control (207 g) (Fig. 1). This difference was noticeable even by the farm staff. It seems that out of protective effect of IgY antibodies the positive effect might be associated

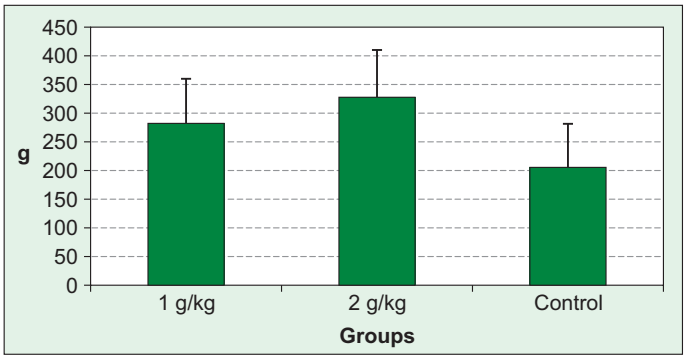

Fig. 1. Average daily gains of piglets in Trial III

Tab. 6. Some selected production parameters (trial IV)

Parameter		Group	
		2 g IgY	Control
Number of piglets: – 28 day of life – 60 day of life	 no/% no/%	 213/100 203/95.3	 647/100 626/96.7
Culled during experiment period	no/%	6/2.8A	2/0.3B
Mortality	no/%	4/1.8	19/2.9
Mean daily gains	kg/%	0.501/106.3	0.471/100
FCR head/day	kg/%	1.49/95.5	1.56/100

with better palatability of the feed containing egg yolk additive.

Trial IV. Table 6 presents selected production parameters to evaluate the effect of application 2 g IgY/kg feed during 3 weeks after weaning. Piglets fed the mixture containing egg yolk plasma presented higher daily gains. In experimental group mortality rate was lower than in control one, but 1.1% difference wasn't statistically important. Significantly ($p \leq 0.05$) more piglets from experimental group showed poorer growth rate and were culled during the study. Calculated FCR was about 5% more efficient in experimental animals.

IgY doses used in presented study (all experiments) were at lower range of doses utilized by other authors (0.2-5%) who used IgY from eggs of as well as non-immunized hens was applied (10, 12, 13, 15, 19, 20, 25, 27). The confirmation of protective effect of low IgY doses in piglets rearing is very important from practical point of view, because of production costs of such feed additive.

Different feed additives may influence the production results by two ways: 1) stimulation of growth rate through metabolism improvement and/or increasing feed intake; 2) stabilization and enhance the protective barrier against gastrointestinal tract disturbances. Application of the IgY in weaned piglets may be joined to both types.

Based on the results obtained in different farms that differed at maintenance system, technology level and number of animals it may be concluded, that higher growth rate was observed in piglets obtaining IgY with feed. Applied additive positively affected the palatability of the feed (trial III), and it might improve stabilization and growth of normal/desirable gut microflora (14). Morbidity and mortality evaluation differed between trials and between groups fed with different IgY doses. Similarly in study of Zuo et al. (28), although no full protection against morbidity and mortality was received, the survival rate increased when 2 g IgY were added to the feed. The possible explanation may be that the presence of IgY within the gut lumen limited the extend of gastrointenstinal tract injury in experimental animals. The morbidity and mortality rate, the occurrence of cachectic piglets were limited in piglets obtained feed containing 2 g IgY/kg. This dose could be recommended for field conditions.

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Corresponding author: prof. dr hab. Tadeusz Stefaniak, Katedra Immunologii, Patofizjologii i Prewencji Weterynaryjnej, Uniwersytet Przyrodniczy we Wrocławiu, ul. C. K. Norwida 31, 50-375 Wrocław; e-mail: tadeusz.stefaniak@up.wroc.pl