Influence of iron and immunomodulators on growth performance and immunohematological status of piglets

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Abstract

This study was conducted to determine the effects of supplementation with two different products, containing iron and immunomodulatory components, that are used in piglets to prevent anemia and to improve their immunity and growth performance up to weaning. Twenty four piglets from three litters were randomly assigned to one of the 3 groups (n = 8 per group) representing control group (no supplementation), group with orally administrated iron and group receiving injectable form of iron. The growth performance (mean daily gain weight, weight at birth and at weaning), hematological (erythrocyte counts, hemoglobin, hematocrit, mean cell volume) and immunological (white blood cell counts, percentage of lymphocytes and granulocytes) parameters were evaluated. Both formulations used in this study improved the growth performance of piglets. Both treated groups did not differ significantly in their somatic development, additionally supplementation with both formulations improved all investigated parameters, however Suiferrovit (injectable form) had better antianemic effect, while Imunoferr (oral form) was more effective as an immunostimulatory agent.

Key words: *piglets, immunomodulation, iron, immunity, hematology, growth performance.*

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Introduction

Maintaining a good immune status helps animals to protect itself against pathogens [1, 2]. Low immune status may causes problems in animal production with high influence on production efficiency [2]. It is especially important in young animals, when passive immunity is lowered and adaptive immunity has not yet fully developed [3]. Nutritional means, including dietary supplementation with glutamine [4, 5] zinc [6], probiotics [7] or herbs [8] have been used to improve immunity of piglets. However, due to low feed intake in the first weeks of life, these approaches could be difficult to obtain [5, 9]. In modern intensive pork production system, pigs are weaned between 15 and 28 days of age [8, 10]. Weaning is associated with growth retardation as well as an increase in both, morbidity and mortality in piglets [8, 11]. This has led to the development of feed additives with high efficiency and low toxicity, in order to boost nonspecific the immune systems and improve the host defenses of young pigs during weaning [12].

Additionally, iron deficiency anemia (hypochromic, microcytic anemia) is well-known in piglets in early life [13, 14]. Anemia occurs because piglets are born with unusually small iron stores, milk contains low levels of iron, and pigs have a very rapid growth rate. During the first 4 weeks of life, the body weights of the piglets increase by about 4-fold. The dietary requirement for iron during this period is 7 mg per day, and only 1 mg per day can be supplied by the sow's milk [13, 15-17]. The need to provide the piglets with an adequate amount of iron before weaning, is therefore essential because sow's milk alone will not meet the iron requirements of rapid growth and expanding blood volume [15]. Anemia interferes with growth, and anemic pigs are lethargic and more susceptible to infection than healthy ones [11]. For that reason, beside iron

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supplementation, lots of products intended for pigs to prevent anemia contain also immuno-modulating agents.

Several different methods can be used to offer iron to young piglets. The most common administration method, is through an intramuscular or subcutaneous injection of iron. However, this method is very stressful to the piglets. Furthermore, it may cause trauma to the muscles [15]. That is why, an alternative methods need to be considered i.e. supply iron orally [15, 16].

This study was conducted to determine the efficacy of new commercial product (for oral administration) intended for pigs to prevent anemia and to increase piglets immunity, with comparison to untreated group (negative control), and to group treated parenterally.

Materials and methods

Products

Imunoferr (Biofactor). It is a new product intended for pigs, recommended for piglets, sows, weaners and fatteners to prevent anemia and as an immunostimulant. According to the manufacturer, it improves also the weight gain. It contains microelements (manganese sulfate, cobalt sulfate, copper sulfate and iron sulfate) and vitamins B_1, B_2, B_6, B_{12}, L-carnitine, taurine, methionine, lizine, and glucose.

Suiferrovit (Biowet Puławy). It is a product that is intended for piglets to prevent anemia and increase immunity. It contain an inactivated pig serum, enriched with iron dextran, microelements (copper chloride II, cobalt chloride II, calcium pantothenate) and vitamins B_1, B_2, B_6, P.

Animals

A total of 24 piglets from three litters, derived from local farm were used. The farm has closed production cycle and the basic herd consists of 60 sows. Batches of 9 sows were formed every 21 days. Complete management and health data for the sows and their offspring were maintained. The prophylactic program at the time of pregnancy consists of vaccination the sows with inactivated vaccines against atrophic rhinitis and colibacillosis. Production was in all in-all out procedure with a thorough cleaning between batches. Ten days before partum the sows were moved to the individual farrowing pens (2 × 2.5 m). The piglets were weaned at approximately 26-28 days.

Piglets were divided into 3 groups (n = 8 per group). The piglets from the control group (C) did not receive any iron or immunomodulatory product, beside PBS intramuscularly as placebo (2 ml/animal). The piglets from group A received Imunoferr orally, in 3 and 10 day of life (respectively, 1.5 ml and 3 ml per animal). The piglets from group B received Suiferrovit, according to producer recommendation (5 ml per animal subcutaneously in 5th day of life and 5 ml 10 days later).

Blood samples

Blood samples from piglets were collected from *vena cava cranialis* in vacuum tubes, containing EDTA-K3 as an anticoagulant (Medlab, Poland) in 3, 7, 14, 21 and 28 day of life. Local Ethical Commission approved all procedures involved in the study.

Evaluation of hematological and immunological parameters

Whole blood samples were analyzed for different leukocyte proportions and concentrations on a Celoscope-AutoCounter AC 920 (Swelab Instrument AB, Sweden) calibrated for porcine blood, within 1 h after blood samples were obtained. Proportion of lymphocytes and granulocytes were calculated as a percentage of leukocyte concentration. Moreover, levels of hemoglobin (Hb), hematocrit (HCT), and mean cell volume (MCV) were also determined.

Evaluation of production parameters

In clinical evaluation a harmlessness of both products used in the study, as well as a basic production parameters (average daily gain weight, weight at birth and at weaning), were evaluated. Body weights were measured at the beginning of the trial and at 26th day of life.

Statistical analysis

Data were expressed as mean ± SD. Results were statistically analyzed using one way ANOVA (StatSoft, Poland). Tukey post-hoc test was used to compare differences among the treatment groups. A p-value less than 0.05 was taken to indicate statistical significance.

Results

No adverse local or systemic reactions were evidenced in all animals, after administration of both formulation used.

Effect of supplementation on growth performance of piglets

Growth performance results are presented in Table 1. There were no significant differences ($p > 0.05$) for mean body weight at day of farrowing and weaning between piglets from all groups. The average weight gains till weaning during the study were greater in pigs treated either with Imunoferr and Suiferrovit, in comparison to the controls, but it was significantly differ only with respect to group C ($p < 0.01$). The piglets from group C had lower daily body weight gain compared to groups A and B, but this difference was significant only between group C and B ($p < 0.05$). Weight gains did not differ significantly between pigs treated orally and parenterally. In group A the differences in individual body weight at weaning (26 day of life) were the smallest.

Table 1. Characteristic of selected production parameters in piglets from different groups

	C	A	B
Body weight (kg) at day of farrowing (x ± SD)	1.45 ±0.15	1.44 ±0.26	1.49 ±0.13
Body weight (kg) at day of weaning (x ± SD)	5.94 ±1.59	7.08 ±1.14	7.40 ±1.74
Body gain till weaning (kg) (x ± SD)	4.49 ±0.52**	5.21 ±1.09	5.91 ±0.98**
Daily body gains (g) from farrowing to weaning (x ± SD)	172.69 ±18.5*	200.61 ±48.9	227.06 ±56.1*

**p ≤ 0.05, ** p ≤ 0.01*

Effect of supplementation on total and differential counts of blood leucocytes

Data on immunological parameters levels, for different treatment groups of pigs, are shown in Table 2. In all tested groups the level of leukocytes decreases in the first seven days of life, but only in piglets receiving Imunoferr the decrease was not significant ($p > 0.05$). In piglets from group A the levels of WBC were the highest during the whole period of study in comparison to remaining groups. This group was the only group in which the increase of WBC after weaning was observed. The relative number of lymphocytes at 14 and 21 days of life, were the highest in group A, but significantly only with respect to negative control ($p < 0.05$). There were no significant differences between levels of leukocytes and granulocytes in group A and B, but significantly higher relative number of lymphocytes was observed at 28 days of life ($p < 0.05$), between mentioned groups.

Table 2. Characteristics of immunological parameters in treated and control groups (mean ± SD)

Parameters	**unit**	**C**	**A**	**B**
			3 days old piglets	
WBC	10^9/l	14.40 ±1.02	14.9 ±1.84	14.47 ±4.40
Lymphocytes	%	44.75 ±4.50	42.5 ±6.19	50.0 ±4.24
Granulocytes	%	40.00 ±3.56	43.25 ±5.68	36.0 ±4.32
			7 days old piglets	
WBC	10^9/l	8.45 ±1.28*	12.7 ±6.25*	9.45 ±1.53
Lymphocytes	%	41.50 ±8.35	49.2 ±18.90	47.25 ±13.20
Granulocytes	%	39.25 ±10.24	38.8 ±19.51	41.25 ±15.28
			14 days old piglets	
WBC	10^9/l	8.72 ±1.09**	12.4 ±2.45**	10.22 ±1.09
Lymphocytes	%	45.21 ±5.14*	54.0 ±4.70*	52.25 ±5.74
Granulocytes	%	38.00 ±4.10	34.8 ±4.27	35.0 ±4.69
			21 days old piglets	
WBC	10^9/l	8.02 ±1.65	11.6 ±4.40	8.10 ±1.37
Lymphocytes	%	54.73 ±4.47*	64.5 ±3.10*	60.75 ±6.60
Granulocytes	%	28.40 ±6.31	25.75 ±3.95	29.75 ±5.68
			28 days old piglets	
WBC	10^9/l	10.10 ±2.85*	15.22 ±3.3*	12.40 ±1.51
Lymphocytes	%	51.52 ±6.85[a]**	63.50 ±4.4[a,b]	54.25 ±1.89[b]*
Granulocytes	%	30.75 ±4.35	32.25 ±7.85	33.5 ±1.29

WBC – white blood cells
*Values in the same row with the same superscripts (a, b) are significantly different at *p < 0.05, **p < 0.01*

Effect of supplementation on hematological parameters

Data on basic hematological parameters levels for different treatment groups of pigs are shown in Table 3. The initial levels of all investigated parameters were not statistically significant ($p > 0.05$) for different groups of piglets at day 3 post-partum. Starting from day 14 the levels of erythrocytes were significantly higher in treated groups, than in negative control ($p < 0.001$). There were no significant differences ($p > 0.05$) for the erythrocyte counts between group A and B during whole period of study. At 7 days of age, the mean Hb levels for piglets from group C were significantly lower than in piglets from A ($p < 0.05$) and B ($p < 0.01$) groups. The Hb level, lower than physiological ranges, was observed only in group C, at 28 days of life. Starting from day 14, Hb concentrations were the highest in group B, but significant difference between both treated groups was observed only at the 28 days of life. In untreated group the levels of Hb decreased gradually from the start to the end of study. On days 14 and 21 HCT were significantly lower in control pigs than in treated groups.

Discussion

Results of this study indicate, that supplementation with both formulations tested, in the first weeks of life, improved the overall growth performance in weaned piglets. Notably, in the treated groups, average daily gains were about 16% and 31% higher, respectively for Imunoferr and Suiferrovit, compared with non-additive control. These results indicate the growth-promoting effect of both formulations. The average daily body gain, body gain till weaning and body weight at day of weaning, were the highest in group supplemented parenterally.

From data shown in Table 3, it is clear, that supplementation of iron in the first weeks of piglets life is necessary. The supplementation of iron in newborn piglets is very important and effectively prevent anemia [11, 15, 18-20]. Markowska-Daniel *et al*. [18- 20] reported that health condition and production parameters of animals receiving iron were superior than in non treated piglets, moreover, this prophylactic program resulted in a significant decrease of deaths amongst the piglets. Hematological analysis demonstrated that the application of iron was very effective.

Table 3. Characteristic of hematological parameters in treated and control groups (mean ± SD)

Parameters		Untreated	Oral	Injectable
	unit		**3 days old piglets**	
Erythrocytes	10^{12}/l	4.59 ±0.60	4.98 ±0.80	4.49 ±0.70
HCT	l/l	0.29 ±0.03	0.31 ±0.05	0.28 ±0.03
MCV	fl	63.00 ±2.94	64.2 ±3.30	63.20 ±3.20
Hb	mmol/l	9.20 ±1.07	9.60 ±1.80	9.00 ±1.09
			7 days old piglets	
Erythrocytes	10^{12}/l	4.52 ±0.67	4.76 ±1.23	5.13 ±0.42
HCT	l/l	0.27 ±0.02^{a**}	0.28 ±0.07^{b*}	0.34 ±0.02^{a,b}
MCV	fl	60.00 ±5.32	60.20 ±4.91	67.50 ±5.92
Hb	mmol/l	8.00 ±0.13^{a,b}	8.20 ±2.46^{a*}	9.82 ±0.33^{b**}
			14 days old piglets	
Erythrocytes	10^{12}/l	3.90 ±0.40^{a,b}	5.10 ±0.25^{a***}	5.20 ±0.39^{b***}
HCT	l/l	0.22 ±0.06^{a,b}	0.31 ±0.03^{a***}	0.33 ±0.02^{b***}
MCV	fl	60.20 ±4.91	60.30 ±1.16	65.2 ±5.30
Hb	mmol/l	7.50 ±0.28^{a,b}	8.90 ±0.95^{a***}	9.90 ±0.28^{b***}
			21 days old piglets	
Erythrocytes	10^{12}/l	3.50 ±0.17^{a,b}	5.40 ±0.80^{a***}	5.60 ±0.11^{b***}
HCT	l/l	0.19 ±0.05^{a,b}	0.32 ±0.05^{a**}	0.35 ±0.01^{b***}
MCV	fl	57.50 ±0.62^{a,b}	59.50 ±1.29^{a***}	64.00 ±0.82^{b***}
Hb	mmol/l	6.40 ±0.10^{a,b}	9.60 ±1.60^{a**}	10.40 ±0.30^{b***}
			28 days old piglets	
Erythrocytes	10^{12}/l	3.71 ±0.10^{a,b}	5.09 ±0.30^{a***}	5.33 ±0.05^{b***}
HCT	l/l	0.16 ±0.05^{a}	0.26 ±0.05^{a}	0.32 ±0.01^{a***}
MCV	fl	47.2 ±1.26^{a***}	48.50 ±0.58^{b***}	61.25 ±1.30^{a,b***}
Hb	mmol/l	5.92 ±0.06^{a**,b}	6.80 ±0.63^{a,c***}	9.47 ±0.10^{b***,c}

*Values in the same row with the same superscripts (a, b, c) are significantly different at *p < 0.05, **p < 0.01, ***p < 0.001*

In piglets, that did not receive iron in the first weeks of life, the levels of all hematological parameters were below the physiological ranges at 28 days of life [21]. The level of Hb and concentration of the erythrocytes in groups supplemented with iron (orally or parenterally), were much better, than in the control group, and did not drop below physiological ranges up to weaning. However, in piglets received iron parenterally, all hematological parameters had higher values, what indicate that this way of iron supplementation was more efficient, than the oral one, what is in agreement with data obtained by Svoboda *et al*. [13], and contrary to data obtained by Petrichev *et al.* [14]. Effects of per os iron supplement on development of hematological profile of piglets was evaluated by Svoboda *et al.* (2004), who found that on day 7 and 14 of experiment piglets who received iron lactate orally had significantly higher levels of erythrocytes than untreated and intramuscularly treated (iron-dextran) animals. However, according to findings present in recent study, at 28 days of life the higher values of most hematological indices were recorded in the group with parenteral administration. In the previous study [13], similarly to our results, two groups receiving iron by different routs of administration (orally or intramuscularly), did not differ in their somatic development. On the contrary, Petrichev *et al.* [14] reported, that oral supplementation with iron sulfate in complex with methionine, was more efficient in anemia prophylaxis than iron dextran given intramuscularly.

The level of HCT, in groups treated with iron, did not changes significantly during the period of study, while in untreated control the value of this parameter was significantly lower, especially at 28 days of life, when it reached only 0.16 L/L. In group receiving iron orally the levels of HCT and MCV also were below physiological values, but HCT was significantly higher than in negative control ($p < 0.05$). Up to day 21 there were no significant differences between group treated orally and parenterally, with respect to values of all hematological parameters.

The time around weaning is a stressful event, associated with a period of underfeeding, changes in intestinal morphology, and higher susceptibility to infections [1-3]. Because of the total band on use of antibiotic growth promoters (AGP), many producers are looking for alternative growth promoters or management strategies, but at this point, no "magic bullets" are available. An important finding from the present study is, that orally supplementation with Imunoferr, enhance the immunity of piglets at the time of weaning. The number of leukocytes was the higher in group A during whole period of study, but differences were significant only with respect to untreated control. The percentage of lymphocytes in group A was significantly higher than in remaining groups. Only in piglets received Imunoferr the increase of leukocyte number at weaning was observed.

The results concerning immunological parameters tested in present study, point to positive effect of both products on piglets immunity. However, it should be noted that WBC numbers and percentage of lymphocytes were much higher in piglets received Imunoferr, especially at weaning.

On the basis of our recent finding, we summarized, that supplementation with both formulations improved all investigated parameters. However Suiferrovit had better antianemic effect, while Imunoferr was more effective as an immunostimulatory agent. Both formulations used in this study improved the growth performance of piglets. Two treated groups did not differ significantly in their somatic development.

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