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Field evaluation of the influence of garlic extract and probiotic cultures on sows and growing pigs[1)]

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Summary

The present study aimed to evaluate the influence of a garlic extract and probiotic strains, *Enterococcus faecium*, *Lactobacillus rhamnosus* and *Lactobacillus fermentum*, on sows and their offspring. A total of 24 sows and the 301 piglets they farrowed were used in this trial. The animals (sows and piglets) were assigned to 3 groups: group A, supplemented with garlic extract; group B, supplemented with probiotics; and the control group C, without supplementation. The trial lasted from the 80th day of gestation to the weaning day for sows. For piglets, results were analyzed during two periods, from the 8th day to the 56th or the 147th day of life, in order to determine the more desirable duration of supplementation. Morphological and biochemical blood parameters, as well as body weight and piglet losses, were recorded. At weaning, the piglets supplemented with probiotics were heavier than the rest of experimental population ($P \leq 0.01$). On the day of sale for slaughter, the pigs from the control group were on average 15 kg lighter ($P \leq 0.01$) than those from both supplemented groups. Differences in blood parameters were also noted between the groups. Our findings suggest that the use of a garlic extract and probiotic bacteria may help improve pig performance at various stages of the production cycle.

Keywords: pigs, garlic extract, probiotics, rearing results, blood parameters

The ban on antibiotic growth promoters (AGP) and the tendency towards a decrease in the therapeutic use of antibiotics prompted search alternative strategies (24, 25, 34). Currently, various feed additives present very promising options. Because of their immunoprotective or immunostimulating properties, they can be given to pigs from different production groups, depending on farm conditions. Those with immunoprotective effects are involved in maintaining the organism's homeostasis and do not stimulate the host's immunological system. They include probiotics, immunoglobulins (derived from blood plasma, colostrum, or egg yolk) and bacteriophages, which can be administered from birth. Acidifiers could be introduced before weaning, and enzymes after weaning as well (2, 4, 27-29). The role of immunostimulating additives is to activate the host's immune system, and that is why they are usually introduced around the weaning period or later. This group of additives includes probiotics, symbiotics and, conditionally, bioplexes (Zn, Cu, Mn, Se), which are administered when an animal is lacking minerals. An interesting group of feed additives is also yeast, yeast derivatives and herbs that demonstrate immunoprotective and immunostimulating properties (17, 26, 30).

Probiotics are most often identified on the basis of a live culture of microorganisms that improve the host's indigenous microbial balance (9, 36). Positive effects of the use of probiotics that increase the host's immunity to infectious agents include greater accessibility of nutrients, competitive inhibition of epithelial adherence, inhibition of epithelial invasion by pathogenic bacteria and production of antimicrobial substances (1, 9, 21).

Natural bioactive compounds obtained from plants are referred to as phytobiotics, phytochemicals or phytogenics. Main attention is paid to their antimicrobial and antioxidant characteristics. Phytobiotics also have an immunomodulatory effect through the enhancement of immune cell proliferation, modulation of cytokines and antibody titers. Some authors suggest that they

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can be used as growth promoters instead of banned AGPs (14, 22). Garlic preparations have been used in medicine for many years. Most of them show a broad spectrum of antibacterial activity. Garlic inhibits the growth of Gram-negative and Gram-positive bacteria. It also exhibits antifungal activity, which is based on the inhibition of mycotoxin production (5).

Search for growth promoters that could optimise large-scale farming has continued incessantly since the ban on AGPs. However, an ideal substitute still has not been found, and research in this area seems to be much needed, especially under field conditions, since optimum nutrition, health and management are the basis of optimal productivity. Although many reports on the use of probiotics or garlic derivatives in pigs can be found in the literature, there is still little information on such dietary supplementation in practice. This field study was undertaken to evaluate the effects of garlic extract and cultures of *Enterococcus faecium*, *Lactobacillus rhamnosus* and *Lactobacillus fermentum* on sows and piglets that received them as feed additives in terms of their health status and production results, and to determine the optimal administration time and period for such preparations.

Material and methods

All procedures were approved by the Local Ethics Committee at the University of Environmental and Life Sciences, Wrocław, Poland (application No. 22/2014).

The study was performed on 24 crossbreed sows (Polish Large White × Polish Landrace) and their 301 offspring (Polish Large White × Polish Landrace × Pietrain) at a com-

Tab. 1. Composition and nutritive value of diets

	Empty and pregnant sows	Lactating sows	Suckling piglets	Weaners	Fatteners up to 50 kg	Fatteners over 50 kg
Ingredients [%]						
Barley	50	50	27	25	18.5	19
Wheat		30	40	35	30	30
Triticale	22.5				30	30
Rye	20					
Soybeans	5	15	2.5	13.5	17	17
Fish meal			2.8			
Soybean oil		0.7	2	1.5	1.8	1.3
Acidizer		0.3	0.3		0.2	0.2
Zinc oxide			0.4			
	Premix[a] 2.5	Premix[b] 4	Concentrate[c] 25	Concentrate[d] 25	Premix[e] 2.5	Premix[f] 2.5
Nutritive value						
Energy [MJ/kg]	12.84	12.95	14.35	14.27	13.63	13.51
Total Protein [g/kg]	124.82	162.85	202.54	225.38	168.83	169.41

Explanations: Composition of the mineral and vitamin mixture per 1 kg:
[a] Polfamix LP TOP1: Lysine 4 g, Methionine 2 g, Threonine 2 g, Total Calcium 297 g, Total Phosphate 69 g, Sodium 62 g, Magnesium 10 g, Vitamin A 480000 IE, Vitamin D3 65,000 IE, Vitamin E 3000 mg, Vitamin K 80 mg, Vitamin B1 80 mg, Vitamin B2 200 mg, Vitamin B6 150 mg, Vitamin B12 1200 mcg, Biotin 8,000 mcg, Niacin 1250 mg, Folic Acid 200 mg, Ca Pantothenate 600 mg, Choline 12,000 mg, Manganese 3100 mg, Zinc 5000 mg, Iron 4000 mg, Copper 990 mg, Iodine 30 mg, Selenium 12 mg, Betaine 5500 mg, Antioxidant 1000 mg;
[b] Polfamix LK TOP2: Lysine 50 g, methionine 7 g, Threonine 8 g, Total calcium 230 g, Total Phosphorus 60 g, Sodium 55 g, Magnesium 10 g, Vitamin A 300000 IE, Vitamin D3 50000 IE, Vitamin E 3000 mg, Vitamin K 100 mg, Vitamin B1 55 mg, Vitamin B2 225 mg, Vitamin B6 100 mg, Vitamin B12 1000 mcg, Biotin 10,000 mcg, Niacin 700 mg, Folic Acid 50 mg, Ca Pantothenate 500 mg, Choline 12,000 mg, Manganese 2500 mg, Zinc 2500 mg, Iron 3000 mg, Copper 625 mg, Iodine 30 mg, Selenium 12 mg, Betaine 5500 mg;
[c] Vitarex PW3: Crude protein 400 g, Crude oils and fats 24.1 g, Metabolic energy 12.43 MJ, Crude ash 140.2 g, Crude fibre 45.2 g, Lysine 37 g, Methionine 16 g, Methionine + cystine 22.05 g, Threonine 18 g, Tryptophan 5.35 g, Calcium 30 g, Phosphorus 13 g, Sodium 9.5 g, Magnesium 1.82 g, Vitamin A 48000 IU, Vitamin D3 8000 IU, Vitamin E 140 IU, Zinc mg 480 mg, Copper mg 400 mg;
[d] Vitarex PW4: Crude protein 350 g, Crude oils and fats 24 g, Metabolic energy 11.65 MJ, Crude ash 170.2 g, Crude fibre 50.3 g, Lysine 33.2 g, Methionine 9.7 g, Methionine + cystine 14, 54 g, Threonine 18 g, Tryptophan 4.62 g, Calcium 35 g, Phosphorus 19.5 g, Sodium 8.5 g, Magnesium 2.02 g, Vitamin A 60000 IU, Vitamin D3 8000 IU, Vitamin E 200 IU, Zinc mg 500 mg, copper mg 604 mg;
[e] Polfamix PT1: Lysine 90 g, Methionine 17 g, Threonine 19 g, Total Calcium 254 g, Total Phosphorus 70 g, Sodium 67 g, Magnesium 8 g, Vitamin A 260000 IE, Vitamin D3 70,000 IE, Vitamin E 3,000 mg, Vitamin K 70 mg, Vitamin B1 55 mg, Vitamin B2 200 mg, Vitamin B6 80 mg, Vitamin B12 1200 mcg, Biotin 6000 mcg, Niacin 800 mg, Folic Acid 20 mg, Ca Pantothenate 500 mg, Choline 6000 mg, Manganese 1000 mg, Zinc 4000 mg, Iron 3000 mg, Copper 980 mg, Iodine 25 mg, Selenium 12 mg;
[f] Polfamix PT2: Lysine 80 g, Methionine 10 g, Threonine 14 g, Total Calcium 245 g, Total Phosphate 69 g, Sodium 66 g, Magnesium 8 g, Vitamin A 260000 IE, Vitamin D3 60,000 IE, Vitamin E 3,000 mg, Vitamin K 60 mg, Vitamin B1 50 mg, Vitamin B2 150 mg, Vitamin B6 65 mg, Vitamin B12 1200 mcg, Biotin 2000 mcg, Niacin 750 mg, Folic Acid 15 mg, Ca of Pantothenate 500 mg, Choline 5000 mg, Manganese 1000 mg, Zinc 3000 mg, Iron 2000 mg, Copper 900 mg, Iodine 25 mg, Selenium 10 mg, Iron 3000 mg, Copper 980 mg, Iodine 25 mg, Selenium 12 mg, Antioxidant 1000 mg.

mercial farm. The farm operates in a closed cycle with a one-week production rhythm, and the basic herd consists of 130 sows. Animals included in the study received routine care and veterinary treatment at the farm. After the completion of the study, sows were kept for breeding, and fatteners were intended for sale. Two weeks before mating, sows were vaccinated with Eryseng Parvo (Laboratories Hipra S.A., Spain) and Porcilis PRRS (Intervet, Netherlands), and 4 months later the Porcilis PRRS vaccination was repeated. Additionally, 2 weeks before parturition, Biotropina® (Biowet Drwalew S.A., Poland) was administered to sows. Iron in the form of Ferran 200 (Vet-Agro sp. z o.o., Poland) was given to piglets on the 2nd day of life in a dose of 1 ml/animal as an intramuscular injection. The piglets were vaccinated against *Porcine Circovirus-2* with Circovac (Merial, France) on the 21st day of life.

After weaning, sows were moved to the reproduction sector and kept in individual unbedded pens until gestation was confirmed, and then they were moved to group bedded pens with a feed station for up to 30 heads (Wolbrink System NEDAP, Netherlands). On the 80th day of gestation, 24 multiparous sows from one technological group were randomly chosen for the trial and divided into 3 groups: C – control, A – fed with garlic extract, and B – fed with a composition of probiotic bacteria. All sows were in a similar body condition, and they were between the 2nd and 5th parities. Only a negative control was included in the study. A positive control (sows or piglets supplemented with antibiotics) was not included in this research because of the EU-wide ban on the use of antibiotics as antimicrobial growth promoters (AGP) in animal feed (introduced on January 1, 2006).

Sows from Groups A and B were individually administered additives from the 80th day of gestation to the weaning day. Piglets were housed with their dams in triple-slatted pens in the farrowing unit. All piglets were divided into groups like the sows.

After weaning (on the 23rd day of life) piglets were moved to the nursery unit and kept in grouped slatted pens for 20 weaners. At the 11th week of life, they were moved to the fattening sector and kept in grouped deep-litter pens for 50 fatteners.

Animals were fed with mixtures prepared at the farm. Feed mixture formulas for all production groups are presented in Table 1. The scheme of the study is shown in Tables 2 and 3. In Table 3, the labels B+ and A+ denote the supplementation of additives to piglets from the 8th day of life to the end of the trial (for 147 days) whereas B– and A– mean that the additives were administered up to the 56th day of life. Garlic extract and probiotic bacteria were given to each animal individually by direct oral administration. Allivet™ (Centaur, Poland, authorization number PL2408001p) is a complementary feed composed of 6 : 1 garlic extract, which means that one litre of Allivet is obtained from 6 kg of garlic. The preparation contains more than 100 allyl sulfides, and one of them is allicin in a concentration below 1%.

Strains of the probiotic bacteria *Enterococcus faecium* CCM 6226 (serial number 030714), *Lactobacillus rhamnosus* CCM 1825 (serial number 010914) and *Lactobacillus fermentum* CCM 7192 (serial number 011014) were selected from a collection of microorganisms of PharmaGal-Bio (Slovakia). All strains were admitted to trading as feed additives (EC Regulation 1831/2003) and are on the updated list of 24.10.2018. Every kilogram of the supplemented feed comprised 690 g of corn starch, 50 g of maltodextrin, 250 g of protein and 10 g of the probiotic bacteria (1×10^9 CFU/g). Before administration, 2 g of this mixture was suspended in 10 ml of water. The strains were selected on the basis of positive results reported in numerous publications for each strain (6, 7, 40).

Tab. 2. Study scheme for sows

Group C (n = 8)	**Control sows with standard feeding**
Group B (n = 8)	**Standard feeding with the addition of probiotic bacteria *Enterococcus faecium*, *Lactobacillus rhamnosus* and *Lactobacillus fermentum*; 2 g/sow/day from the 80th day of gestation to the weaning day**
Group A (n = 8)	**Standard feeding with the addition of garlic extract; 10 ml/sow every 3 days from the 80th day of gestation to the weaning day**

Tab. 3. Study scheme for offspring. Within experimental groups B+ and A+ on the 56th day; litters were divided into 2 subgroups: B+ and B–, A+ and A–

Group C (n = 90)	**Control pigs with standard feeding**
Group B+ (n = 42)	**Standard feeding with the addition of probiotic bacteria from the 8th day of life to the end of the experiment; 2 g/day/head**
Group B– (n = 42)	**Standard feeding with the addition of probiotic bacteria from the 8th day of life to the 56th day of life; 2 g/day/head**
Group A+ (n = 46)	**Standard feeding with the addition of garlic extract from the 8th day of life to the end of the experiment; 1 ml every 3 days per 10 kg of body weight**
Group A– (n = 46)	**Standard feeding with the addition of garlic extract from the 8th day of life to the 56th day; 1 ml every 3 days per 10 kg of body weight**

Data and sample collection. Sows: Blood samples were collected via venepuncture of the *vena cava cranialis* into 2 tubes containing K_2EDTA (dipotassium salt of ethylenediaminetetra-acetic acid) as an anticoagulant and with polypropylene pearls to obtain serum (on the 3rd day before parturition).

Offspring: Body weight was estimated on the 2nd (start of the experiment), 23rd (weaning), 56th (additional regrouping of piglets depending on feed additive supplementation) and 147th days of life (sale). Because of the feeding technology used at the farm (feed for each production group was stored in an individual silo) and the fact that piglets were fed *ad libidum*, FCR (feed conversion ratio) was not calculated in this trial. Losses and health disorders were recorded. Blood samples were collected via venepuncture of the *vena cava cranialis* into 2 tubes containing K_2EDTA and polypropylene pearls during the 1st, 3rd, 4th, 8th and 11th weeks of life.

Laboratory analysis. A morphological analysis was performed using a CELL-DYN 3700 analyser, manufactured by Abbot Laboratories, Polska Sp. z o. o. The following parameters were determined: total number of white blood cells (WBC), neutrophils (NEU), lymphocytes

(LYM), monocytes (MONO), eosinophils (EOS), basophils (BASO), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean red cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (PLT). Total serum protein (TP) and its fractions: albumins (ALB), alpha-globulins (α), beta-globulins (β), gamma-globulins (γ), as well as the albumin to globulin ratio, were estimated on SAS-MX Serum Protein SB agar gels (Helena Biosciences, Europe) with an ELITE 3000 Power Supply (by WEALTE) and in an SAS-MX chamber.

Statistical analysis. Supplementation results were compared by analysing variance procedures for a completely randomised design using the Statistica 12.5 statistical package (StatSoft Inc., Tulsa, OK). Differences between means were determined by the post-hoc Tukey test at 0.05 and 0.01 levels of significance. The results are presented in tables as the mean ($\bar{x}$) and standard deviation (± SD).

Tab. 4. The average body weight of piglets during the experiment ($\bar{x}$ ± SD)

Day of life	Group				
	C	B+		A+	
2	1.70 ± 0.22	1.85 ± 0.23		1.73 ± 0.21	
23	7.41^{B} ± 0.72	7.92^{A} ± 0.81		7.25^{B} ± 0.72	
	C	B+	B–	A+	A–
56	24.95^{B} ± 1.43	29.99^{AC} ± 1.67	28.08^{AD} ± 1.71	28.97^{A} ± 1.31	28.03^{AD} ± 1.53
177	104.08^{B} ± 12.58	119.02^{A} ± 9.01	121.19^{A} ± 7.81	119.04^{A} ± 9.31	119.08^{A} ± 9.32

Explanation: Significant differences are marked with pairs of letters ab, cd; small letters at $P \leq 0.05$, and capital letters at $P \leq 0.01$

Tab. 5. White blood cells (WBC) in sows 3 weeks before delivery ($\bar{x}$ ± SD)

Parameter	Group C	Group B+	Group A+
WBC [g/l]	12.09^{a} ± 1.23	12.55^{a} ± 2.50	8.98^{b} ± 2.83
NEU [g/l]	5.84^{A} ± 0.71	5.28^{A} ± 1.25	3.36^{B} ± 1.05
LYM [g/l]	4.22 ± 0.50	5.49 ± 1,15	4.14 ± 1.54
MONO [g/l]	0.92^{B} ± 0.001	1.07^{A} ± 0.14	0.84^{B} ± 0.07
EOS [g/l]	0.71 ± 0.28	0.61 ± 0.18	0.57 ± 0.41
BASO [g/l]	0.03^{b} ± 0.00	0.07^{a} ± 0.04	0.04^{b} ± 0.00

Explanation: as in Tab. 4.

Tab. 6. Morphological blood indicators for piglets in their 1st, 3rd and 4th weeks of life ($\bar{x}$ ± SD)

Parameter	Group C			Group B+			Group A+		
	Week of life								
	1st	3rd	4th	1st	3rd	4th	1st	3rd	4th
WBC [g/l]	11.56^{aA} ± 2.30	12.52^{aA} ± 3.02	13.53^{a} ± 5.33	8.79^{bd} ± 2.39	8.34^{b} ± 1.94	7.72^{b} ± 2.76	5.43^{cB} ± 1.45	7.28^{B} ± 2.90	9.29 ± 2.58
NEU [g/l]	8.56^{a} ± 2.11	4.25^{a} ± 2.65	4.28^{A} ± 1.07	5.62^{bd} ± 1.67	1.48^{b} ± 0.64	1.95^{B} ± 0.94	2.82^{bc} ± 1.07	2.89 ± 1.14	2.40^{B} ± 0.59
LYM [g/l]	2.00 ± 0.41	6.96^{A} ± 1.85	6.10 ± 1.97	2.00 ± 0.59	6.28^{A} ± 0.99	4.66 ± 2.93	1.53 ± 0.29	3.24^{B} ± 1.50	5.79 ± 2.78
MONO [g/l]	0.86 ± 0.31	1.22^{a} ± 0.68	1.14^{A} ± 0.38	0.99 ± 0.27	0.56^{b} ± 0.23	0.48^{bB} ± 0.25	0.93 ± 0.48	0.73 ± 0.17	0.95^{a} ± 0.28
EOS [g/l]	0.05 ± 0.02	0.08 ± 0.06	0.15^{a} ± 0.07	0.12 ± 0.08	0.08 ± 0.06	0.06^{b} ± 0.03	0.08 ± 0.06	0.08 ± 0.04	0.10 ± 0.05
BASO [g/l]	0.10 ± 0.03	0.10^{A} ± 0.04	0.05 ± 0.02	0.07 ± 0.02	0.02^{B} ± 0.01	0.04 ± 0.02	0.08 ± 0.02	0.05^{B} ± 0.02	0.07 ± 0.05
RBC [T/l]	4.29 ± 0.32	5.29^{A} ± 0.46	5.67 ± 0.69	3.68 ± 0.65	4.02^{B} ± 0.53	5.30 ± 0.77	3.92 ± 0.79	4.49 ± 0.86	5.39 ± 0.68
HGB [g/l]	8.81 ± 4.82	105.77 ± 5.76	110.75 ± 4.17	70.78 ± 11.99	101.85 ± 7.15	114.21 ± 13.46	76.64 ± 13.70	97.85 ± 10.70	110.71 ± 7.62
HCT [l/l]	0.29^{a} ± 0.02	0.36 ± 0.02	0.38 ± 0.03	0.24^{b} ± 0.04	0.33 ± 0.03	0.38 ± 0.04	0.26 ± 0.05	0.32 ± 0.05	0.38 ± 0.03
MCV [pg]	66.83 ± 3.93	69.17^{B} ± 3.24	67.26^{b} ± 3.13	64.76 ± 2.91	82.29^{A} ± 8.77	72.19^{a} ± 3.57	66.63 ± 2.87	72.02^{B} ± 4.38	70.21 ± 3.62
MCH [g/l]	19.33 ± 1.09	20.04^{B} ± 0.94	19.57^{B} ± 1.41	19.16 ± 1.01	25.99^{A} ± 3.01	21.66^{A} ± 0.89	19.61 ± 0.75	22.01^{B} ± 1.79	20.70 ± 1.28
MCHC [g/l]	289.5 ± 5.07	290^{bB} ± 5.61	291.37 ± 15.22	295.75 ± 7.83	312.75^{A} ± 10.47	300.38 ± 4.87	294.25 ± 2.82	305.75^{a} ± 17.50	294.63 ± 7.07
PLT [g/l]	320.63 ± 132.02	400.75^{a} ± 161.53	242.95 ± 102.25	282.5 ± 117.78	170.62^{bB} ± 66.17	253.96 ± 131.95	326.88 ± 78.93	424^{A} ± 170.91	374.25 ± 210.06

Explanation: as in Tab. 4.

Results and discussion

The average body weight on the 2nd day of life was nearly equal in all groups and amounted to 1.76 kg (Tab. 4). On the weaning day, considerable differences in the body weight were noted between groups. Piglets from Group B+ were heavier than the rest of experimental population ($P \leq 0.01$). On days 56 and 147, pigs from Group C were the lightest, which was confirmed statistically. During the fattening period, daily gains were noticeably higher in Group B–, which did not receive supplementation from day 56. Both groups A (A+, A–) showed the same growth as B+. The average number of piglets in experimental litters was similar, and amounted to 12.5. Piglet losses in groups A+, B+ and C were respectively 9.6%, 10.1%, 5.0% by the weaning day and another 2.1%, 5.6%, 3.2% by the day of transfer to the fattening sector. There were no losses during the fattening period. Throughout the trial, the pigs were in good health and did not require additional treatment.

The morphological blood analysis in sows confirmed statistically significant results only in the white blood cell patterns (Tab. 5).

Tab. 6 presents the morphological blood indicators for piglets in the 1st, 3rd and 4th weeks of life. There were considerable differences in the WBC patterns for piglets: the highest number of WBC was always found in Group C, which differed from the other groups ($P \leq 0.05$, $P \leq 0.01$). The same tendency was observed for NEU, LYM and MONO, but with smaller differentiation. Few differences were found between the groups for EOS and BASO. Similarly, for RBC, HGB and HCT on control days, the highest values were found in Group C, but statistically confirmed differences were noted only for RBC and HTC. The opposite relationship was observed for MCV, MCH and MCHC: the average value of these parameters was the lowest for control piglets. The lowest value of PLT was found in the 3rd week in Group B+, and it differed significantly from those in Groups A+ ($P \leq 0.01$) and C ($P \leq 0.05$).

Tab. 7 presents morphological blood indicators of weaners in the 8th and 11th weeks of life. The concentration of WBC in the first control term was highest in Group B+, and this result differed significantly from the WBC count in Group C ($P \leq 0.05$). The count of WBC in the second control term was highest in Group B–, which differed significantly from Groups C, A+, A– and B+ ($P \leq 0.01$ and $P \leq 0.05$). A detailed analysis of NEU, LYM and MONO as well as other morphological blood parameters is presented in Table 7. The

Tab. 7. Morphological blood indicators for weaners in their 8th and 11th weeks of life ($\bar{x} \pm SD$)

Parameter	Group C		Group B+		Group B–		Group A+		Group A–	
	Week of life									
	8th	11th	8th	11th	8th	11th	8th	11th	8th	11th
WBC [g/l]	15.95^{b} ± 4.21	17.68^{B} ± 1.60	23.60^{a} ± 7.94	17.98^{b} ± 1.92	21.93 ± 2.13	20.70^{aA} ± 1.65	18.08 ± 0.96	17.53^{B} ± 1.75	19.63 ± 3.03	17.60^{B} ± 1.46
NEU [g/l]	7.15^{bB} ± 1.46	7.48 ± 1.13	10.49^{ac} ± 2.47	7.24 ± 1.19	12.18^{cA} ± 2.03	8.01 ± 1.35	7.43^{bB} ± 2.65	6.50 ± 0.32	8.89^{d} ± 0.32	7.11 ± 1.73
LYM [g/l]	5.82^{b} ± 1.88	7.50^{B} ± 1.38	9.56^{a} ± 3.55	8,47 ± 1.35	6.85 ± 0.35	10.92^{aA} ± 1.08	8.84 ± 2.35	8.68 ± 1.71	8.17 ± 2.75	8.13^{b} ± 2.52
MONO [g/l]	2.58 ± 0.73	2.37^{A} ± 0.27	3.00^{A} ± 1.26	1.88 ± 0.29	2.86^{a} ± 1.03	1.38^{bB} ± 0.79	1.41^{bB} ± 0.25	2.04 ± 0.58	2.25 ± 0.68	2.12^{a} ± 0.11
EOS [g/l]	0.31 ± 0.06	0.27 ± 0.03	0.44^{A} ± 0.15	0.37 ± 0.11	0.31 ± 0.11	0.35 ± 0.19	0.31 ± 0.09	0.24 ± 0.02	0.25^{B} ± 0.07	0.22 ± 0.09
BASO [g/l]	0.09 ± 0.05	0.06 ± 0.03	0.10 ± 0.04	0.01^{b} ± 0.01	0.11^{a} ± 0.03	0.07 ± 0.02	0.11^{a} ± 0.03	0.09^{a} ± 0.07	0.04^{b} ± 0.05	0.03 ± 0.005
RBC [T/l]	5.65^{B} ± 0.29	5.93 ± 0.37	5.88 ± 0.31	6.29 ± 0.22	6.16 ± 0.53	6.23 ± 0.35	6.29 ± 0.63	5.86 ± 0.32	6.52^{A} ± 0.47	6.17 ± 0.38
HGB [g/l]	102.48 ± 5.64	105.33^{aB} ± 4.47	107.00 ± 6.46	113.50^{A} ± 2.88	106.00 ± 5.55	104.25^{B} ± 4.74	106.25 ± 3.89	100.23^{bB} ± 2.06	109.75 ± 7.03	104.75^{B} ± 0.89
HCT [l/l]	0.33^{Bb} ± 0.02	0.35^{B} ± 0.03	0.36^{a} ± 0.02	0.39^{A} ± 0.02	0.35 ± 0.03	0.36^{B} ± 0.01	0.35 ± 0.02	0.34^{B} ± 0.01	0.37^{A} ± 0.02	0.36^{B} ± 0.01
MCV [pg]	57.70 ± 3.60	59.75 ± 2.30	61.10 ± 2.04	62.45^{aA} ± 0.70	56.68 ± 2.44	57.85^{B} ± 2.09	56.48 ± 4.67	58.95^{b} ± 2.44	57.15 ± 2.45	58.11^{B} ± 2.74
MCH [g/l]	18.18^{a} ± 0.82	17.80^{c} ± 0.61	18.20^{a} ± 0.42	18.05^{aA} ± 0.35	17.25 ± 0.60	16.75^{dB} ± 0.19	17.10 ± 1.19	17.13 ± 0,49	16.88^{b} ± 0.63	17.00^{b} ± 1.16
MCHC [g/l]	314.25^{aA} ± 3.37	298.00^{a} ± 4.11	298.00^{B} ± 5.29	288.75^{b} ± 3.45	304.50 ± 6.99	290.00^{b} ± 9.86	302.25^{b} ± 11.69	290.50 ± 1.38	295.75^{B} ± 5.90	292.25 ± 4.33
PLT [g/l]	366.15 ± 202.08	278.75 ± 126.91	392.68 ± 203.62	189.98 ± 165.75	398.75 ± 114.40	222.85 ± 142.83	230.93 ± 109.97	231.75 ± 52.90	187.85 ± 118.95	275.65 ± 154.17

Explanation: as in Tab. 4.

average level of NEU was statistically more different in the 8th week of life. The lowest concentration of LYM was observed in both control terms in Group C, which was significantly different in this respect from Groups B+, B– and A– ($P \leq 0.01$ and $P \leq 0.05$). The level of MONO in the first term was lowest in Group A+, and it was significantly different from that in Groups B+ and B– ($P \leq 0.01$ and $P \leq 0.05$). In the second term, the lowest level of MONO occurred in group B–, and it was significantly different from MONO levels in Groups C and A– ($P \leq 0.01$ and $P \leq 0.05$). With regard to red cell indices in the second control term, statistically significant differences were noted in HGB and HTC, which were the highest in Group B+. The highest value of MCV was found in Group B+, and it was significantly different from MCV values in Groups B–, A– and A+ ($P \leq 0.01$ and $P \leq 0.05$). High statistical differences between groups in MCH and MCHC were observed in both control terms.

The average concentration of TP in the serum of sows 3 weeks before delivery was 70.36 g/L, and the difference of 9 g/l between Groups C and A was important (Tab. 8). Statistically meaningful differences were noted in globulin fractions between Group C (in which they were the highest) and the other groups. The lowest value of A/G was noted in the control group, and this result differed statistically from results in Groups A ($P \leq 0.01$) and B ($P \leq 0.05$); a significant difference was also confirmed between groups B and A ($P \leq 0.05$).

The average value of TP and its fractions in piglets' and weaners' serum is shown in Tables 9 and 10. In the first control term, the highest concentration of TP was found in the control group, and the difference of 7 g/l between the control group and Group A+ was statistically important. In the subsequent control terms, the concentration of TP in Group C was the lowest, and in the 3rd week of life the differences between Group C and Groups B+ and A+ were statistically confirmed. A similar tendency was observed for albumin concentration.

An analysis of body weight changes during the experimental period allowed us to conclude that growth in all pigs was comparatively high (11, 20, 30), and except for the suckling period, the growth rate in the experimental groups was distinctly higher than it was in the control group. Usually the positive effect of probiotics on growth is reported in suckling and weaned piglets (15, 23) and rarely in fatteners (3, 26). Many authors (16, 23) report that probiotics, as well as garlic preparations, could be used as growth stimulants, but in older healthy pigs no noteworthy effects were usually observed. In the present trial, the use of both feed additives made it possible to obtain a satisfactory final body weight of fatteners. It is important for pig producers because this can shorten the occupation of the facilities, thus leading to better productivity and potentially higher income. Tatara et al. studied the effect of perinatal administration of aged garlic extract (AGE) and allicin (ALL) to pregnant sows on the growth and development of piglets. Sows were given AGE or allicin daily from the 91st day of pregnancy

Tab. 8. Total protein and its fractions in sow serum 3 weeks before delivery ($\bar{x} \pm SD$)

Parameter	Group C	Group B	Group A
Total protein [g/l]	74.61^{a} ± 6.08	70.90 ± 6.06	65.58^{b} ± 6.62
Albumin [g/l]	23.42 ± 1.21	25.41 ± 3.39	26.00 ± 1.91
α-globulin [g/l]	14.07^{Aa} ± 1.93	11.79^{b} ± 1.87	10.55^{B} ± 1.42
β-globulin [g/l]	14.96^{a} ± 2.85	11.98^{b} ± 1.04	12.63 ± 2.41
γ-globulin [g/l]	22.21^{a} ± 3.54	21.11 ± 3.11	17.05^{b} ± 3.89
Albumin/globulin	0.46^{Bb} ± 0.04	0.56^{ad} ± 0.08	0.67^{Ac} ± 0.08

Explanation: as in Tab. 4.

Tab. 9. Total protein and its fractions in piglet serum ($\bar{x} \pm SD$)

Parameter	Group C			Group B+			Group A+		
	Week of life								
	1st	3rd	4th	1st	3rd	4th	1st	3rd	4th
Total protein [g/l]	55.09^{a} ± 6.15	41.67^{B} ± 4.82	47.45 ± 5.72	49.08 ± 5.86	55.65^{A} ± 5.79	47.88 ± 3.05	48.00^{b} ± 2.85	53.87^{A} ± 7.49	49.57 ± 3.00
Albumin [g/l]	17.20 ± 1.89	19.47^{b} ± 1.95	23.25 ± 3.24	14.50 ± 2.39	22.78^{a} ± 2.58	24.87 ± 1.65	15.49 ± 2.54	22.40 ± 2.55	24.18 ± 1.87
α-globulin [g/l]	10.58 ± 0.82	9.65^{B} ± 1.39	11.46^{Aa} ± 1.12	9.99 ± 1.73	14.79^{A} ± 4.81	7.24^{B} ± 1.16	9.83 ± 1.65	7.94^{B} ± 1.12	9.15^{b} ± 2.13
β-globulin [g/l]	11.17 ± 1.39	8.00^{B} ± 1.54	7.21 ± 1.47	10.01 ± 3.09	8.89^{B} ± 1.53	9.85 ± 1.14	10.05 ± 1.48	12.30^{A} ± 2.38	10.29 ± 1.68
γ-globulin [g/l]	16.14 ± 4.05	4.51^{b} ± 2.11	5.51 ± 1.39	14.59 ± 1.18	9.42 ± 1.46	5.91 ± 0.89	12.65 ± 3.85	10.94^{a} ± 7.48	5.95 ± 1.73
Albumin/globulin	0.46 ± 0.07	0.80 ± 0.31	0.96 ± 0.07	0.42^{b} ± 0.05	0.70 ± 0.18	1.08 ± 0.04	0.50^{a} ± 0.08	0.75 ± 0.27	0.97 ± 0.19

Explanation: as in Tab. 4.

Tab. 10. Total protein and its fractions in weaner serum ($\bar{x}$ ± SD)

Parameter	Group C	Group B+	Group B–	Group A+	Group A–
	8th week of life				
Total protein [g/l]	40.62 ± 2.77	41.43 ± 3.64	41.83 ± 2.02	41.23 ± 1.78	41.43 ± 0.62
Albumin [g/l]	16.45^{B} ± 1.81	18.63 ± 3.87	18.90 ± 1.54	18.60 ± 0.85	20.72^{A} ± 0.78
α-globulin [g/l]	9.75^{A} ± 1.28	9.76^{A} ± 1.09	8.80 ± 0.82	8.60 ± 0.74	7.91^{B} ± 0.39
β-globulin [g/l]	8.44 ± 1.53	8.26 ± 0.62	8.80 ± 0.82	9.15 ± 0.93	8.06 ± 0.49
γ-globulin [g/l]	6.06 ± 2.20	5.72 ± 3.66	4.74 ± 0.92	4.89 ± 0.44	4.73 ± 0.86
Albumin/globulin	0.69^{b} ± 0.22	0.83 ± 0.30	0.84 ± 0.30	0.83 ± 0.06	1.00^{a} ± 0.09
	11th week of life				
Total protein [g/l]	45.14 ± 2.39	48.61 ± 3.32	44.95 ± 5.74	43.40 ± 5.33	47.17 ± 2.79
Albumin [g/l]	17.40 ± 0.85	21.87^{a} ± 3.97	19.28 ± 3.32	17.02^{b} ± 4.42	19.93 ± 1.28
α-globulin [g/l]	6.62 ± 0.56	12.98 ± 1.44	14.18 ± 3.34	13.80 ± 4.56	13.25 ± 2.25
β-globulin [g/l]	6.62 ± 0.56	7.60 ± 1.57	6.62 ± 0.67	6.31 ± 3.18	7.12 ± 1.51
γ-globulin [g/l]	8.55 ± 2.22	6.17 ± 3.82	4.90 ± 1.09	6.24 ± 1.77	6.87 ± 3.65
Albumin/globulin	0.63 ± 0.13	0.83 ± 0.22	0.76 ± 0.09	0.65 ± 0.30	0.73 ± 0.09

Explanation: as in Tab. 4.

up to weaning on the 28th day of piglets' life. Those author's results confirmed that the administration of the garlic preparations to the mother improved nutrition at early stages of postnatal development and had positive effects on body weight determined at birth and during 56 days of postnatal development of piglets. Similarly, our results showed a relationship between the garlic extract and improved weight gain compared to that in the control group. On the 3rd and 56th days of piglets' life, Tatara's experimental groups were heavier than the control group by respectively 0.5 kg and 4.6 kg on average (32). In our study, piglets in group A+ (receiving garlic) were heavier than the control group on the 2nd and 56th days of life by 0.03 kg and 4 kg, respectively.

With regard to bacteria supplementation, Delia et al. (9) conducted a trial under extensive pig farming conditions. After weaning, piglets were fed for 8 weeks with a basic feed enriched with 1000, 1500 and 2000 mg/kg of probiotic treatment. This probiotic preparation contained *Lactobacillus plantarium* ATCC 14917 1×10^{11} cfu/kg, *Lactobacillus fermentum* DSM 20016 1×10^{11} cfu/kg and *Enterociccus faecium* ATCC 19434 1×10^{11} cfu/kg. In the above study, a significant difference in daily weight gain (DWG) was documented four weeks after probiotic supplementation. After 56 days, pigs supplemented with 1000 mg/kg of probiotic preparation were heavier by 3.11 kg than the control group. This was consistent with the results of our trial, in which the most noticeable differences in piglets' growth appeared after weaning.

The average white blood cell counts in sows from all experimental groups were within the range of reference values given by Elbers et al. (12). However, the lowest count of WBC among all sub-populations of sows 3 weeks before delivery was found in Group A, which received the garlic extract. It may indicate their better health status. This result correlates with that from a study by Kaiser et al. (19), who found that healthy sows expressed lower values of WBC and NEU than sows with diagnosed dysgalactia syndrome. And this observation coincides with the interpretation of the concentration of globulin fraction presented below.

The average concentration of TP in all sows was satisfactory according to recommendations from Elbers et al. (12) and Verheyen et al. (37). Serum albumin could be used as a predictor of the nutritional status in animals, and its slightly higher concentration in experimental groups may suggest a better feed digestibility – especially in connection with a lower concentration of all globulin fractions, which can attest to the improved health status of sows in the trial. This observation results from an evaluation of the alb/glob value. However, the average values are generally low (37). The lowest value was noted in the control sows, which can be explained by the greater risk of bacterial infection in this group.

Our findings concerning the influence of probiotic supplementation on pigs' haematology show that Group B+ had the lowest levels of NEU and BASO in the 4th week of life. This result is similar to that presented by Dlamini et al. (10), who described the beneficial effects of a probiotic bacteria mixture given to pigs from the 4th to the 8th week of life. The abovementioned authors suggest that a high level of NEU and BASO may be a sign of an ongoing infection, which in our study correlates with results from the control group. In our study, the levels of WBC and LYM in Group A+ at weaning were lower than in the control group, but higher than in Group B+. In contrast, in a study by Yan and Kim (39) concerning the use of garlic, the concentration of WBC and LYM increased after its administration. These authors suppose that this may have been due to increased phagocytic function in macrophage and lymphocyte proliferation or to overstimulation. Dlamini et al. (10) showed that piglets supplemented with probiotics had a higher concentration of IgG. They believe that probiotic bacteria can act on the immune system as an adjuvant and enhance IgG production. Our results could confirm this, but we also suggest another explanation. The higher concentration of globulins in piglets supplemented with probiotics could have resulted from the immunoprotective proper-

ties of this additive. However, our hypothesis is that, because of the presence of the additional probiotic bacteria which take part in the host's defence, the immune system of piglets may not be stimulated or may be slightly stimulated to develop an active immune response.

The concentration of TP and its fraction in the first week of a piglet's life can indicate strong protection imparted by maternal immunity. Our results show convergence with those of Szymeczko et al. (31), particularly with regard to the control group. Around the 3rd week of life, when a peak of milk yield can usually be expected (18) along with a decrease in piglets' passive immunity, a higher concentration of TP was observed in Groups A+ and B+, and it differed significantly from that in the control group. Moreover, these animals had a higher concentration of ALB, which can be explained by a better conversion of feed and milk. On the other hand, piglets from the control group were not additionally protected by feed additives, and they had the lowest γ-globulin concentration and the highest decrease in this fraction. Tatara et al. (33) investigated the effects of postnatal administration of aged garlic extract (AGE) and allicin on the performance and development of the piglets' gastrointestinal tract (GI). They concluded that AGE and allicin given postnatally to piglets helped in the development of GI and supported an immunostimulating effect on non-specific defence mechanisms. In our study, during the weaning time, protein electrophoresis results were more equal in all population. However, the higher level of α-globulin fraction in Group C may suggest development of new inflammations or infections because this fraction contains, among others, acute-phase proteins (APPs) that appear at the beginning of such disturbances (8, 35).

Determination of blood parameters, such as proteinogram and morphology, may be useful in pigs in detecting inflammation and unfavourable health conditions or in confirming the absence of health disorders as a result of a good animal management and protection programme (8, 26). Decreased values of serum albumin, leucocytosis and increased gamma-globulin levels indicate an ongoing inflammatory process (13). When comparing these results with blood parameters of fatteners in our study, the least favourable results were obtained in the control group and in group A+ (supplemented with the garlic extract). It seems that the effect of probiotics on blood indicators is best when they are administered until the 8th week of piglets' life, especially in terms of albumin and WBC levels.

The results indicate that supplementation with a garlic extract and probiotic bacteria is most beneficial when it lasts until the 8th week of a pig's life, for both economic and health reasons.

To the best of our knowledge, this is the first study in which the efficacy of a garlic extract and probiotics was evaluated simultaneously, in the same field trial and under the same environmental farm conditions. A distinct relationship between pigs' body weight and blood parameters, influenced by the feed additives, was found under field conditions. The results suggest that the use of the garlic extract and the probiotic bacteria *Enterococcus faecium*, *Lactobacillus rhamnosus* and *Lactobacillus fermentum* may help improve pigs' performance at various stages of the production cycle. Supplementation of these additives to piglets until the 56th day of their life improved their average body weight. Continued supplementation of garlic and probiotics (up to 147 days) does not seem to have any notable effect. Animals from groups supplemented for a longer period (A+, B+) were not heavier than those that received both additives only until the 56th day of life. At the end of the fattening period, the control pigs were lighter by 15 kg than the others.

Since the growth rate of animals differs strongly between the growing and fattening periods, the ultimate determination of the optimal time for supplementing these additives may require further studies.

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