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Effect of probiotics, prebiotics and herb oil on performance and metabolic parameters of broiler chickens

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Summary

Probiotic bacteria are used to balance a disturbed intestinal microflora and related dysfunctions of the gastrointestinal tract (GIT). They could be an effective alternative to the use of synthetic substances in nutrition and medicine. This study has investigated the effect of probiotics, prebiotics and potentiated probiotics on the health and production of broiler chickens, ROSS 308 hybrid, 1-42-days-old. The chickens were divided into six groups, 30 chickens in each. Group K served as a control, chickens from group L were supplied probiotics, those from groups O and E received prebiotics, and the diet of chickens from groups L+O and L+E was supplemented with potentiated probiotics. Weight gain, health of the chickens and selected biochemical and hematological blood parameters were observed. This experiment has found that the application of probiotics and prebiotics significantly improved the weight gain of broiler chickens and also affected some biochemical parameters. The study has recorded a significant decrease in concentration of serum cholesterol, as well as the activity of alanine aminotransferase. The individual groups of chickens differed in the content of total lipids, proteins, calcium, phosphorus and the activity of alkaline phosphatase. Results of our experiment indicate that supplementation of the broiler chicken diets with potentiated probiotics has a positive effect on weight gain and the health of chickens.

Keywords: broiler chicken, probiotic, prebiotic, potentiated probiotic

Enteric diseases are a subject of considerable concern in the poultry industry because they result in reduced productivity, increased mortality and the associated contamination of poultry products intended for human consumption. After the ban on the subtherapeutic use of antibiotics in the European Union induced by increased antibiotic-resistance, an increased effort has been put into discovering some alternative to antimicrobial growth promoters in poultry production. The use of probiotics and prebiotics is one of several approaches that has the potential to reduce enteric diseases in poultry and the subsequent contamination of poultry products (16). The word probiotic is of Greek origin and means „for life". Today's probiotics are defined as biopreparations that contain living cells or metabolites of stabilized autochthonous micro-organisms that optimize the colonization and composition of gut microflora in both animals and humans and have a stimulative effect on digestive processes and the immunity of the macro-organism (5). Micro-organisms to be considered for probiotic use must be able to pass the stomach–duodenum barrier in a viable condition. They have to multiply at the site of destination in the intestine (17). Prebiotics are defined as „non-digestible food ingredients that have a beneficial effects on the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (6). Combinations of prebiotics and probiotics are known as synbiotics. The live microbial additions may be used in conjunction with a specific substrate for their growth, e.g. fructo-oligosaccharides with a *Bifidobacterium* strain. Synbiotics are effective only in the colon, therefore it is necessary to look for products that could protect the entire GIT. Potentiated probiotics provide effective protection in both the small and large intestine. Potentiated probiotics are defined as bioproducts containing productive bacterial strains and synergistically acting components of natural origin, such as oligosaccharides, plant extracts, PUFA, etc. (2).

The aim of the current study was monitoring the effect of probiotics, prebiotics and herb oil on the performance and metabolic parameters of broiler chickens.

Material and methods

Broilers, treatment and diet. The experiment was carried out on 180 clinically healthy 1-day-old broiler chickens of the

ROSS 308 hybrid. The chickens were reared in a deep litter in separate sections at identical microclimate conditions. During the first week of the experiment the temperature was in the range 32-34°C, during the second 30-32°C and for the remaining weeks it ranged between 20 and 25°C. The relative humidity was kept in a range of 70-75% up to the 24th day and after this day at 65-70%. During the first two weeks the light was on for 24 hours and after that it was turned off for one hour every day. Commercial feed for broilers was supplied *ad libitum* as follows: weeks 1-3 HYD 01 (NL 200 $g.kg^{-1}$, ME 12 $MJ.kg^{-1}$, ash 70 $g.kg^{-1}$, fibre 35 $g.kg^{-1}$), weeks 4-5 HYD 02 (NL 180 $g.kg^{-1}$, ME 12 $MJ.kg^{-1}$, ash 70 $g.kg^{-1}$, fibre 40 $g.kg^{-1}$), 6th week HYD 03 (NL 170 $g.kg^{-1}$, ME 12 $MJ.kg^{-1}$, ash 70 $g.kg^{1}$, fibre 40 $g.kg^{-1}$). The chickens had free access to water.

The chickens were divided to six groups, 30 chickens in each: group K was the control group, chickens from group L were given *Lactobacillus fermentum* 2I3 in the form of broth added to drinking water at a dose of 0.2 ml per head and day, the diet of chickens from the O group consisted of complete feed with the addition of oligosaccharides, E group chickens were supplied feed supplemented with essential oils from herbs, and those from L+O group were supplemented with a combination of lactobacilli and oligosaccharides, and chickens from L+E group were given a combination of lactobacilli and essential oils.

Probiotics and prebiotics. Probioticum *Lactobacillus fermentum* 2I3, resistant to rifampicin, was cultivated in MRS broth (50 µg/l) for three days to the required concentration of 10^9 CFU (colony forming units) per gram and the obtained stock solution was refrigerated. The required inoculum was withdrawn aseptically and transferred to drinking water which was supplied to chickens from groups L, L+O and L+E.

Essential oils *Thymus vulgaris* and *Origanum vulgaris* (Calendula, Ltd., Nová Ľubovňa, Slovak Republic) were mixed with feed to a 0.05% concentration (5 ml origanum + 5 ml thymus per 10 kg feed). 10 ml of oil mixture (5 ml origanum + 5 ml thymus) was mixed in a mixer with 250 g of rice. Then 750 g of feed was added and mixed again to obtain an 0.5% concentration. The premix obtained in this manner was mixed first with 4 kg feed and then an additional 5 kg of feed to obtain 10 kg of feed with 0.05% concentration of essential oils intended for groups E and L+E.

Oligasaccharides Maldex 150 and Raftifeed OPS (ORAFTI Tienen, Belgium) were added to the feed and mixed to obtain 0.4% concentration – 40 g Maldex 150 and 40 g Raftifeed OPS in 10 kg food for groups O and L+O.

Determination of weight. The chickens were weighed on scales once a week.

Sampling of blood and haematological and biochemical analysis. On day 42 blood samples were taken from the vena cutanea ulnaris of 15 chickens selected at random after 24-hours of fasting. The blood for hematology was sampled into heparinised tubes.

A differential blood count was determined by the panoptical method according to Pappenheim. The number of erythrocytes (Ec) and leucocytes (Lc) was determined in a Bürker chamber after dilution with a medium for birds (13). Haematocrit (Hk) was determined by the microhaematocrit centrifugate method.

The serum levels of cholesterol (Chol), bilirubin (Bil), total lipids (TL), total proteins (TP), glucose (Glu), calcium (Ca), phosphorus (P) and activity of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined photometrically using Bio-La kits (Pliva-Lachema a.s., Brno, Czech Republic). The activity of aspartate aminotransferase (AST) and concentration of hemoglobin (Hb) were determined in blood sera employing a spectrophotometric analyzer Reflotron (Boehringer Manheim, Germany).

The chickens were rendered unconscious by a blow to the head and then killed by cervical dislocation. The cadavers were sent to pathological anatomy for dissection.

The results from experimental groups were compared with those from the control group and the differences were evaluated statistically by the one-way ANOVA test (Tukey's Multiple Comparison Test).

Results and discussion

Clinical examinations during the experiment showed no changes in the state of health of control and experimental chickens. There was no record of any morbidity or mortality of the chickens. Similar findings were reported by many authors: Koščová (10) used lactobacilli potentiated with essential oils from origanum and thyme and three days later infected the chickens orally with salmonellae. They proved that supplementation of chick diet with combined administration of *Lactobacillus fermentum* and essential oils from oregano and thyme resulted in a reduction of pathogens in the crop and caecum. Bailey (1) proved the incre-

Tab. 1. Mean weight of chickens during the experiment

Day		Group					
		K	L	O	E	L+O	L+E
1	$\bar{x}$	64.17	71.17	68.50	68.50	69.00	69.17
	SEM	1.224	1.349	1.226	1.226	1.135	1.176
	n	30	30	30	30	30	30
7	$\bar{x}$	180.5	183.5	217.8**	216.2**	226.0**	212.2**
	SEM	4.265	3.852	4.101	3.095	4.085	4.171
	n	29	30	30	30	30	30
14	$\bar{x}$	337.1	346.7	392.3**	409.7**	433.2**	385.5**
	SEM	5.434	7.894	8.626	6.455	7.277	6.317
	n	29	30	30	30	30	30
21	$\bar{x}$	626.4	657.3	741.3**	765.8**	786.7**	688.7***
	SEM	13.65	15.70	15.65	12.73	14.26	12.88
	n	29	30	30	30	30	30
28	$\bar{x}$	1001.0	1036.0	1117**	1208**	1187**	1113****
	SEM	17.78	17.08	15.61	14.16	14.80	12.76
	n	29	30	30	30	30	29
35	$\bar{x}$	1493	1559	1684*	1767**	1715**	1671*
	SEM	27.39	17.83	23.62	12.42	16.87	18.44
	n	29	30	30	30	30	29
42	$\bar{x}$	2054	2136*	2265*	2329**	2291****	2243*
	SEM	20.82	25.37	21.86	20.71	22.35	18.93
	n	29	30	30	30	30	29

Explantations: statistical significance: * – $P < 0.05$, ** – $P < 0.001$, *** – $P < 0.005$, **** – $P < 0.01$

ased survival of chickens infected by salmonella when their diet was supplemented with probiotic bacteria and fructo-oligosaccharides. In the present experiment with feeding probiotics and prebiotics to chickens the lowest daily weight gains were recorded in the control group throughout the experiment. The body weight of chickens in experimental groups was higher. When comparing the groups of broilers fed the diet with lactobacilli, the lowest weight gains throughout the experiment were observed in chickens fed only pure probiotic, higher in the group with a combination of probiotic and essential oils and the highest in the group fed a combination of probiotic and oligosaccharides. Many other authors reported increased efficacy of probiotics when supplemented together with synergically acting components (14, 15). The potentiating of probiotics with oligosaccharides and essential oils results in an increased intake of feed and a subsequent increase in weight gain. Higher body weight, better feed conversion and a decrease in mortality to a minimum in broiler chicks after supplementation of feed with probiotics was also described by Jin (8) and Timmerman (19). The weight of chickens supplemented solely with the probiotic (tab. 1) increased significantly ($P < 0.05$) in comparison with the control chickens only at the end of the experiment. In the groups in which the diet was supplemented with oligosaccharides, essential oils and their combinations with the probiotic, the weight of chickens increased significantly from 7-days-of-age onwards in comparison with the control birds. The mean weight gain per day reached 48.53 g in the control, 50.46 g in group L, 56.73 g in group O, 55.13 g in group E, 54.19 g in group L+O and 53.02 g in group L+E.

The pathological-anatomical examination of chickens at the end of the experiment failed to reveal any organ changes.

No significant differences in hematological parameters were observed between individual groups (tab. 2).

Biochemical examination of blood at the end of the experiment (tab. 3) showed a decrease in serum cholesterol level in all experimental groups of chickens in comparison with the control; however, the differences were significant ($P < 0.001$) only in groups supplemented with lactobacilli and oligosaccharides and in the group fed diets containing lactobacilli and essential oils. Chickens from experimental groups L+O and L+E showed a significant increase ($P < 0.001$) in the level of total proteins in comparison with control chickens. The level of total lipids was decreased significantly in the chickens from group E and increased in group L+O in comparison with the control (K) group. Only small differences between control and experimental groups of chickens were observed for bilirubin, glucose and phosphorus. The activity of alanine aminotransferase (ALT) was lower in all experimental groups in comparison with the control, the difference being significant only in group L, E and L+E. The activity of aspartate aminotransferase (AST) was increased significantly ($P < 0.05$) in chickens from group E

Tab. 2. Hematological parameters determined in chicken blood on day 42 of the experiment

Parameter		Group					
		K	L	O	E	L+O	L+E
Ec T/l	$\bar{x}$	2.111	2.266	2.051	2.112	2.123	2.291
	SEM	0.0477	0.1005	0.0832	0.0532	0.0772	0.0867
	n	14	15	15	15	15	15
Lc G/l	$\bar{x}$	28.070	22.730	22.470	26.730	25.730	30.200
	SEM	0.1692	0.1007	0.1037	0.1544	0.1325	0.1401
	n	14	15	15	15	15	15
Hk l/l	$\bar{x}$	0.357	0.363	0.330	0.353	0.356	0.370
	SEM	0.0068	0.0074	0.0061	0.0086	0.0055	0.0120
	n	14	15	15	15	15	15
Hb mmol/l	$\bar{x}$	6.453	5.373	5.325	7.349	7.199	7.239
	SEM	0.5724	0.3728	0.3339	0.7082	0.5902	0.6278
	n	14	15	15	15	15	15

Tab. 3. Biochemical parameters in chicken serum on day 42 of the experiment (n = 15)

Parameter		Group					
		K	L	O	E	L+O	L+E
ALT µkat/l	$\bar{x}$	0.119	0.050**	0.096	0.078****	0.091	0.075****
	SEM	0.0059	0.0092	0.0082	0.0071	0.0072	0.0087
ALP µkat/l	$\bar{x}$	120.7	93.03	159.9	48.26***	108.0	106.6
	SEM	20.46	14.64	16.34	7.264	21.57	17.44
AST µkat/l	$\bar{x}$	5.618	5.855	5.762	6.327*	6.213	5.784
	SEM	0.1129	0.2110	0.1596	0.1528	0.1300	0.0862
Bil mmol/l	$\bar{x}$	5.198	3.805	4.655	4.198	3.807	8.909
	SEM	0.5116	0.7328	0.5606	0.8865	0.8119	1.632
Chol mmol/l	$\bar{x}$	3.396	3.226	3.089	2.776	1.657**	1.842**
	SEM	0.2426	0.1901	0.2896	0.1429	0.2892	0.1873
Glu mmol/l	$\bar{x}$	11.42	10.62	10.35	10.51	12.39	9.847
	SEM	0.9158	0.3320	1.065	0.3824	0.7435	0.7146
TL g/l	$\bar{x}$	9.379	6.587	7.133	4.813****	13.46***	7.427
	SEM	0.7971	1.093	0.5562	1.030	0.7051	0.7092
TP g/l	$\bar{x}$	19.73	25.73	27.57	31.97	53.48**	60.45**
	SEM	1.354	1.944	1.932	2.013	3.153	4.851
Ca mmol/	$\bar{x}$	1.704	1.939	1.738	1.767	1.775	1.949
	SEM	0.1023	0.0521	0.0281	0.0379	0.0615	0.0570
P mmol/l	$\bar{x}$	2.702	2.597	2.362*	2.975	2.689	2.948
	SEM	0.0806	0.0705	0.0512	0.0633	0.0937	0.1094

Explantations: as in tab. 1.

compared to the control ones. In all remaining groups the differences were insignificant. The activity of alkaline phosphatase (ALP) in chickens from group E was significantly lower ($P < 0.05$) than that in the control group K.

The results of biochemical parameters showed a decreased level of cholesterol, total lipids and activity of ALT. The study also observed a decrease in the activity of alkaline phosphatase and aspartate aminotransferase which can indicate the improved metabolism of osteogenous mineral substances. This was reflected in the insignificant increase in blood calcium and phosphorus in comparison with control chickens. Strompfová (18) fed probiotic and enterocin A, produced by a strain of *Enterococcus faecium* EK, to 13 piglets and observed a significant decrease in serum cholesterol and an increase in the level of total proteins, calcium, haemoglobin and haematocrit. Liong (12) used various strains of lactobacilli in combination with prebiotics (FOS or MOS) in rats and also observed decreased levels of cholesterol and total lipids. In another study it was observed that the blood parameters, blood protein and serum cholesterol of the treated groups with probiotics were not significantly different from the control (7). Link and Kováč (11) in their study involving piglets observed a decrease in total lipids, cholesterol and urea in the serum. Similar results were obtained by Jin (8), where serum cholesterol levels were significantly lower in broilers fed the diets with probiotics. On the basis of his study conducted in 1995 Farell pointed to the fact that consumption of eggs from hens with an adjusted ratio of ω-6 and ω-3 polyunsaturated fatty acids (PNMK) resulted in a decrease in LDL cholesterol in the final consumers. In the present experiment the lipids decreased significantly in the group receiving feed supplemented with essential oils, which supports the theory about changes in lipid metabolism through the influence on composition and ratio of PUFA in the diet (9). Better feed conversion and utilization of proteins from the food by the proteolytic action of probiotics was reflected in the higher level of total protein in the blood serum in treated groups of chickens.

For practical purposes it is important that probiotics have many beneficial effects, such as an inhibitory effect against pathogens, an optimising effect on digestive processes, an immunostimulatory effect, an anti-tumour effect and anticholesterol activity. The mode of action of probiotics has not been fully explained as yet. The mechanisms behind the specific health benefits are related to gut micro flora modification and strengthening the gut mucosal barrier, e.g. adherence of probiotics to intestinal mucosa with a capacity to prevent pathogen adherence, pathogen inactivation, modification of dietary proteins by intestinal micro flora, modification of bacterial enzymatic activity, influence on gut mucosal permeability and regulation of the immune system (17).

Contrary to mammals, poultry start to consume solid feed a few hours after hatching. Thus high doses of probiotic cells (about 10^6-10^7 viable cells/g feed) reach the intestine directly after birth, when microbial communities are still undeveloped (20). In agriculture and veterinary medicine probiotics may be effectively used to optimize digestive processes, stimulate growth and prevent diseases of the digestive tract in the young. The effect of probiotics under different conditions may be due to the probiotic preparation itself or may be caused by other factors. Probiotic bacteria offer new dietary alternatives for the management of such conditions through stabilization of the intestinal microflora, promotion of colonization resistance, regulation of the immune response and preservation of intestinal integrity (17). The combination of probiotics and prebiotics could improve the survival of the probiotic organism because its specific substrate is readily available for its fermentation and result in advantages to the host that the live microorganism and prebiotic offer (3).

Results of the experiment confirmed the favorable effect of probiotics, prebiotcs and potentiated probiotics on growth and state of health of broiler chickens. The observations indicated that the potentiating of probiotics increases their effect and improves the productivity and health of animals. On the basis of our observations we could state that supplementation of feed with probiotics, prebiotics and plant oils is one of the possibilities of how to favorably affect the productivity of animals in the future.

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