Investigation of the effect of probiotics and potentiated probiotics on productivity of laying hens

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ABSTRACT: Probiotic bacteria are used to balance a disturbed intestinal microflora and dysfunctions of the gastrointestinal tract (GIT). They could be an effective alternative to the use of synthetic substances in nutrition and medicine. We investigated the effect of probiotics and potentiated probiotics on the productivity of laying hens. An experiment was carried out on 30 hens of the Hyssex layer hybrid starting from week 30 of laying. The hens were divided into three groups, 10 hens in each (K – control group, L – hens supplemented with probiotics and L + E – hens fed probiotics potentiated with essential oils). No statistically significant differences among the groups were observed in the quality of eggs. Biochemical determination of yolk cholesterol showed an insignificant difference after 25 days of feeding the probiotics. By day 50 of the experiment, yolk cholesterol decreased significantly ($P < 0.05$) in group L + E and insignificantly in group L of layers. Biochemical examination of blood serum detected an insignificant decrease in the activity of ALT, serum cholesterol and total lipids. Our results allowed us to conclude that probiotics and potentiated probiotics favourably affected the investigated hens and their products.

Keywords: laying hen; probiotic; potentiated probiotic; egg cholesterol

Poultry farms oriented on laying hens and production of hen eggs are forced to keep up with the latest trends in nutrition and management of laying hens. The effort of the breeders used to focus particularly on increasing egg yield, weight of eggs and increasing industrialisation of their production while an insufficient attention was paid to the quality of eggs and eggshells. When trying to improve the quality of eggs and their shells, one should also consider the mechanism of egg production. The composition of mixed feed supplied to hens should also comply with the above-mentioned efforts. The study of the action of probiotics on quality and quantity of produced eggs has opened a new way of affecting the productivity of animals (Yoruk et al., 2004). When using a balanced diet supplemented with natural substances of varying character and composition that stimulate specifically the growth, metabolic and immunological factors of macroorganisms, one can expect improved quality of products and maximum utilisation of genetic potential of an individual at the lowest possible consumption of feed per unit of weight. In practical life it means that one can reach high productivity and profitability in poultry rearing.

With the increasing development of resistance of pathogens to antibiotics and the effort to ban the subtherapeutic use of feed antibiotics in the European Union and potentially also in the USA, an increased effort has been made to find some alternative ways resulting in higher productivity and better health of animals and preventing the contamination of products of animal origin. The use of probiotics and prebiotics is one of several ap-

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proaches that can exert a positive effect on animal health and thus also on the economic effectiveness of animal production (Patterson and Burkholder, 2003). The positive biomedicinal effects of probiotics consist in the inhibition of digestive tract pathogens, optimisation of digestion, stimulation of the immune system and in their antitumoural, antiallergenic and anticholesterol action. The word probiotic is of Greek origin and means "for life". Today's probiotics are defined as biopreparations which contain living cells or metabolites of stabilized autochthonous micro-organisms that optimise colonization and composition of gut microflora in both animals and humans and have a stimulative effect on digestive processes and immunity of the macroorganism (Fuller, 1992). Microorganisms 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 (Salminen et al., 1998). Potentiated probiotics provide effective protection in both the small and the large intestine. They are defined as bioproducts containing productive bacterial strains and synergistically acting components of natural origin, such as oligosaccharides, plant extracts, PUFA, etc. (Bomba et al., 2002).

The present experiment investigated the effect of dietary supplementation of laying hens with probiotics and probiotics potentiated with herb oils. We focused on their health and on selected productive parameters.

MATERIAL AND METHODS

Animals, treatment and diet. An experiment was carried out on 30 laying hens of the Hyssex laying hybrid at the 30th week of laying. The experiment lasted for 50 days. It was divided into two 25-day stages. The layers were divided into three groups, 10 layers in each. Each hen was kept in an individual cage in an experimental facility of the Internal Clinic of UVM. During the experiment the light was on for 15 hours per day and was turned off for the remaining 9 hours. Artificial ventilation in the room was ensured to keep the internal temperature between 13 and 18°C. The hens were provided feed and water *ad libitum* by automatic water-basin. They were fed complete mixed feed HYD 10 (crude protein 153 g/kg, ME 11.5 MJ/kg, ash 160 g/kg, fibre 60 g/kg, lysine 7 g/kg, methionine + cysteine 6 g/kg, Ca 28 to 45 g/kg, P 5 g/kg, Na 1.2–2.5 g/kg, Mn 40 mg/kg, Zn 60 mg/kg, vitamin A 8 000 IU/kg, vitamin D_3 1 600 IU/kg, vitamin E 10 mg/kg). Before the beginning of the experiment the hens were allowed to acclimatise for one week.

The division of hens into groups was as follows: group K – control, group L – supplemented with *Lactobacillus fermentum* CCM 7158 added to water in the form of broth at a dose of 0.2 ml per head and day, group L + E – feed supplemented with the combination of lactobacilli and essential oils at 0.05% concentration.

Probiotics and prebiotics. The probiotic *Lactobacillus fermentum* CCM 7158, isolated from the ileum of healthy poultry, was cultivated in MRS broth for three days to the required concentration of 10^9 CFU/ml and the obtained stock solution was refrigerated. The required inoculum was withdrawn aseptically and dosed individually to experimental layers assigned to groups L and L + E.

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

The health of hens and their intake of feed and water were checked daily.

Collection of eggs. Eggs laid by the experimental hens were collected four times per day. The eggs for yolk cholesterol determination (5 eggs from each group) were collected on days 0, 25 and 50. Hard boiled and separated egg yolks were stored in a freezer and examined later for the level of cholesterol using the method by Hammad et al. (1996) and Berio and Hebert (1990). The concentration of cholesterol in 1 g of egg yolk was determined spectrophotometrically by the Bio-La tests (Pliva-Lachema a.s. Brno, Czech Republic) employing a Shimadzu UV-1501 UV-VIS spectrophotometer (Shimadzu, Japan).

Sampling of blood and haematological and biochemical analysis. On days 0 and 50 of the experiment blood samples were taken from the vena cutanea ulnaris. The blood for haematology was sampled to heparinised tubes.

The number of erythrocytes (Ec) and leucocytes (Lc) was determined in a Bürker chamber after dilu-

Table 1. Number of laid eggs and their mean weight

	Group	Weight of eggs (g)			Quantity of laying
		$\bar{x}$	SD	n	
1st phase	K	55.87	2.059	136	154
	L	58.19	2.887	152	172
	L + E	56.49	4.910	82	112
2nd phase	K	60.19	1.859	170	186
	L	59.92	4.442	149	173
	L + E	57.81	3.937	53	106
Total period	K	58.03	2.923	306	340
	L	59.05	4.159	301	345
	L + E	57.12	4.405	135	218

n = number of examined eggs

tion with a medium for birds (Lukačová and Fried, 1962). Haematocrit (Hk) was determined by the microhaematocrit centrifuge method.

The serum level of cholesterol (Chol), total lipids (TL), total proteins (TP), calcium (Ca), phosphorus (P), activity of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined photometrically using Bio-La test kits (Pliva-Lachema a.s., Brno, Czech Republic). The activity of aspartate aminotransferase (AST), uric acid (UA) and concentration of haemoglobin (Hb) were determined employing a Reflotron spectrophotometric analyser (Boehringer Manheim, Germany).

The hens were stunned with a blow to the head and then killed by cervical dislocation. The cadavers were sent for the *post mortem* patho-anatomical examination.

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

RESULTS

Clinical examination showed no changes in the health of control and experimental hens. Control hens laid 154 eggs in the first stage and 186 eggs in the second stage of the experiment. The egg yield of hens from group L reached 172 in the first and 173 in the second stage while layers from group L + E laid 112 eggs during the first 25 days and 106 eggs during the remaining 25 days of the experiment. The mean weight of eggs in the first stage was 55.87 g in the control group and 58.19 g and 56.49 g in groups L and L + E, resp. In the second stage, the mean weight of eggs laid by control hens increased to 60.19 g, in hens supplemented with lactobacilli to 59.92 g and in the group receiving potentiated probiotics to 57.81 g. All changes in the mean weight of eggs were insignificant (Table 1).

On day 0 of the experiment the level of cholesterol in egg yolk of control hens reached 26.38 mg per 1 g egg yolk, which amounts to 2.64% of the total egg yolk content, in group L the respective value was 25.86 mg/g (2.58%) and in group L + E it reached 25.39 mg/g (2.54%). Within 25 days, the level of cholesterol decreased insignificantly in all layer groups reaching 22.39 mg/g (2.24%) in group K, 17.23 mg/g (1.72%) in group L and 21.46 mg/g (2.15%) in group L + E. The last measurement on day 50 showed an insignificant decrease in this parameter in group L (16.87 mg/g; 1.69%) and increase in control hens (25.69 mg/g; 2.57%). After 50 days of feeding the potentiated probiotics to the hens from group L + E they laid eggs with a significantly ($P < 0.05$) decreased cholesterol level per 1 g egg yolk (14.38 mg/g; 1.44%) (Figure 1).

No significant differences in haematological parameters were observed among the groups (Table 2).

The values of biochemical parameters determined in the blood serum of hens (Table 3) were evaluated statistically between the groups and between days 0 and 50. The activity of ALT in the control hens decreased by the second sampling from initial 0.244 μkat/l to 0.074 μkat/l. A similar decrease in ALT activity from 0.213 μkat/l to 0.067 μkat/l

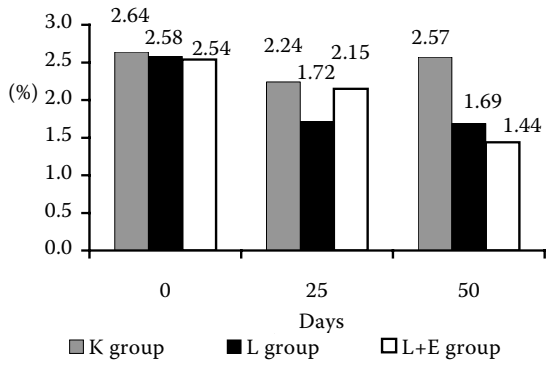

Figure 1. Percentage proportion of cholesterol in the entire egg yolk in individual groups of hens throughout the experiment

was observed in group L and this decrease was significant ($P < 0.05$). The group L + E also showed a downward trend in ALT activity from initial 0.141 μkat/l to final 0.059 μkat/l. All differences were insignificant. The comparison of all groups showed an insignificantly lower activity of ALT in experimental groups. The level of AST activity on day 0 was lower in the control group (5.58 μkat/l) in comparison with groups L (6.14 μkat/l) and L + E (6.02 μkat/l). By day 50, the level of AST increased in group K to 6.36 μkat/l (compared to that on day 0), decreased in group L to 4.94 μkat/l and increased in group L + E to 6.5 μkat/l. When comparing the ALP activity on days 0 and 50, we observed an insignificant decrease in this parameter (group K 70.61 μkat/l; group L 59.31 μkat/l; group L + E 62.48 μkat/l). The activity of ALP on day 50 was insignificantly lower in experimental hens (group L + E 55.79 μkat/l, group L 52.68 μkat/l) compared to the control (58.16 μkat/l). The level of total proteins increased significantly ($P < 0.05$) by day 50 from initial 37.64 g/l (day 0) to 76.7 g/l. In the group supplemented with lactobacilli we also observed a significant increase ($P < 0.001$) in TP from 39.74 g/l to 112.1 g/l. An increase in TP level was observed also in the experimental group L + E from initial 39.87 g/l to 55.6 g/l but the difference was insignificant. The concentration of uric acid showed a downward trend. During the first measurement it reached 240.3 μmol/l in group K and 188.4 μmol/l and 197.2 μmol/l in groups L and L + E, resp. The second measurement showed an insignificant decrease to 218.7 μmol/l and 129.8 μmol/l in groups K and L + E, resp., and an increase to 208.4 μmol/l in group L. A comparison of the results among groups showed a significant decrease ($P < 0.001$) in group L + E. Total lipids decreased insignificantly from 13.45 g/l to 9.29 g/l in group K and from 10.46 g/l to 8.17 g/l in group L. The group L + E showed a significant decrease ($P < 0.01$) in TL between the first (15.56 g/l) and second sampling (6.01 g/l). The cholesterol level was lower in hens supplemented with probiotics and potentiated probiotics in comparison with the control hens and decreased insignificantly in the course of the experiment. On day 0 of the experi-

Table 2. Haematological parameters of hens on days 0 and 50 of the experiment

		K		L		L + E	
		0 day	50 day	0 day	50 day	0 day	50 day
Ec (T/l)	*x*	1.988	2.050	2.131	2.364	2.010	2.268
	SD	0.3450	0.3001	0.2401	0.4897	0.2173	0.3283
	n	10	10	10	10	10	10
Lc (G/l)	*x*	27.3	31.2	31.4	26.8	27.7	23.0
	SD	7.349	15.140	7.919	6.989	6.343	8.869
	n	10	10	10	10	10	10
Hk (l/l)	*x*	0.307	0.316	0.360*	0.327	0.313	0.340
	SD	0.0225	0.0376	0.0228	0.0373	0.0316	0.0683
	n	10	10	10	10	10	10
Hb (mmol/l)	*x*	5.032	4.483	4.578	4.903	4.792	5.044
	SD	0.7726	0.5651	1.5300	1.0350	0.9092	0.7113
	n	10	10	10	9	10	9

*$P < 0.001$; *n* = number of samples per group

Table 3. Biochemical parameters of hens on days 0 and 50 of the experiment (n = 10)

		K		L		L + E	
		0 day	50 day	0 day	50 day	0 day	50 day
ALT (μkat/l)	$\bar{x}$	0.2439	0.07389	0.2129	0.06689	0.1410	0.05956
	SD	0.1524	0.03871	0.1289	0.03211	0.0947	0.01792
AST (μkat/l)	$\bar{x}$	5.5790	6.35800	6.1430	4.93800*	6.0220	6.50400
	SD	0.5415	1.58900	0.6344	1.21250	1.8110	1.33900
ALP (μkat/l)	$\bar{x}$	70.6100	58.16000	59.3100	52.68000	62.4800	55.79000
	SD	16.3200	15.22000	13.5600	14.17000	15.7800	14.93000
TL (g/l)	$\bar{x}$	13.4500	9.29200	10.4600	8.17300	15.5600	6.01400
	SD	4.7780	3.07200	3.04400	3.66700	7.6440	1.98900
Chol (mmol/l)	$\bar{x}$	1.7820	1.37600	1.4750	1.05900	1.2460	1.05700
	SD	1.4940	0.48050	0.5417	0.57480	0.6166	0.48830
TP (g/l)	$\bar{x}$	37.6400	76.70000	39.7400	112.10000*	39.8700	55.60000
	SD	8.3640	36.39000	13.2400	39.17000	6.4330	25.32000
UA (μmol/l)	$\bar{x}$	240.3000	218.70000	188.4000	208.40000	197.2000	129.80000**
	SD	83.1300	62.26000	34.1900	37.70000	28.8300	21.19000
Ca (mmol/l)	$\bar{x}$	1.6560	2.28000	1.4660	2.32500	1.5310	2.33300
	SD	0.1734	0.23880	0.0639	0.20380	0.2440	0.24530
P (mmol/l)	$\bar{x}$	1.7500	1.36900	2.2330	1.54100	1.7740	1.88200*
	SD	0.4006	0.23010	0.8269	0.33760	0.4151	0.50770

*$P < 0.05$; **$P < 0.001$

ment it reached 1.78 mmol/l in control hens and decreased to 1.37 mmol/l by day 50. The initial level of cholesterol in group L hens was 1.47 mmol/l and decreased to 1.06 mmol/l within 50 days. The cholesterol level in group L + E started at 1.246 mmol/l and decreased to 1.06 mmol/l at the second sampling. The determination of serum calcium showed an insignificant increase in all groups at the second sampling. It increased from 1.66 mmol/l to 2.28 mmol/l in control hens, from 1.47 mmol/l to 2.33 mmol/l in hens from group L and from 1.53 mmol/l to 2.33 mmol/l in group L + E. The level of phosphorus decreased from 1.75 mmol/l to 1.37 mmol/l in control hens, from 2.23 mmol/l to 1.54 mmol/l in hens from group L and increased from 1.77 mmol/l to 1.88 mmol/l in hens from group L + E.

DISCUSSION

With regard to human nutrition, consumer eggs belong to the basic and valuable sources of animal proteins. They are a source of all essential nutrients and one should also stress their high content of vitamins (particularly vitamin B_{12}), minerals (particularly iron) and presence of amino acids which are found in yolk and white in proportions considered optimal for human nutrition. However, egg yolk is also one of the most important sources of cholesterol and, as such, one of the highly risky factors in human nutrition. The level of yolk cholesterol in hen eggs may be decreased by targeted nutrition of hens and selection of genetically suitable breeds (Baumgartner et al., 2001).

Our experiment focused on the influence of probiotics and potentiated probiotics on health and productivity of laying hens and, at the same time, on potential improvement in the quality of hen eggs. From the medical point of view, no health changes were observed in control and experimental hens. We also failed to observe any differences in feed consumption among the groups. Panda et al. (2003) reported that probiotics had no effect on feed conversion, weight of eggs and serum level of phosphorus and ALP but reduced the level of serum and egg yolk cholesterol and increased egg yield, weight and thickness of eggshell and blood calcium

level. Similar results were presented by Balevi et al. (2001), who conducted a study on hens from week 22 of egg laying and failed to observe any significant changes in conversion and uptake of feed, egg index, weight of eggs and egg yield. The physical quality of eggs was not affected significantly in our experiment. The weight of eggs increased insignificantly in the second stage of our experiment in all observed groups (K, L and L + E).

With regard to the biochemical composition of egg yolk, we observed an insignificant decrease in yolk cholesterol after 25 days of supplementation of the probiotic. By day 50 of the experiment, the yolk cholesterol level decreased significantly in the group receiving a combination of probiotic and prebiotic. Similar results were described by Kurtoglu et al. (2004) and Li et al. (2006), who observed, besides a decrease in yolk cholesterol, also a decrease in serum cholesterol.

The supplementation of probiotics caused no significant changes in haematological parameters. The values of respective parameters were in the physiological range.

Statistical processing of biochemical data showed significant changes in some biochemical parameters. The activity of ALT decreased insignificantly in all groups of hens at the second sampling. The activity of AST increased insignificantly in the control and L group and decreased significantly in the experimental L + E group of hens. The activity of alkaline phosphatase showed no significant changes but we observed a decrease in the experimental groups in comparison with the control. This fact may suggest an improvement in the metabolism of osteogenous mineral substances indicated by an increase in serum calcium in all groups of laying hens. The group L + E also showed a significant increase in blood phosphorus. Panda et al. (2003) similarly observed an increased level of calcium in blood after the application of probiotics but failed to detect any changes in the level of phosphorus and ALP activity. Šály et al. (1994) reported that the metabolism of osseous calcium begins to decrease after 6 months of laying, which explains the decreasing quality of eggshell with increasing age of laying hens. After the administration of vitamin E aimed to increase the weight of eggs, the quality of eggshell decreased. Therefore the authors recommended that similar measures in older hens should be accompanied by supplementation of suitable additives to hen diet. When studying the effect of probiotics, cholesterol is the most frequently observed parameter. A number of authors reported a decrease in the level of serum cholesterol in laying hens whose diet was supplemented with probiotics (Mohan et al., 1995; Kurtoglu et al., 2004; Li et al., 2006 and others). The layers observed in our experiment also showed an insignificant decrease in serum cholesterol at the second sampling. The level of total lipids decreased in all observed groups. Among changes in the other biochemical parameters we should mention an increase in total proteins in all laying hens, which was significant in group L. The level of uric acid decreased significantly after the application of potentiated probiotics but increased insignificantly after the application of pure probiotics.

The *post mortem* patho-anatomical examination confirmed the good nutritional status of hens and failed to detect any pathological changes in their organs. Our observations allowed us to conclude that probiotics and potentiated probiotics positively affected the metabolism of hens and improved egg production parameters. Through supplementation of probiotics to hen diet one can regulate many parameters in animal products and improve the composition of food supplied to the consumer.

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