THE IMPACT OF PHYTOBIOTICS *ORIGAMI AETHEROLEUM* AND PROBIOTICS *PEDIOCOCCUS ACIDILACTICI* MA 18/5 M ON BIOCHEMICAL BLOOD INDICES AND PRECEACAL AVAILABILITY OF CALCIUM AND PHOSPHORUS IN BROILER CHICKENS.

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ABSTRACT

The aim of our work was to evaluate the effect of phytobiotics vegetable oils *Origami aetheroleum* and probiotics, based on *Pediococcus acidilactici* MA 18/5 M on growth performance, availability of preceacal P, Ca and blood biochemical parameters of broiler chickens. The feeding trial on 450 chickens was performed, where all chickens were allocated to three treatment groups with 3 replications each. First group was negative control, second group received diet supplemented with vegetable oils *Origani aetheroleum* and third group diet was supplemented with *Origami aetheroleum* and probiotics, based on *Pediococcus acidilactici* MA 18/5 M. The highest body weight gain improvement (5% *vs.* control), where diet was supplemented with *O. aetheroleum* and *Pediococcus acidilactici* MA 18/5 M on the 8th day of age was estimated. Feed conversion ratio was reduced by 10% *vs.* control at the period 9–21 days in the group where diet was supplemented with *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M. Combination of *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M, compared to control showed best effectiveness in reduction of blood cholesterol, HDL-cholesterol and LDL-cholesterol. Preceacal availability of Ca and P had tendency to decrease when *O. aetheroleum* or *Pediococcus acidilactici* MA 18/5 M were added.

Keywords: *Origami aetheroleum*, *Pediococcus acidilactici*, blood indices, Ca, P, broiler chicken

INTRODUCTION

Phytogenic feed additives (often also called phytobiotics or botanicals) are mainly herbs (flowering, nonwoody, and nonpersistent plants), spices (herbs with an intensive smell or taste), essential oils (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation), or oleoresins (extracts derived by nonaqueous solvents). Essential oils are characterized by inflammatory and bacteriostatic effect and act as antimicrobial agents in the digestive system of broilers. Many important variables affecting the production of broilers as digestion of nutrients, increases of body weight, feed palatability, improve feed conversion as well as sensory properties of meat **(Močár et al., 2010).**

Beneficial effects of phytobiotics in poultry may arise from activation of feed intake and secretion of digestive enzymes, immune stimulation, antibacterial, coccidiostatic, anathelmic, antiviral or anti-inflammatory activity and inhibition or particularly antioxidant properties. Turmeric, a powder of the rhizome of *Circuma longa*, improves feed intake in poultry when used at 0.25% level in feed (**Wenk and Messikommer, 2002**). Eugenol, a component of the essential oil from cloves, thymol, curcumin, piperin or *Sanguinaria canadensis* has been shown to inhibit *Salmonella typhimurium*, *Clostrodium perfringens* and bacterium that cause necrotic enteritis in broiler chickens (**Karapinarand and Aktug, 1987; Newton et al., 2002; Burt, 2004**). Polysaccharides derived from many plants (*Lentinus edodes, Astragalus membraneaceusradix)* play important role in stimulating the growth of immune organs such as spleen, thymus and bursa, increasing the number and activities of many interdependent cell types such as T, B lymphocytes, macrophages and natural killer cells and enhancing cellular and humoral immune response (**Guo et al., 2003**).

Within phytogenic feed additives, the content of active substances in products may vary widely, depending on the plant part used (e.g., seeds, leaf, root, or bark), harvesting season, and geographical origin. The technique for processing (e.g., cold expression, steam distillation, extraction with nonaqueous solvents, etc.) modifies the active substances and associated compounds within the final product (**Windisch et al., 2008**).

The purpose of the study was to evaluate the effect of phytobiotics vegetable oils *Origami aetheroleum* and probiotics, based on *Pediococcus acidilactici* MA 18/5 M on growth performance, availability of preceacal P, Ca and blood biochemical parameters of broiler chickens.

MATERIAL AND METHODOLOGY

Feeding trial with 450 one-day-old broiler chickens was performed, where all chickens were allocated to three groups with 3 replications each. The birds were fed basal diet without supplementation for 5 weeks *ad libitum* (control group), basal diet supplemented with vegetable oils *Origani aetheroleum* 0.05 g.kg^{-1} (second group) and basal diet supplemented with combination of vegetable oils *O. aetheroleum* 0.05 g.kg^{-1} and probiotics *Bactocell®* (3x10^{6} cfu.kg^{-1}) (third group). Vegetable oil *O. aetheroleum* is produced from plant *Origanum vulgare* L. (*Lamiaceae*). *O. aetheroleum* – essential oil was determined by the use of gas chromatography (chromatograph type 8015-91-6): relative density 0.915 – 0.975 g.cm^{-3}, effective compound carvacrol 57.0% **(Močár et al., 2010).** *Bactocell®* is a probiotic formulated with a specific strain of lactic acid bacteria *Pediococcus*

acidilactici registered at the National Collection of Micro-organism Culture (CNCM, Pasteur – France), number MA 18/5 M. The basal diet was formulated for broiler chickens in a way to meet its nutrient and energy requirements **(NRC, 1994).** The birds were kept on a deep litter and had free access to water from stationary watering containers.

Table 1 Calculated nutrient composition of diet

Ingredients	Composition
Metabolizable energy, (MJ/kg)	13.23
Crude protein*, %	20.91
Digestible protein, %	14.99
Crude fat*, %	6.51
Crude fiber* %	2.45
Crude ash*, %	2.83
Lysine, %	1.16
Methionine/Cysteine, %	0.97
Tryptophan, %	0.26
Linoleic acid, %	1.83
Threonine, %	0.85
Cystine, %	0.37
Methionine*, %	0.62
Lysine (av.) %	1.15
Starch, %	44.93
Sugar, %	4.26
NaCl, %	0.24
Ca*, %	0.96
P (total)*, %	0.66
Na*, %	0.17
Cl*, %	0.20
K, %	0.79
Mg, %	0.17
P (av.), %	0.44

* Analyzed

Productivity of broiler chickens was tested during the feeding study. 8 broiler chickens at age of 35 days from each group were killed at the end of trial, following the recommendations for euthanasia of experimental animals **(Close et al., 1997)**. Preceacal digestibility of Ca and P using Cr_2O_3 as marker by atomic absorption spectrophotometer Perkin Elmer EDL System 2 were estimated. Cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterols, triglycerides as described in **Pearson et al. (1953)**, total protein as described in **Tietzs (1998)**, protein fractions (globulin α1, globulin α2, globulin β, globulin γ and total albumin) by electrophoresis in blood serum of broiler chickens were estimated.

The results were estimated by statistical data program Statistica 5.5. Statistically significant differences between the groups were established with *Duncan* test. The differences between the control and experimental groups are deemed to be statistically significant, when $P \leq 0.05$.

RESULTS AND DISCUSION

The data of broiler chickens body weight is presented in Table 2. Body weight of broiler chickens increased by 4% ($P \geq 0.05$) on the day 8th where vegetable oils *Origami aetheroleum* were added, but *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M had better effect – 5% ($P \leq 0.05$), compared to control. On day 21st BW was improved compared to control by 2%, where vegetable oils *Origami aetheroleum* ($P \geq 0.05$) were added and by 1 % where *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M were added ($P \geq 0.05$). At the age of 35 days the BW of chickens had tendency to decrease compared to control group by 1% and 2%, respectively where *Origami aetheroleum* or it combination with *Pediococcus acidilactici* MA 18/5 M were added ($P \geq 0.05$).

Table 2 The effect of *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M on body weight of chickens (g)

Chickens age in days	Control	*Origami aetheroleum*	*Origami aetheroleum + Pediococcus acidilactici*
1	42.57 ± 0.16	42.59 ± 0.12	42.57 ± 0.10
8	159.5^{a} ± 2.12	165.4 ± 2.13	167.8 ± 2.03^{b}
21	851.88 ± 12.93	872.32 ± 10.42	858.32 ± 10.02
35	2303.22 ± 21.88	2285.16 ± 32.60	2248.11 ± 27.42

$^{a, b}$ – values within each row with different superscripts are different at $P \leq 0.05$

The feed conversion ratio (FCR) of broiler chickens at different ages, expressed as kg feed/kg weight gain, is shown in Table 3. At the period of 1–8 days FCR compared to control was 9 % lower in chickens, fed diet supplemented with vegetable oils *Origami aetheroleum* and 11% lower in chickens fed diet supplemented with vegetable oils *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M, ($P \geq 0.05$). At the period of 22–35 days, the FCR decreased compared to control only where *O. aetheroleum* and *P. acidilactici* MA 18/5 M were added, ($P \geq 0.05$). FCR was 1–2% lower in both groups, compared to control during all experimental period.

The highest 10% mortality in broiler chickens was estimated in control group and one where *Origami aetheroleum* was added.and 6% respectively in group where *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M were added.

The study data of biochemical blood serum indices is presented in Table 4. Total cholesterol, HDL-Cholesterol and LDL-Cholesterol level decreased in both experimental groups, compared to control, but statistically significant differences were found only in *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M group ($P \leq 0.05$). Supplementation of vegetable oils *Origami aetheroleum* alone and in combination with *Pediococcus acidilactici* MA 18/5 M to diet of chickens reduced concentration of triglycerides, α2 globulins and γ, but it was not statistically significant ($P \geq 0.05$). Total protein and β globulins levels increased in both experimental groups, compared to control ($P \geq 0.05$).

Table 3 The effect of *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M on FCR (kg feed.kg^{-1} weight gain) in broiler chickens

Trial periods in days	Control	*Origami aetheroleum*	*Origami aetheroleum* + *Pediococcus acidilactici*
1 – 8	1.63 ± 0.08	1.48 ± 0.03	1.45 ± 0.05
9 – 21	1.38 ± 0.05	1.40 ± 0.02	1.24 ± 0.07
22 – 35	1.95 ± 0.07	1.94 ± 0.20	1.94 ± 0.07
1 – 35	1.70 ± 0.02	1.69 ± 0.10	1.68 ± 0.03

Table 4 The effect of *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M on biochemical blood indices in broiler chickens

Blood indices	Control	*Origami aetheroleum*	*Origami aetheroleum* + *Pediococcus acidilactici*
Cholesterol, mmol.l^{-1}	4.32^{a} ± 0.16	3.92 ± 0.21	3.63 ± 0.15^{b}
HDL-cholesterol, mmol.l-1	3.10^{a} ± 0.00	2.83 ± 0.14	2.74 ± 0.12^{b}
LDL-cholesterol, mmol.l^{-1}	0.73 ± 0.20	0.66 ± 0.13	0.52 ± 0.06
Triglycerides, mmol.l^{-1}	0.92 ± 0.30	0.74 ± 0.20	0.82 ± 0.15
Total protein, g.l^{-1}	37.60 ± 1.33	39.82 ± 2.20	38.26 ± 2.21
Albumin,%	55.60 ± 3.14	52.70 ± 1.38	56.08 ± 2.48
Alpha 1 globulins, %	4.0 ± 0.73	3.60 ± 0.61	4.82 ± 0.36
Alpha 2 globulins, %	9.76 ± 1.02	7.62 ± 0.32	8.72 ± 0.41
Beta globulins, %	18.84 ± 5.21	27.16 ± 3.02	25.88 ± 2.66
Gamma globulins, %	11.78 ± 2.84	8.88 ± 2.03	4.50 ± 0.71

$^{a, b}$ – values within each row with different superscripts are different at P≤0.05

Albumin and α1 globulins levels decreased in group which received diet with *Origami aetheroleum* alone, while increase compared to control was estimated in group supplemented with *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M (P≥0.05). **Lee et al. (2003)** reported that dietary carvacrol versus thymol at the concentration of 200 ppm lowered weight gain and feed intake, but improved the feed gain ratio when birds were fed the respective diet for 4 weeks. We suggest that the effect of dietary carvacrol on feed gain ratio could be related to increased efficiency of feed utilization and/or altered carcass composition.

It is widely believed that elevated cholesterol levels in the blood represent a risk factor for heart disease, with low-density lipoproteins (LDL) being most concern. There is evidence that phytobiotics may be able to reduce total LDL cholesterol levels. **Tollba et al. (2010)** reported that plasma total cholesterol and total lipids were decreased (P<0.05) in groups fed aromatic herbal extract either alone or blended with organic acids compered with control groups under cold temperature conditions.

The data of preceacal availability of P and Ca is presented in Table 5. The preceacal availability of Ca and P decreased in both experimental groups, compared to control.

Table 5 The effect of *O. aetheroleum* and *P. acidilactici* MA 18/5 M on preceacal P and Ca availability in broiler chickens

Parameter	Control	*O. aetheroleum*	*O. aetheroleum* + *P. acidilactici*
Ca	82.29 ± 8.14	81.78 ± 5.84	80.95 ± 8.08
P	86.50 ± 8.95	82.19 ± 6.72	85.04 ± 5.85

CONCLUSION

The highest effect of *Origami aetheroleum* and its combination with *Pediococcus acidilactici* MA 18/5 M on body weight of broiler chickens was estimated till 21st day. Addition of *Origami aetheroleum* and *Pediococcus acidilactici* MA 18/5 M during period of 9–21 days had the most effective influence on FCR. Feed additives positively reduced the concentration of blood cholesterol, HDL-cholesterol and LDL-cholesterol. Preceacal availability of Ca and P had tendency to decrease when phytobiotics or probiotics were added.

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