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Original Contribution

EFFECT OF FEEDING LACTINA® PROBIOTIC ON PERFORMANCE, SOME BLOOD PARAMETERS AND CAECAL MICROFLORA OF MULE DUCKLINGS

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ABSTRACT

A study was carried out to determine the effect of dietary probiotic Lactina® on production performance, some blood parameters and caecal microflora of male mule ducklings raised in 93 days under field conditions. The probiotic preparation Lactina® consisted of freeze-dried pure cultures of *Streptococcus thermophilus, Enterococcus faecium* and 4 strains of *Lactobacillus*. Each gram of Lactina® contained $0.1x10^9$ CFU. Day-old birds were randomly allocated to -L (n = 2240) and +L (n = 2330) groups. The difference between treatments on these birds was the supplementation of the probiotic in the feeds (300 g/t) of +L group. Lactina® significantly improved body weight gain, feed conversion ratio and liver weight of mules. The probiotic supplementation did not affect the intestine length, the weight of gizzard, heart, and blood constituents comprising, haemoglobin, total protein and cholesterol concentrations. Lactina® fed ducklings had reduced total counts of bacteria, *E. coli* and *Salmonella* and elevated number of *Lactobacilli* in the caecal digesta. In addition, lower cost of body weight yield and lower mortality rate was found for the same treatment.

Key words: probiotic Lactina®, ducklings, performance, microbial populations, caecum, blood constituents

INTRODUCTION

In modern poultry production, different types of growth promoters are being applied*. The public concern about pathogenic resistant bacteria in humans (1) determines the increasing pressure by the consumer for a reduction or ban on use of nutritive antibiotics. This situation then calls for active search for alternative products that would replace the antibiotic growth promoters. Some of these new products -probiotics – are live microbes, which grow in the gastrointestinal tract (2) and create beneficial conditions for nutrients' utilisation (3, 4, 5), inhibit pathogenic bacteria (6, 7) in the host (8). Utilising probiotics in animal nutrition provides not only economic and health benefits (9, 10, 11) they produce also safe foods.

Information on the use of probiotics in the waterfowl is scarce. On account of this we have embarked on a study to evaluate the effect of feeding probiotic preparation Lactina® on production performance, some blood parameters and caecal microbial population of the mule duckling.

MATERIALS AND METHODS

Male mule ducklings were randomly allocated to -L (n = 2240) and +L (n = 2330) groups. The difference between the treatments on both groups was the supplementation of the probiotic Lactina® to the feeds of +L group at inclusion rate of 300 g/t. Lactina® is a probiotic preparation of freeze-dried pure cultures of *L. bulgaricus, L. acidophilus, L. helveticus, L. lactis, Streptococcus thermophilus* and *Enterococcus faecium*. Each gram of Lactina® contained $0.1x10^9$ CFU. The birds were fed diets formulated according to INRA (1989) (12) recommendations. The composition and nutrient content of starter and finisher are given on **Table 1.**

Birds were kept in rooms with concrete floor covered with pine wood shavings litter

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to a depth of 5 cm and the stocking rate (birds/m^2) was as follows: Week One - 25; Week Two – 15; Week Three – 10 and from Week Four – 5-6. After Week Five ducklings had free access to open area. The ambient temperature was maintained at 27-28°C during Week One, 23-24°C during Week Two and 18-22°C during Week Three and subsequently. The mules received continuous artificial lighting according to the following regimen: Week One-Three - 24 h light, Week Four - 18 h light: 6 h darkness, Week Five and subsequently - 16 h light: 8 h darkness. Feed and drinking water were supplied *ad libitum*. Starter was fed as mash and finisher as pellets with diameter ∅ = 4 mm.

Table 1*: Composition and nutrient content of starter and finisher*

Item	***Starter*** 1-21 days	***Finisher*** 22-93 days
Ingredients, %:		
Maize	*50.44*	*52*
Wheat	*15*	*22.4*
Soya bean meal	*22*	*9*
Sunflower meal	*6*	*10*
Fish meal	*3*	*3*
L-lysine	*0.1*	*0.05*
DL-methionine	*0.11*	*-*
Sodium chloride	*0.2*	*0.2*
Limestone	*1.4*	*1.8*
Dicalcium phosphate	*1.2*	*1*
Toxibind	*0.3*	*0.3*
Vitamin and mineral premix[1]	*0.25*	*0.25*
Nutrient value:		
Metabolizable energy[3]*, MJ/kg*	*11.9*	*12.1*
Crude protein[2]*, %*	*19.2*	*16.8*
Calcium[2]*, %*	*0.96*	*1.07*
Phosphorus[2]*, %*	*0.67*	*0.62*
Lysine[3]*, %*	*1.08*	*0.77*
Methionine[3]*, %*	*0.47*	*0.33*
Methionine+cystine[3]*, %*	*0.80*	*0.63*

[1]*Premix provided per 1 kg of compound feed: vitamin A – 9500 IU, vitamin* D_3 *– 3050 IU, vitamin E – 30 mg, thiamine - 1 mg, riboflavin – 6,5 mg, pyridoxine - 2 mg, biotin – 0,05 mg, folic acid – 0,375 mg, Ca-panthotenate - 10 mg, choline chloride – 350 mg, Fe –54 mg, Zn – 50 mg, Cu – 5 mg, Mn – 80 mg, I – 1 mg, Se – 0,2 mg*
[2]*Proximate analysis*
[3]*Calculated analysis*

The average body weight (BW) of ducklings at 93 days of age was calculated after group weighing. From Day 93 to Day 110 the birds from both groups were force-fed but no probiotic was applied.
Mortality rate for each group was calculated for 93-day period.

The ingredients and nutritional value of diets are given on **Table 1**.

The proximate analysis of feeds was performed using procedures detailed by the Association of Official Analytical Chemistry (AOAC, 1990) (12).

Starter and grower were analysed for lactic acid bacteria and *Enterococcus faecium*. At Days 54 and 93 of the experiment 10 ducklings of each treatment were weighed before the morning feeding and decapitated. Afterwards the intestine length, liver weight, gizzard and heart weights were measured.

The caeca were removed and opened after treatment of their surface with 79% ethanol. One gram of caecal contents was homogenised in 9 ml peptone water (0.1% peptone, wt/vol) and serial dilutions were prepared and plated on Nutrient agar, double layer Hydrolised milk agar with China blue, Endo agar and Brilliant-green Phenol-red agar for determination of total bacteria count, number of lactic acid bacteria, coli and salmonella bacteria, respectively.

The blood samples were taken from *v. jugularis* of the same decapitated birds. The serum was separated by centrifugation for 10 min at 2000x g. Total cholesterol content was determined using CHOP-PAP enzymatic colorimetric method by means of Cholesterol Liquicolor kit (*Human GmbH, Germany*).

Whole blood samples were analysed for ASAT, ALAT and triglycerides concentrations by diagnostic kits (*Roche Diagnostics GmbH, Manheim, Germany*) using an automated biochemical analyser (*Reflotron Manual, Germany*). Haemoglobin levels were determined following the cyanhaemoglobin method. Total protein concentrations were measured by the Biuret reaction.

Breast fillet (magret), thighs and foie gras were measured after slaughtering of birds at age 110 days and average yield was calculated on total basis.

The data for growth performance, weight of internal organs, microbial population and blood parameters were expressed as means and standard deviations and were subjected to statistical analysis using the software package STATISTICA (1994) (14).

RESULTS AND DISCUSSION

The count of *Lactobacilli* and *Enterococci* in the starter (mash form) was 1 x 10^6 and 0.2 x 10^6 CFU/g and 0.1 x 10^6 and 0.05 x 10^6 CFU/g in the finisher (pellets), respectively. Apparently, heat treatment and high pressure during pelleting process has decreased the counts of *Lactobacilli* ten-fold and *Enterococcus faecium* – four-fold. These results showed the higher resistance of *Enterococcus faecium* to the pelleting treatment, which makes these bacteria a suitable compound of probiotic preparations for inclusion in pelleted feeds.

Feed intake for 93-day period was 17.9 kg/bird for +L group and 0.214 kg higher compared to -L treatment. The higher feed consumption resulted in nearly 0.200 kg higher average BW of probiotic group. Feeding Lactina® improved FCR with 4% compared to the non-supplemented group **(Table 2).**

In similar experiments with Muskovy ducks, but at higher inclusion rate of Lactina® (1 and 0.5 kg/t starter and finisher, respectively), Penkov et al. (15) reported better FCR for probiotic fed birds compared to the non-supplemented group. Penkov and Hristova (2004) (16) found more efficient energy utilisation and amino acids digestibility after probiotic administration. According to (17) and (5) improved feed conversion might be explained by the increased intestinal amylase activity when lactic acid bacteria are fed to fowl.

As expected, the weight of liver, gizzard and heart of 93-day mules was higher compared to 54-day ducklings. The highest increase was mainly at the extent of liver, which doubled its weight for the period from 54 to 93 days of age. Lactina® fed birds had significantly heavier liver than –L group: 28 and 39% at age of 54 and 96 days, respectively. Penkov et al. (2004) (15) have also found higher yield of giblets of male Muskovy ducklings receiving the same probiotic, compared to the control group. In the experiment of (18) probiotic supplementation resulted in 24% heavier gizzard and 20% heavier liver than non-supplemented broilers.

Adding lactic acid bacteria has led to increased BW yield and reduced FCR of 40-day old broiler chickens (19). Similar favourable effects of *Lactobacillus* have been found by other authors (20, 21).

According to (22) and (11) probiotics stimulate the immune function and this might be the possible reason for the lower mortality rate of Lactina® fed ducklings in our experiment **(Table 2).**

Table 2*: Body weight, feed consumption, feed conversion ratio and mortality rate of 93-day old ducklings, fed diets with Lactina® (+L, n=2330) or without Lactina® supplementation (-L, n=2240)*

Items	***Treatments***	
	+L	***-L***
Average body weight, kg	3.932	3.743
Total feed consumption, kg	18.115	17.901
Feed conversion ratio, kg/kg	4.678	4.860
Mortality rate for 93 days, %	1.7	3

The body weight of mules at 54 days of age was not improved by the probiotic treatment, but significant difference was found at the end of experiment (93 days) **(Table 3).** The length of intestines was similar and was not affected either by the age, or by Lactina® feeding. Apparently, by the day 54 ducklings already have well developed intestines. In contrast, laying hens without lactic bacteria supplementation had longer intestines (Nahashon et al., 1996), explained by the presence of unfavorable microflora. Ivanov (2004) has found that feeding probiotics significantly improved the length, weight and volume of small intestines and caecum of broiler chickens.

Table 3: *Body weight, intestine length and internal organs weight (mean±SD) of 54 u 93–day old ducklings (n=10), fed diets with Lactina® (+L) or without Lactina® supplementation (-L)*

Items	**Age, days**			
	54		**93**	
	+L	**-L**	**+L**	**-L**
Body weight, g	3581±162.4[a]	3488±247.5[a]	4017±169.0[b]	3883±204.2[c]
Intestine length, cm	260±10.7	253±16.7	278±26.4	265±18.7
Internal organs, g	200.8±15.6[a]	173.2±11.1[b]	314.3±33.9[c]	262.4±30.6[d]
Liver, g	91.9±8.76[a]	72.4±7.19[b]	186.7±20.76[c]	135.3±20.55[d]
Gizzard, g	91.5±9.49[a]	84.8±6.54[a]	100.3±13.88[a]	97.2±15.18[a]
Heart, g	17.4±1.79[a]	16.0±0.64[a]	27.3±1.37[b]	29.5±3.27[b]

[a,b]*Means without a common superscripts within the same line differ at P<0.05*

Blood parameters of mules were within the physiological ranges and were not influenced either by Lactina® inclusion or by the age of the birds **(Table 4).** The only exception was the decreased concentration of blood triglycerides in ducklings from -L group and the reason for this was not known.

Table 4*: Some blood parameters (mean±SD) of 54 u 93-day old ducklings (n=10), fed diets with Lactina® (+L) or without Lactina® supplementation (-L)*

Items	***Age, days***			
	54		***93***	
	+L	***-L***	***+L***	***-L***
Whole blood				
Haemoglobin, g/L	133.2±6.9	124.7±9.3	128.8±6.69	127.2±12.3
ASAT, U/L	20.9±5.18	25.6±8.80	22.3±4.74	24.8±9.12
ALAT, U/L	11.9±3.68	14.8±3.88	9.2±2.91	13.5±3.23
Triglycerides, mmol/L	2.4±0.30[a]	1.6±0.38[b]	4.5±1.01[a]	3.5±1.49[a]
Blood serum				
Total protein, g/100 g	3.92±0.21	4.03±0.25	4.78±0.39	4.26±0.65
Total cholesterol	4.36±0.78	4.10±0.31	4.33±0.30	4.14±0.43

[a,b]*Means without a common superscripts within the same line differ at P<0.05*

The probiotic tested did not significantly affect the serum levels of cholesterol of 54 and 93-day old ducklings **(Table 4),** confirming the results of previous trials with broiler chickens (23). The cholesterol concentrations were in the range of 4.1-4.4 mmol/L regardless of the age of the birds.

Feeding 0,05 or 0,1% *Lactobacillus* cultures to broilers has decreased the serum cholesterol levels (24). Similar cholesterol depressing effect has been found in rats (25), broilers (26) and laying hens (27, 28).

The review of (29) indicated that the effects of probiotics on serum cholesterol in humans are inconclusive. Rossouw et al. (1981) (30) has demonstrated even elevated cholesterol levels in humans on diets supplemented with probiotic bacteria.

The mechanisms for reduction of serum cholesterol levels are still not clear. Some authors (31, 32, 33) suggested that *L. acidophilus* was able to assimilate the cholesterol molecules. Such traits of *Lactobacillus* and *Bifidobacteria in vitro* conditions has been reported by (34).

Lactobacillus and *Bifidobacteria* could contribute to the regulation of serum cholesterol concentrations by deconjunction of bile acids. Since the excretion of deconjugated bile acids is enhanced and cholesterol is its precursor, more molecules are spent for recovery of bile acids (35). As a result of increased synthesis of this acids, it is expected the level of serum cholesterol to be reduced.

Klaver and van der Meer (1993) (36) suggested that co-precipitation with bile acids might be of importance for decreasing of serum cholesterol concentrations.

Significant changes in microbial populations of caecal content of mules were found (**Table 5**). Apparently, the inclusion rate was high enough and the strains of Lactina® preparation were suitable to resist the pelleting process and acidic conditions and to grow in the intestine. Six-fold increase in concentration of lactic acid bacteria in the caecal content of 54-day old ducklings was found in +L group compared to –L group. In addition, significant reduction of bacteria counts in +L group was found as follows: total counts - 1.6-fold, *Coli*

- three-fold and *Salmonella* - 2.2-fold. These changes in microbial populations showed the favourable effect of added probiotic on *Lactobacillus* concentrations in the caeca of birds. While *Lactobacilli* in caecal content of –L group represented only 5.8%, in +L group they reached 56% of total bacterial counts. Presumably, the predominance of lactic acid bacteria was related to the better performance of Lactina® fed ducklings. The same preparation has suppressed *Enterococci* and *Salmonella* growth in Muskovy ducks (37) and decreased *Salmonella* releasing in Japanese quails (38).

Table 5: *Bacteria counts (mean±SD) in the caecal digesta of 54-day old ducklings (n=10), fed diets with Lactina® (+L) or without Lactina® supplementation (-L)*

Items	***Age, days***	
	54	
	+L	**-L**
Total bacteria counts, x 10^7/g	$16.7±5.47^a$	$27.1±10.27^b$
Lactobacilli, x 10^6/g	$90.3±9.50^a$	$15.7±3.77^b$
Coli, x 10^6/g	$2.7±0.91^a$	$8.3±1.98^b$
Salmonella, x 10^6/g	$3.0±0.78^a$	$6.6±1.64^b$

^{a,b}Means without a common superscripts within the same line differ at P<0.05

Table 6: *Average yield of magret, thighs and foie gras and cost of body weight (BW) yield of 110-day old force-fed ducklings after 93-day feeding with Lactina® (+L) or without Lactina® supplementation (-L)*

Items	***Treatments***	
	+L	**-L**
Magret, g	800	815
Thighs, g	720	708
Foie gras, g	690	700
Cost of feed, BGL/t	459	450
Cost of BW yield, BGL/t*	2147	2187

*Calculated on the basis of feed cost

Ivanov (2004) (18) has also found 5-fold reduction in *Coli* counts and increase in Gram-positive bacteria in the large intestine of broiler chickens after feeding lactic acid bacteria.

Expectations that after force-feeding of heavier birds with larger liver, more foie gras will be produced, were not satisfied. Significant differences in the yield of magret, thighs and foie gras were not found **(Table 6).** Presumably, the most possible reason for these results was the human factor: after the day 93 +L and –L groups were force-fed by different teams.

Taking into account the market price of the probiotic (30 BGL/kg) and its inclusion rate (0.3 kg/t), addition of Lactina® increased the feed cost with 9 BGL/t (Table 6). Regardless of this, due to the lower FCR, one tone of BW yield was achieved with 40 BGL cheaper compared to the non-supplemented group.

In addition to this beneficial effect, 1.3 points reduction in mortality rate was found for Lactina® fed ducklings.

CONCLUSIONS

Feeding the probiotic preparation Lactina® to mule ducklings resulted in elevated concentrations of *Lactobacillus* in the caecum at the extent of inhibition of *Salmonella* and *Coli*-forms. In addition, increased BW, 4% reduction in FCR and lower mortality rate were found compared to the non-supplemented group.

Blood haemoglobin, total protein and total cholesterol concentrations were not significantly affected by the probiotic.

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