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Original Contribution

EFFECT OF DIET FORMULATION ON BASIS OF DIGESTIBLE AMINO ACIDS AND SUPPLEMENTATION OF PROBIOTIC ON PERFORMANCE OF BROILER CHICKS

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ABSTRACT

The aim of the study was to investigate the effect of diet formulation on digestible amino acids (DAA) basis and supplementation of probiotic Lactina® on performance of broiler chickens. Four treatments were tested: TAA - total amino acids diet formulation, DAA - digestible amino acids diet formulation, TAA+L and DAA+L - diets with Lactina® supplementation. 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. Formulation of diets for broilers on DAA basis promoted the existence of increased levels of poor quality protein sources (sunflower meal and meat and bone meal) and resulted in reduced feed cost per 1 kg of yield without negative influence on performance traits. Lactina® fed chickens had decreased feed conversion ration, increased body weight and yield of carcass, meat, liver, gizzard and heart. The tested probiotic preparation did not affect the total cholesterol serum content and the small intestine morphometry but tended to reduce the total counts of bacteria and *E. coli* and to increase *Lactobacilli* concentrations in the caecal digesta.

Key words: digestible amino acids, diet, formulation, probiotic Lactina®, broilers, performance, economic effect

INTRODUCTION

High efficiency in broiler production requires maximising the feed utilisation and minimising the losses due to mortality and/or growth retardation. For maintaining an optimal productivity and good health status the following factors play some vital roles:

1. Feeding diets balanced on digestible amino acid (DAA) basis, instead of total amino acids (TAA) content
2. Supplementation of feeds with probiotics.

In the past, poultry producers and nutritionists have been formulating compound feeds on TAA basis However, in recent decade there has been an increasing awareness on the need to balance diets on DAA basis (1, 2, 3, and 4). The switch to the DAA basis follows from the fact that, for synthesis of their proteins, birds utilise not the protein per se, but this part of amino acids which is available (digestible). Consequently, it seems logical to determine requirements of DAA on one hand and to meet them in providing properly balanced feeds on the other hand. An increasing amount of data, regarding DAA requirements and DAA content of feedstuffs, has been published (1, 2, 4, 5).

After deprivation of nutritive antibiotics as growth promoters attempts to test alternatives have been made. One of possibilities is water or diet supplementation with probiotics – mono- or mixed cultures of live microbes (bacteria, fungi and yeast) – that affect the microbial balance in the host gastrointestinal tract (GIT) (6). A large amount of experiments on chicken has demonstrated positive influence on the immune response (7, 8), enzymatic activity in the small intestines (9) and feed utilisation (10, 11). In addition, probiotics may contribute to the improvement of health status of birds, suppressing the growth of *E. coli, Salmonella, Shigella* (12), *Pasteurella and Staphylococcus,* neutralising their endotoxins (13) and reducing ammonia production in the

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intestines (14).

No significant effect on growth rate and feed efficiency has been found in other studies on broiler chickens (15, 16, 17). Most likely, the inconsistent results in using probiotics might be due to differences in species and strains of microbes, in combinations, in inclusion rate and way of application.

The present experiment aimed to study the effect of diet formulation on basis of digestible amino acids and supplementation of probiotic Lactina® on performance of broiler chickens.

MATERIALS AND METHODS

A trial with broiler chickens (hybrid Ross 500) was carried out from one to 42 days of age. The chickens were distributed into four groups and each group consisted of 8 000 birds:

TAA – fed diets balanced on total amino acid basis

TAA+L – fed diets balanced on total amino acid basis and supplemented with probiotic Lactina®

DAA – fed diets balanced on digestible amino acid basis

DAA+L – fed diets balanced on digestible amino acid basis and supplemented with probiotic Lactina®.

Compound feeds were formulated according to the guidelines of Ross Breeders Limited (18). The composition and nutrient content of starter, grower and finisher are given on **Table 1** and **Table 2**.

Table 1: *Composition and nutrient content of starter (S), grower (G) and finisher (F), balanced on total amino acid basis*

Item	S	G	F
	0-10 days	11-28 days	29-42 days
Ingredients, %:			
Maize	48.89	50.21	56.51
Soya bean meal	29	27	21
Sunflower expeller	7	7.9	9
Meat and bone meal	5	6	6
Fish meal	4	3	1
Sunflower oil	3.5	3.8	4.5
L-lysine	0.1	0.1	0.1
DL-methionine	0.15	0.13	0.1
Sodium chloride	0.25	0.25	0.25
Limestone	0.3	0.2	0.13
Dicalcium phosphate	0.9	0.5	0.5
Vitamin and mineral premix[1]	0.5	0.5	0.5
Roxazim G®	0.01	0.01	0.01
Coccidiostat[2]	0.05	0.05	0.05
Mycofix®	0.15	0.15	0.15
Selacid®	0.2	0.2	0.2
Nutrient value:			
Metabolisable energy[4], MJ/kg	12.8	13.3	13.7
Crude protein[3], %	24.9	23.2	21.0
Calcium[3], %	1.10	1.00	0.97
Available phosphorus[4], %	0.52	0.48	0.44
Lysine[3], %	1.38	1.26	1.06
Methionine[3], %	0.54	0.50	0.43
Methionine+cystine[3], %	0.95	0.91	0.86
Treonine[3], %	0.92	0.90	0.78
Digestible lysine[4], %	1.17	1.10	0.90
Digestible methionine[4], %	0.50	0.47	0.40
Digestible methionine+cystine[4], %	0.85	0.81	0.71
Digestible treonine[4], %	0.81	0.78	0.67

[1]*Premix provided per 1 kg compound feed: vitamin A – 14000 IU, vitamin D_3 – 4000 IU, vitamin E – 80 mg, thiamine - 5 mg, riboflavin – 8 mg, pyridoxine - 4 mg, biotin – 0.14 mg, folic acid – 2 mg, Ca-panthotenate - 25 mg, Fe – 50 mg, Zn – 80 mg, Cu – 12 mg, Mn – 100 mg, I – 1 mg, Se – 0.2 mg*
[2]*Withdrown during the last week of fattening*
[3]*Proximate analysis,* [4]*Calculated analysis*

The single difference between groups TAA and DAA on one hand and groups TAA+L and DAA+L on the other was the presence of the probiotic Lactina® in the feeds for the last two groups. Lactina was included at rate of 600 g/t of starter and 300 g/t of grower and finisher. Lactina is a probiotic preparation of freeze-dried pure cultures of *Lactobacillus bulgaricus, L. acidophilus, L. helveticus, L. lactis, Streptococcus thermophilus* and *Enterococcus faecium*. Each gram of Lactina® contained $0.1x10^9$ CFU.

Table 2: *Composition and nutrient content of starter (S), grower (G) and finisher (F), balanced on digestible amino acid basis*

Item	S	G	F
	0-10 days	11-28 days	29-42 days
Ingredients, %:			
Maize	51.45	52.07	56.24
Soya bean meal	25	25	19
Sunflower expeller	9	9	11.4
Meat and bone meal	7	7	7
Fish meal	2	1	-
Sunflower oil	3.3	3.8	4.5
L-lysine	0.26	0.24	0.22
DL-methionine	0.18	0.18	0.18
Sodium chloride	0.25	0.25	0.20
Limestone	0.15	0.15	0.15
Dicalcium phosphate	0.5	0.4	0.3
Vitamin and mineral premix[1]	0.5	0.5	0.5
Roxazyme G®	0.01	0.01	0.01
Coccidiostat[2]	0.05	0.05	0.05
Mycofix®	0.15	0.15	0.15
Selacid®	0.2	0.2	0.2
Nutrient value:			
Metabolisable energy[4], MJ/kg	12.8	13.3	13.7
Crude protein[3], %	23.8	22.1	20.9
Calcium[3], %	1.13	1.07	0.95
Phosphorus[3], %	0.92	0.88	0.83
Available phosphorus[4], %	0.52	0.48	0.45
Lysine[3], %	1.29	1.22	1.01
Methionine[3], %	0.50	0.47	0.40
Methionine+cystine[3], %	0.90	0.86	0.79
Treonine[3], %	0.87	0.85	0.76
Digestible lysine[4], %	1.16	1.08	0.92
Digestible methionine[4], %	0.51	0.45	0.40
Digestible methionine+cystine[4], %	0.82	0.80	0.72
Digestible treonine[4], %	0.75	0.73	0.65

[1]*Premix provided per 1 kg compound feed: vitamin A – 14000 IU, vitamin D_3 – 4000 IU, vitamin E – 80 mg, thiamine - 5 mg, riboflavin – 8 mg, pyridoxine - 4 mg, biotin – 0.14 mg, folic acid – 2 mg, Ca-panthotenate - 25 mg, Fe – 50 mg, Zn – 80 mg, Cu – 12 mg, Mn – 100 mg, I – 1 mg, Se – 0.2 mg*
[2]*Withdrown during the last week of fattening*
[3]*Proximate analysis*
[4]*Calculated analysis*

Birds were kept in rooms with concrete floor covered with pine wood shavings to a depth of 5 cm. The stocking rate was 14 chickens/m^2. The birds received continuous artificial lighting daily according to the following regimen: 0-6 days - 23 h light: 1 h dark, 7-35 days - 5 h light: 1 h dark (4 cycles/day), 36-42 days - 23 h light: 1 h dark. Feed and drinking water were supplied for *ad libitum* consumption.

Live weight (LW) of 200 randomly selected broilers of each group was registered at 14-day intervals until 42 days of age.

Mortality rate for each group was calculated for the 42-day period.

The chemical analyses of feeds were performed following the procedures of AOAC (19). Amino acids content was determined by means of Amino-analyser after hydrolysis of feed samples. Digestible amino acids were calculated using coefficients of true ileal digestibility, published by Degussa (20).

Broilers were slaughtered at age of 42 days and for every 1000 chickens the following weights were measured: carcass, breast fillet, thighs, liver, gizzard and heart.

At day 42 of the experiment six chickens from groups TAA and TAA+L were decapitated. Samples (0.5 cm) of jejunum and ileum were immediately taken and washed by tap water. Afterwards they were treated with a 10% solution of formaldehyde for 24 h and with a 5% solution of formaldehyde for 6 days. The samples were treated with haematoxylin-eosin solution and villi height and layers' thickness was measured using the light microscope.

At the end of experiment the caeca were removed after slaughtering of 20 randomly selected broilers from groups DAA and DAA+L. Caeca were opened after treatment of their surface by 79% ethanol solution. 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 hydrolysed 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 20 randomly selected chickens of groups DAA and DAA+L. The serum was separated by centrifugation at 2000g for 10 min. Total cholesterol content was determined using CHOP-PAP enzymatic colorimetric method by means of Cholesterol liquicolor kit (*Human GmbH, Germany*).

The experimental data were subjected to statistical evaluation by analysis of variance for probiotic supplementation and for amino acid formulation using the software package STATISTICA (21).

RESULTS AND DISCUSSION

Formulating diets on TAA basis (**Table 1**) or on DAA basis (**Table 2**) resulted in lower total amino acids concentration in feed for group DAA. The inclusion rate of high protein but expensive ingredients (soya bean meal and fish meal) in diet DAA was reduced to the extent of available and cheaper sunflower and meat and bone meal. Regardless of the treatment, the feed intake remained within the range of 3.7-3.8 kg per 42-day period. The growth rate of chickens of group DAA, the yield of carcass, breast fillet, thighs, liver, gizzard and heart were similar to those of group TAA (**Table 3** and **Table 4**).

Table 3: *Body weight, feed consumption, feed conversion ratio and mortality rate of chicken fed diets balanced on total (TAA) or digestible amino acid (DAA) basis and with Lactina® supplementation (TAA+L, DAA+L)*

Items	Groups				
	TAA	TAA+L	DAA	DAA+L	SEM
Body weight, g					
age, days					
0	46.1	45.7	45.0	46.4	1.68
14	320.8	325.2	318.6	330.0	10.5
28	1005.6	1038.4	1016.3	1040.5	22.0
35	1615.2	1688.9	1609.7	1692.3	36.1
42	1943.2[a]	2017.8[b]	1930.5[a]	2028.1[b]	42.8
Feed consumption, kg	3.78	3.77	3.81	3.74	
Feed conversion ratio, kg/kg	2.00	1.91	2.02	1.89	
Mortality rate for 42 days, %	3.9	2.1	4.6	2.9	

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

Similar results have been obtained by Marinov (22). The author found that the partial replacement of soya bean meal by sunflower meal and supplementation of diet with synthetic lysine and methionine did not negatively affect the performance of broilers. At present, this effect might be explained by the concept for digestible amino acids (3, 4, 23).

It seems reasonable in estimating the biological value of feeds for poultry to take into account DAA rather than TAA contents (24). Digestible amino acid levels more closely represent this portion of protein, which is available to birds for maintenance and production. An increasing number of publications has demonstrated the beneficial effect of using DAA in diet formulations to increase the inclusion levels of poorly digestible ingredients. Uzu (25) successfully replaced part of soya been meal by sunflower and rape seed meal, maintaining broilers' growth rate and feed conversion ratio when diets were formulated on digestible but not on total amino acid basis. The same approach allowed utilisation of canola meal by up to 20% in compound feed (26). Rostagno et al. (27) compared the effect of typical maize-soya bean meal diet for chickens with formulations based on rice bran, feather meal and meat and bone meal. Data have shown again that applying of TAA as a criterion for diet optimisation gave worse results compared to utilising DAA values.

Table 4: *Slaughtering traits of chicken fed diets balanced on total (TAA) or digestible amino acid (DAA) basis and with Lactina® supplementation (TAA+L, DAA+L)*

Items	Groups				
	TAA	TAA+L	DAA	DAA+L	SEM
Carcass, g	1340.0[a]	1420.5[b]	1322.0[a]	1438.1[b]	29.6
Carcass yield, %	68.96	70.40	68.48	70.91	2.65
Breast fillet, g	355.3[a]	397.6[b]	360.4[a]	390.2[b]	12.3
Thighs, g	468.2[a]	488.1[ab]	459.8[a]	492.0[b]	14.6
Internal organs, g	82.0[a]	91.0[b]	80.3[a]	92.3[b]	2.18
Liver, g	41.5[a]	45.3[b]	40.1[a]	47.2[b]	1.15
Gizzard, g	32.4[a]	35.4[b]	32.3[a]	34.0[ab]	0.82
Heart, g	8.5[a]	10.7[b]	7.9[a]	11.1[b]	0.22

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

The yield of meat and internal organs for human consumption from group DAA was more profitable than from group TAA (**Table 7**). These results were possible due to reduced protein levels of feeds and inclusion of cheaper ingredients for group DAA without depression in broilers' performance.

Lactina® supplementation to broiler diet had no significant effect on feed consumption (**Table 3**) but LW and carcass, breast fillet, thighs, liver, gizzard and heart weights were improved (**Table 4**). FCR of groups TAA+L and DAA+L was 4-6% lower compared to control birds (groups TAA and DAA). Feed utilisation might be enhanced by the increased amylolytic activity but not due to proteolytic and lipolytic activity in the small intestine under the influence of added lactic bacteria (28, 29).

Inclusion of *L. acidophilus* or mixture of 12 strains of *Lactobacillus* resulted in increased BW gain and reduced FCR in broilers at the age of 40 days (29, 30). Similar beneficial effect of *Lactobacillus* has been observed by other authors (31, 32).

Table 5 : *Serum total cholesterol and bacteria counts (X±SD) in the caecal digesta of chicken fed diets balanced on digestible amino acid (DAA) basis and with Lactina® supplementation (DAA+L)*

Items	Groups	
	DAA	DAA+L
Serum total cholesterol, mmol/L	3.04±0.37	3.06±0.45
Bacreria counts		
Total, x 10^8/g	3.43±1.44	0.42±0.17
Lactobacilli, x 10^7/g	0.27±0.09	0.42±0.11
Coli, x 10^7/g	1.07±0.26	0.34±0.08

After 42 days of probiotic feeding (DAA+L) the villi height and the thickness of jejunum and ileum walls had values similar to these of control group (**Table 6**). In contrast, other experiments have shown that the treatment of birds with *Lactobacillus* has led to changes in small intestinal mucosa, associated with increased IgA and IgM production. Dunham et al. (33) observed higher villi and deeper crypts, and Nahashon et al. (10) observed increased cellularity of Peyer's patches. Salminen et al. (34) and Rautava and Isolauri (35) also found a positive effect of probiotics on defence function of intestinal wall and immune system. This type of changes might be the possible explanation of lower mortality rate in chickens from group TAA+L and group DAA+L (**Table 3**) compared to the groups without probiotic supplementation.

In our experiment, probiotic supplementation did not significantly affect the level of total cholesterol in the serum of 42-day aged broilers (**Table 5**) and remained within the physiological range of 3 mmol/L.

In contrast, the addition of 0.05 and 0.1% *Lactobacillus* cultures to feed led to significant reduction in the cholesterol levels in the serum of broilers (36). Similar depressing effect of probiotics on cholesterol serum concentrations has been found in rats (37), broilers (31) and layer hens (38).

Decreased level of cholesterol might be associated with its co-precipitation with bile acids (39) or assimilation by *Lactobacillus* cells (40).

The lack of effect of probiotic supplementation in the present experiment could be explained by the pathway of regulation of cholesterol level in blood serum. Most likely, the feedback for maintaining cholesterol metabolism could have masked the possible influence of probiotic.

Added probiotic preparation tended to influence the microbial populations in the caecal digesta (**Table 5**). Total counts of bacteria, and specifically of *coli*-forms, were suppressed by the microbes of Lactina included in diet DAA+L. To that extent more *Lactobacilli* were found but differences between supplemented (DAA) and non supplemented (DAA+L) groups did not reach significance due to high variability of individual samples (p>0.05). Inhibitory effect of *Lactobacilli* on *coli* counts has been demonstrated in experiments of Samarai and All-Attar (41).

Table 6: *Morphometry (X±SD) of jejunum and ileum of chickens at age of 42 days fed diets balanced on digestible amino acid (DAA) basis and with Lactina® supplementation (DAA+L)*

Items	Groups	
	DAA	DAA+L
Jejunum		
Villi height, µm	10.74±3.36	10.25±1.51
Muscle layer thickness, µm	2.52±0.66	2.29±0.37
Propria thickness, µm	2.74±0.71	2.98±0.41
Total wall thickness, µm	5.26±1.37	5.27±0.78
Ileum		
Villi height, µm	6.82±3.05	6.67±1.07
Muscle layer thickness, µm	2.80±0.39	2.45±0.37
Propria thickness, µm	2.03±0.48	1.69±0.36
Total wall thickness, µm	4.83±0.87	4.11±0.73

Improved performance of chickens fed probiotics might be associated with the partial replacement of intestinal microflora by *Lactobacilli*. Jin et al. (29) succeeded in decreasing β-glucuronidase content in GIT of chickens by means of *Lactobacilli* strains, which is related to reduced hydrolysis of glucuronides - toxic substances. The latter compounds could be a possible reason for deteriorated nutrient utilisation and decreased performance of birds.

It has been stated that lower concentration of pathogens in the GIT of birds is due to "competitive exclusion": adhesion of *Lactobacilli* to intestinal mucosa to the extent of *E. coli* and *Salmonella* (28, 42).

Table 7: *Economic effect of diets balanced on total (TAA) or digestible amino acid (DAA) basis and with Lactina® supplementation (TAA+L, DAA+L)*

Items	Groups			
	TAA	TAA+L	DAA	DAA+L
Feed cost, euro/kg	0.245	0.250	0.223	0.229
Gain cost, euro/kg*	0.487	0.478	0.451	0.432
Additional income**				
Compared to TAA group, euro/kg	0	0.028	0.020	0.032

**Calculated on the basis of feed cost*
***From fillet, thighs, livers, gizzards and hearts*

Results of "competitive exclusion" are not always consistent. Fuller (43) decreased *E. coli* counts in the crop and small intestines using *Lactobacillus* strains, but not in the caeca of chickens. In contrast, Adler and DeMassa (44) and Watkins and Kratzer (45) reported reduced *E. coli* in caecal content of broilers after *Lactobacilli* supplementation in feed.

The economic effect of diet formulation on DAA basis and Lactina® supplementation is given on **Table 7**. As expected, inclusion rate of 0.6 kg/t starter and 0.3 kg/t grower and finisher contributed to more expensive feed (with 5 euro/t) in groups TAA+L and DAA+L. This effect was offset by the reduced FCR and improved yield of meat and internal organs for consumption. In addition, balancing diets on DAA basis and increased inclusion rate of sunflower meal and meat and bone meal resulted in cheaper feed formulation for groups DAA and DAA+L compared to groups TAA and TAA+L.

Among all treatments, the lowest cost of yield (432 euro/t) and the highest additional income (32 EUR/t) were obtained from broilers of group DAA+L. This was a consequence of the combined positive effects of balancing feeds on DAA basis and Lactina® supplementation on performance of chickens.

CONCLUSIONS

Formulation of diets for broilers, on DAA basis, leads to reduced feed cost per 1 kg of yield without negative influence on performance traits.

Lactina® fed chickens have improved BW gain, FCR and yield of carcass, meat, liver, gizzard and heart. The tested probiotic preparation does not affect the total cholesterol serum content and the small intestine morphometry but tends to reduce the total counts of bacteria and *E. coli* and to increase *Lactobacilli* concentrations in the caecal digesta.

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