Effect of dietary *Lactobacillus spp*. and *Enterococcus faecium* supplementation on muscle amino acid profile and protein properties in broilers

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SUMMARY

This experiment was conducted on 400 broiler chicks to evaluate the amino acids (AAs) profile in response to feeding diets containing *Lactobacillus* species. Birds were randomly segregated into 5 groups. During the first experimental period (1-21 days of age), group T1 was fed the diet that contained 4 x 10^6 CFU.g^{-1} the group T2 contained 1.2 x 10^7 CFU.g^{-1} and for group T3 contained 2 x 10^7 CFU.g^{-1} of *L. plantarum* and *L. acidophilus*, which represents 1%, 3%, and 5% Kashik of total amount of diet respectively. Group T4 contained (2.5 x 10^6.g-1 E. *faecium*) and the group C considered as control. From d 22-40 day of age all groups were fed diets without any probiotics additives. Probiotics improved amino acids of breast, and the legs muscle, however the influence of probiotics on muscles AAs was not similar. The results of fed probiotics at day 21 of age observed improvement in T1 and T2 groups more than other treatments. During this period of age, the best positive influence observed in treatment T1. At 40 day old, the positive influence of probiotics on AAs in breast were significantly ($P < 0.01$) more in T1 and T3 than other treatments. The results were disproportionate and variable between two age stages. Probiotics significantly improved of AAs in the legs in treatment T2 at 21 day old but in other treatments were not improved significantly ($P < 0.01$). At 40 days old, probiotics significantly improved all legs AAs in treatments T1 and T2 ($P < 0.01$). The influence of probiotics on the legs muscle was more than breast muscles. In generally, the probiotics did not uniquely determined the content of AAs on the breast and legs muscles

Keywords: broiler; dried yogurt; meat quality; amino acids; *Lactobacillus* species; *E. faecium*

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INTRODUCTION

Probiotics are being developed for use in animal feed to enhance production performance and prevent gastrointestinal infections. The ban on using antibiotics as growth promoters, antibiotic resistance and the inherent problems of developing new vaccines make a compelling case for developing alternatives to antibiotics. Among the probiotics in use today, *Lactobacillus* (LAB) has been shown to play a vital role in disease prevention, immune enhancement, improved growth and carcass yield in poultry (Apata, 2008). D'Mello (2003) reported that the responses of growing poultry to individual amino acids (AAs) are influenced by a wide range of factors. They include: environmental temperature, immunological stress, sex age, species, and several dietary factors. The 10 AAs including alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine are produced. Tyrosine is produced from phenylalanine, so if the diet is deficient in phenylalanine, tyrosine will be required as well. The essential AAs are arginine (required for the young, but not for adults), histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Addition of LAB to broiler chick diets significantly improved growth performance, increased nutrient digestibility and stimulated humoral immune response (Apata, 2008). Lactic acid bacteria such as Lactobacilli, Streptococci and Bifidobacteria are the most common organisms used in probiotic preparations. Zhang (2007) reported that the probiotic improved AAs of breast, and the flavor AAs were higher than that of the other group ($P < 0.01$), meanwhile Pelicano et al. (2005) reported that the addition of growth promoters did not influence the qualitative and quantitative parameters of carcass and breast meat of broiler chickens. Mahmood et al. (2005) reported that the results showed that there were significant differences in broiler carcass chemical composition between the probiotic-treated groups and the control. However, Zhou et al. (2010) found no significant difference among the groups with and without probiotics in the above chemical composition of Guangxi Yellow chickens. Kroliczewska et al. (2008) reported that in the chemical leg muscle analyses, the legs crude protein percentage was not significantly affected by the probiotics. The inclusion of probiotics in the diets at early ages 1 to 21 days of chicks might improve chick performance at slaughter age (42 days) by reducing pathogenic microorganisms and improving the utilization of nutrients (Khosravi et al., 2008). The aim of the experiment was to confirm how the probiotics additives influence the quality of broiler´s breast and legs muscles, when the birds fed with probiotics only for first 3 weeks of age.

MATERIALS AND METHODS

The experiment was conducted on 400 day-old broiler chicks Strain ROSS 308 were randomly divided into five groups (C, T1, T2, T3 and T4) with four replicates of 20 birds each and reared for 40 days (Table 1). This experiment conducted at the Zvolen Research Centre in Slovakia. Feed and water were offered *adlibitum*. During the first experimental period (1-21 days of age), the feed for group T1 contained 4 x 10^6 CFU.g^{-1} the group T2 contained 1.2 x 10^7 CFU.g^{-1} and for group T3 contained 2 x 10^7 CFU.g^{-1} of *L. plantarum* and *L. acidophilus*, which represents 1%, 3%, and 5% Kashik (dried yogurt) of total amount of diet respectively (Table 1), group T4 contained (2.5x 10^6.g^{-1} E. *faecium*) and the group C considered as control. All treatment groups from 22-40 d of age were fed diets without probiotic additives.

Table 1. Design of the experiment

Treatment	Replications	Birds	Total
C[1]	4	20	80
T1 [2]	4	20	80
T2[3]	4	20	80
T3 [4]	4	20	80
T4 [5]	4	20	80
Total (Birds)	400		

[1]C = Control group none used probiotics; [2]T1 = Contains 4x 10^6.g^{-1} *L. plantarum* and *L. acidophilus;* [3]T2 = Contains 1.2x 10^7.g^{-1} *L. plantarum* and *L. acidophilus;* [4]T3 = Contains 2x 10^7.g^{-1} *L. plantarum* and *L. acidophilus*; [5]T4= Contains 2.5x 10^6.g^{-1} *E. faecium*

In the experiment a 4-stage feeding program was used; first pre-starter for the first week, second: starter for 2nd and 3rd week of age, during these stages treatment groups were supplied with probiotics (Table 2). While the treatment bird diets (grower and finisher) were not supplied with probiotics from 22-40 day of age (Tables 1 and 2). On d 21, 20 birds (4 from each group) were slaughtered, and the same number of birds were slaughtered on d 40 (all birds slaughtered during the experiment were 40 birds as sample for analysis). For amino acids analysis, the ion exchange chromatography method was used in amino acid analyzer (AAA 400 by INGOS, Prague, Czech R.). ISO 13903 (2005) standard method was applied. The following principle was preceded Amino acid analysis: 1. Oxidation; 2. Hydrolysis; 3. Adjustment of pH; 4. Chromatography; 5. Calculation of results. All data from the experiments were analyzed with using a one-way ANOVA (SPSS, 1997) and Duncan Multiple Range test (Duncan, 1955) used to study the differences between the mean values (mean ± SD of 20 chicks related to meat quality). In addition, the (a, b, c, d) means with different superscript within row are significantly different (P < 0.01).

Table 2. Rations and calculated content nutrition in pre-starter, starter, grower and finisher ration

Components (%)		Pre-starter					Starter					Grower	Finisher
		C[1]	T1[2]	T2[3]	T3[4]	T4[5]	C[1]	T1[2]	T2[3]	T3[4]	T4[5]		
Corn		41.50	41.50	41.50	41.75	41.50	43.50	43.50	43.50	43.80	43.50	43.00	44.80
Soybean meal (46%)		33.50	32.50	30.50	28.50	33.50	31.00	30.00	28.00	26.00	31.00	30.00	26.00
Wheat		12.50	12.50	12.50	12.50	12.50	17.00	17.00	17.00	17.00	17.00	20.00	23.00
Fish meal		4.50	4.50	4.50	4.50	4.50	2.70	2.70	2.70	2.70	2.70	-	-
Rapeseed oil		4.50	4.50	4.50	4.50	4.50	2.50	2.50	2.50	2.50	2.50	3.20	2.55
CaCO3		0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	1.45	1.10
Monocalicum phosphate		1.20	1.20	1.20	1.20	1.20	1.10	1.10	1.10	1.10	1.10	1.00	1.00
Vitamin- mineral premix (BR1) 1.0 %*		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-
Vitamin- mineral premix (BR2) 1.0 %*		-	-	-	-	-	-	-	-	-	-	1.00	-
Vitamin- mineral premix (BR3) 1.0 %*		-	-	-	-	-	-	-	-	-	-	-	1.00
Salt		0.25	0.25	0.25	-	0.25	0.30	0.30	0.30	-	0.30	0.30	0.33
Methionine 99%		0.15	0.15	0.15	0.15	0.15	-	-	-	-	-	0.05	0.12
Lysine 78		-	-	-	-	-	-	-	-	-	-	-	0.10
Kashik %		-	1.00	3.00	5.00	-	-	1.00	3.00	5.00	-	-	-
IMB52 %		-	-	-	-	0.0025	-	-	-	-	0.0025	-	-
Crude Protein	$g.kg^{-1}$	235.6	236.6	238.5	240.7	235.6	217.2	218.1	220.1	222.3	217.2	196.8	184.5
Metabolisable energy	$MJ.kg^{-1}$	13.0	13.0	13.1	13.1	13.0	12.6	12.6	12.6	12.7	12.6	12.6	12.6
Ash	$g.kg^{-1}$	63.5	64.4	66.2	65.5	63.5	58.6	59.5	61.3	60.2	58.6	57.3	52.2
Crude Fibre	$g.kg^{-1}$	27.0	26.6	25.8	25.0	27.0	27.4	27.0	26.2	25.4	27.4	27.5	26.9
L – lysine	$g.kg^{-1}$	15.0	15.1	15.5	15.8	15.0	13.4	13.6	13.9	14.3	13.4	12.1	10.1
Methionine + Cysti	$g.kg^{-1}$	11.7	11.7	11.8	11.9	11.7	9.7	9.7	9.8	9.9	9.7	9.4	9.4
Ca	$g.kg^{-1}$	10.2	10.2	10.3	10.4	10.2	9.1	9.2	9.2	9.3	9.1	7.7	7.9
Phosphorus	$g.kg^{-1}$	7.7	7.7	7.8	7.8	7.7	7.0	7.0	7.1	7.1	7.0	6.0	5.9
Na	$g.kg^{-1}$	1.7	2.3	3.2	3.4	1.7	1.7	2.2	3.2	3.0	1.7	1.3	1.4

*The premix compositions are shown in Table 3; [1]C = Control group none used probiotics; [2]T1 = Contains 4x $10^6.g^{-1}$ *L. plantarum* and *L. acidophilus;* [3]T2 = Contains 1.2x $10^7.g^{-1}$ *L. plantarum* and *L. acidophilus;* [4]T3 = Contains 2x $10^7.g^{-1}$ *L. plantarum* and *L. acidophilus;* [5]T4= Contains 2.5x $10^6.g^{-1}$ *E. Faecium*

Table 3. The composition of supplementary compound premixes

		Premix BR1	Premix BR2	Premix BR3
Index	Unit	1.0 %	1.0 %	0.5 %
Vitamin A	$IU.kg^{-1}$	1 500 000	1 200 000	2 000 000
Vitamin D3	$IU.kg^{-1}$	500 000	500 000	500 000
Vitamin E (alpha-tocopherol)	mg/kg	7 000	5 000	4 000
Vitamin K3	$mg.kg^{-1}$	400	200	400
Vitamin B1	$mg.kg^{-1}$	400	200	400
Vitamin B2	$mg.kg^{-1}$	800	600	1 000
Vitamin B6	$mg.kg^{-1}$	500	330	600
Vitamin B12	µg/kg	3 000	2 400	3 000
Vitamin B9	$mg.kg^{-1}$	200	84	100
Ca Panthotenate	$mg.kg^{-1}$	2 500	1 650	2 000
Niacin	$mg.kg^{-1}$	6 000	3 000	4 000
Biotin	$mg.kg^{-1}$	20	12	10
Choline – chloride	$mg.kg^{-1}$	50 000	20 000	40 000
Lysine	$g.kg^{-1}$	140	180	-
Methionine	$g.kg^{-1}$	240	240	200
Co	$mg.kg^{-1}$	40	40	65
I	$mg.kg^{-1}$	110	110	190
Mn	$mg.kg^{-1}$	11 300	11 300	18 500

Cu	mg.kg^{-1}	1 500	1 540	1 900
Se	mg.kg^{-1}	30	30	50
Zn	mg.kg^{-1}	8 200	8 200	13 700
Fe	mg.kg^{-1}	7 600	12 500	12 400
Endox	mg.kg^{-1}	12 500	5 000	25 000
Robenidin	mg.kg^{-1}	3 300	-	-
Salinomycinat Na E766	mg.kg^{-1}	-	6 000	-

RESULTS AND DISCUSSION

Breast muscles AAs composition

The results showed that there were significant differences in the breast AAs composition at day 21 of age among the probiotic-treated groups and the control (Table 4). The composition of breast muscles AAs at day 21 of age (Table 4) indicated that the influence of probiotics on AAs is not similar.

Table 4. Effects of treatments on AAs composition of breast muscles at day 21 of age

Essential AAs (g/kg AAs/DM)	Treatments C[1]	T1[2]	T2[3]	T3[4]	T4[5]
Arginine	58.62±0.228 a*	57.09±0.284 b	55.72±0.327 c	53.87±0.160 d	57.04±1.126 b
Histidine	36.41±2.798	37.75±0.202	38.27±0.633	35.08±0.289	37.07±1.401
Isoleucine	32.66±0.251 d	36.30±0.185 a	34.27±0.669 c	35.47±0.493 ab	34.97±0.407 bc
Leucine	60.64±0.622 a	60.77±0.282 a	58.90±0.210 b	59.40±0.308 ab	59.45±1.481 ab
Lysine	68.04±0.330 b	67.80±0.217 b	65.30±0.827 c	70.71±0.572 a	67.80±1.395 b
Methionine	23.93±0.314 b	25.54±0.509 a	25.12±0.557a	23.66±0.071 b	23.74±0.415 b
Phenylalanine	29.59±1.040	30.49±0.310	29.46±0.396	30.31±0.830	29.72±1.016
Threonine	36.55 ± 0.199 b	37.47 ± 0.381a	34.48± 0.362c	36.51 ± 0.459 b	35.15 ± 0.458 c
Valine	37.40±0.710 abc	38.64±0.410 a	35.95±0.288 c	37.98±0.333 ab	36.98±1.284 bc
Non-essential AAs (g/kg AAs/DM)					
Alanine	44.21±0.8092 ab	45.04±0.2962 a	41.82±0.617 c	43.66±0.348 ab	43.56±0.939 b
Asparagine	76.98±0.263 a	76.33± 0.316 a	72.94 ±1.593 bc	74.68 ± 1.122 ab	71.85 ± 1.777 c
Cystine	9.21±0.128 c	13.27±0.650 a	12.65±0.290 a	10.63±0.271 b	8.65±0.235 c
Glutine	98.15±0.181 b	102.76±0.437 a	97.44±0.380 b	101.47±0.363 a	97.96±1.379 b
Glycine	32.31±0.462 b	35.48±0.462 a	31.96±0.246 b	32.63±0.301 b	33.09±1.126 b
Proline	31.33±0.415 b	34.22±0.195 a	31.17±0.841 b	34.31±0.228 a	31.59±0.778 b
Serine	32.24±0.392 a	32.15±0.304 a	30.03±0.317 c	30.93±0.088 b	30.51±0.419 bc
Tyrosine	25.73±0.588 ab	26.90±0.477 a	25.36±0.195 b	25.41±0.413 b	26.26±1.071 ab

* Values are expressed as mean ±SD of 20 chicks, (a, b, c) means with different superscript within row are significantly different ($P < 0.01$); [1]C = Control group none used probiotics; [2]T1 = Contains 4x 10^6.g^{-1} *L. plantarum* and *L. acidophilus;* [3]T2 = Contains 1.2x 10^7.g^{-1} *L. plantarum* and *L. acidophilus;* [4]T3 = Contains 2x 10^7.g^{-1} *L. plantarum* and *L. acidophilus*; [5]T4= Contains 2.5x 10^6.g^{-1} *E. faecium*

Probiotics significantly improved lysine in T3 (2.67 g/kg higher than in C group), isoleucin (3.64 and 1.61 g) and methonine (1.61g and 1.19 g resp.) in T1 and T2 of breast muscles at 21 day in compare with C group, however arginine (4.75g in T3) and threonine (2.07g in T2) were decreased. Histidine and phenylalanine were not affected by probiotics, while leucine and valine decreased in T2 (Table 4). Nonessential AAs were affected by probiotics;

cystine, glutine and proline were improved. These findings corroborate the results reported by Zhang (2007) who reported that the probiotic improved AAs of breast, but it is in contrast with Pelicano et al. (2005), while alanine, asparagines and serine reduced. Glycine was significantly higher in T1 compared with C samples, but tyrosine was not affected by probiotics at 21day (Table 4). During this stage of age, in general, the positive influence of using of LAB showed in treatment T1 was more than other treatments in majority of essential and nonessential AAs in breast (Table 4), for example; isoleucine (36.30), methionine (25.54), phenylalanine (30.49), threonine (37.47), alanine (45.04), cystine (13.27), glutine (102.76), glycine (35.48), proline (34.22).

Table 5. Effects of treatments on AAs composition of breast muscles at day 40 of age

Essential AAs (g/kg AAs/DM)	Treatments				
	C[1]	T1[2]	T2[3]	T3[4]	T4[5]
Arginine	59.06±0.331 ^{a}*	58.10±0.458 ^{b}	56.24±0.279 ^{c}	59.51±0.229 ^{a}	58.90±0.269 ^{a}
Histidine	35.41±0.155 ^{b}	38.51±0.370 ^{ab}	40.69±0.213 ^{a}	40.56±5.052 ^{a}	42.27±0.826 ^{a}
Isoleucine	34.42±0.194 ^{cd}	37.35±0.243 ^{a}	34.17±0.299 ^{d}	36.08±0.245 ^{b}	35.06±0.679 ^{c}
Leucine	61.56±0.125 ^{a}	61.29±0.269 ^{a}	59.40±0.313 ^{b}	61.85±0.380 ^{a}	60.03±0.983 ^{b}
Lysine	69.06±0.282 ^{a}	69.29±0.201 ^{a}	65.65±0.270 ^{b}	69.13±0.222 ^{a}	69.44±0.356 ^{a}
Methionine	24.48±0.242 ^{c}	25.96±0.348 ^{a}	25.18±0.257 ^{b}	24.26±0.259 ^{cd}	23.83±0.305 ^{d}
Phenylalanine	30.22±0.506 ^{bc}	31.01±0.287 ^{ab}	29.76±0.296 ^{c}	31.63±0.387 ^{a}	30.65±0.807 ^{abc}
Threonine	37.42±0.260 ^{ab}	38.72±0.391 ^{a}	35.28±0.101 ^{c}	33.22±0.348 ^{d}	36.25±1.742 ^{bc}
Valine	38.04±0.175 ^{a}	39.82±0.320 ^{a}	36.89±0.382 ^{b}	38.59±0.334 ^{a}	37.31±0.841 ^{b}
Non-essential AAs (g/kg AAs/DM)					
Alanine	45.89±0.192 ^{a}	45.91±0.209 ^{a}	43.33±0.393 ^{b}	45.42±0.309 ^{a}	43.08±1.079 ^{b}
Asparagine	76.85±0.238 ^{a}	76.71±0.250 ^{a}	75.31±0.249 ^{c}	75.80±0.204 ^{b}	74.37±0.164 ^{d}
Cystine	11.68±0.181 ^{c}	13.78±0.247 ^{a}	13.29±0.230 ^{b}	11.80±0.113 ^{c}	8.71±0.190 ^{d}
Glutine	99.92±0.112 ^{c}	103.64±0.335 ^{b}	105.67±0.277 ^{b}	112.26±0.363 ^{a}	103.98±2.072 ^{b}
Glycine	32.92±0.094 ^{c}	35.89±0.257 ^{a}	33.81±0.142 ^{b}	33.22±0.348 ^{bc}	32.07±0.706 ^{d}
Proline	32.10±0.330 ^{d}	35.13±0.194 ^{b}	33.16±0.147 ^{c}	35.71±0.228 ^{a}	31.78±0.106 ^{d}
Serine	33.36±0.320 ^{a}	32.99±0.198 ^{ab}	31.27±0.244 ^{c}	31.85±0.259 ^{bc}	31.24±1.393 ^{c}
Tyrosine	21.98±0.770 ^{c}	26.88±0.293 ^{a}	25.29±0.271 ^{b}	27.40±0.247 ^{a}	26.98±0.496 ^{a}

*Values are expressed as mean ±SD of 20 chicks, (a, b, c) means with different superscript within row are significantly different ($P < 0.01$). [1]C = Control group none used probiotics; [2]T1 = Contains 4x $10^6.g^{-1}$ *L. plantarum* and *L. acidophilus;* [3]T2 = Contains 1.2x $10^7.g^{-1}$ *L. plantarum* and *L. acidophilus;* [4]T3 = Contains 2x $10^7.g^{-1}$ *L. plantarum* and *L. acidophilus*; [5]T4= Contains 2.5x $10^6.g^{-1}$ *E. faecium*

The results of fed probiotics on breast muscle essential AAs composition at 40 day of age as in (Table 5) showed significant differences among treatments; histidine, isoleucine, methionine, phenylalanine and valine were improved especially in T1 group (3.10g, 2.93g, 1.48g, 0.79g and 1.78g higher than in C group respectively), these results are in agreement to Zhang, (2007) and Mahmood et al. (2005), but in contract with Zhou et al. (2010), while in T3 group; arginine, histidine, isoleucine, leucine, lysine, phenylalanine and valine were improved (0.45g, 5.15g, 1.66g, 0.29g, 0.07g, 1.41g and 0.55g higher than in C group resp.), the results are in contract with found by Pelicano et al.(2005), however in agreements to Zhang (2007). The positive effects of probiotics on histidine of breast T2 and T3 groups were significantly higher than in control, while arginine was lower in T1 and T2 compared with control (Table 5). Leucine and valine were not affected by probiotics (expect in T2 and T4 which significantly decreased), while lysine was not affected by probiotics, expect in T2 which significantly decreased (Table 5). Probiotics able to change the types and numbers of gut microflora. LAB have proven to be effective in broiler performance. For re-establishing, the natural conditions which have been disrupted by modern trends in conditions used for rearing young Birds. However, the high level of LAB not always has the positive effect.

Nonessential AAs were affected by probiotics; glutine and tyrosine were improved. In agreement to Zhang (2007) and contrast with Pelicano et.al. (2005), while cystine and Glycine were improved in T1 and T2, but both decreased in T4, and proline was improved in T1, T2 and T3, but not affected in T4 (Table 5), in agreement with findings of Zhang (2007), (Khosravi *et al*., 2008), but in contrast with Pelicano et.al. (2005) and Zhou et al. (2010), while asparagines and serine in T2, T3, and T4 were decreased in comparison with the control group at 40 d old (Table 5). During this stage of age, the positive influence of using 1% (T1) and 5% (T3) of LAB on isoleucine, pheylalanine and valine in breast were more than other levels of LAB. The results were disproportionate and variable between two age stages.

Table 6. Effects of treatments on AAs composition of legs muscle at day 21 of age

Essential AAs	Treatments				
(g/kg AAs/DM)	C[1]	T1[2]	T2[3]	T3[4]	T4[5]
Arginine	44.71±0.341 ^{b}	41.82±0.455 ^{c}	46.04±0.660 ^{a}*	41.91±0.290 ^{c}	42.55±0.580 ^{c}
Histidine	20.98±0.870 ^{a}	19.44±0.355 ^{b}	21.29±0.529 ^{a}	19.24±0.242 ^{b}	20.87±0.765 ^{a}
Isoleucine	23.82±1.070 ^{b}	24.05±0.340 ^{ab}	25.43±0.611 ^{a}	23.06±0.533 ^{b}	20.08±0.895 ^{c}
Leucine	43.42±0.556 ^{b}	42.25±0.805 ^{c}	45.76±0.293 ^{a}	40.55±0.226 ^{d}	41.09±0.370 ^{d}
Lysine	48.29±0.693 ^{b}	47.47±0.768 ^{bc}	50.43±1.636 ^{a}	45.89±0.448 ^{c}	45.98±0.838 ^{c}
Methionine	19.13±0.326 ^{b}	20.69±0.326 ^{a}	19.25±0.154 ^{b}	19.65±0.296 ^{b}	19.11±0.240 ^{b}
Phenylalanine	22.67±0.496 ^{b}	22.31±0.309 ^{b}	23.97±0.361 ^{a}	21.16±0.509 ^{c}	21.10±0.626 ^{c}
Threonine	25.63±0.443 ^{b}	24.48±0.022 ^{c}	27.47±0.428 ^{a}	23.13±0.438 ^{d}	25.11±0.190 ^{bc}
Valine	25.04±0.952 ^{b}	25.00±0.506 ^{b}	27.10±0.408 ^{a}	24.26±0.211 ^{b}	21.30±1.637 ^{c}
Non-essential AAs (g/kg AAs/DM)					
Alanine	32.78±0.476 ^{b}	32.13±0.394 ^{b}	34.06±0.477 ^{a}	30.74±0.189 ^{c}	32.57±0.447 ^{b}
Asparagine	52.26±0.628 ^{b}	51.74±0.193 ^{bc}	57.91±0.690 ^{a}	48.55±0.346 ^{d}	51.12±0.214 ^{c}
Cystine	9.20±0.090 ^{b}	9.88±0.309 ^{a}	8.53±0.159 ^{c}	10.12±0.091 ^{a}	9.36±0.176 ^{b}
Glutine	78.31±1.174 ^{b}	78.88±0.970 ^{ab}	80.09±0.495 ^{a}	79.19±0.149 ^{ab}	76.47±0.213 ^{c}
Glycine	28.67±0.575	28.45±0.620	30.49±0.758	28.09±0.458	27.81±3.001
Proline	26.80±0.285 ^{b}	24.42±0.355 ^{d}	27.78±0.585 ^{a}	26.66±0.085 ^{b}	25.44±0.686 ^{c}
Serine	23.76±0.487 ^{a}	22.43±0.370 ^{b}	24.80±0.767 ^{a}	21.54±0.405 ^{b}	24.04±0.621 ^{a}
Tyrosine	19.71±0.353 ^{ab}	19.44±0.360 ^{b}	20.46±0.445 ^{a}	19.03±0.298 ^{bc}	18.84±0.344 ^{c}

*Values are expressed as mean ±SD of 20 chicks, (a, b, c) means with different superscript within row are significantly different ($P < 0.01$). [1]C = Control group none used probiotics; [2]T1 = Contains 4x 10^{6}.g^{-1} *L. plantarum* and *L. acidophilus;* [3]T2 = Contains 1.2x 10^{7}.g^{-1} *L. plantarum* and *L. acidophilus;* [4]T3 = Contains 2x 10^{7}.g^{-1} *L. plantarum* and *L. acidophilus*; [5]T4= Contains 2.5x 10^{6}.g^{-1} *E. faecium*

Legs muscle AAs composition.

Analysis of variance showed significant difference among different treatment groups. The effect of LAB on the leg muscle is presented in Table 6. At 21 day of age, a positive effect was observed in essential AAs between treatment T2 ($P > 0.01$) when compared with control group (expect in histidine and methionine). However, probiotics significantly decreased arginine, histidine, leucine, lysine and phenylalanine in the leg muscles in treatments T1, T3 and T4, whereas significantly increasing of arginine, isoleucine, leucine, lysine, phenylalanine, threonine and valine in T2 was observed (Table 6). Probiotics significantly increased alanine, asparagine, glutine and proline in the leg muscles in treatment T2 was observed in 21 day old (Table 6), whereas cystine was decreased. However, other treatments were lower compared with control (expect in cystine and serine). In 40 day old, probiotics significantly improved all legs muscle AAs in T1 and T2 groups (**e**xpect proline), when compared with group C, as in (Table 7). All AAs in T1 were higher than C group. Results are in agreement with findings by Mahmood et al. (2005) and Zhang (2007), but in contract with that reported by Kroliczewska et al. (2008) and Zhou et al. (2010). The positive effects of probiotics on the legs muscle AAs in T1 and T2 groups were higher than in T3 and T4 (Table 7).

Table 7. Effects of treatments on AAs composition of legs muscle at day 40 of age

Essential AAs	Treatments				
(g/kg AAs/DM)	C[1]	T1[2]	T2[3]	T3[4]	T4[5]
Arginine	44.08±0.505 ^{b}*	47.32±0.128 ^{a}	47.02±0.266 ^{a}	42.30±0.183 ^{c}	43.65±0.936 ^{b}
Histidine	20.56±0.469 ^{c}	24.89±0.308 ^{a}	25.54±0.133 ^{a}	20.24±0.160 ^{c}	21.86±0.772 ^{b}
Isoleucine	23.84±1.423 ^{cd}	29.42±0.215 ^{a}	27.20±0.584 ^{b}	23.32±0.321 ^{d}	25.11±0.190 ^{c}
Leucine	43.40±0.029 ^{c}	48.96±0.498 ^{a}	47.29±0.440 ^{b}	40.97±0.363 ^{d}	43.42±0.352 ^{c}
Lysine	48.17±0.131 ^{c}	54.26±0.575 ^{b}	55.25±0.128 ^{a}	47.38±0.156 ^{d}	48.58±0.375 ^{c}
Methionine	19.30±0.215 ^{b}	20.46±0.109 ^{a}	19.53±0.212 ^{b}	19.61±0.439 ^{b}	19.30±0.132 ^{b}
Phenylalanine	22.33±0.555 ^{b}	25.27±0.242 ^{a}	24.41±0.332 ^{a}	21.99±0.277 ^{b}	22.62±0.561 ^{b}
Threonine	23.53±0.547 ^{bc}	27.58±0.416 ^{a}	27.75±0.611 ^{a}	22.82±0.975 ^{c}	24.91±0.904 ^{b}
Valine	24.51±0.264 ^{d}	30.70±0.453 ^{a}	29.19±0.292 ^{b}	25.43±0.289 ^{cd}	26.00±0.877 ^{c}
Non-essential AAs (g/kg AAs/DM)					
Alanine	31.11±0.294 ^{d}	34.71±0.241 ^{b}	35.31±0.050 ^{a}	32.40±0.302 ^{c}	32.31±0.288 ^{c}
Asparagine	50.00±0.382 ^{b}	56.65±0.326 ^{a}	57.56±0.577 ^{a}	47.64±2.261 ^{c}	52.19±0.390 ^{b}
Cystine	9.19±0.127 ^{d}	9.96±0.137 ^{b}	10.50±0.134 ^{a}	9.55±0.243 ^{c}	9.65±0.069 ^{bc}
Glutine	79.63±0.436 ^{d}	92.84±0.524 ^{b}	94.11±0.323 ^{a}	80.14±0.450 ^{d}	84.18±0.667 ^{c}
Glycine	27.37±0.300 ^{b}	30.63±0.354 ^{a}	27.53±0.446 ^{b}	26.96±2.317 ^{b}	28.10±1.108 ^{b}
Proline	27.06±0.142 ^{ab}	27.49±0.334 ^{a}	26.22±0.482 ^{c}	26.70±0.174 ^{bc}	25.44±0.351 ^{d}
Serine	21.16±0.828 ^{d}	24.54±0.315 ^{b}	27.75±0.611 ^{a}	22.82±0.975 ^{c}	22.67±0.340 ^{c}
Tyrosine	19.43±0.135 ^{c}	20.93±0.299 ^{a}	20.98±0.130 ^{a}	20.49±0.440 ^{ab}	19.94±0.496 ^{bc}

*Values are expressed as mean ±SD of 20 chicks, (a, b, c) means with different superscript within row are significantly different ($P < 0.01$). [1]C = Control group none used probiotics; [2]T1 = Contains 4x $10^{6}.g^{-1}$ *L. plantarum* and *L. acidophilus;* [3]T2 = Contains 1.2x $10^{7}.g^{-1}$ *L. plantarum* and *L. acidophilus;* [4]T3 = Contains 2x $10^{7}.g^{-1}$ *L. plantarum* and *L. acidophilus*; [5]T4= Contains 2.5x $10^{6}.g^{-1}$ *E. faecium*

CONCLUSIONS

In conclusion, 1% (T1) of probiotics had most positive effects on both breast and legs muscle AAs at 40 day of age. In compare between probiotics influence breast and legs AAs compositions. The inclusion of probiotics in the diets might improve chick performance by reducing pathogenic microorganisms and improving the utilization of nutrients (Khosravi et al., 2008). Probiotics affected on legs muscle more than breast muscles. Feeding broilers with probiotics improved breast AAs profile at day 21 of age, and also the positive influence of probiotics on breast and legs AAs in T2 and T3 at 40 day old. Broilers in general require further research to clearly understand the functional mechanism between *lactobacillus spp.* and broiler meat amino acids profile.

The present study showed that LAB is an alternative to the treatment of broiler AAs profile improvement. Thus, a certain feed additives take over the role of antibiotics. Further studies are needed to investigate the effect of supplementation a mixture of Lactobacillus sp. on broiler, to obtain new and more accurate results. In addition, the investigations in this field it is very rear, hardly we found some papers similar of current experiment.

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