Bulgarian Journal of Agricultural Science, 31 (No 2) 2025, 390–396

Effect of dietary probiotic and prebiotic supplementation on meat amino acid, fatty acid and organic acid profiles in Ile-de-France lambs

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Abstract

Slavov, I., Ivanov, N. & Laleva, S. (2025). Effect of dietary probiotic and prebiotic supplementation on meat amino acid, fatty acid and organic acid profiles in Ile-de-France lambs. *Bulg. J. Agric. Sci., 31*(2), 390–396

A total of 45 Ile-de-France lambs were divided into three groups with similar characteristics. The lambs from experimental group I were individually supplemented with 8 g Immunobeta prebiotic and lambs from experimental group II − with the same amount of prebiotic + 4 g probiotic Zoovit.

When a live weight of 23-25 kg was attained, five male lambs from each group were slaughtered, and samples from *musculus Longissimus Lumborum (m. LL)* were collected.

The purpose of the study is to determine the effect of the added prebiotic and the prebiotic+probiotic combination on fatty acids, amino acids, cholesterol and organic acids. To fulfill the objective, the fatty acid and amino acid profiles of the meat, the content of cholesterol, organic acids, ribose and inosine were analyzed.

A statistically insignificant increase in all amino acids was demonstrated in lamb meat from experimental groups I and II as compared to controls. The amino acid content of *m. LL* of lambs whose ration was supplemented with Immunobeta prepbiotic was lower than that of animals that received the Zoovit + with Immunobeta combination.

The content of analysed fatty acids in lambs supplemented with the Immunobeta prepbiotic were inconsistently lower than respective values in control lambs. A similar tendency was found out for meat cholesterol.

The dietary supplementation with prebiotic and prebiotic + probiotic had not statistically significant effect on the content of organic acids, ribose and inosine in *m. LL.*

Keywords: Ile-de-France; probiotic; prebiotic; amino acids; fatty acids; cholesterol

Introduction

The meat of sheep and goats is considered as a high-quality and gourmet product in many countries worldwide. These meats are a good source of protein and are outlined with relatively low total saturated fatty acids and cholesterol content. This makes them healthy products and increases their demand and consumption (Mazhangara et al., 2019; Saha et al., 2023).

According to Liu et al. (2016) the dietary supplementation of broiler chickens with a *Lactobacillus johnsonii* probiotic improves the growth performance and meat quality; what is more, influences the meat content of the amino acid proline and total amino acids associated with meat taste and flavour. The authors affirmed that the dietary probiotic may change the fat deposits and fatty acid profile.

Cramer et al. (2018) demonstrated that the inclusion of a probiotic in the ration of chickens may alleviate the harmful effects of oxidative and heat stress. A number of researchers showed that the probiotic supplementation can increase the antioxidant capacity, reduce lipid peroxidation and reactive oxygen species in meat (Bai et al., 2016, 2017; Kim et al., 2016).

In their experiments with one control and three experimental groups of rams, Arilov et al. (2019) observed a higher content of the amino acid tryptophan in *m. LL* that received a probiotic compared to controls (P<0.05). The authors found out also higher levels of oxyproline in meat of experimental groups whose feed was supplemented with a probiotic (P<0.05), as well as higher values of the protein quality indicator.

Chang et al. (2018) concluded that the supplementation of a *Lactobacillus plantarum* based probiotic in the diet of pigs increased the amino acid content of *m. LL* vs non-supplemented pigs. The authors reported also an effect on concentrations of monounsaturated and polyunsaturated, linolenic and linoleic fatty acids, and physicochemical parameters of meat. The probiotic may influence the fatty acid profiles of meat and milk; with possible beneficial effect of lower fat and cholesterol content on consumer's health (Elghandour et al., 2024). Osman et al. (2023) found out that the dietary probiotic dose increase was inversely associated with *m. LL.* content of lauric, arachidonic, behenic and lignoceric acids in goats. The authors stated that the interaction of concentrate and the probiotic was important, and specified that arachidonic and lignoceric acids concentrations were influenced only by the probiotic but not by the concentrate feed.

It was proved that yeast-containing probiotic supplements enhance the immunity via their immunomodulatory effect on animals, reduce cholesterol, adhesion properties of intestinal mucosa and stimulate intestinal microbiota, thus contributing to gut health improvement (Elghandour et al., 2024). Milewski & Zaleska (2011) reported increased meat concentrations of 9-tetradecenoic (myristoleic), linolenic, docosahexaenoic and conjugated linoleic acid in lambs supplemented with yeast probiotic (P<0.05). The authors also stated that the meat of lambs supplemented with the *Saccharomyces cerevisiae* probiotic was outlined with higher dry matter and muscle fat content compared to control lambs (P<0.01). Furthermore, the arachidonic acid content in the intramuscular fat of probiotic-fed lambs was lower (P<0.01). The use of a yeast probiotic in lamb nutrition had a substantial effect on intramuscular fat fatty acid profiles of *m. LL* and *m. Quadriceps femoris (m. QF)*, with higher stearic acid levels in *m. LL* vs *m. QF* (P<0.05) (Milewski & Zaleska, 2011).

Benamirouche et al. (2020) reported statistically significantly lower stearic acid content and substantially higher linoleic and polyunsaturated fatty acids content (p<0.05) in breast and thighs of probiotic-supplemented broiler chicken compared to controls whereas Hossain et al. (2012) established that the addition of a probiotic decreased linoleic acid, PUFA, PUFA/SFA and n-6 levels.

Additional investigations are necessary to characterise specific strains, to identify the optimum dosage and to elucidate the interactions between probiotics and intestinal microbiota (Al-Shawi et al., 2020).

Literature data on probiotic effects on lamb amino acid and fatty acid composition are relatively few. The aim of the present study was to analyse the effects of dietary supplementation of Ile-de-France lambs with the Immunobeta prebiotic and the combination of Zoovit probiotic and Immunobeta prebiotic on *m. LL* fatty acid and amino acid profiles, the content of cholesterol, organic acids, and organic compounds ribose and inosine.

Material and Methods

The experiments were performed in the experimental base of the Agricultural Institute, Stara Zagora. A total of 45 Ile-de-France lambs divided into 3 groups – one control and two experimental − of 15 animals each were included. The groups were with similar initial live body weight, sex ratio and birth type.

The lambs were reared in group boxes supplied with feeders for hay and concentrate and drinkers with constant access to fresh tap water as stipulated by Ordinance No. 40 on the conditions for raising of agricultural animals, considering their physiological and behavioural characteristics. The animals were fed *ad libitum* (+ 5 to 10% residue) a ration corresponding to their age that met all requirements for nutrients and biologically active substances. The ration included concentrate and alfalfa hay (Table 1 and Table 2).

Table 1. Composition of compound feed for Ile-de-France lambs

Ingredient	Content, %
Soybean meal	4.00
Limestone	3.00
Salt	0.50
Wheat	42.00
Premix 16-97-K	0.20
Sunflower meal	20.00
Maize	30.30

Table 2. Nutritional composition of the compound feed

Ingredient	Content, %
Protein	15.90
Fat	2.40
Fibre	5.43
Moisture	11.40
Ca	1.20
P	0.50
Salt	0.570

The compound feed contained 1.12 units for growth, 2778.25 kcal/kg energy and TDN 0.174.

The animals from experimental group I were individually supplemented once daily with 8 g prebiotic Immunobeta, and those from experimental group II: with the same amount of the prebiotic plus 4 g probiotic Zoovit.

The probiotic preparation Zoovit consisted of four lactic acid bacterial strains: *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus salivarius* subsp. *thermophilus, Lactobacillus acidophilus, Lactobacilus lactis* and one strain *Propinibacterium*.

The Immunobeta prebiotic is an immunostimulant produced from selected *Saccharomyces cerevisiae* yeast strains through enzymatic autolysis. It consists of 30% β-glucans, 25% mannan-oligosaccharides and 7% nucleotides.

The trial lasted until a live weight of 23-25 kg was attained. After that, five male lambs from each group were slaughtered. The slaughter took place in a licensed slaughterhouse in the Stara Zagora region in line with all requirements for humane handling of animals during transport and slaughtering according to Ordinance No. 26 on the conditions for the protection and welfare of animals during their transport and Ordinance No. 22 on minimisation of animal suffering during slaughter or killing. Samples from *m. LL* were collected from all animals. After collection of samples, they were transported in a cool bag to the physico-chemical analysis lab of the AgroBioInstitute at the Agricultural Academy, Sofia.

The content of the following compounds (in g/100 g) were determined in meat: amino acids, fatty acids and cholesterol, organic acids, as well as organic compounds ribose and inosine.

For determination of meat polar metabolites hydrolysis and derivatisation were applied prior to the gas chromatography using centrifugal vacuum concentrator (Labconco Centrivap) at 60°C via rapid vortexing after 2-hour incubation in Thermoshaker (TS-100 Analytik Jena AG, Germany) at 80°C at 300 rpm and a repeated one-hour incubation and subsequent gas chromatographic analysis.

To this end, a system including Agilent GC 7890 gas chromatograph and Agilent MD 5975 mass-selective detector; HP-5MS column with the following parameters: length 30 m, diameter 0.32 mm and film thickness 0.25 µm was used.

The compounds were identified by comparison of retention times and relative retention indices (RT and RRI) with those of standard substances and mass-spectral data from libraries of The Golm Metabolome Database and NIST'08 (National Institute of Standards and Technology, USA), determined by the internal standard method.

For analysis of lipids, a thermal incubator Thermoshaker TS-100 Analytik Jena AG, Germany was used for one hour at 96ºC and 300 rpm, then the combined organic layers, after cooling and extraction of solution were vacuum-dried in a centrifugal vacuum concentrator (Labconco Centrivap) at 40ºC and 1.0 µL µL (fraction of methyl esters of tetradecanoic acid, hexadecanoic acid, 9-octadecenoic acid (E), octadecanoic acid and 9,12-octadecanoic acid (Z, Z), analyzed by the GS system.

Results were analyzed using IBM SPSS Statistics 19 statistical software using one-way ANOVA.

Results and Discussion

Table 3 presents the results about lamb amino acid profile in control, experimental group I (supplemented with Immunobeta prebiotic) and experimental group II (supplemented with Immunobeta prebiotic plus Zoovit probiotic).

The relatively narrow range of variation of amino acids levels with respect to the average determines the lack of consistent differences among the groups.

The highest content in the lamb meat from the I experimental group of the essential amino acids leucine (2.44 g/100 g), valine (1.80 g/100 g), lysine (1.75 g/100 g), threonine (1.72 g/100 g), phenylalanine (1.68 g/100 g). The differences between the I experimental group and the control group in terms of these amino acids are 19.61%, 19.21%, 19.86%, 19.44%, 20.00% with better values in the group receiving Immunobeta.

Among the amino acids with the highest content in the meat of experimental group I is glutamic acid (3.84 g/100 g), aspartic acid (3.09 g/100 g), the amino acid arginine (2.02 g/100 g), alanine (1.97 g/100 g), glycine (1.61 g/100 g) and tyrosine (1.40 g/100 g). The content of these amino acids exceeded the corresponding mean values of the unsupplemented lamb by 20.00%, 19.77%, 20.24%, 20.12%, 20.15%, 19.66%.

In the lambs from the II experimental group, which received a combination of prebiotic + probiotic, compared to the control and the I experimental group, all amino acids registered higher growth, and the differences were statistically unproven. Increased content of amino acids phenylalanine and tryptophan in breast meat of broiler chickens fed a ration with probiotic + prebiotic was reported by Haščík et al. (2020). Higher levels of free amino acids glycine, proline, valine, leucine and isoleucine in the meat of pigs fed probiotic-supplemented feed were reported by Chang et al. (2018), whereas the concentrations of aspartic and glutamic acids, lysine, methionine, phenylalanine, arginine and tyrosine were higher in the non-supplemented control group.

Table 3. Amino acid profiles of *m. LL* in Ile-de-France lambs, g/100 g

Amino acids	Групи животни						
	Control group		Experimental group I		Experimental group II		p-value
	n	$\bar{x}$ ±SD	n	$\bar{x}$ ±SD	n	$\bar{x}$ ±SD	
Alanine	5	1.64±0.23	5	1.97±0.23	5	2.24±0.42	0.134
Valine	5	1.51±0.21	5	1.80±0.22	5	2.05±0.38	0.137
Leucine	5	2.04±0.29	5	2.44±0.30	5	2.77±0.52	0.139
Isoleucine	5	1.30±0.19	5	1.56±0.19	5	1.77±0.33	0.135
Glycine	5	1.34±0.19	5	1.61±0.19	5	1.83±0.34	0.135
Proline	5	1.16±0.16	5	1.39±0.17	5	1.57±0.29	0.137
Serine	5	1.27±0.18	5	1.52±0.18	5	1.73±0.32	0.138
Threonine	5	1.44±0.21	5	1.72±0.21	5	1.96±0.36	0.136
Methionine	5	0.70±0.10	5	0.84±0.10	5	0.96±0.18	0.135
Aspartic acid	5	2.58±0.36	5	3.09±0.37	5	3.52±0.66	0.136
Glutamic acid	5	3.20±0.45	5	3.84±0.46	5	4.36±0.82	0.137
Phenylalanine	5	1.40±0.20	5	1.68±0.20	5	1.90±0.36	0.139
Tyrosine	5	1.17±0.16	5	1.40±0.17	5	1.59±0.30	0.137
Arginine	5	1.68±0.24	5	2.02±0.24	5	2.29±0.43	0.134
Histidine	5	0.60±0.08	5	0.72±0.09	5	0.82±0.15	0.132
Lysine	5	1.46±0.21	5	1.75±0.21	5	1.99±0.37	0.137
Cysteine	5	0.25±0.04	5	0.30±0.04	5	0.34±0.07	0.156
Tryptophan	5	0.31±0.05	5	0.38±0.04	5	0.43±0.08	0.129
Creatine	5	0.40±0.06	5	0.48±0.06	5	0.55±0.10	0.123
Hypoxanthine	5	0.32±0.05	5	0.39±0.05	5	0.44±0.08	0.135
Total amino acids	5	25.79±3.59	5	30.88±3.70	5	35.11±6.56	0.137

Higher meat levels of lysine, methionine and glutamic acid were also reported by Tang et al. (2021) in broiler chickens fed probiotic-supplemented feed.

The analysis of results showed that the values of tryptophan, creatine and hypoxanthine in lamb meat from the three groups had comparatively close values, despite the fact that average values in supplemented groups were higher than those of controls. Higher meat content of tryptophan was reported by Arilov et al. (2019) in rams supplemented with a probiotic.

In the analysis of the fatty acid profile of the meat (Table 4), we find that all fatty acids have higher values in the II experimental group compared to the I group and the control group, and the results are mathematically unproven. Chang et al. (2018) reported similar results to ours in pigs.

In experimental group I, the content of stearic acid reached 2.06 g/100 g, and palmitic acid – 1.85 g/100 g. The average content of the same fatty acids in experimental group II was 2.63 g/100 g and 2.36 g/100 g, respectively, and was statistically unproven higher than that of the control group.

The content of oleic and linoleic fatty acids in the meat of experimental group I was 0.38 g/100 g and 0.43 g/100 g. The average values of the same fatty acids (oleic, linoleic) in ex-

Table 4. Fatty acid profiles and cholesterol of *m. LL* in Ile-de-France lambs, g/100 g

Fatty acids	Groups of animals						
	Control group		Experimental group I		Experimental group II		p-value
	n	$\bar{x}$ ±SD	n	$\bar{x}$ ±SD	n	$\bar{x}$ ±SD	
Myristic (C14:0)	5	0.23±0.04	5	0.22±0.08	5	0.28±0.08	0.587
Palmitic (C16:0)	5	1.98±0.28	5	1.85±0.66	5	2.36±0.68	0.557
Stearic (C18:0)	5	2.21±0.31	5	2.06±0.74	5	2.63±0.76	0.559
Oleic (C18:1)	5	0.40±0.06	5	0.38±0.13	5	0.48±0.14	0.574
Linoleic (C18:2)	5	0.46±0.07	5	0.43±0.16	5	0.55±0.16	0.574
Cholesterol	5	0.52±0.08	5	0.51±0.04	5	0.51±0.09	0.961
Total fatty acids	5	5.29±0.74	5	4.93±1.77	5	6.29±1.82	0.562

Table 5. Organic acid profiles of *m. LL* in Ile-de-France lambs, g/100 g

Organic acids	Groups of animals						
	Control group		Experimental group I		Experimental group II		p-value
	n	x̄ ±SD	n	x̄ ±SD	n	x̄ ±SD	
Succinic acid	5	0.12±0.02	5	0.15±**0.02**	5	0.17±**0.03**	0.137
Fumaric acid	5	0.16±0.03	5	0.19±0.02	5	0.22±0.04	0.125
Malic acid	5	0.24±0.04	5	0.29±0.04	5	0.33±0.06	0.151
Pyroglutamic acid	5	0.56±0.08	5	0.67±**0.08**	5	0.76±0.14	0.145
4-aminobutyric acid	5	0.71±**0.10**	5	0.85±**0.10**	5	0.96±0.18	0.145
Total organic acid	5	1.79±0.25	5	2.14±0.25	5	2.44±0.46	0.140

perimental group II were 0.48 g/100 g and 0.55 g/100 g, respectively, and exceeded the levels compared to the control by 20.00% and 19.57%. The obtained values were supported by other researchers that demonstrated reduced content of saturated palmitic and stearic fatty acids, yet increased content of oleic acid and of polyunsaturated linoleic in probiotic-fed broilers (Saleh et al., 2012, 2013; Benamirouche et al., 2020). Conversely, Hossain et al. (2012a) reported increased content of myristic, palmitic, oleic acids along with lower stearic and linoleic fatty acids in breast muscles of probiotic-supplemented chickens.

Tang et el. (2021) established higher content of palmitic, oleic and unsaturated fatty acids in breast meat of broilers fed a ration with probiotic (P<0.05). In contrast, Hossain et al. (2012a) reported increased values for myristic, palmitic, oleic, and linoleic fatty acids, while the amount of stearic and linoleic fatty acids decreased after probiotic supplementation.

Goluch et al. (2023) in an experiment with pigs found a decrease in the content of myristic and stearic fatty acids and an increase in palmitic acid in *m. LL* in pigs after administration of probiotics.

The cholesterol content in the meat (table 4) does not show significant differences in its dynamics in the three groups of animals, and the obtained results are very close. In both experimental groups, the cholesterol content (0.51 g/100 g) was 1.92% lower than that of the control group. Bidura et al. (2019) found a significantly lower cholesterol content in the meat of ducks fed a probiotic compared to the control group. Ahmed et al. (2023) also found that the cholesterol content of *m. LL* in pigs was lower in animals fed probiotic supplementation compared to those fed standard feed.

The concentrations of studied organic acids: succinic, fumaric, malic, pyroglutamic and 4-aminobutyric, are presented in Table 5. All levels were higher in both prebiotic – and probiotic + prebiotic supplemented groups, without statistically significant differences.

Data presented on Figure 1 and Figure 2 showed that the concentrations of organic compounds ribose and inosine in meat were the highest in experimental group II with insignificant differences vs those in controls and experimental group I.

Ribose content of lamb meat from experimental group II (0.59 g/100 g) exceeded those in the control group (0.43

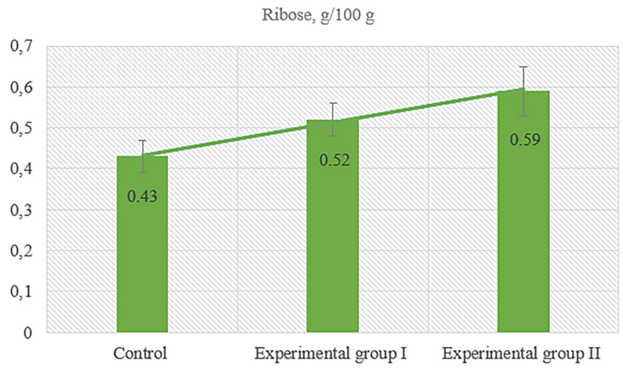

Fig. 1. Ribose content **of *m. LL* in Ile-de-France lambs, g/100 g**

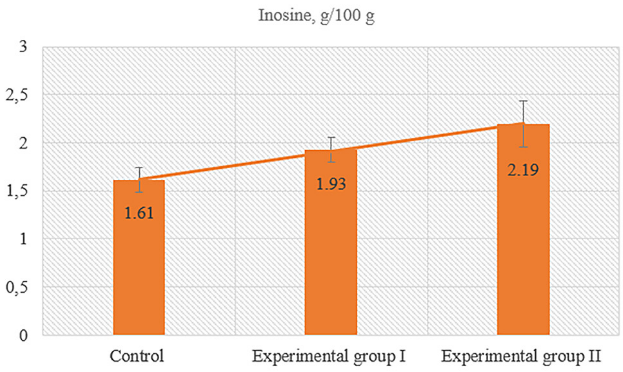

Fig. 2. Inosine content of *m. LL* in Ile-de-France lambs, g/100 g

g/100 g) and experimental group I (0.52 g/100 g). Chang et al. (2018) demonstrated higher levels of nucleotides and inosine in the meat of pigs supplemented with a probiotic than in the non-supplemented animals.

The average inosine levels were increased in experimental groups I and II (1.93 g/100 g and 2.19 g/100 g respectively) as compared to control lambs (1.61 g/100 g).

Conclusions

The study allowed concluding that:

- The addition of Immunobeta prebiotic and the combination Zoovit + Immunobeta prebiotic the ration of Ile-de-France lambs leads to an unreliable increase in the levels of all investigated amino acids in *m. LL*.
- In lambs receiving the prebiotic Immunobeta, the levels of all fatty acids in *m. LL* were marginally lower than the control group. The addition of prebiotic Immunobeta + probiotic Zoovit leads to marginally higher values of all fatty acids in *m. LL*, compared to those in the control group.
- The cholesterol content in *m. LL* in both experimental groups registered a marginally lower level than the control group of lambs.
- There are no statistically significant differences in the content of organic acids, ribose and inosine in *m. LL* as a result of the addition of the prebiotic Immunobeta as well as the combination of the probiotic Zoovit and the prebiotic Immunobeta in Ile de France lambs.

Acknowledgements

The research was carried out under project No. КП06ПН76/42, financed by the Scientific Research Fund.

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Received: **February, 14, 2024**; *Approved*: **August, 30, 2024;** *Published:* **April, 2025**