FEEDING TECHNOLOGY IMPACT ON BROILER PRODUCTIVITY AND INTESTINAL TRACT MICROFLORA

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Abstract. A trial was carried out with broiler chicks 1 to 42 days old of cross Ross-308 with an aim of establishing the impact produced by the combination of the dry form of prebiotics obtained from Jerusalem artichoke and the probiotic bacteria *Lactobacillus reuteri* L. and *Pediococcus pentosaceus* L. on productivity of broilers and quality of their meat as well as on feed conversion, bacteria of the family *Enterobacteriaceae* and lactic bacteria counts in the *ileum* part of intestinal tract. As the result of the trial it was established that supplementation of the broiler basic feed with 0.5 % dry powder prebiotics obtained from JA (*Helianthus tuberosus* L.) in combination with probiotics 1 x 10^8 cfu·g^{-1} *Lactobacillus reuteri* L. and 1 x 10^8 cfu·g^{-1} *Pediococcus pentosaceus* L., both the productivity of broiler chicks and the meat quality increased in comparison with the control group. The live weight of the broiler chicks increased by 2.1 %, the feed consumption for obtaining 1 kg of live weight decreased by 3.2 %, the level of the favourable lactic bacteria in *ileum* part of the intestinal tract was increased by 0.85 log cfu·g^{-1}, the cholesterol level in the meat of broiler chicks was reduced by 22.7 mg·$(100\ g)^{-1}$, moreover, the meat had higher nutritional value: higher level of essential amino acid tryptophan and lower level of connective tissue protein – oxiproline.

Keywords: Jerusalem artichoke; Lactobacillus reuteri; Pediococcus pentosaceus; broiler chicken; feed conversion; productivity; Enterobacteriaceae.

Introduction

Because of developing cross-resistance and multiple-antibiotic resistance of pathogenic bacteria [1], antibiotics are more and more being removed from poultry diets around the world as well as in Latvia. Due to prohibition of antibiotics, attempts were made to find novel, alternative feed additives [2], boosting disease resistance of the animal system and promoting growth and optimum feed conversion of animals. Fructooligosaccharides can substitute antibiotics to enhance the growth and production efficiency of broilers [3]. One of the sources of fructooligosaccharides is Jerusalem artichoke *Helianthus tuberosus* L. (JA). The principal storage carbohydrate of JA is inulin, and therefore carbon in the tubers is found predominantly in the form of inulin. At the same time, the tubers of JA are a good source of vitamins, especially the vitamin B complex (thiamine, riboflavin, niacin, B6, pantothenic acid, biotin, and cobalamin), vitamin C, and β-carotene [4]. JA are grown also in Latvia (*Helianthus tuberosus* L.), the optimum agrotechnical conditions for their cultivation and the inulin content are accordingly studied [5]. According to the data of Lepse and Bite [5], the sorts of JA cultivated in Latvia contain 51.88-61.50 % inulin.

The other feed additive is probiotics, a powerful microbial feed supplement promoting the activity of beneficial bacteria that outcompete other bacteria for nutritional resources and adhesion sites on the intestinal wall produce antibacterial substances that impact harmful bacteria, and modify the host's immune response [6]. The group of probiotics includes individual micro-organisms, fungi, and yeasts the operating mechanism of which ensures the growth of broilers, increase the feed conversion [7] and decrease the death of birds [8]. It is proved in research literature that *Pediococcus acidilacti* L. and *Lactobacillus reuteri* isolated from healthy chicken gut inhibited the growth of *Campylobacter jejuni* L. [9]. By enhancing the humoral immunity when included at 0.1 % of the broiler diet *Pediococcus acidilacti* L. effectively enhances the resistance of birds and partially protects them against the negative effects on growth associated with coccidiosis [10; 11]. Damayanti et al. [12] proved that *Pediococcus pentosaceus* L. Db9 found in duodenum of broiler chicken had tolerance in low pH (1, 2 and 3), gastric juice pH 2 and bile salt 0.3, therefore this micro organism has a potential as a probiotics candidate for chicken. Other researchers found that *Pediococcus pentosaceus* L. strain TMU457 had to be considered as potential ingredient for chicken probiotic feed formulation [13].

Up to now, there has been no research published in Latvia as to the impact of synbiotics – JA dry powder in combination with lactic bacteria *Lactobacillus reuteri* L. and *Pediococcus pentosaceus* L. – on productivity and meat quality of broilers.

Our working assignment under the current research study was to investigate changes of productivity, feed conversion, microbiology of the intestinal tract and eventually the meat quality of broilers receiving synbiotics (JA dry powder mixed with lactic bacteria *Lactobacillus reuteri* L. and *Pediococcus pentosaceus* L.) as a supplement to their basic feed.

The following hypothesis was set forth for the purpose of the above research: The dry form of JA in combination with the lactic bacteria *Lactobacillus reuteri* L. and *Pediococcus pentosaceus* L. increases the productivity and decreases the feed conversion, improves the intestinal micro flora and the quality of the obtained meat.

Materials and methods

The trial was carried out at the vivarium of the Research Institute of Biotechnology and Veterinary Medicine SIGRA with broiler chicken of cross *Ross-308* at the age of 1 to 42 days according to the scheme in Table 1.

Table 1

Scheme of the triale

Group	Basic feed (BF)	Feeding programme	
		Prebiotic	Probiotic
1st – control	BF	-	-
2nd – trial	BF	0.5 % dray concentrate *Jerusalem artichoke* (JA)	1x 10^8 cfu·g^{-1} *Lactobacillus reuteri* + 1x10^8 cfu·g^{-1} *Pediococcus pentosaceus* to 1 kg BF
3rd – trial	BF	1.0 % dray concentrate *Jerusalem artichoke* (JA)	1x 10^8 cfu·g^{-1} *Lactobacillus reuteri* + 1x10^8 cfu·g^{-1} *Pediococcus pentosaceus* to1 kg BF

The content of broiler chicken basic feed (BF) has been balanced according to the commercial recommendations for the particular cross.

The BF ingredient composition was the same for all broiler chicken groups, namely: wheat, soybean meal, maize, sunflower meal, soy oil, calcium carbonate, monocalcium phosphate, salt, complex of premixes, DL – Methionine, L – Lysine HCl, L – Threonine.

Acquired on 25.01.2011 from the company *Herbe* Ltd, dry concentrated JA powder, certificate No.157, was added to the basic feed of broiler chicks as a source of prebiotics. Its inulin content was 50 % oligosaccharides.

According to the analogue principle the broiler chickens were distributed in 3 groups (n = 50). BF of the 1st – control group – broiler chicken did not contain either prebiotic or probiotic. BF of the 2nd and 3rd trial groups of broiler chicken was supplemented with different amount of dry concentrated *Jerusalem artichoke* L. (JA) and probiotic *Lactobacillus reuteri* L. 1x 10^8 cfu·g^{-1} and *Pediococcus pentosaceus* L. 1x10^8 cfu·g^{-1}. Over the trial period, the productivity of broiler chicken and feed conversion rate was investigated. The content of *ileum* was bacteriologically tested for counts of lactic acid bacteria and *Enterobacteriaceae*. At the end of the trial, the chemical composition of meat was biochemically tested.

Productivity efficiency (PEF – production efficiency factor) [14] was calculated by using the formula:

$$\frac{L \times LW}{A \times FCR} \times 100 \quad (1)$$

where L – liveability, %;
LW – live weight, kg;
A – age, days;
FCR – feed conversion, kg·kg^{-1}

The protein concentrate samples were analysed for dry matter, (ISO 6496-1999), crude protein (LVS EN ISO 5983-1:2005), total ash (ISO 5984-2002). The meat samples of broiler chicken at the age of 42 days were analysed for protein (LVS ISO 937:1978), total fat (LVS ISO 1443:1973). Detection of *Enterobacteriaceae* in the contents of *ileum* part of the intestinal tract of broilers carried out pursuant to standard LVS ISO 21528-2:2007 "Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count method" by use of media and reagents: violet red bile glucose agar (VRBG; Biolife, Italy, 4021882), nutrient agar (Biolife, Italy, 401810), glucose agar (Biolife, Italy, 401970) and oxidase reagent (BD Medical Systems, Mexico, 261181). The bacteria of genus *Enterobacteriaceae* down to the species level were detected by use of BBL Crystal biochemical kits of gram-negative bacteria (BD, USA, 245000). The lactic acid bacteria count in the contents of broiler intestines was carried out with the help of the methods provided by René L. van Winsen et al. [15], using Rogosa agar (BD, US, CM0627).

The statistical analysis was performed by using SSPS 17 statistic programme package (SPSS Inc. Chicago, IL, USA). Statistical significance was declared at $P < 0.05$. The data were presented as mean and standard errors. The data obtained as the result of microbiological analysis were evaluated with the help of descriptive statistics and T tests [16].

Results and discussion

The efficiency of prebiotics – the dry form of JA in combination with probiotics – lactic acid bacteria *Lactobacillus reuteri* L. 1 x 10^8 and *Pediococcus pentosaceus* L. 1 x 10^8 was evaluated measuring the productivity, feed conversion, intestinal microbiology and meat quality of broilers. The productivity of the broilers over the trial period reflected in Table 2 was found to be high.

Table 2

Productivity of broiler chickens in the trial

Parameters	Groups		
	1st group – control	**2nd group – trial**	**3rd group – trial**
Live-weight at the age of 42 days, g	$3227.0^a \pm 80.0$	$3295.0^b \pm 122.3$	$3198.0^a \pm 89.1$
% to control	100.0	102.1	99.1
Feed conversion (FCR), kg·kg^{-1}	$1.58^a \pm 0.02$	$1.53^b \pm 0.04$	$1.59^a \pm 0.06$
% to control	100.0	96.8	100.6
Production efficiency factor (PEF)	486.3^a	512.7^b	478.8^c
± to control	-	+26.4	-7.5

[a, b,c] – statistically significant diference ($p < 0.05$)

Comparing the live weight increase among the trial groups, it was found that Group 2 having received JA in concentration 0.5 % in combination with probiotics 1x 10^8 *Lactobacillus reuteri* L. and 1 x 10^8 *Pediococcus pentosaceus* L. at the age of 42 days produced higher live weight than that established for Group 3 and the control group. At the age of 42 days, it was accordingly by 2.1 % and 3.0 % higher ($p < 0.05$).

The above finding indicates a higher feed conversion in the gastro-intestinal tract due to more optimum intestinal micro flora achieved with the help of the supplement under trial: the lowered pH level enhanced the growth of beneficial lactic bacteria in the *ileum* part of intestinal tract [17; 18].

A lower live weight was found for broiler chicks having received 1.0 % JA in 1.0 % concentration in combination with the probiotics supplement (Group 3).

An improved feed conversion was found for the chicks of the trial group having received JA in 0.5 % concentration in combination with probiotics 1x 10^8 *Lactobacillus reuteri* L. and 1 x 10^8 *Pediococcus pentosaceus* L. as a supplement to their basic feed – it was by 1.53 kg lower, i.e., by 3.2 % and 3.9 % lower than for Trial group 3 and the Control Group. Consequently, the 0.5 % JA powder concentrate applied in combination with *Lactobacillus reuteri* L. and *Pediococcus*

pentosaceus L., improved the feed conversion. Inulin incorporated in the JA powder together with probiotics added to the ration ensured a complex impact on feed utilisation by the animal system [19; 20].

The economic expression of interrelations among the live weight, meat quality, feed consumption for production of 1 kg of meat and distribution of meat is called the productivity index. The productivity index for Trial Group 2 was by 26.4 units higher than that of the Control Group. This confirms the assumption that a diet version well adapted to the requirements of the organism, provides it with the required feed stuffs, enhances the live weight gain and reduces the feed conversion [21; 17].

Dynamics of bacteria of genus Enterobacteriacea are reflected in Table 3. At the beginning of the trial, for 7 days old chicks the *Enterobacteriaceae* count in the ileum part of their intestine was 4.60 log cfu·g^{-1} on the average and the lactic bacteria count – 5.68 log cfu·g^{-1} on the average.

Table 3

Dynamics of bacteria of genus *Enterobacteriaceae* and lactic bacteria counts in the *ileum* part of intestinal tract of broilers

Group	Bacteria of genus *Enterobacteriaceae* log cfu·g^{-1}	Lactic bacteria count, log cfu·g^{-1}
	Ileum	*Ileum*
Background (at 7 days of age)		
	4.60 ± 0.032	5.68 ± 0.062
At 28 days of age		
Group 1	7.73 ± 0.09	6.88 ± 0.048
Group 2	6.83 ± 0.05	8.18 ± 0.048
Group 3	7.45 ± 0.06	7.75 ± 0.029
At 42 days of age		
Group 1	7.98 ± 0.05	7.88 ± 0.085
Group 2	6.80 ± 0.04	8.73 ± 0.138
Group 3	7.85 ± 0.06	7.83 ± 0.149

Analysing the data of Table 3, it is evident that at the age of 28 days, both the counts of *Enterobacteriaceae* and lactic acid bacteria counts in the *ileum* part of intestinal tract have increased. At the same time, the highest increase of the favourable lactic acid bacteria is observed for Group 2, where the basic feed of the broiler chicks was supplemented with 0.5 % dry concentrated JA powder together with stock culture of 1x 10^8 *Lactobacillus reuteri* L. and 1x 10^8 *Pediococcus pentosaceus* L. In the Control Group, the same indicator has grown 1.21 times, in Trial Group 2 – 1.44 times while in Trial Group 3 – 1.36 times in comparison with the lactic bacteria counts in the ileum part of intestines for 7 days old broiler chicks. At the same time, for 28 days old broiler chicks, the count of bacteria of genus *Enterobacteriaceae* had least increased for Group 2 (1.48 times) in comparison with the Control Group (1.68 times) and Trial Group 3 (1.62 times). Thus, JA dry concentrated powder supplement in 0.5 % concentration in combination with probiotic bacteria for 28 days old chicks has enhanced the growth of the favourable lactic acid bacteria and hindered the increase of the unfavourable bacteria of genus *Enterobacteriaceae* in the *ileum* part of intestines where much of the digestion of the feed and all of the absorption of the nutrients take place.

The lactic acid bacteria count in the *ileum* part of intestine at the age of 42 days is also the highest for Trial Group 2 broilers reaching 8.73 log cfu·g^{-1}, while the *Enterobacteriaceae* count in this part of intestine has reduced 6.83 to 6.80 log cfu·g^{-1} in comparison with the ileum content of chicks at the age of 28 days.

The results of meat chemical tests are reflected in Table 4. It is seen that addition of supplement containing a combination of prebiotics and probiotics to broiler feed has enhanced the improvement of the meat quality for broilers of Trial Group 2. The crude protein level is higher and the crude fat level is lower by accordingly 0.40 % and 0.55 %, in comparison with the broilers of the Control Group. The biological substances contained in Jerusalem artichoke (inulin) in combination with probiotics improve the digestibility of crude protein and crude fat [19].

Table 4

Biological value of broiler meat (in breast muscle mass)

Parameters	1st group	2nd group	3rd group
Total protein, %	$20.72^{a} \pm 0.35$	$21.11^{a} \pm 0.46$	$20.90^{a} \pm 0.39$
Total fat, %	$4.25^{a} \pm 0.17$	$3.70^{b} \pm 0.19$	$4.18^{a} \pm 0.14$
Cholesterol, mg·(100 g)$^{-1}$	$84.77^{a} \pm 0.82$	$62.11^{b} \pm 0.56$	$66.41^{b} \pm 0.65$
Tryptophan, g·kg^{-1}	$3.24^{a} \pm 0.11$	$3.44^{a} \pm 0.12$	$3.30^{a} \pm 0.12$
Oxiproline, g·kg^{-1}	$0.57^{a} \pm 0.02$	$0.48^{b} \pm 0.02$	$0.52^{a} \pm 0.02$
Tryptophan/oxiproline relation	5.68^{a}	7.16^{b}	6.34^{a}
Energy value of meat, ccal·(100 g)$^{-1}$	126.30^{a}	115.33^{b}	118.90^{b}

$^{a, b}$ – statistically significant difference ($p < 0.05$)

The cholesterol level of meat is one of the most important indicators of the meat quality. The cholesterol level depends upon the nutrition of broilers, especially on the contents of the feed applied. Including a prebiotic supplement (JA) in combination with probiotics *Lactobacillus reuteri* L. and *Pediococcus pentosaceus* L. in chicken feed, it is possible to reduce the cholesterol level of the outcoming meat. Inulin contained in Jerusalem artichoke hinders the absorption of the cholesterol in the intestines and reduces formation of the endogenous cholesterol. Inulin is not absorbed in the upper part of the digestive system: stomach. A part of it is split in the acid environment of the stomach and later absorbed through the small intestine wall into the blood circulation system. The remaining part is fermented in the large intestine and binds a large amount of the toxic, chemical elements as cholesterol, heavy metals and is eventually excreted from the system [22; 23]. It is seen in Table 4 that the lowest cholesterol level in the mass of muscle tissue of broilers was found for Trial Group 2 which received 0.5 % JA concentrated powder in combination with probiotics1x 10^{8} *Lactobacillus reuteri* L. and 1x 10^{8} *Pediococcus pentosaceus* L. – 62.1 mg·(100 g)$^{-1}$, i.e. by 22.7 mg·(100 g)$^{-1}$ and 18.4 mg·(100 g)$^{-1}$ accordingly in comparison with the Control Group and Trial Group 3.

The meat quality of broilers is characterized by amino acids tryptophan and oxiproline as well as their interrelation. With increase of the connective tissue protein in the broiler meat the nutritive value of meat is reduced as connective tissue contains more oxiproline (because of more cartilages, sinews, etc.) and essential amino acid tryptophan, essential amino acid of higher value characterizes the muscle tissue amount in meat. As it is seen in Table 5, the best nutritive value of meat was found in Group 2 which received basic feed + 0.5 % JA + 1 x 10^{8} *Lactobacillus reuteri* L. + 1 x 10^{8} *Pediococcus pentosaceus* L.. The connective tissue protein for this group is lower than 0.10 g kg^{-1}, tryptophan and the oxiproline ratio is higher than 1.48 g kg^{-1}, in comparison with the Control Group.

An essential indicator is energy value of meat which is inversely proportional to its quality. Meat of higher quality was obtained from Trial Group 2 broilers: its energy value was 115.3 kg·(100 g)$^{-1}$, i.e. by 8.8 % lower than for the broilers of the Control Group.

The highest energy value was found in meat obtained from the Control Group broilers which did not receive prebiotic/probiotic supplement to their basic feed, i.e. by 10.97 – 7.40 kg·(100 g)$^{-1}$ because of the elevated crude fat level in their meat.

Conclusions

The optimum supplementation to broiler basic feed would be dry powder of JA (Helianthus tuberosus) in 0.5 % concentration in combination with probiotics 1 x 10^{8} *Lactobacillus reuteri* L. and 1 x 10^{8} *Pediococcus pentosaceus* L. stock culture.

The above new feeding formulation increased the productivity of broilers as well as the quality of the final product in comparison with the control group as follows:

- Increased the live weight by 2.1 %;
- Decreased the feed consumption required for production of 1 kg live weight by 3.2 %;
- Improved the intestinal micro flora;
- Increased the amount of favourable micro flora *Lactobacillus* spp. log cfu·g^{-1} in the *ileum* part of the intestinal tract;

- Decreased the amount of bacteria of genus *Enterobacteriaceae*. Decreased the cholesterol level in meat by 22.7 mg·(100 g)$^{-1}$;
- Achieved a higher nutritive value of meat (a higher amount of essential amino acid tryptophan, less connective tissue protein: oxiproline).

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