FEED ADDITIVES IN CHICKEN FEED AND THEIR IMPACT ON MICROFLORA OF DIGESTIVE TRACT

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Abstract. The study was conducted to test the effect of *Jerusalem artichoke* (JA) in dry form on the digestive tract of broiler chicken. The birds were divided in seven groups. The feed ration of the first group chicken was without supplement, the other six groups – supplemented with additive JA and *Lactobacillus reuteri* in different concentrations. Two birds from each group were slaughtered at the trial beginning on the 7th, further – 28th and 42nd days of age. The content of *ileum* was bacteriologically tested for counts of lactic acid bacteria and *Enterobacteriaceae*. The results confirm that intestinal homeostasis is most favourably influenced by adding dried JA in 0.5 % concentration to the basic feed or a symbiotic – dried JA in combination with $1 \cdot 10^8$ *Lactobacillus reuteri* in 0.5 % concentration.

Keywords: broiler chicken, *Enterobacteriaceae*, *Jerusalem artichoke, Lactobacillus reuteri*, lactic acid bacteria.

Introduction

Over the last decades, probiotics, prebiotics, enzymes, feed acidifiers, anti-oxidants and different additives of plant origin have commonly served as feed additives [1; 2]. Each of the above groups has a specific impact: promote better feed conversion, growth and development of the animal's organism, regulating the microflora of the intestinal tract, etc. [3].

As pointed out by Cebra et al. (1999) [4], microorganisms found on the intestine wall serve as promoters of anatomic, physiologic and immunologic development of the host organism. In their study, Lee et al. [5] have discovered that the micro-flora residing in broiler intestines in 68.85 % of cases consists of bacteria of genus *Lactobacillu*s, incl. *Lactobacillus reuteri* (*L. reuteri*), *L. salivarius*, *L. delbruckii*, *L. cripatus*, *L. acidophilus.* The remaining part of the bacterial microflora is made up of bacteria of genera *Clostridium*, *Bacillus*, *Streptococcus*, *Enterococcus*, *Fusobacter*, *Bifidobacter*, *Ochrobactrum*, *Alcaligenes*, *Campylobacter*, *Escherichia*, *Salmonella* and *Bacteroides* [6]. Bacteria are capable of aggravating the lipid digestion and modifying the digestion of carbohydrates and proteins. They can increase the demand for amino acids and energy. Bacteria might have an adverse effect on vitamin consumption by the animal system. Favourable bacteria at the same time defend the system of birds against pathogens: the mutual interaction of bacteria and the lymphoid tissue of the mucous membrane is the underlying mechanism for the animal system to fight the pathogenic microorganisms having penetrated the intestinal tract [7].

The amount of bacteria in small intestine and *caecum* for 1 day old chicks increases to 9 lg cfu$\cdot g^{-1}$ and 11 lg cfu$\cdot g^{-1}$ of the contents of the intestinal tract accordingly. Such amount of bacteria persists for the following 30 days [8]. In the small intestine of 10 day old chicks, Shakouri et al. [9] have found 6.91 lg cfu$\cdot g^{-1}$ bacteria of genus *Enterobacteriaceae* and 9.18 lg cfu$\cdot g^{-1}$ lactic acid bacteria.

As pointed out by Patterson & Burkholder [10] probiotics both act as antagonists producing substances like bacteriocins, organic acids and hydrogen peroxide which hinder development of microorganisms and in the way of exclusion by competition when they attach themselves to the mucous membrane of the intestine wall and thus hinder access and attachment to it for pathogenic microorganisms thus excluding the impact of unfavourable microorganisms.

Prebiotics are part of feed or feed ingredients remaining indigested in the gastro-intestinal tract while they are used by certain microflora colonizing it for its growth and development, by the same token laying a favourable impact on the system of the host [11].

Inulin is one of the most widely applied prebiotics [12]. Inulin is a polysaccharide: D-fructose polymer with β (2-1) linkages in the main chain and D-glucose as a terminal molecule. JA and chicory are used as sources for acquisition of inulin. According to the data of Lepse & Bite [13], the varieties of JA cultivated in Latvia contain 51.88-61.5 % inulin. The Polish researchers point out [14] that JA enjoys also a high protein content which moreover contains essential amino acids in ideal proportions.

Research literature is poor of data on application of JA as a feed additive for feeding of the broiler chicken [15].

As indicated by Patterson & Brukholder [10] the operational efficiency of probiotics in the system of broilers is determined by both, the combinations of micro-organisms involved, their proportions and the environmental impact at the time of feeding as well as the genetic and health status of the birds. Also, prebiotics as additives to broiler feed have not produced unequivocal results. In Latvia no attempts have been made so far to use the concentrate of JA (a prebiotic) or its combination with *L. reuteri* (a probiotic) as additives of broiler feed.

In order to improve microbiological eubiosis of broilers, our task within the present research study was to apply prebiotics (dry powder of JA) and synbiotics (dry powder of JA in combination with *L. reuteri)* establishing along the way the optimum dose of the above additives.

The hypothesis of the current research is that use of the concentrate of JA (a prebiotic) or its combination with *L. reuteri* (a probiotic) as additives for basic feed favourably influence intestinal homeostasis of broilers.

Materials and methods

140 cross-breed ROSS 308 broiler chicks were included in the trial. The basic feed (BF) used for all groups of chicks in the trial was age-consistent feed commercially produced by the feed company *Riga Mixed Feed Plant Ltd*:

- Granulated TMR feed for the age group 0-10 days (Batch No. 293),
- Granulated TMR feed *Grower* for the age group 11-27 days (Batch No. 305),
- Granulated TMR feed *Finisher I* for the age group 27-35 days (Batch No. 278).

The chicks were distributed in 7 groups, 20 chicks per group. During the trial, starting with day 7 up to day 42, prebiotics and synbiotics were added to the basic feed as reflected in Table 1.

Table 1

Application of additives to BF in broiler trial groups

Group	Additives applied	
	Prebiotic	Probiotic
CG (control)	without	without
G2	0.5 % dry concentrated JA	without
G3	1.0 % dry concentrated JA	without
G4	0.5 % dry concentrated JA	1 x 10^8 *Lactobacillus reuteri*
G5	1.0 % dry concentrated JA	1 x 10^8 *Lactobacillus reuteri*
G6	2.0 % dry concentrated JA	1 x 10^8 *Lactobacillus reuteri*
G7	3.0 % dry concentrated JA	1 x 10^8 *Lactobacillus reuteri*

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

L. reuteri, the probiotics applied, were obtained from the company *SIA Anima Lab* accompanied by a conformity certificate (*L. reuteri* 10 billion cfu·g^{-1}, produced 08.11.2011., shelf-life: 07.11. 2013).

To acquire *ileum* content samples for microbiological testing, 3 control slaughters were performed in the control group and each of the trial groups on 7, 28 and 42 days of life, which corresponds to the time of shifting diets to ensure the growth and development for the corresponding groups of chicken.

<u>*Microbiological investigations*</u>. The contents of the small intestine in the *ileum* part of the digestive tract from 63 birds were microbiologically tested in the Latvia University of Agriculture Agency *Sigra* Biotechnology and Veterinary Medicine Research Institute, Biochemistry and Microbiology Research Laboratory (LATAK-T-038-09-99).

Detection of *Enterobacteriaceae* in the contents of *ileum* part of the intestinal tract of the broilers carried out pursuant to the 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” [16] 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 the use of BBL Crystal biochemical kits of gram-negative bacteria (BD, USA, 245000).

The lactic acid bacteria count in the contents of thebroiler intestines was carried out by the help of the methods provided by René L. van Winsen et al. [17]. The intestine contents were aseptically placed in a sterile Stomacher's bag, weighed on the scales (accuracy ± 0.01 g) m g (measurement uncertainty ± 5 %), which accordingly represented the tested sample. To obtain the primary dilution, buffered peptone water was added (Biolife, Italy, 401278) 9 x m ml. The samples were homogenized in *BagMix* homogenizer (Interscience, France) for 2 minutes. Subsequent dilutions were performed up to the dilution stage 10^{11}.

10 ml of Rogosa agar, chilled down to 44-47 ºC (BD, US, CM0627), were poured on inoculate on each sterile Petri dish. Inoculate was mixed with medium with horizontal movements, allowing the medium to harden and form an upper layer of 15 ml volume. After hardening, the dish were turned over and incubated at 42 ºC in the thermostat for 48 ± 2 hours.

After incubation, the lactic acid bacteria colonies were counted (*Lactobacillus* spp., *L.* casei, *L. delbrueckii* and *Streptococcus cremoris*).

The number (*N*) of the colony forming units (cfu) of the lactic acid bacteria per 1 g of the intestine contents was calculated according to formula (1):

$$N = \frac{\sum C}{V \times 1.1 \times d} \quad (1)$$

where $\sum C$ – sum of the colonies counted on the two dishes retained from two successive dilutions, at least one of which contains a minimum 10 colonies;
V – volume of inoculums placed in each dish, in milliliters;
d – dilution corresponding to the first dilution retained.

Determination of *Salmonella enteritidis* in the broilers intestinal content was carried out **i**n accordance with the standard LVS EN ISO 6579/A1:2007 "Microbiology of food and animal feeding stuffs. – Horizontal method for the detection of *Salmonella* spp. – Amendment 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in samples from the primary production stage (ISO 6579:2012/Amd 1:2007) [18].

Isolation of *Campylobacter jejuni* was performed in accordance with Manual of Clinical Microbiology [19] by making use of clinical media and their additives.

Statistical Analysis. The data obtained as the result of the microbiological analysis were evaluated by the help of descriptive statistics and T tests [20], using *SPSS* 17.0 statistic programme package (SPSS Inc. Chicago, IL, USA).

Results and discussion

At the beginning of the trial, on day 7, for chicks of all groups the count of both *Enterobacteriaceae* and lactic acid bacteria in the contents of *ileum* part was similar, varying accordingly from 4.76 (G6) to 5.34 lg cfu·g^{-1} (G4) and from 6.52 (G6) to 7.30 lg cfu·g^{-1} (G4) Table 2.

Total count of the *Enterobacteriaceae* family bacteria in *ileum* part of the digestive tract for the CG group chicken statistically significantly changed ($p = 0.001$) from 5.0 ± 0.29 lg cfu·g^{-1} starting from day 7 up to 6.80±0,84 lg cfu·g^{-1} on day 42. The lactic acid bacteria count, on its turn, in *ileum* part of the digestive tract for the CG group chicken underwent statistically significant changes ($p = 0.004$) from 7.20 ± 0.45 lg cfu·g^{-1} on day 7 to 7.38 ± 0.78 lg cfu·g^{-1} on day 42. From small intestines of the digestive tract of the control group chicken *Salmonella enteritidis* were isolated starting with day 28. For 28 days old chicken of the group G2 the increase of bacteria from the genus *Enterobacteriaceae* in the contents of *ileum* was 0.84 lg cfu·g^{-1} in comparison with the same indicator on day 7 which is statistically significantly ($p = 0.013$) higher than for the chicken of the CG group. If in the CG group the lactic bacteria count on day 28 had decreased by 0.14 lg cfu·g^{-1}, then for the group G2 it had increased by 0.36 ($p = 0.002$). In the same way, for the broiler chicken on day 42 the count

of both *Enterobacteriaceae* and lactic acid bacteria in the *ileum* part of the intestinal tract was higher than for the chicken of the control group, 2.22 and 1.88 lg cfu·g^{-1} accordingly. At the same time, *Salmonella enteritidis* were not found in the digestive tract of the birds having received dry concentrated JA as feed additive in 0.5 % dose. *Campylobacter coli* were not isolated from the digestive tract of any group of birds.

Table 2

Microbiological characteristics of *ileum* part of broilers digestive tract

Group	Age, days	lg cfu·g^{-1} of *Enterobac-teriaceae* number	lg cfu·g^{-1} of *lactic acid bacteria* number	*Salmonella enteritidis* occurrence	*Campylobacter coli* occurrence
CG	7	5.00 ± 0.29	7.20 ± 0.45	not detected	not detected
	28	5.56 ± 0.49	7.06 ± 0.21	found on	not detected
	42	6.80 ± 0.84	7.38 ± 0.78	found on	not detected
G2	7	5.12 ± 0.13	7.24 ± 0.11	not detected	not detected
	28	5.96 ± 0.34	7.60 ± 0.12	not detected	not detected
	42	7.34 ± 0.05	9.12 ± 0.04	not detected	not detected
G3	7	4.88 ± 0.04	6.76 ± 0.11	not detected	not detected
	28	6.98 ± 0.45	8.10 ± 0.10	found on	not detected
	42	5.32 ± 0.04	5.18 ± 0.04	found on	not detected
G4	7	5.34 ± 0.05	7.30 ± 0.10	found on	not detected
	28	5.84 ± 0.11	8.62 ± 0.04	not detected	not detected
	42	4.98 ± 0.08	10.60 ± 0.10	not detected	not detected
G5	7	4.90 ± 0.22	6.82 ± 0.08	not detected	not detected
	28	6.82 ± 0.08	7.12 ± 0.13	found on	not detected
	42	6.56 ± 0.40	8.94 ± 0.05	found on	not detected
G6	7	4.76 ± 0.11	6.52 ± 0.13	not detected	not detected
	28	6.38 ± 0.08	8.20 ± 0.12	not detected	not detected
	42	6.38 ± 0.08	8.94 ± 0.10	not detected	not detected
G7	7	4.78 ± 0.09	7.02 ± 0.05	not detected	not detected
	28	6.80 ± 0.07	7.78 ± 0.08	found on	not detected
	42	5.10 ± 0.20	8.66 ± 0.11	found on	not detected

For the trial group G4, the lactic acid bacteria count (Table 2) in the contents of *ileum* for 42 days old birds significantly differed ($p = 0.028$) from all other trial groups reaching 10.60 ± 0.10 lg cfu·g^{-1}. For the group G4, on days 28 and 42 the lactic acid bacteria count in the contents of *ileum* in comparison with the same count on day 7 increased by 1.32 and 3.3 lg cfu·g^{-1} , respectively ($p = 0.003$). The count of bacteria from the genus *Enterobacteriaceae* for the birds on day 42 was low- 4.98 ± 0.08 lg cfu·g^{-1}.

In the trial group G5, on day 42, the lactic acid bacteria count in *ileum* contents was by 1.66 lg cfu·g^{-1} lower than for the group G4 at the respective age. At the same time, the count of *Enterobacteriaceae* for the trial group G5 on day 42 in the contents of *ileum* was by 1.58 lg cfu·g^{-1} higher than for the birds of the group G4. For the trial group G6 on day 42, the lactic acid bacteria count in the contents of *ileum* was by 1.66 lg cfu·g^{-1} lower than for the group G4 at the respective age and the *Enterobacteriaceae* count in contents of *ileum* for the group G6 on day 42 was by 1.40 lg cfu·g^{-1} higher than for the birds of the group G4. In the trial group G7 both, on day 28 and on day 42, the lactic acid count in the content of *ileum* was lower, while *Enterobacteriaceae* count was higher than for the group G4. The application of synbiotics as an additive to the feed ration at 1.0 % and 3.0 % concentration did not enhance the proliferation of lactic acid bacteria beneficial for digestive processes to such a high extent as when it was added in 0.5 % dose. In the trial group, having received a symbiotic as a feed additive (dry concentrated JA and 1 x 10^8 *Lactobacillus reuteri*) 0.5 % dose from the digestive tract of which on day 7 *Salmonellas enteritidis* were isolated, in further examinations on day 28 and day 42 they were not found. The application of synbiotics in 1.0 % and 3.0 % dose as feed additive did not preclude the proliferation of *Salmonellas enteritidis* in the digestive tract. From the digestive tract, *Campylobacter coli* were not isolated.

The main carbohydrate in JA is inulin which can be deemed excellent growth substrate for the intestinal microflora, incl. the lactic acid bacteria [21]. In research of Kunova et al. [22] the average number of vital lactic acid cells after cultivating different *Lactobacillus* species and separate clones in culture medium with inulin added for 24 hours, increased from 4.29 to 8.20 lg cfu·ml^{-1} proving the ability of lactic acid bacteria to use inulin in life processes. Briandet et al. [23] have established that capability of bacteria of genus *Lactobacillus* of inhibiting the attachment of *Salmonella spp.* to epithelium cells of the intestine wall depended upon the amount of these bacteria in the intestinal tract. For probiotics to be able to lay a favourable impact on the digestive tract functions, the applied bacteria should colonize the intestinal tract. Successful colonization of probiotics is dependent upon the vitality and stability of the probiotic bacteria clone applied, its suitability for the system of birds, the applied dose, frequency of application, health status of the bird, age of the bird as well as genetic pre-requisites [24]. Not all clones of species *Lactobacillus* are capable of attaching themselves to enterocytes, which are the absorptive cells of the intestine wall. Therefore, probiotic bacteria not always colonize the epithelium of the intestinal tract and they can be easily washed out of it [25]. The research study proved that dried JA powder in 0.5 % concentration influenced the development of lactic acid bacteria in *ileum* content more favourably, which is essential to ensure the dominance of favourable microflora in the intestinal contents. Having multiplied, the favourable lactic acid bacteria prevented proliferation of *Salmonella enteritidis* in the intestinal tract.

Conclusions

1. The supplementation of JA in a dried form and 0.5 % concentration enhances proliferation of both the bacteria of genus Enterobacteriaceae and the lactic acid bacteria in the ileum part of the digestive tract. The dried form of JA added to the feed in 1.0 % concentration first enhances the multiplication of the lactic acid bacteria, while on day 42 of life a reduction of the amount of the above bacteria in the ileum part of the digestive tract is observed.
2. The addition of JA in 0.5 % concentration in combination with Lactobacillus reuteri 1 x 10^8 considerably increases the number of the favourable lactic acid bacteria in the ileum part of the digestive tract of birds, at the same time not increasing the number of bacteria of genus Enterobacteriaceae.
3. The dried form of the JA added to the basic feed of birds in 0.5% concentration and the JA additive in 3.0 % concentration in combination with Lactobacillus reuteri $1 \cdot 10^8$ ensured a favorable morphofunctional status of the digestive tract of broilers by intensifying the exchange of cells and absorption of feed, thus activating the digestive processes.

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