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# Assessment of the Effect of α-galactosides Injected During Embryogenesis on Selected Chicken Traits*

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The effect of different doses of α-galactoside (RFOs) preparations from *Pisum sativum* L. cv. Opal, injected into eggs during embryogenesis, on maintaining a high number of bifidobacteria, selected chicken broiler traits and the lipoprotein level of blood were studied. Two independent experiments were conducted. In the first, Ringer water solution containing 1.763 mg/egg of fructooligosaccharides (FOS) (I group), 2.1158 mg of pea RFO preparation containing 20% sucrose (II group) and 0.4232 mg of sucrose (III group) were injected into Hubbard broiler breeder eggs containing 12-day old embryos. Only Ringer water solution was applied to the eggs of the control group (IV group). The number of bifidobacteria determined in faeces of two-day old chicken of groups I and II was significantly higher in comparison with the sucrose and control groups. The high level of bifidobacteria of groups I and II was maintained during 6 weeks. The dose of both preparations had no influence on the body weight, carcass, breast muscle, leg and abdominal fat ratio, total cholesterol, HDL and LDL serum concentrations. Broiler mortality and breast muscle cholesterol concentration was highest ($P<0.05$) for the control group. On the other hand, the European Production Index, as well as serum triglycerides, were the lowest for this group. The second experiment was performed on Hybro G chicken breeder eggs. 0.69, 3.43 and 6.87 mg/egg of pea RFO preparation doses containing 20% sucrose were injected into the experimental groups. The number of bifidobacteria in the caecum and selected meat traits of broilers were determined. The results of this experiment confirmed that RFO injection *in ovo* causes the long-time maintenance of a high level of bifidobacteria. The dose of the preparations does not have any effect on the selected broiler meat traits, except that the highest dose increases the percent of carcase in body weight. However, this dose reduced the hatchability of the treated embryos.

Key words: α-galactosides, fructooligosaccharides, embriogenesis, bifidobacteria, hatching, meat traits.

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The colonic microflora is a complex mixture of hundreds of different harmful, toxic and beneficial bacteria. Metabolically active cells constitute about half of the mass of large intestinal contents. In this respect, the colonic microflora should be considered an important factor influencing metabolism and health (TOMOMATSU 1994). In general, available energy is a limiting factor for the growth of colonic microflora. Hence, enrichment of a diet with non-digestible carbohydrates (prebiotics) results in an increase of metabolic activity and the development of beneficial bifidobacteria in the colon (MITSUOKA *et al.* 1987; GIBSON *et al.* 1994, 1998; TOMOMATSU 1994; VAN LOO *et al.* 1999;

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GULEWICZ *et al*. 2002). Up to now, fructooligosaccharides (FOS) have been the best known and most commonly applied oligosaccharides (HIDAKA *et al*. 1986; SPIGEL *et al.* 1994; JONG WON 1996; SARSSONG *et al.* 2000). In this aspect, compounds such as α-galactosides, also called raffinose family oligosaccharides (RFOs) are known to a lesser extent. These compounds are α- (1→6) galactosides linked to carbon C-6 of the glucose moiety of sucrose. These saccharides occur mainly in plants belonging to the *Fabaceae* family, where they perform a very important physiological function (LARSSON *et al*. 1993; HORBOWICZ & OBENDORF 1994; GÓRECKI *et al*. 1997). From the nutritional point of view, RFOs have been considered thus far as antinutritional factors because they are not hydrolyzed by mucosal enzymes in the small intestine of monogastric animals and are fermented in the lower gut with liberation of gas causing arduous flatulence (CRISTOFARO *et al*. 1974; SAINI & GLADSTONES 1986; PRINCE *et al*. 1988).

As a result of adding FOS to the diet, a decrease of the number of *Salmonella typhimurium* and decrease of pH, caused by the increase of volatile fatty acids, in intestines of broiler chicken has been reported (CHOI *et al.* 1994). By means of *in vitro* and *in vivo* studies, it was observed that certain metabolites of bifidobacteria, unknown thus far, inhibit the development of pathogenic microorganisms such as *Clostridium perfringrens* and *Escherichia coli*, and also *Salmonella*, *Veillonella*, *Shigella*, *Listeria*, etc. (GIBSON *et al*. 1995; OYARZABAL & CONNER 1995). According to AMMERMAN *et al.* 1988, the addition of FOS to the feed improves economically important traits of broilers. The studies performed by JAMROZ *et al*. 1997 showed that an addition of mannose oligosaccharides to the diet caused essentially curbed weight gain and an increase (ca. 6%) of consumption of feed by the tested birds in comparison to the control. Similarly, WALDROUP *et al*. (1993) did not confirm the beneficial influence of FOS on body mass, utilization of feed and level of circumintestinal fat. The authors suggest that this effect may be due to the disadvantageous interaction between various fodder additions such as oligosaccharides, antibiotics, growth hormones and coccidiostatics.

Up to now, studies on prebiotic properties of oligosaccharides have been focused on the determination of bifidobacteria number in faeces of two-day old chicken when these compounds were applied during embryogenesis (VILLALUENGA *et al*. 2004). In the light of these results, interesting questions can be raised: does a single injection of oligosaccharides during embryogenesis cause maintenance of a high level of intestine bifidobacteria in the late period of bird development ?, and, does a single injection of oligosaccharides during embryogenesis affect selected broiler chicken traits? This matter is important both theoretically and also in an economical aspect, as expensive feeding oligosaccharides added to fodder might be replaced with a single injection of a low dose of these compounds during chicken embryogenesis. The aim of this work was to answer these questions.

## Material and Methods

### Samples and chemicals

Seeds of pea *Pisum sativum* L. cv. Opal were supplied by Dr S. Stawiński from the Plant Breeding and Acclimatization Station at Przebędowo near Poznań, Poland. FOS were provided by Orafti S.A. Belgium. TPY – solid medium for isolation of bifidobacteria was purchased from BTL Łódź, Poland. Mupirocin was purchased from Oxoid, Great Britain.

### Preparation of RFOs

α-galactoside preparation was isolated and purified from pea seeds according to the method described by GULEWICZ *et al.* (2000).

### HPLC analysis of RFOs

The analysis (separation and quantification) of pea RFO preparation was carried out by high performance liquid chromatography HPLC-RI (FRIAS *et al*. 1994; GULEWICZ *et al*. 2002).

The influence of pea RFO preparation injected during embryogenesis was determined on:

1. the number of bifidobacteria in the later period of development and selected parameters of blood;

2. broiler chicken meat traits.

Experiment (1) was conducted with Hubbard broiler breeder eggs from a commercial flock of hens. The eggs were incubated in a pas-Reform incubator at 37.8 °C and 60 RH. On the 12th day of incubation, the eggs were candled, and the infertile ones or those containing only dead embryos were removed. Then, the eggs were divided into four groups of fifty eggs each, and a hole (0.8 mm) in the shell was made above the air chamber. The oligosaccharide preparations were injected into the eggs on the 12th day of embryogenesis with the following doses: FOS – 1.763 mg/egg (group I) and pea RFO containing 20% sucrose – 2.1158 mg/egg (group II) in 0.2 ml of Ringer water solution. The dose 2.1158 mg/egg corresponded to the

dose 1.763mg/egg of pure α-galactosides present in pea preparations. Pure sucrose at a dose of 0.4232 mg/egg (20% of 2.1158 mg dose of RFO) was injected into group III. 0.2 ml of Ringer water solution (group IV) was applied to the eggs of the control group. The hatchability of the treated eggs was registered. The chickens were wing-banded and placed in the conventional poultry house of the Experimental Station at Mochełek. The chickens were kept under continuous lighting, with water and food (a commercial diet) *ad libitum* until 12 h before slaughtering, when food was withdrawn. At the end of the experiment, at the age of 6 weeks, all birds were weighed, slaughtered and dissected (HAAHN & SPINDLER 2002).

Experiment (2) was conducted with Hybro G broiler breeder eggs from a commercial flock of hens. The eggs were incubated in a pas-Reform incubator at 37.8 °C and 60 RH. On the 12th day of incubation, the eggs were candled, and the infertile ones or those containing only dead embryos were removed. Then, the eggs were divided into four groups of one hundred eggs each, and a hole (0.8 mm) in the shell was made above the air chamber. The eggs of experimental groups II, III and IV were injected by the following doses of α-galactoside preparations: 0.69; 3.43 and 6.87 mg/egg, respectively, in 0.2 ml of Ringer water solution, while the control (group I) was injected with 0.2 ml of Ringer water solution only. The hatchability of the treated eggs was registered. The birds were kept in cages of 7 birds each. Eleven cages, i. e. 77 chicken, constituted one group. The treatment of broilers was the same as in the experiment 1.

Determination of a number of bifidobacteria

Fecal matter was collected for the analysis from 30 two-day old chickens from each group into the tared test tubes with 10 ml of sterile 0.85% sodium chloride solution and stored in a $CO_2$ atmosphere. Next, the tubes were weighed in order to determine the fecal mass. Decimal dilutions from $10^{-6}$ up to $10^{-9}$ were made from the suspensions. From each dilution, 1 ml was applied on a Petri dish covered with modified TPY agar with glacial acetic acid (1 ml/l) and mupirocin (100 mg/l) (RADA & PETR 2000; RADA *et al*. 2001). The number of bifidobacteria was estimated on the above-mentioned medium. The incubation was carried out in $CO_2$ rich atmosphere for 72 h at 37 °C. The results were calculated in colony forming units CFU/g of faeces. The same method was used for the determination of bifidobacteria in caecum sampled immediately after slaughtering the broilers.

Determination of serum lipids and breast muscle cholesterol concentration

Blood was collected in non-heparinized blood collection tubes from 65 randomly selected chickens (15 per group) at the age of 21 and 42 days. The blood samples were centrifuged at 2000 g for 3 min, and the serum was transferred into vials and stored at -20 °C until use. The serum samples were analyzed for total cholesterol, HDL cholesterol and triglycerides by enzymatic diagnostic kits (Alpha Diagnostic). The LDL cholesterol was calculated by the differences between total cholesterol and HDL cholesterol.

Determination of breast muscle cholesterol

Breast muscle total cholesterol was determined by spectrophotometry using a UV-Vis 3100 Shimadzu model.

Statistical analysis

The hatchability results were compared using the $\chi^2$ test, adjusted for the estimation of percentage values (LAUGHLIN & LUNDY 1976). The other chicken traits were compared using the Student's *t*-test at the significance level of 0.05 and 0.01.

## Results

As shown in Table 1, the main sugar of the α-galactoside preparation is stachyose (48.32%). The raffinose and verbascose content is at a level of 11.60 and 16.92%, respectively. Also, the preparation contains 20% sucrose.

Table 1

The chemical composition of pea α-galactoside preparation

| Sugars | Percentage Content |
|---|---|
| sucrose | 20.00 |
| raffinose | 11.60 |
| stachyose | 48.32 |
| verbascose | 16.92 |
| Total | 96.84 |

Figure 1 shows the influence of FOS and RFO preparations on a number of bifidobacteria in faeces of two-day old chicken. Administration of both oligosaccharides on the 12th day of embryogenesis increased the number of bifidobacteria in faeces of two-day old chicken in comparison with the control group. The effect of sucrose injection, in the

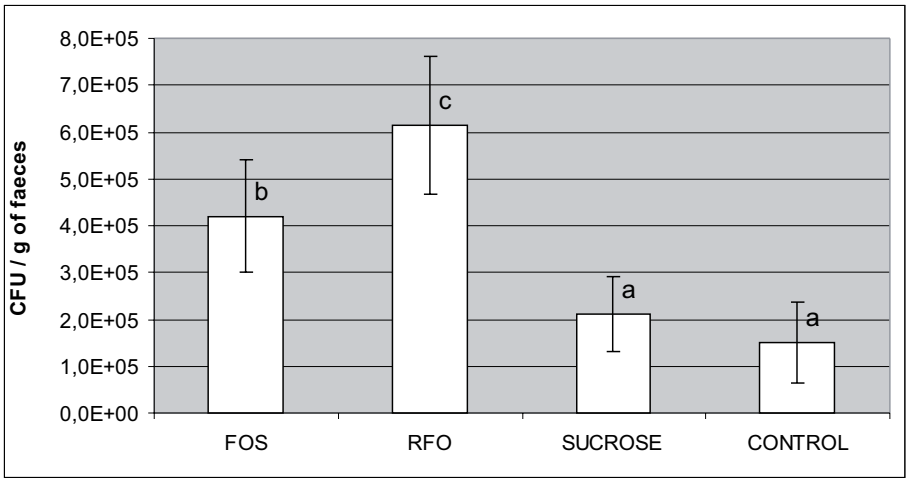


Fig. 1. Number of bifidobacteria in faeces of two-day old chicken. Letters a-c indicate statistical significance at P<0.01.

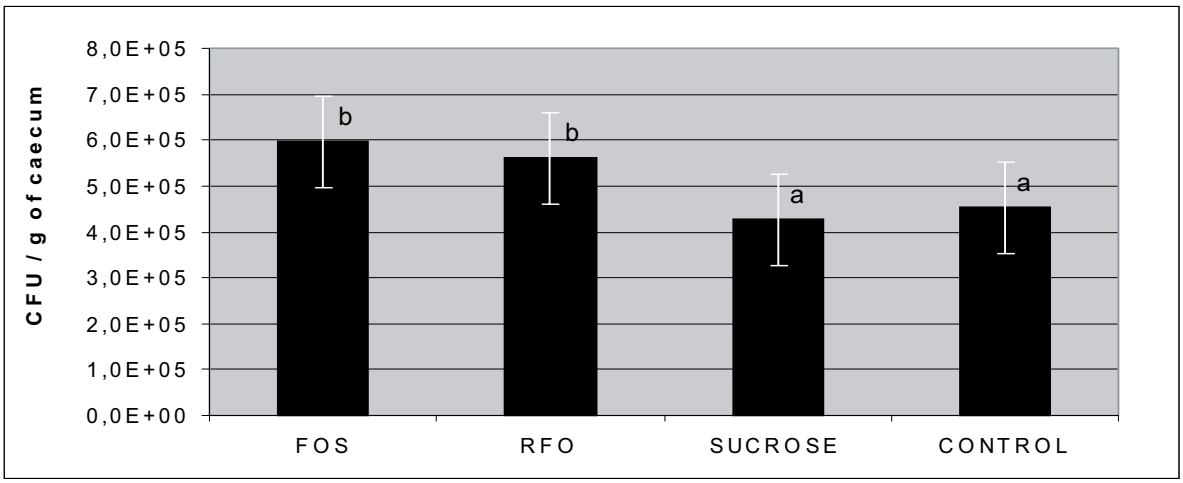


Fig. 2. Number of bifidobacteria in caecum of six-week old chicken. *Letters a-b indicate statistical significance at P<0.05.

amount corresponding to the content in the RFO preparation, was at the control level. Notably, the RFO preparation influenced the growth of bifidobacteria more beneficially than the FOS one.

On the 42nd day of the experiment, the difference in the number of bifidobacteria remained statistically significant in comparison with the control and sucrose groups. The difference between FOS and RFO preparations disappeared (Fig. 2).

The effect of both oligosacharides and sucrose on body weight, mortality and the European Production Index is presented in Table 2. The injection of sucrose significantly decreased body weight of 21-day old chicken in comparison with

Table 2

Effect of oligosaccharides *in ovo* injection on selected traits of one-, twenty one- and forty two-day old broiler chickens

| Traits | | Groups | | | |
|---|---|---|---|---|---|
| | | FOS | RFO | Sucrose | Control |
| Body weight (g) | 1st day | 35.5 ± 0.43 a | 35.9 ± 0.44 a | 35.8 ± 0.69 a | 35.9 ± 0.44 a |
| | 21st day | 814.5 ± 11.4 a | 807.0 ± 11.0 a | 741.1 ± 17.6 b | 798.0 ± 11.4 a |
| | 42nd day | 2491.9 ± 46.8 a | 2424.4 ± 46.4 a | 2438.3 ± 77.3 a | 2418.2 ± 48.3 a |
| Mortality (%) | | 9.1 | 7.4 | 9.1 | 13.2 |
| European Production Index | | 289 | 288 | 283 | 268 |

Table 3

Effect of oligosaccharides injected *in ovo* on carcasses, breast muscle, legs and abdominal fat of broiler chickens

| %* | Groups | | | |
|---|---|---|---|---|
| | FOS | RFO | Sucrose | Control |
| Carcass | 75.6 ± 0.55 a | 76.7 ± 0.55 a | 75.3 ± 0.55 a | 76.5 ± 0.55 a |
| Breast muscles | 17.8 ± 0.38 a | 17.9 ± 0.38 a | 17.8 ± 0.38 a | 17.9 ± 0.38 a |
| Legs | 15.2 ± 0.27 a | 15.2 ± 0.27 a | 14.7 ± 0.27 a | 15.0 ± 0.27 a |
| Abdominal fat | 1.51 ± 0.19 a | 1.75 ± 0.19 a | 1.40 ± 0.19 a | 1.52 ± 0.19 a |

* carcasses – the percent of body weight, breast muscles, legs and abdominal fat – the percent of carcass.

Table 4

Effect of oligosaccharides injected *in ovo* on serum lipids and breast muscle cholesterol content of twenty one- and forty-day old broiler chickens

| mg/dl | Groups | | | |
|---|---|---|---|---|
| | FOS | RFO | Sucrose | Control |
| Total cholesterol | | | | |
| 21st day<br>42nd day | 218.4 ± 17.8 a<br>200.4 ± 19.2 a | 209.9 ± 13.3 a<br>184.8 ± 19.2 a | 172.3 ± 21.0 a<br>224.2 ± 19.2 a | 177.4 ± 16.6 a<br>203.2 ± 19.2 a |
| HDL | | | | |
| 21st day<br>42nd day | 91.2 ± 4.8 a*<br>87.9 ± 5.2 a | 83.1 ± 3.6 a<br>91.3 ± 5.2 a | 82.3 ± 5.7 a<br>86.5 ± 5.2 a | 91.1 ± 4.5 a<br>82.3 ± 5.2 a |
| LDL | | | | |
| 21stday<br>42nd day | 90.0 ± 17.8 a<br>101.8 ± 19.2 a | 99.3 ± 13.6 a<br>82.9 ± 19.2 a | 60.0 ± 21.1 a<br>126.6 ± 19.2 a | 63.0 ± 16.6 a<br>110.0 ± 19.2 a |
| Triglycerides | | | | |
| 21st day<br>42nd day | 186.0 ± 12.6 a<br>53.5 ± 13.6 a | 161.4 ± 9.4 a<br>52.8 ± 13.6 a | 150.2 ± 14.9ab<br>55.5 ± 13.6 a | 116.5 ± 11.8 b<br>54.3 ± 13.6 a |
| Breast muscle total cholesterol | 49.3 ± 1.8 a | 50.3 ± 1.8 ab | 52.3 ± 1.8 ab | 54.7 ± 1.8 b |

*Letters a-b indicate statistical significance at P<0.01.

Table 5

Effect of pea RFO preparation applied during embryogenesis on selected broiler chicken performance. Letters a-b following entries indicate statistical significance at P<0.05

| Performance* | Group I control | Group II 0.69 mg/egg | Group III 3.43 mg/egg | Group IV 6.87 mg/egg |
|---|---|---|---|---|
| Final body weight (g) | 1755 ±95 a | 1853 ±86 a | 1817±98 a | 1808 ±92 a |
| Feed conversion (kg/kg) | 1.90 ±0.2 a | 1.82 ±0.1b | 1.87 ±0.1a | 1.89 ±0.3 a |
| Carcass | 70.8 ±2.8 a | 72. 8 ± 2.7 a | 71.6 ±2.7 a | 75.1 ±1.7 b |
| Breast muscles | 18.2 ±1.2 a | 19.3 ±1.2 a | 19.8 ±2.4 a | 20.4 ±1.7 a |
| Abdominal fat | 1.74 ± 0.2 a | 1.85 ± 0.6 a | 1.55 ± 0.5 a | 2.16 ± 0.9 a |
| Eur. Production Index | 225 | 230 | 222 | 217 |

*carcass – the percent of body weight; breast muscles and abdominal fat – the percent of carcass.

the other groups. However, these differences disappeared on the 42nd day of the experiment. The highest mortality was observed for the control group (13.2%), while the lowest for the RFO group (7.4%). The mortality of the remaining groups was at the level of 9 %. The lowest European Production Index was recorded in the case of the control group. The results presented in Table 3 show that oligosacharides administrated *in ovo* at a dose of 1.763 mg/egg did not have a statistically signifi-

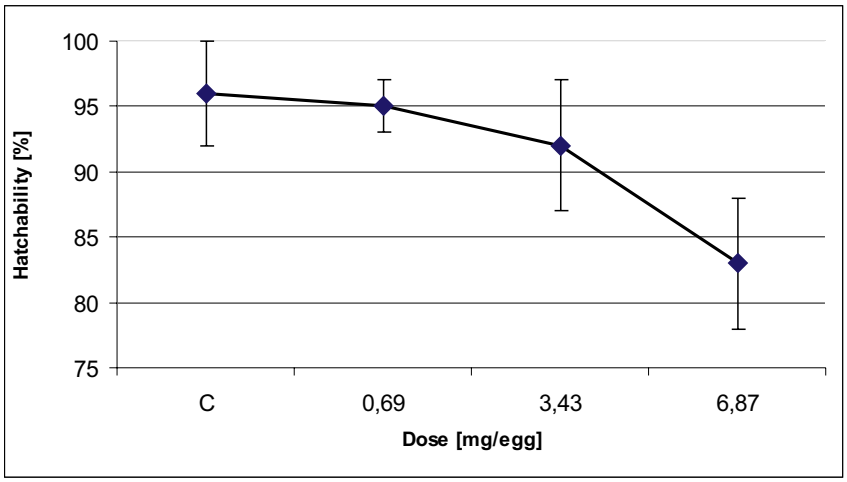


Fig. 3. Effect of different doses of pea RFO preparation on chicken hatchability.

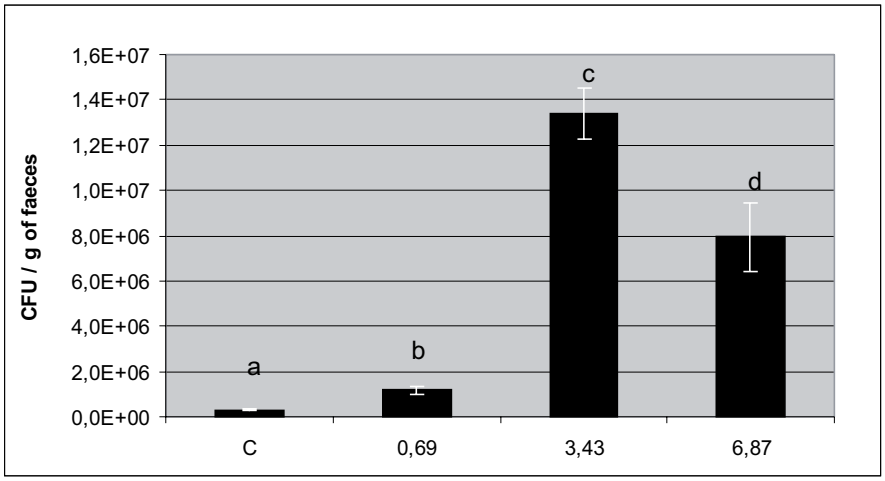


Fig. 4. Effect of different doses of pea RFO preparation on the number of bifidobacteria in caecum of six-week old chicken.

cant effect on the mass of the carcass, breast muscle, legs and abdominal fat ratio of broiler chicken. Both oligosacharides had no influence on total serum cholesterol and its HDL and LDL fraction in 21- and 42-day old chicken (Table 4). The content of triglicerides in experimental groups was statistically increased in 21-day old chicken in comparison to the control. However, this difference disappeared on the 42$^{nd}$ day. The breast muscle cholesterol content was significantly different between FOS and the control group only.

The effect of different doses of pea RFO preparation applied *in ovo* on the selected broiler chicken meat traits is presented in Table 5. The doses of pea preparations had no effect on the tested meat traits, except a dose of 6.90 mg /egg that significantly influenced the percent of carcase in body weight. Unfortunately, the hatchability of chicken at this dose distinctly decreased to the level of 82% (Fig. 3). Similarly, the number of caecum bifidobacteria at this dose was clearly lower than at 3.43 mg/egg (Fig. 4).

## Discussion

The evaluation of prebiotic activity of oligosaccharides has been based on their effect on two-day old chicken intestinal colonization by bifidobacteria (VILLALUENGA *et al*. 2004). Although bifidobacteria modified medium TPY, highly selective for the isolation and growth of bifidobacteria (RADA & PETR 2000), was used for determination in this study – it should be acknowledged that: (i) all accessible media used by the authors were not 100% selective for elimination of growth of other bacteria, (ii) not all bifidobacteria have the same ability for colonization of the alimentary tract. For this reason a control group and numerous numbers of repetitions were introduced into this study. Here it was demonstrated that a single *in ovo* injection of oligosaccharides leads to the long-term maintenance of a high level of intestine bifidobacteria, which may beneficially influence meat traits of a broiler. It is known that meat traits are influenced by both genetic and environmental factors,

accounted for in this study by diversified genetic material (Hubbard and Hybro G broiler breeder eggs) and by rearing in cages or in a poultry house, and also by the application of different oligosaccharides and doses during embryogenesis.

The effect of FOS and RFO preparations injected *in ovo* on prebiotic activity maintenance

The maintenance of prebiotic activity by different oligosaccharides administered during embryogenesis was determined as a first step in the experiments. Only one dose (1.763 mg/egg) of FOS and pea RFO preparations (VILLALUENGA *et al*. 2004) was used. The oligosaccharides were injected into Hubbard broiler breeder eggs and birds were reared in a poultry house. Taking into account that the pea RFO preparation contains 20% sucrose, the role of sucrose in the activity of the RFO preparation was also determined. The results clearly showed that the injection of FOS and RFO *in ovo* causes an increase and the maintenance of a high level of bifidobacteria up to the end of the experiment, at the age of six weeks. Simultaneously, sucrose alone had no effect on the number of bifidobacteria and gave results at the level of the control. On the other hand, an oligosaccharides dose of 1.763 mg/Hubbard and the environmental conditions in which birds were reared had no statistically significant influence on the measured meat traits (Table 2, 3). This result is not surprising because other authors, who used much higher doses administrated *per os* or as a fodder supplement, did not observe any effect either (LISOWSKI *et al*. 2003; WALDROUP *et al*. 1993). An unquestionable benefit of the application of oligosaccharides was a decrease in mortality (13.2% for control, 7.4% for pea RFO). The application of both preparations *in ovo* had no significant effect on the lipoprotein level, which confirms the results of blood analysis of six-week old broilers. However, oligosaccharides caused a distinct decrease of breast muscle cholesterol in comparison to the control group (Table 4). The results of this experiment suggested that both oligosaccharides differing in structures are a very good carbon source for bifidobacteria and the bonds β (2→1) in FOS and α (1→6) in RFO are recognized by enzymes produced by these bacteria.

The influence of pea RFO preparation injected in different doses during embryogenesis on selected chicken broiler meat traits

In order to show whether a dose of pea RFO preparation may effect meat traits of broilers, an experiment was performed for three experimental groups with 0.69, 3.43 and 6.87 mg/egg doses. In this manner, the range of doses included the dose 1.763 mg/egg used for the first experiment. Additionally, different genetic material (Hybro G egg) was included and the rearing of broilers was carried out in cages this time. This change of experimental conditions was purposeful and created major investigative possibilities. The results showed that all three administrations increased the number of bifidobacteria in a statistically significant way in comparison to the control group. However, the number of bifidobacteria in control and experimental groups was distinctly higher in comparison to the first experiment (Figs 1, 2, 4). This is probably due to a change of both genetic and environmental factors. The strongest beneficial influence on the bifidobacteria population was observed at the dose 3.43 mg /egg (Fig. 4). The highest dose (6.87 mg/egg) caused a decrease of bifidobacteria level and chicken hatchability (Fig. 3). This observation is in agreement with earlier data (VILLALUENGA *et al*. 2004). In spite of many changes in experimental conditions (genetic material, environmental conditions and different doses of RFO), α-galactosides had no effect on meat traits of broilers except the highest dose of 6.87 mg/egg that influenced slaughter percentage (Table 5). Unfortunately, as far as hatchability is concerned, this dose is undesirable.

Although this study did not reveal any beneficial effect of the tested oligosaccharides injected *in ovo* on chicken broiler meat traits, the fact of maintaining a high bifidobacteria number during the experimental time has multiple effects on health. As it is known from the literature, oligosaccharides as prebiotics contribute to health in many ways: (i) they influence the proliferation of bifidobacteria and reduce the numbers detrimental microorganisms; (ii) they reduce toxic metabolites and detrimental enzymes; (iii) they prevent pathogenic and autogenous diarrhea; they prevent constipation by stimulating intestinal peristalsis and by increasing faecal moisture with osmotic pressure; (iv) they alleviate the detoxifying load of the liver; (v) they reduce the serum cholesterol level; (vi) they reduce blood pressure; (vii) they show anticancer activity; (viii) they influence the production of vitamins $B_1$, $B_2$, $B_6$, $B_{12}$, nicotic and folic acids (AMMERMAN *et al*. 1988; BAILEY *et al.* 1991; CHOI *et al.* 1994; CORRIER *et al.* 1990; WALDROUP *et al.* 1993; TOMOMATSU and papers cited therein, 1994). In the light of the present study, the application of oligosaccharides to the chicken diet can be successfully replaced by injecting these compounds *in ovo* in very low doses. Moreover, taking into consideration health benefits, this approach is of high significance and, therefore, should not be underestimated.

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