

PREBIOTICS AND SYNBIOTICS IN BROILER CHICKEN PRODUCTION: *IN VIVO* PERFORMANCE AND MEAT QUALITY ASPECTS: A REVIEW

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ABSTRACT

A large amount of antibiotics has long been used to control pathogenic diseases and as growth promoters to improve performance in livestock. However, this approach had significant and unwanted side-effects, such as development of antimicrobial resistance and carry-over of the antibiotic residues to poultry products. In this light, the use of antibiotics as growth promoters (AGPs) was banned by the European Union since 2006, based on their possible negative consequences for animal health and food safety. This ban has led to animal performance problems and the increased incidence of enteric diseases in farms, with serious economic damage. In the post-antibiotics era, probiotics, prebiotics and synbiotics are proposed as alternatives to AGPs in poultry production. To be effective, these compounds have to be administered to the animals under fully controlled conditions and as early as possible. *In ovo* technology enables delivery of sustainable bioactives, such as pre-/probiotics and their combination, directly into the egg air chamber at day 12 of embryonic incubation. Previously, different types of prebiotics and the routes of delivery, as well as their synergistic combinations with probiotics, were tested in field and laboratory trials also by our research groups. Some of the obtained results (*in vivo* performance, slaughter and meat quality traits) are described hereinafter.

Key words: broiler chicken; prebiotic and synbiotic; *in ovo*; performance; meat quality

INTRODUCTION

Over the last fifty years the world's poultry production has almost quadrupled. Moreover, over the last eight years, the costs of poultry feed ingredients have increased considerably. This has been due to a greater global feed grain demand and an increased use of corn for ethanol production. Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in economics in broiler industry. In fact, the 70 % of total cost of production is contributed by feed (Willems *et al.*, 2013). Therefore, improvement of feed conversion ratio (FCR) will considerably increase the margin of profit. Between 1950 and 2000, the majority of poultry feeds contained antibiotic growth promoters (AGPs) used as a tool for the control of pathogenic diseases and

for the efficient livestock production. AGPs act by modifying the intestinal microflora, especially against Gram-positive bacteria, which are associated with animals' poorer health performance. However, this approach had significant and unwanted side-effects, such as development of antibiotic-resistant pathogens and carry-over of the antibiotic residues to poultry products, such as meat and eggs. Therefore, the role of AGPs in the emergence of antibiotic resistance in humans has been questioned, and on the basis of the 'precautionary principle' (Turndige, 2004) the European Commission decided to ban AGPs. The last phase of the EU-wide ban on AGPs in animal feed took effect some years ago (EC Regulation No. 1831/2003).

The ban of antibiotics at sub-therapeutic level contributed to increased incidence of enteric diseases

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in farms, with serious economic consequences. Many alternatives have been investigated to replace antimicrobials without any loss of productivity or negative influence on health. Probiotics, prebiotics and synbiotics are one of the proposed solution, as alternatives to AGPs, to prevent enteric disease and increase performance in poultry. As claimed by some authors, alternative for AGPs are of practical significance, when they improve animal performance at levels comparable to AGPs. There is a growing interest in the use of a variety of probiotics and prebiotics in several feeding trials in broiler chickens to promote animal health by altering the intestinal microbial community (Awad *et al.*, 2008).

Probiotics are live microorganisms which, when administered in adequate amounts, exhibit a health benefits on the host, including: regulation of bacterial homeostasis, stabilization of gastrointestinal barrier function (Salminen *et al.*, 1996; Gaggia *et al.*, 2010), expression of bacteriocins (Mazmanian *et al.*, 2008; Gaggia *et al.*, 2010), immunomodulatory effects (Salzman *et al.*, 2003; Gaggia *et al.*, 2010). Prebiotic (fructooligosaccharides, inulin, galactooligosaccharides, transgalacto-oligosaccharides, raffinose family oligosaccharides) has been defined as “non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (Gibson and Roberfroid, 1995), especially bifidobacteria and lactobacteria (Baffoni *et al.*, 2012). They are not hydrolysed or absorbed in the upper tract of digestive system. Prebiotics are a source of carbon and energy for the friendly strains of bacteria already inhabiting in the colon, where bacterial fermentation processes of some nutrients occurs (Dankowiakowska *et al.*, 2013). Some studies have suggested that synbiotics, a mixture of probiotics and prebiotics, is the best option to active the metabolism of one or a limited number of health promoting bacteria and/or by selectively stimulating their growth improving the host’s welfare and thus the growth (Gibson and Roberfroid, 1995; Slawińska *et al.*, 2014; Kamel and Mohamed, 2016). The main importance of this form of synergism is that a probiotic alone, i.e. without a source of nourishment, which can be represented by a prebiotic, cannot survive well in the digestive system. Some researchers reported the importance and benefits of this kind of synergy between probiotics and prebiotics and the effectiveness in helping young animals to achieve better growth performance (Patterson and Burkholder, 2003). There are different ways to deliver these bioactive substances into avian gastrointestinal tract. Conventionally, in-feed or in-water supplementation has been used at first hours/days post-hatching. This approach relies on amount of feed and/or water intake, the quality of water (chlorinated) and other experimental

factors (Bednarczyk *et al.*, 2016). As a consequence, consumed dose of prebiotics varies in the first hours/days after hatching. Furthermore, during early post-hatching period, infection of chicks by detrimental bacteria is also possible. Therefore, to be effective, these compounds have to be administered to the animals under fully controlled conditions and as early as possible. In fact, some recent research tends to exclude the unwanted effects of several factors that may affect the action of supplements.

In ovo technology enables delivery of sustainable bioactives, such as pre-/probiotics and their combination, directly into the egg air chamber at day 12 of embryonic incubation; it allows for a precise delivery of the bioactive substance to all embryos, which equalizes the effects across the flock and assures proper development of the gut microflora in all chicks. Previously, different types of prebiotics and their synergistic combinations with probiotics were tested in field and laboratory trials also by our research groups.

Bioactive substances used and results obtained

During the last years, different prebiotics and their synergistic combinations with probiotics were tested in field and laboratory trials by our research groups:

- a) commercial prebiotics, as DN (DiNovo[®], BioAtlantis Ltd, Tralee, Co., Kerry, Ireland) a *Laminaria* spp. seaweed extract containing laminarin and fucoidan, and BI (Bi²tos, Clasado Ltd, Sliema, Malta) a non-digestive trans-galactooligosaccharides (GOS) from milk lactose digested with *Bifidobacterium bifidum* NCIMB 41171; RFO (raffinose family oligosaccharides) in-house extracted from lupin (*Lupinus luteus*) seeds (Gulewicz *et al.*, 2000).
- b) different synbiotic preparations (SYN1: BI+*Lactobacillus salivarius*; SYN2: RFO + *Lactobacillus plantarum*).

Some of the obtained results (*in vivo* performance, slaughter and meat quality traits) in different trials are reported below.

Trial 1

A trial was performed to evaluate the effect of different prebiotics (DN, BI and RFO) and mode of their administration on *in vivo* performance, carcass and meat quality traits in Ross 308 broiler chickens (Bednarczyk *et al.*, 2016). The prebiotics were used for comparison between different routes of delivery: *in ovo* injection (T1), *in ovo* injection combined with in-water delivery (T2) and in-water delivery (T3). Control group (C) was injected *in ovo* with physiological saline only and did not receive any prebiotic in-water. Hatching eggs were collected from the same breeder flock and incubated in the commercial broiler hatchery. At day 12

of incubation 1500 eggs, containing viable embryos, were randomly allotted into four experimental groups (375 eggs per group). Eggs were injected *in ovo* with 0.2 mL solution containing: 3.5 mg.embryo⁻¹ BI, 0.88 mg.embryo⁻¹ DN and 1.9 mg.embryo⁻¹ RFO. The C group was injected *in ovo* with physiological saline only and did not receive any prebiotic in-water. Following injection, each hole was sealed with hot glue and the egg incubation was continued until hatching. Solutions of prebiotics were injected *in ovo* using dedicated automatic system (Bednarczyk *et al.*, 2011). After hatching chicks were sexed and 600 males (42.0 g average weight) were randomly assigned to ten experimental groups (60 males per group): T1 (DN, BI and RFO), T2 (DN, BI and RFO), T3 (DN, BI and RFO) and C. Chicks from T1 and C groups were raised without any additional supplementation with prebiotic. T2 and T3 groups were supplemented in-water with respective prebiotic (DN, BI or RFO) for first seven days of life. Those animals received 12 ml of the prebiotics dissolved in water per pen (20 mg of prebiotic.ml⁻¹). Birds were grown up to 42 days of age in collective cages (n = 6 replicate cages, 10 birds in each cage). Broilers were fed commercial diets *ad libitum* according to age. Amounts of feed offered to each cage were recorded. Feed intake (FI) and feed conversion ratio (FCR) were calculated on a cage basis. The prebiotics increased body weight gain (BWG), especially during the first 21 days of life, irrespective of route of delivery (T1, T2 or T3), as compared with the C group (P < 0.05). These results provide further support for the hypothesis concerning well-established growth promoting effect of dietary prebiotics, attributed to their ability to strongly bind the pathogenic bacteria and decoy pathogens away from the intestinal lining. Prebiotic-treated chickens showed trend for increased FI and FCR; this could be due to the stimulation of the intestinal microbiota expansion in the chicken guts by the injection of prebiotics during the *in ovo* development. In fact, it has been suggested that the effect of prebiotics on chicken growth performance could be related to metabolism modification linked to an increase in the digestive enzymes activity (Pruszyńska-Oszmala *et al.*, 2015), the decrease in bacterial enzymes activity and ammonia production along with the improved feed intake and digestion (Kabir, 2009). Our results indicate a positive stimulation of the broiler body weight (BW) expressed as soon as in the starter period (1-21 days), which might be explained by early supplementation of chicken embryos with prebiotics using *in ovo* method. However, injection of prebiotics *in ovo* combined with in-water supplementation did not express synergistic effects on broiler performance compared to *in ovo* injection only. These results confirm that single *in ovo* prebiotic injection into the chicken embryo can

successfully replace prolonged in-water supplementation post-hatching.

Carcass weight and yield were unaffected by prebiotics. However, *in ovo* administration significantly increased carcass weight and yield compared to in-water administration. On the contrary, pectoral muscle (PM) weight was significantly higher in all prebiotic groups, regardless of the mode of administration, compared to the C group. All prebiotics increased significantly fiber diameter (μm) when compared with the untreated control. No differences were observed with respect to mode of application. The histological observations showed a trend towards intramuscular fat infiltration in the DN group when compared with the C group (P = 0.07), whilst no differences were found among the other groups and the different methods of administration. These differences in the intramuscular fat content could be related to different growth rate and feed conversion efficiency. Cholesterol levels in PM were unaffected by prebiotics or methods of application. The total saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA) amount was affected neither by prebiotics nor by mode of administration. The obtained results on SFA content are consistent with the study of Rule *et al.* (2002) conducted on broiler chicken. The total polyunsaturated fatty acid (PUFA) content was similar in all experimental groups, however, prebiotic groups had a slightly higher (P = 0.082) amount of PUFA compared to C group. Regarding the selected fatty acid ratios, only the ratio of PUFA to SFA (P/S) was significantly different among experimental groups with higher (P < 0.01) value for DN group compared to the control one. The obtained value of P/S ratio is a little bit higher than the recommended value of 0.4–0.7, even if it is lower than values of other meat species (Wood *et al.*, 2003). Anyway, the obtained data showed a particularly lower n-6/n-3 ratio due to the higher incidence of n-3 fatty acids, probably due to the inclusion of n-3 fatty acids into the diet administered to the birds. This is a positive aspect for a nutritional point of view, because the obtained value is assigned between the ideal value of 1 and the maximum value of 4. In addition, meat from all experimental groups is characterized by low values of atherogenic index (AI) and the thrombogenic index (TI), even though are similar among groups. These indices, calculated according to the formulas suggested by Ulbricht and Southgate (1991), take into account the different effects, which the single fatty acid might have on human health and, in particular, on probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation (Ulbricht and Southgate, 1991).

In conclusion, *in ovo* administration of prebiotics was associated with the improvement of body weight, PM weight and PM fiber diameter, which are relevant

for commercial poultry production. Prebiotics significantly improved fatty acid profile and nutritional ratios of meat. Delivery *in ovo* combined with in-water supplementation of prebiotics did not show synergistic effects on broilers performance compared to *in ovo* injection only. These results confirm that a single injection of prebiotics into the chicken embryo can successfully replace prolonged in-water supplementation post-hatch. At the same time, the amount of the prebiotic used was at least ten times lower in case on *in ovo* method (3.5 mg BL.embryo⁻¹ *in ovo* vs. 40 mg BL.chick⁻¹ in-water). As such, *in ovo* method should be further recommended to the poultry industry.

Trial 2

The study was carried out to evaluate effect of two different synbiotics (SYN1 and SYN2) on *in vivo* performance and meat quality traits in broiler chickens (Cobb 500FF). Hatching eggs were collected from the same breeder flock and incubated in the commercial broiler hatchery. On day 12 of incubation, 5850 eggs were divided into 3 experimental groups treated with different bioactives, *in ovo* injected: SYN1, the group injected with 0.2 ml of a synbiotic formulation containing 2 mg.embryo⁻¹ of Bi²tos (ClasadoBioSciences Ltd.), trans-galactooligosaccharides enriched with 105cfu/embryo of *Lactobacillus salivarius* IBB3154; SYN2, group injected with 0.2 ml of a synbiotic formulation containing 2 mg.embryo⁻¹ of raffinose family oligosaccharides (RFO) enriched with 105 cfu. embryo⁻¹ of *Lactobacillus plantarum* IBB3036; C, control group injected with 0.2 ml of physiological saline solution. The injection hole was covered with a drop of organic glue and the incubation was continued until hatching. Among the hatched chickens, 2040 males (680 per each group) were randomly chosen and reared in a commercial poultry house (PiastPasze Sp. z.o.o., Olszowa, Poland). Chickens were raised in pens (n = 75 per pen) with 8 pen replicates per treatment for effect on performance. Moreover, separate pens for sampling (n = 10 birds per pen: 8 replications per each experimental group) were included in the experimental design. Animals were fed *ad libitum* with commercial diets according to their age and had free access to water. The FI and FCR were calculated on a pen basis. At 42 days of age, two birds per pen (16 birds per treatment) were randomly chosen from the separate pens for sampling and slaughtered. At slaughter, hot carcass weight was recorded and carcass yield was calculated. The PM was removed from each carcass and weighed; its percentage was calculated basing on hot carcass weight. At 24 hours *post-mortem*, pH, colour and water holding capacity (WHC) were recorded on the right PM. The left PM was vacuum packaged and frozen until chemical

analysis for total lipids, cholesterol and fatty acids.

In ovo synbiotic administration had no significant effect on mortality, growth performance and slaughter traits (carcass weight and yield, breast weight and yield). Similarly, physicochemical characteristics (pH, color, WHC), intramuscular collagen content and the degree of collagen maturation (hydroxylsilypyridinoline crosslink/collagen) of PM were not significantly affected by synbiotics. Differently, synbiotic administration had a significant effect on total lipid and fatty acid composition, but it depended on the kind of bioactivities administered. SYN2 lowered (P = 0.06) the muscle lipid content. The results on fatty acid (FA) composition showed a marked difference in the the proportion of several FA among the experimental groups. Meat from SYN1 group, compared with that of C and SYN2 groups, displayed an unfavorable FA profile due to: i) higher (P < 0.01) content of total saturated fatty acids (SFA); ii) lower monounsaturated fatty acids (MUFA) (P < 0.05 compared to SYN2); iii) lower (P < 0.01) polyunsaturated fatty acids (PUFA); and iv) lower n-6 PUFA (P < 0.01) and n-3 PUFA (P < 0.01 and P < 0.05 compared to C and SYN2, respectively). From the nutritional point of view, a higher P/S ratio is recommended; indeed it should be increased over 0.4. Atherogenic and thrombogenic indexes were significantly lower in SYN2 and C groups compared to SYN1. Total cholesterol content was similar among groups (41.10 ± 1.70 mg.100 g⁻¹).

In conclusion, the results of this study indicate that *in ovo* administration of synbiotics did not negatively affect productive performance and physiochemical properties of meat. However, the meat from C and SYN2 birds showed a preferable fatty acid profile, with a positive effect on nutritional properties of the chicken meat.

CONCLUSION

Thanks to the experience and the new knowledge acquired by our team over the years, during field and laboratory studies, we are able to give a fairly complete application of the innovative *in ovo* technology of bioactive compound delivery for improvement of the multiple production and health traits in broiler chickens, including growth rate, feed intake and nutrient digestibility, as well as meat quality. Nevertheless, future studies need to delve more into the mode of action of these bioactive substances in order to promote the use of pre-/synbiotics, which are consumer- and environment-friendly and contribute to the reduction of antibiotic use for therapeutic treatment in poultry production. This will open *in ovo* injection for a large scale application in different production systems.

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