In ovo validation model to assess the efficacy of commercial prebiotics on broiler performance and oxidative stability of meat

Giuseppe Maiorano,*[,1] Katarzyna Stadnicka,[†] Siria Tavaniello,* Cinzia Abiuso,* Joanna Bogucka,[†] and Marek Bednarczyk[†]

*Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy; and [†]Department of Animal Biochemistry and Biotechnology, UTP University of Science and Technology, 85-064 Bydgoszcz, Poland

ABSTRACT The purpose of this study was to examine the effect of in ovo injection of 2 different prebiotics, DiNovo (DN; *Laminaria* spp., extract containing laminarin and fucoidan) and Bi^{2}tos (BI; non-digestive transgalactooligosaccharides from milk lactose digested with *Bifidobacterium bifidum* NCIMB 41171), on growth, slaughter traits, intramuscular fat percentage (IF) and muscle fiber diameter, and lipid oxidation of meat in chickens reared under commercial conditions, following an in ovo trial protocol. On d 12 of embryonic incubation, 350,560 Ross 308 eggs were randomly divided into 3 experimental groups and automatically injected in ovo with: physiological saline (control group), BI at dose of 3.5 mg/embryo and DN at dose of 0.88 mg/embryo. Hatched chicks (males and females) were allocated dependent on treatment group into 3 poultry houses on each farm (3 farms in total) with a stocking density of 21.2 to 21.5 chicks/m^2. At 42 d of age, 14 randomly chosen birds (7 males and 7 females), per each treatment from each farm, were individually weighed and slaughtered. The results showed no significant differences of final number of chickens/chicken house, mortality, BW per treatment, stocking density (kg/m^2), feed intake, feed conversion rate (FCR), and European Broiler Index among 3 experimental groups. Treatments with BI and DN were associated with slight increases ($P > 0.05$) in average BW and a minor improvement ($P > 0.05$) of FCR in BI group. Slaughtered chickens from DN and BI treated groups had significantly increase of BW, carcass weight, carcass yield, and breast muscle weight compared with the control group. IF and muscle fiber diameter were similar among groups. Males had significantly higher slaughter traits compared to females, except for breast muscle yield. The prebiotic treatments led to a higher lipid oxidation in meat, even if the detected TBA reactive substances were below the critical value recognized for meat acceptability. In conclusion, in ovo administration of prebiotics was associated with improvements in a number of parameters of relevance to commercial poultry production.

Key words: chickens, in ovo technology, prebiotics, performance traits, lipid oxidation

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INTRODUCTION

The recent ban of antibiotics as growth promoters has become a challenge for animal nutrition. Alternative methods are required to control and prevent the occurrence of certain animal diseases. In particular, enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality, and the associated contamination of poultry products for human consumption (Patterson and Burkholder, 2003). Application of prebiotics and probiotics alone or in synergistic combination as synbiotics is believed to reduce incidence of those diseases and subsequent contamination of poultry products (Fioramonti et al., 2003; Siragusa and Ricke, 2012). The use of natural bioactive substances in poultry production can meet expectations of consumers, who are willing to pay a higher price for antibiotic-free animal products (Ewing and Tucker, 2009). Prebiotics can be defined as non-digestible feed ingredients with selective effects on intestinal microbiota; thus conferring benefits upon host health. They are commonly used in poultry production to achieve optimum productivity and welfare in poultry (Yegani and Korver, 2008). In ovo injection of prebiotics at an early stage of development increases the population of beneficial microflora on the day of hatch compared to dietary inclusion (Bednarczyk et al., 2016). This leads to a high and stable level of *Bifidobacteria* throughout the growing period of broiler chickens (Villaluenga et al., 2004). In ovo technology enables delivery of sustainable bioactives such as pre/synbiotics as early as possible, namely at d 12 of embryonic incubation, and influences the microbiome structure in newly

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[1]Corresponding author: maior@unimol.it

hatched chicks (Pilarski et al., 2005; Bednarczyk et al., 2011; Slawinska et al., 2014; Madej et al., 2015; Płowiec et al., 2015; Madej and Bednarczyk, 2016).

Broilers lack enzymes galactosidases. Supplementation of non-digestible galacto-oligosaccharides to the diet shows functional response in performance and promotes colonization of the gastrointestinal tract by Bifidobacteria (Jung et al., 2008). A bioactive mixture of galactooligosaccharides (**B-GOS**) produced through the enzymatic activity of β-galactosidase from *Bifidobacterium bifidum* NCIMB 41171 was earlier proposed as prebiotic for human treatment (Clasado Biosciences Ltd., Jersey, UK). B-GOS possess bifidogenic and immunomodulatory properties which were earlier proven in the monogastric model (Tzortzis, 2009). Here, a derivative candidate, non-digestive transgalactooligosaccharides (**GOS**) in a novel formulation of Bi^{2}tos (Clasado BioSciences Ltd., Jersey, UK) are proposed for a routine treatment in poultry.

A recent study conducted on broiler chickens (Bednarczyk et al. 2016) has shown that GOS injected in ovo (3.5 mg/embryo) increased the number of lactobacilli and bifidobacteria in the chicken excreta, as well as the body weight gain. Differently, feed intake (**FI**) and feed conversion rate (**FCR**) were negatively affected by GOS. Still, results of performance parameters including BW and FCR are inconsistent among studies conducted on small cohorts (200 to 500 animals) with different prebiotic treatments (Hanning et al., 2012; Kim et al., 2011; Mookiah et al., 2014), provided that the breed, sex, and environmental conditions are respected. Nevertheless, Ricke (2015) concluded that the type of host immune responses vs. growth performance may not necessarily be positively correlated with each other. Studies have shown that dietary provision of *Laminaria* spp. derived seaweed extract containing laminarin and fucoidan promotes improved growth and feed efficiency of pigs in the absence of in-feed antibiotics (Gahan et al., 2009; McDonnell et al., 2010). In a previous investigation (Bednarczyk et al., 2016), *Laminaria* spp. derived seaweed extract injected in ovo significantly increased body weight gain compared to the control group, especially during the first 21 d of life; while FI and FCR were not affected by prebiotic injection. Furthermore, previous investigations indicated that the supplementation of a seaweed extract to pigs suppressed enteric populations of *Enterbacteriaceae* (Reilly et al., 2008; Lynch et al., 2010) and increased numbers of lactobacilli (Lynch et al. 2010; McDonnell et al., 2010). Thus, seaweed extracts may provide a means to improve productivity and gut health in monogastric animals.

Quality of broiler meat has been emphasized in several studies. Among meat macronutrients, the lipid fraction has the highest susceptibility to modifications which are the major cause of chicken meat quality deterioration, as the reduction of shelf life of meat and meat products (Funaro et al., 2014). Prebiotics can alter lipid metabolism (Letexier et al., 2003) and enhance the polyunsaturated:saturated fatty acids ratio in chicken meat (Zhou et al., 2009; Velasco et al., 2010) with benefits to human health but a greater risk for the shelf life of the meat. Lipid and pigment oxidation in meat have negative effects on purchase decisions of consumers, which result in substantial economic losses. To our knowledge, there is no available information regarding the potential effect of in ovo injection of GOS and carbohydrates derived from *Laminaria* spp., on the shelf life of chicken meat. This study aims to explore potential of GOS and carbohydrates derived from *Laminaria* spp, injected in ovo, with an automatized system and determine their influence on performance, carcass traits, some meat quality traits (intramuscular fat and muscle fiber diameter), and lipid oxidation of meat in chickens reared under commercial condition.

MATERIALS AND METHODS

Birds, Experimental Design and Rearing Measurements

A total of 350,560 Ross 308 eggs were incubated in a commercial hatchery (Drobex-Agro Sp. z o.o., Solec Kujawski, Poland) a Petersime incubator (vision version, Petersime NV, Zulte, Belgium) for the in ovo protocol. The eggs were randomly divided into 2 prebiotic groups: **DN** (DiNovo, Bioatlantis Ltd, Tralee, Co. Kerry, Ireland; *Laminaria spp.* seaweed extract containing laminarin and fucoidan) and **BI** (Bi^{2}tos, Clasado Ltd., Sliema, Malta; non-digestive GOS from milk lactose digested with *Bifidobacterium bifidum* NCIMB 41171), and a control (**C**) group. The composition of the 2 prebiotics is described in details in Bednarczyk et al. (2016). On the twelfth d of incubation, the eggs were candled and those infertile or containing dead embryos were removed. The fertile eggs were automatically injected in ovo (Bednarczyk et al., 2011), into the air chamber, with 200 μL of physiological saline solution containing BI at dose of 3.5 mg/embryo or DN at dose of 0.88 mg/embryo. The eggs were injected on d 12 of embryonic incubation when the allantochorion is completely developed and highly vascularized (Villaluenga et al., 2004). After injection, each hole was sealed with a natural glue and egg incubation was continued until hatching. The optimal doses of prebiotics were selected in a previous study by evaluating the hatchability and the bacteriological status of the hatched chickens (Bednarczyk et al., 2016). The C group was injected with 200 μL of physiological saline. The research was performed on 3 commercial farms (Drobex Agro Sp. z o.o., Solec Kujawski, Poland), within 20 min driving distance from the laboratory facilities. All treatment groups were represented on the 3 farms. The experimental design is reported in Figure 1. Birds (males and females, approximately ratio 1:1) were reared at stocking density (ranging from

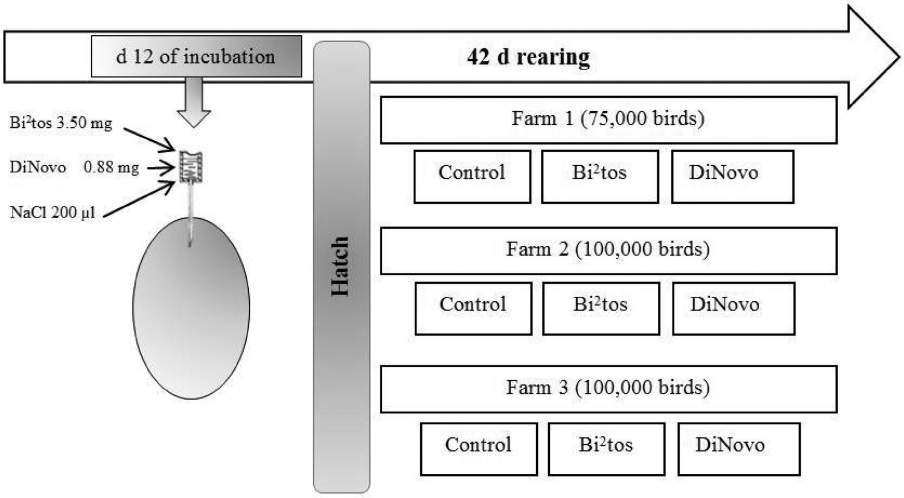

Figure 1. Number of chickens for each chicken house and each treatment.

21.2 to 21.5 chicks/m^2). Temperature was gradually decreased from 33°C on d 0 to 20°C on d 42 and was kept constant thereafter. The lighting program was 23L:1D in the first wk and 18L:6D from the second wk to the slaughter. The management conditions of broiler chickens were the same in all farms. The broilers were fed ad libitum the standard commercial feed mixtures (Table 1): starter (d 1 to 10), grower (d 11 to 35), and finisher (d 36 to 42). Water was provided ad libitum. The animals were reared according to the regulations and permission of the local Ethical Commission (decision No.22/2012 21.06.2012) and in accordance with the animal welfare recommendations of European Union directive 86/609/EEC. All performance data were collected automatically from the slaughterhouse livestock recording system SRPP-MK (*System Rejestracji Procesow Produkcyjnych*, Production Process Registration System, in-house developed by Drobex-Agro Sp. z o.o. Solec Kujawski, Poland): livestock BW, average BW, and final stocking density (kg of live weight/m^2 at slaughter) were calculated. Mortality at first wk of age and for the overall experimental period was calculated. Total FI and FCR were calculated for each treatment (n = 3 replications per experimental group). Moreover, European Broiler Index (**EBI**) was calculated for each chicken house replicate, according to the following formula: EBI = liveability (%) × live weight (kg) × 100/age (d) × FCR.

Slaughter Surveys

At 42 d of age, 14 randomly chosen birds (7 males and 7 females) per treatment from each farm were individually weighed (after a fasting period of 12 h) and transported within 1 h (including careful catching and loading) to a commercial poultry slaughterhouse (PD Drobex, Solec Kujawski, Poland). After careful unloading and hanging in randomized order, all birds were electrically stunned and slaughtered. After evisceration, the hot carcass weight (without head and feet) was recorded, and carcass yield was calculated. In addition, the breast muscle was removed from all carcasses and its percentage based on hot carcass weight was calculated.

Histological Evaluation

From all carcasses, breast muscle samples were removed at 45 min postmortem and quickly frozen in liquid nitrogen (−196°C). Each specimen was microtomed at −25°C into 6 to 8 serial cross-sections of 10 μm (Thermo Shandon microtome, Thermo Fisher Scientific, Runcorn, UK) mounted on glass slides and stained. Hematoxylin and eosin dyes were used to evaluate the general morphology of the tissues, whereas oil red staining was applied to determine the level of intramuscular fat (Dubovitz et al., 1973). The microscopic images of the specimens were taken using Carl Zeiss microscope (Jena, Germany) equipped with a Toup View camera. MultiScan v. 18.03 microscope imaging software (Computer Scanning Systems II Ltd, Warsaw, Poland) was used to measure fiber diameters per 1 mm^2. In brief, the fibers were selected at random and the mean diameter value per each group was calculated. The same method was used to determine the percentage of intramuscular fat per 2 mm^2 area.

Measurement of Oxidative Stability

For oxidative stability evaluation, breast muscle samples were analyzed as raw meat after storage at 4°C for 0, 2, 4, and 6 d. Lipid oxidation was determined by the TBA reactive substances (**TBARS**) method as described by Vyncke (1970, 1975) and with modifications according to Sørensen and Jørgensen (1996). Briefly, 5.0 g of minced meat was homogenized in 15 mL 7.5% trichloroacetic acid with 0.10% propyl gallate and 0.10% EDTA using an Ultra-Turrax T 25 BASIC

Table 1. Ingredients and chemical analysis of diets.

Item (% unless noted)	Period		
	1 to 10 d	11 to 35 d	36 to 42 d
Ingredients			
Corn (7.75% CP)	61.157	65.999	67.932
Soybean meal (47.75% CP)	33.086	28.158	26.032
Soybean oil	1.754	2.057	2.767
Limestone	1.098	0.982	0.698
NaCl	0.200	0.200	0.234
Dicalcium phosphate	1.605	1.504	1.337
Vitamin-mineral premix 1[1]	1.100	-	-
Vitamin-mineral premix 2[2]	-	1.100	-
Vitamin-mineral premix 3[3]	-	-	1.100
Chemical analysis, %			
DM	88.87	88.94	88.91
CP	21.00	19.00	18.00
Lipid	4.61	4.99	5.72
Crude Fiber	2.69	2.63	2.59
Ash	5.82	5.40	5.02
Calculated analysis			
ME, MJ/kg of diet	12.72	13.00	13.30
Lysine, %	1.32	1.19	1.05
Methionine, %	0.65	0.58	0.52
Methionine+cysteine, %	0.98	0.89	0.82
Threonine, %	0.86	0.78	0.71
Tyrosine, %	0.25	0.22	0.21
Calcium, %	0.90	0.84	0.76
Available P, %	0.71	0.68	0.63
Sodium, %	0.16	0.16	0.15
Salt,%	0.35	0.35	0.35
Potassium, %	0.93	0.83	0.79

[1]Supplied per kilogram of diet: vitamin A, 13,000 IU; vitamin D3, 5,000 IU; vitamin E, 80 mg; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 4 mg; vitamin B12, 20 μg; vitamin K, 3 mg; biotin, 0.15 mg; Ca pantothenate, 15 mg; nicotinic acid, 60 mg; folic acid, 2 mg; choline chloride, 0.50 mg; lysine, 2,812 mg; methionine, 3,405 mg; threonine, 745 mg; Ca iodate, 1 mg; Se, 0.35 mg; Fe, 40 mg; Mo, 0.50 mg; Mn, 100 mg; Cu, 15 mg; Zn, 100 mg.

[2]Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 5,000 IU; vitamin E, 50 mg; vitamin B1, 2 mg; vitamin B2, 8 mg; vitamin B6, 3 mg; vitamin B12, 15 μg; vitamin K, 3 mg; biotin, 0.12 mg; Ca pantothenate, 12 mg; nicotinic acid, 50 mg; folic acid, 2 mg; choline chloride, 0.40 mg; lysine, 2,831 mg; methionine, 3,018 mg; threonine, 726 mg; Ca iodate, 1 mg; Se, 0.35 mg; Fe, 40 mg; Mo, 0.50 mg; Mn, 100 mg; Cu, 15 mg; Zn, 100 mg.

[3]Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 5,000 IU; vitamin E, 50 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; vitamin B12, 15 μg; vitamin K, 3 mg; biotin, 0.12 mg; Ca pantothenate, 10 mg; nicotinic acid, 50 mg; folic acid, 1.5 mg; choline chloride, 0.35 mg; lysine, 1,779 mg; methionine, 2,514 mg; threonine, 361 mg; Ca iodate, 1 mg; Se, 0.35 mg; Fe, 40 mg; Mo, 0.50 mg; Mn, 100 mg; Cu, 15 mg; Zn, 100 mg.

(Ika-Werke, Staufen, Germany) and filtered; 1.0 mL of the filtrate was mixed with 1.0 mL of TBA 0.020 M and incubated at 100°C in a water bath for 40 min. Absorbance was measured at 532 nm and 600 nm at room temperature. The TBARS value was expressed as mg of malondialdehyde (**MDA**) per kilogram of sample using a standard curve prepared from 1,1,3,3-tetraethoxypropane.

Statistical Analyses

Statistical analyses of the data were performed using SPSS (SPSS, 2010). Data on performance and mortality were analyzed by one-way ANOVA where prebiotic was the main factor. Scheffé's test was applied to compare the mean values among the experimental groups. Slaughter performance and breast muscle qualitative traits data were evaluated by ANOVA, in a 3 × 2 factorial design that included as factors prebiotic treatment and sex. Scheffé's test was used for comparing mean values. The interactions between the main factors were not significant and are not reported in the results. Lipid oxidation (TBARS values) was analyzed as a repeated-measures, the model included the days of storage and prebiotic treatments, sex effect was not included in the model as it was not significant. The interactions between the main factors were not significant and are not reported in the results. For performance and mortality, the individual chicken house was considered an experimental unit; for slaughter performance and lipid oxidation, the individual bird was considered an experimental unit. All statistical tests were performed for a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

The automatized in ovo technology provided an accurate system for delivering raffinose family oligosaccharides prebiotic directly to the egg and allowed for large scale field investigation (Bednarczyk et al., 2011).

Tables 2 and 3 summarize the housing conditions and productive traits, respectively. At the end of the experiment, the final number of chickens/chicken house (ranging from 22,995 to 23,639) was not different ($P > 0.05$) among the 3 experimental groups. In general, the mortality recorded at first wk of life and at the end of the experiment (ranging from 3.56 to 4.32%) did not differ significantly among the experimental groups. The mortality for the overall experimental period observed in C group (3.56%) was almost similar to the

Table 2. Number and mortality of broiler chickens.

	C[1]	BI[2]	DN[3]	SEM	Significance
n. chick/house at 1 d	24,360	23,933	24,766	344	NS
n. chick/m^2 at 1 d	21.2	21.3	21.5	0.2	NS
n. chicken final/house at 42 d	23,493	22,995	23,639	326	NS
Mortality at 7 d (%)	1.36	2.16	1.85	0.23	NS
Total mortality (%)	3.56	4.15	4.32	0.33	NS

[1]C = Control, in ovo injection of physiological saline.
[2]BI = Bi[2]tos.
[3]DN = DiNovo.
Significance: NS = $P > 0.05$.

Table 3. Productive performance, feed intake (FI), feed conversion ratio (FCR), and European broiler index (EBI) of broiler chickens at 42 d of age from each chicken house.

Item	C[1]	BI[2]	DN[3]	SEM	Significance
Stocking density (kg BW/m^2)	46.04	47.32	46.00	0.86	NS
BW (kg/bird)	2.30	2.36	2.32	0.03	NS
Total FI (g/d/bird)	94.30	94.89	94.65	1.28	NS
FCR (kg/kg)	1.72	1.68	1.72	0.02	NS
EBI	328.2	348.9	323.9	6.60	NS

[1]C = Control, in ovo injection of physiological saline.
[2]BI = Bi^{2}tos.
[3]DN = DiNovo.
Significance: NS = $P > 0.05$.

Table 4. Effect of prebiotics and sex on slaughter performance and breast muscle qualitative traits in broiler chickens.

		Prebiotics (P)		Sex (S)			Significance	
Item	C[1]	BI[2]	DN[3]	Female	Male	SEM	P	S
Final BW (g)	2,151.9^{B,b}	2,360.3^{a}	2,488.1^{A}	2,204.6	2,526.7	0.03	**	**
Carcass weight (CW; g)	1,452.3^{B}	1,646.4^{A}	1,724.7^{A}	1,504.5	1,762.7	0.02	**	**
Carcass yield (%)	67.45^{B}	69.70^{A}	69.30^{A}	68.19	69.76	0.25	*	*
Breast muscle (g)	497.74^{B,b}	559.21^{a}	581.70^{A}	515.81	591.83	10.67	*	**
Breast (% CW)	34.15	33.89	33.79	34.19	33.57	0.31	NS	NS
Fiber diameter (μm)	41.06	34.16	30.25	35.01	35.72	2.86	NS	NS
Intramuscular fat (%)	1.85	2.30	1.83	1.78	2.41	0.30	NS	NS

[1]C = Control, in ovo injection of physiological saline.
[2]BI = Bi^{2}tos.
[3]DN = DiNovo.
Significance: NS = $P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$.
^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).
^{A,B}Means within a row lacking a common superscript differ ($P < 0.01$).

maximum mortality (3.52%) calculated according to European Union (**EU**) Council Directive 2007/43/EC (European Commission, 2007) (1% added to 0.06% multiplied by the age of the birds at slaughter) for broiler slaughtered at 42 d and reared at a stocking density till 42 kg live weight/m^2; instead, BI and DN groups showed a higher mortality than the mortality score of the EU directive. However, it is necessary to point out that despite the high stocking density used in this farm study, the mortality in all experimental groups was reasonable and lower compared to the results obtained in a smaller-scale experimental study (mortality: 5.7% for a stocking density of 47 kg/m^2 and an average BW of 2.67 kg at 39 d of age; Buijs et al., 2009), but similar to the mortality reported in a field study conducted in Poland (stocking density of 46.8 kg/m^2 and an average BW of 2.41 kg at 42 d of age; Utnik-Banaś et al., 2014). In a large field survey, Dawkins et al. (2004) found that for a range of stocking densities, from 30 to 46 kg/m^2 (0.073 to 0.047 m^2/bird), broiler health and welfare were to a great extent determined by the quality of the environment provided by producers. However, the EU Council Directive 2007/43/EC has fixed a maximum stocking density for broilers at 33 kg live weight/m^2 with the possibility to increase this limit to 39 and 42 kg live weight/m^2, if some technical requirements (e.g., feeding, heating, ventilation, disinfection, and mortality) are fulfilled. In any case, farm-level decisions regarding stocking densities for broilers are still driven by cost-effectiveness and today European farms are often operating at high stocking density (e.g., 45 to 48 kg/m^2 in Belgium, Verspecht et al., 2011).

The recorded values for total livestock BW per treatment, stocking density (kg/m^2), average BW, FI, FCR, and EBI were similar ($P > 0.05$) among the experimental groups (Table 3). However, treatment with BI and DN were associated with small increases in average BW (+2.6% and +0.9%, respectively) compared to the C group; even if these increases seem minor, considering the high number of the reared chickens, the economic impact could be interesting. In addition, treatment with BI was associated with a small ($P > 0.05$) improvement in FCR compared to the C and DN groups. A recent study by our group (Bednarczyk et al., 2016) showed that an increase in broiler weight is achieved when DN and BI are administered via in ovo or in water. These results are in agreement with other previous studies which reported enhanced growth performance in birds supplemented with mannooligosaccharides as a growth promoter (Oyofo et al., 1989; Newman, 1994).

Slaughter performance, fiber diameter and intramuscular fat of breast muscle in broiler chickens (males and females) are presented in Table 4. Compared with the C group, chickens from treated groups were significantly heavier (DN = +15.6%, $P < 0.01$; BI = +9.7%, $P < 0.05$) and showed a higher ($P < 0.01$) carcass

weight and carcass yield. There were no statistically significant differences ($P > 0.05$) between the prebiotic groups for the above mentioned traits. Pruszynska-Oszmalek et al. (2015) also found a significant increase in the final BW of 34 d old chickens which were in ovo injected with Bi^{2}tos. Treated birds had a higher breast muscle weight (DN = +16.9%, $P < 0.01$; BI = +12.3%, $P < 0.05$) in comparison with that of the C group; whereas no significant differences ($P > 0.05$) were observed between the prebiotic groups. Breast muscle yield was similar ($P < 0.05$) among the groups. According to our previous study (Maiorano et al., 2012) it can be assumed that the application of prebiotics has a positive impact on muscle weight. Moreover, Maiorano et al. (2012) found a slightly greater thickness (diameter) of muscle fibers in chickens in ovo injected with prebiotics (1.9 mg/embryo of raffinose family oligosaccharides, **RFO**) or synbiotics compared with those of the control group. Similar results (breast muscle weight and fiber diameter) have been noted with Arbor Acres broilers which were supplemented with 200 and 400 mg/kg multistrain probiotics (*Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus natto*) and slaughtered at 42 d of age (Zhou et al., 2015). Differently, thinner muscle fibers were found in prebiotic groups (−26.6% and −16.8% for DN and BI, respectively) compared with the control group in this study; however, the differences were not statistically significant. The smaller thickness of the fibers beneficially affects meat quality, and might be considered an indicator of fibrillarity and a delicate structure of the meat.

Intramuscular fat percentage was slightly higher (+0.5%; $P > 0.05$) in BI group compared to the other experimental groups. To our knowledge no information is available on the effect of prebiotics on intramuscular fat content in current literature. On the other hand, Maiorano et al. (2012) did not find any significant effect of RFO in ovo injected on abdominal fat in Ross broiler chickens. The content of intramuscular fat found in our study is consistent with the results reported by Gornowicz et al. (2009) and Sláková et al. (2009) in Ross chickens slaughtered on d 42 and d 40, respectively.

As expected, males were heavier (+14.6%; $P < 0.01$) and showed a higher carcass and breast weights ($P < 0.01$) and carcass yield ($P < 0.05$); while breast muscle yield, fiber diameter and intramuscular fat were similar between sex ($P > 0.05$).

Figure 2 (a, b) showed the effects of prebiotic treatments on the lipid oxidation of breast meat. Overall, the TBARS values were lower in the meat of C birds in comparison with the prebiotic groups along the entire storage time. However, significant differences ($P < 0.05$) were only observed between the C and BI on d 4 of aerobic storage (Figure 2a). This could be related to the slightly higher intramuscular fat content displayed in the breast muscle from BI treated chickens (Table 4). In all experimental groups, the TBARS values were similar between 0 and 2 d of storage (Figure 2b), followed by an increase of TBARS values after 4 d of storage

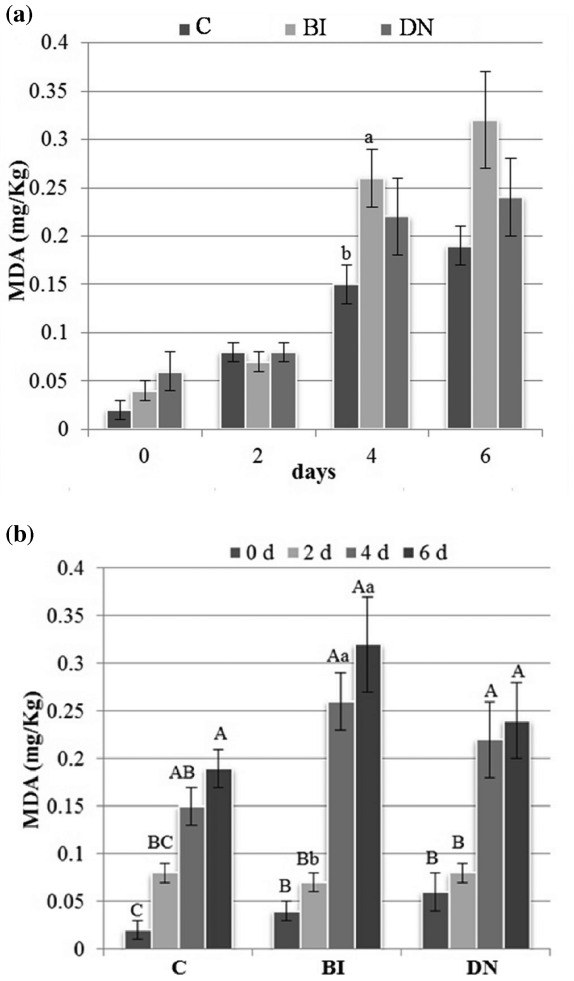

Figure 2. Effect of prebiotic treatment on TBA reactive substances values (mg MDA/Kg of meat) of breast muscle (a) and lipid oxidation development of breast muscle within each treatment (b), during 6 days of storage at 4°C (means ± SE). A, B,C: $P < 0.01$; a,b: $P < 0.05$.

with different magnitude in all groups. In particular, a significant increase of TBARS values were observed in the C group from the fourth d of storage ($P < 0.01$) when compared to time 0, with the highest ($P < 0.01$) TBARS content on d 6 when compared to d 0 and d 2 of storage. Lipid oxidation increased rapidly from d 4 of the aerobic storage in meat of DN and BI groups. In the BI group, the TBARS values on d 4 and 6 of storage were significantly higher when compared to d 0 ($P < 0.01$) and d 2 ($P < 0.05$) of storage. DN group showed a similar trend ($P < 0.01$). Recently, it has been shown that domestic birds are particularly susceptible to oxidative stress as a result of the genetic selection toward larger breast muscles, increased total BW, and the faster growth rates (Fellenberg and Speisky, 2006; Sihvo et al., 2014). This assumption may help to explain why the meat from chickens of prebiotic groups, that had a bigger/heavier breast muscle, showed meat more susceptible to oxidation. Further research is warranted to elucidate and to confirm this result.

In this work, conducted under commercial conditions, new data describing the effect of in ovo administration of BI (non-digestive GOS) and DN (*Laminaria*

spp. extract containing laminarin and fucoidan) prebiotics are reported. Prebiotics were associated with significant improvements in a number of parameters, including BW, carcass weight, carcass yield, and breast muscle weight. Males had significantly higher slaughter traits compared to females, except for breast muscle yield. Meat from prebiotic treated birds displayed a higher lipid oxidation levels compared to that from untreated ones along the entire storage time. However, the highest TBARS detected values are below the TBARS levels associated with meat rancidity (from 0.5 to 1.0 mg/kg muscle for pork and lamb, from 0.6 to 2.3 mg/kg muscle for beef) (Ripoll et al., 2011; Kasapidou et al., 2012).

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