JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.0190100

Effects of Mannan Oligosaccharides and/or *Bifidobacterium* on Growth and Immunity in Domestic Pigeon (*Columba livia domestica*)

Bingjie Ge[1]*, Haiming Yang[1]*, Jun Meng[2], Xiaoshuai Chen[1] and Zhiyue Wang[1]

[1] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, Jiangsu Province, P. R. China
[2] Jiangsu Province Cuigu Pigeon Industry Co., Ltd, Nanjing 211131, Jiangsu Province, P. R. China

The goal of this study was to evaluate the influences of mannan oligosaccharides (MOSs) and/or *Bifidobacterium* on the growth and immunity of pigeons over a 56-day period. One hundred paired adult pigeons were randomly divided into four groups of five paired pigeons. Paired pigeons with two young squabs were housed in a man-made aviary. Parent pigeons in the control group received a basal diet (C), while the other three groups were fed with the basal diet supplemented with 20 g of MOSs/kg of feed (M), 10 g *Bifidobacterium* (1×10^{10} CFU/g) /kg of feed (B), or a combination of M and B (MB). We found higher body weights (BW) in pigeons of the B group than in the C, M, and MB groups. None of the treatments exerted significant effects involving spleen and thymus indices, whereas M birds tended to improve the bursa of Fabricius index. Pigeons fed with the M-supplemented diet exhibited improved serum immunoglobulin M (IgM) concentrations compared with those fed with C and the B- and MB-supplemented diets. In addition, M treatment increased immunoglobulin A (IgA) levels compared with MB treatment. MB treatment improved serum immunoglobulin G (IgG) concentrations compared to that by the C treatment. The concentration of secretory immunoglobulin A (sIgA) was significantly reduced in the duodenum and increased in the ileum in pigeons fed with the MB-supplemented diet. This study indicated that dietary supplementation with *Bifidobacterium* increased the growth performance. Dietary supplementation with MOSs or in combination with *Bifidobacterium* was able to improve immune function in pigeons but exerted no apparent effect on weight gain. Accordingly, in terms of economic benefits, the findings suggested that supplementation with *Bifidobacterium* alone may improve production performance, and that supplementation with MOSs alone may improve immune function in pigeons.

Key words: *Bifidobacterium*, growth performance, immunity, mannan oligosaccharides, pigeon

J. Poult. Sci., 57: 277–283, 2020

Introduction

Domestic pigeons (*Columba livia*) are characterized by an extraordinary growth rate, and are reared for meat production, and are also raised for sporting activities and experiments. Unlike other precocial birds such as chickens and ducks, pigeons, as a type of altricial bird, hatch with unopened eyes and can only be fed by parents with pigeon milk ('crop milk') for the first 3 days after hatching. The characteristic of parental crop milk feeding differentiates pigeons from other poultry. A mixture of crop milk and soaked grains is fed to pigeons from day 4 post hatching, which is gradually replaced by grains (Sales and Janssens, 2003). Pigeons then leave the nest and feed independently from day 28 (Horn and Meleg, 2000). In production, a breeding pair usually feeds two squabs, sometimes three squabs. However, pigeons, especially the squabs, have weak immunity, which compromises growth and production for the above-mentioned purposes.

An increase in bacterial resistance to antibiotics, and the risk of health hazards of consumers due to the presence of residues in animal products, has resulted in a ban on the use of non-therapeutic antibiotics. Therefore, a number of alternative products, such as probiotics, prebiotics, organic acids, essential oils, and oligosaccharides, have been used to enhance the health and the growth performance. Mannan oligosaccharides (MOSs) are derived from the outer cell wall and can shift the gastrointestinal microflora balance towards that of beneficial organisms (Spring *et al*., 2000; Fairchild *et al*., 2001). In contrast to the mode of action of most anti-

Received: September 2, 2019, Accepted: January 16, 2020
Released Online Advance Publication: March 25, 2020
Correspondence: Haiming Yang, College of Animal Science and Technology, Yangzhou University, Wenhui East Road 48#, Yangzhou City, Jiangsu Province 225009, PR China. (E-mail: yhmdlp@qq.com)
* These authors contributed equally to this work.

biotics, MOSs, and possibly other oligosaccharides, serve as alternate attachment sites for Gram-negative pathogens and thereby prevent their attachment to enterocytes and subsequent enteric infection (Van der Wielen *et al*., 2002). In addition, MOSs can improve host health by preventing the multiplication of pathogens in the gastrointestinal tract. These features make MOSs one of the prime candidates to replace antibiotics in animal production. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit to the host" (Fuller, 1989). In this study, *Bifidobacterium* was used as a probiotic. This genus can modulate indigenous intestinal microbiota and improve health via multiple mechanisms, including the direct inhibition of enteric pathogens by decreasing luminal pH, secretion of bactericidal proteins, and stimulation of defensin production by the epithelial cells (Sartor, 2004). This genus may also block pathogen attachment to, or invasion of, epithelial cells by competing for surface receptors, a process termed as colonization resistance (Mack *et al*., 1999; Mattar *et al*., 2002).

Such feed additives have recently been given to broilers as dietary supplements (Higgins *et al*., 2008; Silva *et al*., 2010; Rokade *et al*., 2016; Ghasemian and Jahanian, 2016). In studies conducted in the poultry, MOSs have shown promising effects, such as reduction in the abundance of pathogenic gut microflora, stimulation of strong immune responses, and elevation of the strength of the intestinal mucosae (Hooge, 2004; Rosen, 2007). It appears that the improvements in growth performance due to MOSs are particularly pronounced early in the lifecycle (Yang *et al*., 2005). A previous study showed that the use of oligosaccharides as prebiotics promoted animal health by altering intestinal microbial communities (Rehman *et al*., 2008, 2009). To date, studies investigating the effects of *Bifidobacterium* supplementation in poultry have mainly focused on growth performance and modulation of the intestinal microbiota (Gaggìa *et al*., 2010). Probiotic supplementation has been beneficial for improving chicken performance and immunity under heat stress conditions (Zulkifli e*t al*., 2000; Lan *et al*., 2004). However, few studies have investigated the effects of MOSs and *Bifidobacterium* on immune organ indices, serum immunity, and intestinal mucosal immunity in pigeons. In the present study, we hypothesized that daily supplementation with MOSs and/or *Bifidobacterium* may improve the immunity of pigeons by increasing immune organ indices, the levels of sIgA in the intestinal mucosae, and the concentrations of IgA, IgG and IgM in serum.

The present study was thus conducted using the White King pigeons to thereby provide a theoretical basis for strategies for safe and healthy pigeon production.

Materials and Methods

Ethics Statement

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of the Yangzhou University.

Table 1. **Composition and nutrient levels of basal diets (air-dried basis), %**

Item	Content
Ingredients	**%**
Corn	53.5
Peas	20.0
Wheat	10.0
Soybean meal	5.40
Wheat bran	9.50
NaCl	0.60
Premix[1]	1.00
Total	100
Nutrient levels[2]	
ME (MJ/kg)	12.0
Crude protein	15.0
Ca	0.43
Total phosphorus	0.35
Lysine	0.90
Methionine	0.45

[1] The premix provided the following nutrients per kg of the diet: vitamin A, 125,00 IU; vitamin D3, 4125 IU; vitamin E, 15 IU; vitamin K, 2 mg; vitamin B1, 1 mg; vitamin B2, 8.5 mg; calcium pantothenate, 50 mg; niacin, 32.5 mg; vitamin B6, 8 mg; vitamin B12, 5 mg; biotin, 2 mg; Fe, 60 mg; Cu, 8 mg; Zn, 66 mg; Mn, 65 mg; Se, 0.3 mg; I, 1 mg.
[2] Nutrient levels represent the calculated values.

Birds, Diets, and Management

This study was conducted at the Jiangsu Province Cuigu Pigeon Industry Co., Ltd. The experimental animals were White King pigeons provided by the Jiangsu Cuigu Pigeon Industry Co., Ltd.

Mannan oligosaccharides were purchased from Guangxi Bio-springer Co., Ltd. (Laibin City, Guangxi Prov. China), *Bifidobacterium* species were purchased from Shandong Zhongke Jiayi Bio-engineering Co., Ltd. (Weifang City, Shandong Prov. China). The number of viable *Bifidobacterium* was 1×10^{10} CFU/g.

One hundred paired adult pigeons were randomly divided into four groups of five paired replicates. Paired pigeons with two young squabs were housed in a man-made aviary equipped with a nest and a perch. Water and food were provided *ad libitum*. Young pigeons were raised separately from their relatives from 29 days of age. The experimental period was 56 days. *Bifidobacterium* and MOS were added to the same basal diet. The composition and nutrient levels of the basal diet have been presented in Table 1. The experimental design has been shown in Table 2. All the squabs were maintained unvaccinated and free of antibiotics.

Sampling

All young pigeons were weighed at the end of the 56-day period. Two young pigeons from each replicate were randomly selected and weighed. Peripheral blood from each of these two pigeons was collected from the wing vein, immediately centrifuged at $1200\times g$ and 4℃ for 10 min, then stored at −20℃.

The spleen, bursa of Fabricius, and thymus were isolated

during necropsy. Fat deposits were trimmed away, any moisture on the surface of each organ was dried off, and the organs were then weighed. The mucosae of the duodenum, jejunum, and ileum were also collected and stored at −20℃ until use.

Immune Organ Indices

The organ indices of the spleen, thymus, and bursa of Fabricius were calculated using the following formula: organ index (g/kg)＝organ weight (g)/body weight (kg)×1000.

Measurement of Relative Serum Levels of IgA, IgG, and IgM

Prior to testing, blood serum was thawed at room temperature for 1 h. Serum concentrations of IgA, IgG, and IgM were measured using ELISA kits (Lot 07/2018, Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

Measurement of Relative Abundance of sIgA in Intestinal Mucosa

For measurement of sIgA content, mucosal tissue samples from the duodenum, jejunum and ileum were diluted 10-fold with normal saline (weight/volume＝1/9), homogenized in an ice-water bath, and centrifuged at 1200×*g* and 4℃ for 10 min. After centrifugation, the supernatant obtained from the mucosal homogenate was diluted 5-fold with normal saline (1:4). sIgA levels in the supernatant were measured using a chicken ELISA kit (Jiancheng Bioengineering Institute) according to the manufacturer's instructions. Protein concentration was determined using a Total Protein Quantitative Assay Kit (Jiancheng Bioengineering Institute) to correct for the extent of homogenization.

Statistical Analyses

All data were analyzed by one-way analysis of variance (ANOVA, SPSS 20.0 for Windows). If significant differences were detected, the Tukey's multiple range test with α＝0.05 was applied to perform comparisons among the groups. *P* values less than 0.05 were considered statistically significant.

Table 2. **Experimental design and groups[1] (g/kg)**

Group	MOS	*Bifidobacterium*
Control (C)	—	—
MOS (M)	20	—
Bifidobacterium (B)	—	10
MOS + *Bifidobacterium* (MB)	20	10

[1] Addition of mannose oligosaccharides and *Bifidobacterium* in the different treatment groups. The four groups were as follows: a control group, an MOS group, a *Bifidobacterium* group, and a combination group with a combination of *Bifidobacterium* and MOS.

Results

Body Weight

The effects of MOSs and *Bifidobacterium* supplementation on the body weight of pigeons at the end of the 56-day experimental period have been shown in Table 3. The body weight of the B group was higher than that of the C and the M groups ($P<0.05$). The body weights of the MB group birds were higher than that of the M group ($P<0.05$), but M and MB cohorts had no apparent differences with C.

Immune Organ Indices

The effects of *Bifidobacterium* and MOSs supplementation on immune organ indices of pigeons have been shown in Table 4. None of the treatments had a significant impact on the spleen and thymus indices of pigeons ($P>0.05$), whereas M treatment tended to improve the bursa of Fabricius index ($P=0.094$).

Table 3. **Effects of *Bifidobacterium* and MOS supplementation on body weight in pigeons[1]**

Group	Control	MOS	*Bifido-bacterium*	MOS + *Bifido-bacterium*	SEM[2]	*P* value
Weight (g)	456^{bc}	447^{c}	483^{a}	475^{ab}	8.81	0.003

[a, b] Means with different superscripts in a row differ significantly ($P\leq0.05$)
[1] Data represent means of 5 replicates, with 10 squabs per replicate.
[2] Standard error of the mean.

Table 4. **Effects of *Bifidobacterium* and MOS supplementation on immune organ indices in pigeons[1]**

Group	Control	MOS	*Bifido-bacterium*	MOS + *Bifido-bacterium*	SEM[2]	*P* value
Spleen index[3]	0.720	0.987	1.19	1.11	0.220	0.200
Bursa of Fabricius index	1.03	1.62	1.06	1.01	0.260	0.094
Thymus index	1.41	1.54	1.79	1.45	0.502	0.869

[a, b] Means with different superscripts in a row differ significantly ($P\leq0.05$)
[1] Data represent means of 5 replicates, with 10 squabs per replicate.
[2] Standard error of the mean.
[3] Organ index (g/kg)＝organ weight (g)/body weight (kg)×1000

Table 5. **Effects of *Bifidobacterium* and MOS supplementation on serum levels[1] of IgG, IgA, and IgM (mg/m*l*)**

Group	Control	MOS	*Bifido-bacterium*	MOS + *Bifido-bacterium*	SEM[2]	*P* value
IgG	5.20[b]	5.15[b]	4.37[b]	6.97[a]	0.309	0.000
IgA	1.88[ab]	2.33[a]	1.84[ab]	1.74[b]	0.190	0.032
IgM	0.338[b]	0.612[a]	0.228[bc]	0.174[c]	0.040	0.000

[a, b] Means with different superscripts in a row differ significantly ($P \leq 0.05$)
[1] Data are means of 5 replicates, with 10 squabs per replicate.
[2] Standard error of the mean.

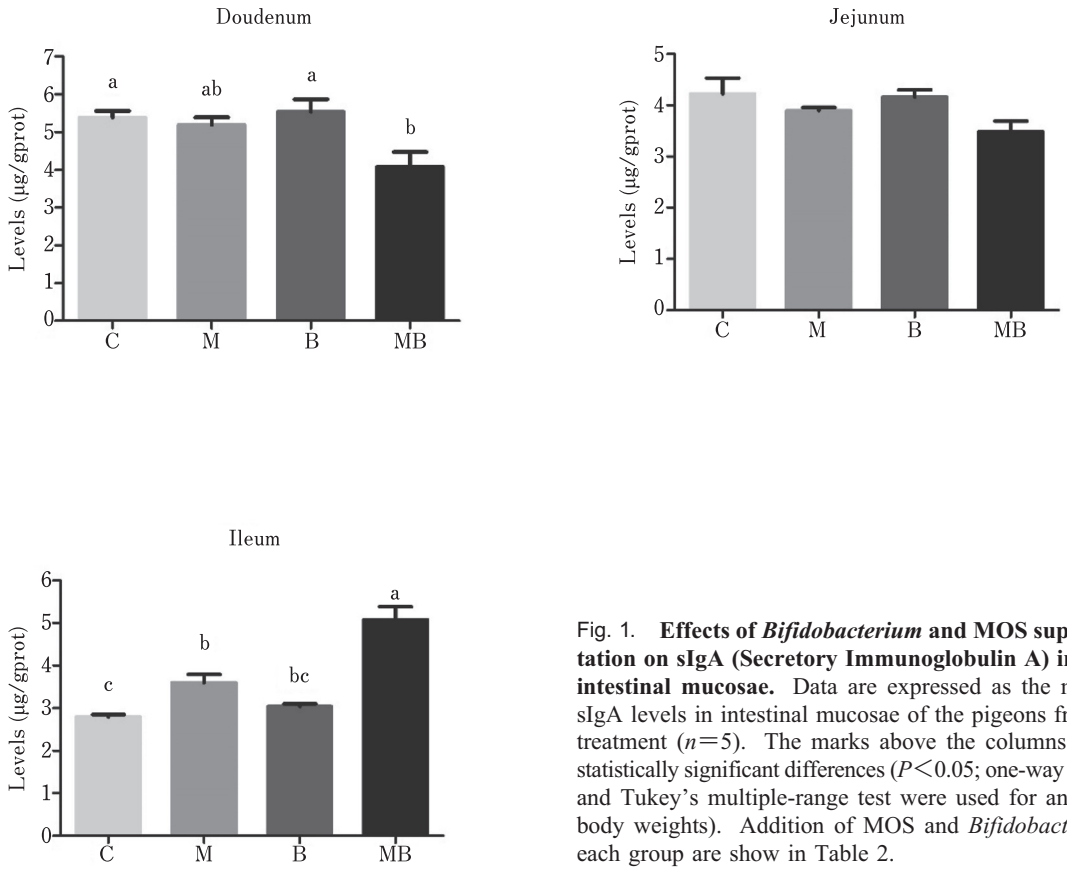

Fig. 1. **Effects of *Bifidobacterium* and MOS supplementation on sIgA (Secretory Immunoglobulin A) in pigeon intestinal mucosae.** Data are expressed as the means of sIgA levels in intestinal mucosae of the pigeons from each treatment ($n=5$). The marks above the columns indicate statistically significant differences ($P<0.05$; one-way ANOVA and Tukey's multiple-range test were used for analysis of body weights). Addition of MOS and *Bifidobacterium* in each group are show in Table 2.

Serum Concentrations of IgA, IgG, and IgM

The effects of *Bifidobacterium* and MOSs supplementation on serum IgA, IgG, and IgM concentrations in pigeons have been shown in Table 5. Dietary supplementation with M elevated the concentrations of IgM in serum compared with the C, the B, and the MB treatments ($P<0.05$). The M treatment also increased the concentration of IgA compared with the MB treatment ($P<0.05$). The concentration of IgG in the MB group birds was higher than that of the other three groups ($P<0.05$).

Concentration of sIgA in the Intestinal Mucosae

The effects of *Bifidobacterium* and MOSs supplementation on sIgA levels in the intestinal mucosae of pigeons have been shown in Fig. 1. A significant decrease in the concentration of sIgA in the duodenal mucosae was obtained with MB treatment compared with that by C treatment ($P<0.05$). The concentration of sIgA in the jejunum was not affected by any dietary supplementation treatments ($P=0.074$). At the end of the 56-day experimental period, the concentration of sIgA in the ileum of pigeons fed with the MB-supplemented diet were significantly improved, compared to with those obtained with the C, the M and the MB treatments ($P<0.05$).

Discussion

Body Weight

MOSs have been hypothesized to play a positive role in

the maintenance of gut integrity by facilitating the growth of beneficial bacteria such as *Lactobacillus* spp. and *Bifidobacterium* spp. (Spring *et al*., 2000). A previous study suggested that the intestinal microbiota may be considered an important determinant of gastrointestinal health (Sohail *et al*., 2011). In addition, Rehman *et al*. (2009) reported that prebiotics effectively elevated the caecal *Lactobacillus* spp. and *Bifidobacterium* spp. levels in broilers. The beneficial intestinal bacteria can promote the digestion and absorption of nutrients, which may explain the increases in body weight observed in the B and in the MB groups. Khan *et al*. (2012) reported that MOSs supplementation exerted no effect on the BW gain of growing pigeons, which was found to be consistent with this study. Our results indicated that the addition of MOSs improved the bursa of the Fabricius index and the serum immunoglobulin levels, and thereby increased the energy consumption of the body. Therefore, supplementation with mannan oligosaccharides cannot induce weight gain.

Immune Organ Indices

Thymus, bursa and spleen are the most important immune organs of broilers, and the development and function of each organ directly affects the immune status of broilers. The weight of the thymus, bursa and spleen can be used to evaluate the immune status of chicks. The greater the absolute and relative weights, the stronger the cellular and humoral immune functions of the body (Rivas and Fabricant, 1988). Our results showed that none of the experimental groups exhibited significant effects involving the immune organ indexes of broilers. This may be related to the quantity of supplementation added.

Concentrations of IgA, IgG and IgM in Serum

Immunoglobulins, which are produced by B lymphocytes, play an important role in the immune system by enhancing the ability to fight viruses and prevent infection (Yan and Polk, 2011). IgM is not only an important product of the primary immune response but also the first antibody isotype that appears during immune responses to infection. IgA is the main immunoglobulin in exocrine secretions and also the main immunoglobulin in colostrum and milk, and plays an important role in defense against pathogens. IgG is the most abundant immunoglobulin in poultry serum and provides resistance to bacteria, viruses, exotoxins and other threats. IgG plays an irreplaceable role in humoral immunity (Barbosa, 2013). In the present study, the concentrations of IgA and IgM in serum were increased in pigeons fed with MOSs. It has been reported that dietary supplementation with 0.05% MOSs increases the mucosal secretion of IgA, and the humoral and cell-mediated immune responses in neonatal chicks (Gómez-Verduzco *et al*., 2009). A previous study also found that prebiotic supplementation had no effect on serum levels of IgG, IgM (Vahabi-Asil *et al*., 2017). The IgG concentration in pigeons belonging to the MB group was significantly higher than the concentrations found in the M and the B groups, which suggested that the probiotic and prebiotics may have acted in an additive or a synergistic manner.

Concentration of sIgA in Intestinal Mucosae

The gastrointestinal tract is not only a site of nutrient absorption and digestion, but also a natural barrier that uses a variety of nonspecific immune mechanisms to prevent harmful substances from invading the organism. When an antigen enters the gastrointestinal tract, it activates the gut-associated lymphoid tissue (GALT) and thereby triggers the production of antibodies that contribute to gastrointestinal mucosal immunity, particularly sIgA. sIgA, the most abundant immunoglobulin in mucosal secretions, is an important shield on the intestinal mucosal surface to protect the intestinal epithelium from enteric toxins and pathogenic microorganisms (Song *et al*., 2019). It can intercept incoming viruses, neutralize invading infectious agents in the lamina propria, shape the mucosal immune system, preserve the integrity of the epithelial cell barrier, and modulate the epithelial and the dendritic cell responsiveness (Wu *et al*., 2016). Intestinal lamina propria lymphocytes include a mixture of several types of lymphocytes, mainly IgA+ plasma cells and CD4+ T helper cells (Th2). The former can produce large quantities of sIgA, whereas the latter can secrete IL-5 and IL-6, which promote the differentiation and maturation of IgA+ B cells into IgA+ plasma cells, and can produce abundant sIgA as part of the immune response.

By measuring sIgA levels in the feces and saliva of infants raised with *Bifidobacterium*, Sjögren *et al*. (2009) showed that *Bifidobacterium* was able to facilitate the secretion of sIgA by the intestinal mucosa. A study conducted by Roller *et al*. (2004) showed that feeding rats *Bifidobacterium* or a mixture of *Lactobacillus rhamnosus*, *Bifidobacterium* and fructo-oligosaccharides was able to increase the content of sIgA in the ileum and regulate immune functions. *Bifidobacterium* reportedly accumulates mainly in the ileum (10^3 CFU/g-10^7 CFU/g) and colon (10^8 CFU/g-10^{12} CFU/g). In this study, the combined addition of *Bifidobacterium* and mannan oligosaccharides increased sIgA levels in the ileum, which indicated that *Bifidobacterium* and MOS were able to jointly improve the local intestinal tract immune function. However, there was a decrease in the duodenal sIgA levels in the MB group pigeons. This was probably because mannose oligosaccharide and *Bifidobacterium* may have played the majority of their roles in the duodenum when the antigen enters the gastrointestinal tract. The decrease in antigen stimulation on the duodenal mucosa may lead to the decreased sIgA secretion.

In order to elucidate the immune mechanisms that *Bifidobacterium* and mannose oligosaccharides exert on intestinal mucosae, the protein levels and gene expression of related immune cytokines and complement components in the intestinal mucosae will be further studied.

In summary, dietary supplementation with *Bifidobacterium* was able to increase growth performance. Dietary supplementation with MOSs, or a combination of *Bifidobacterium* and MOSs, may improve the immune function of pigeons with no apparent effects on weight gain. Therefore, in terms of economic benefits, our results suggested that the addition of *Bifidobacterium* alone may improve production

performance, and the addition of MOS alone may improve the immune function of pigeons.

Acknowledgments

This research was financially supported by the Priority Academic Program Development of the Jiangsu Higher Education Institutions (PAPD) and the Earmarked Fund for the Jiangsu Agricultural Industry Technology System (JATS [2019] 017).

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

Barbosa D. BIOS Instant Notes in Immunology. Journal of Biological Education, 47: 68. 2013.

Fairchild AS, Grimes JL, Jones FT, Wineland MJ, Edens FW and Sefton AE. Effects of hen age, Bio-Mos, and Flavomycin on poultry susceptibility to oral Escherichia coli challenge. Poultry Science, 80: 562−571. 2001.

Fuller R. Probiotics in man and animals. Journal of Applied Bacteriology, 66: 365−378. 1989.

Ghasemian M and Jahanian R. Dietary mannan-oligosaccharides supplementation could affect performance, immunocompetence, serum lipid metabolites, intestinal bacterial populations, and ileal nutrient digestibility in aged laying hens. Animal Feed Science Technology, 213: 81−89. 2016

Gaggìa F, Mattarelli P and Biavati B. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology, 141: 15−28. 2010.

Gómez-Verduzco G, Cortes-Cuevas A, López-Coello C, Ávila-González E and Nava GM. Dietary supplementation of mannan-oligosaccharide enhances neonatal immune responses in chickens during natural exposure to *Eimeria* spp. Acta Veterinaria Scandinavica, 51: 11−17. 2009.

Horn P and Meleg I. Inbreeding effects on production traits in pigeons. Archiv Für Geflügelkunde, 64: 273−277. 2000.

Higgins SE, Higgins JP, Wolfenden AD, Henderson SN, Torres-Rodriguez A, Tellez G and Hargis B. Evaluation of a lactobacillus-based probiotic culture for reduction of Salmonella enteritidis in neonatal broiler chicks. Poultry Science, 87: 27−31. 2008.

Hooge DM. Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993−2003. International Journal of Poultry Science, 3: 163−174. 2004.

Khan AR, Yousaf MS, Rehman H, Zaneb H, Pasha TN, Fatima N, Khushal A, Ijaz A, Ashraf K and Afzal M. Response of maternally isolated rock pigeons (Columba livia domestica) to different dietary concentrations of mannan-oligosaccharide. Poultry Science, 91: 1598−1603. 2001.

Lan PTN, Sakamoto M and Benno Y. Effects of two probiotic *Lactobacillus* strains on jejunal and cecal microbiota of broiler chicken under acute heat stress condition as revealed by molecular analysis of 16S rRNA genes. Microbiology and Immunology, 48: 917−929. 2004.

Mack DR, Michail S, Wei S, McDougall L and Hollingsworth MA. Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression. American Journal of Physiology, 276: 941−950. 1999.

Mattar AF, Teitelbaum DH, Drongowski RA, Yongyi F, Harmon CM and Coran AG. Probiotics up-regulate MUC-2 mucin gene expression in a Caco-2 cell-culture model. Pediatric Surgery International, 18: 586−590. 2002.

Rokade JJ, Bhanja SK, Shinde AS, Sajad, Kapgate M and Mandal AB. Evaluation of mannan-oligosaccharides (MOS) in broiler chicken during hot humid summer using zoo technical, molecular and physio-biochemical tools. Indian Journal of Animal Science, 86: 460−467. 2016.

Rosen GD. Holo-analysis of the efficacy of Bio-MOS in broiler nutrition. British Poultry Science, 48: 21−26. 2007.

Rehman H, Hellweg P, Taras D and Zentek J. Effects of dietary inulin on the intestinal short chain fatty acids and microbial ecology in broiler chickens as revealed by denaturing gradient gel electrophoresis. Poultry Science, 87: 783−789. 2001.

Rehman H, Vahjen W, Kohl-Parisini A, Ijaz A and Zentek J. Influence of fermentable carbohydrates on the intestinal bacteria and enteropathogens in broilers. World's Poultry Science Journal, 65: 75−90. 2009.

Rivas A and Fabricant J. Indication of immunodepression in chicken infected with various strain of Marek's disease virus. Avian Diseases, 32: 1−8. 1988.

Roller M, Rechkemmer G and Watzl B. Prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnoides and Bifidobacterium lactis modulates intestinal immune functions in rats. Journal of Nutrition, 134: 153−156. 2004.

Sales J and Janssens G. Nutrition of the domestic pigeon (*Columba livia domestica*). World's Poultry Science Journal, 59: 221−232. 2003.

Spring P, Wenk C, Dawson KA and Newman KE. The effects of dietary mannan oligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. Poultry Science, 79: 205−211. 2000.

Sartor RB. Probiotic therapy of intestinal inflammation and infections. Curr Opin Gastroenterol, 21: 44−50. 2005.

Silva VK, Torre da Silva JD, Gravena RA, Marques RH, Hada FH and Barbosa de Moraes VM. Yeast extract and prebiotic in pre-initial phase diet for broiler chickens raised under different temperatures. Revista Brasileira de Zootecnia, 39: 165−174. 2010.

Sohail MU, Rehman ZU, Ijaz A, Yousaf MS, Ashraf K, Yaqub T, Zaneb H, Anwar H and Rehman H. ingle or combined effects of mannan oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers. Poultry Science, 90: 2573−2577. 2011.

Song B, Zheng C, Zha C, Hu S, Yang X, Wang L and Xiao H. Dietary leucine supplementation improves intestinal health of mice through intestinal SIgA secretion. Journal of Applied Microbiology, early access.

Sjögren YM, Tomicic S, Lundberg A, Böttcher MF, Björkstén B, Sverremark-Ekström E and Jenmalm MC. Influence of early Gut microbiota on the maturation of childhood mucosal and systemic immune responses. Clinical & Experimental Allergy, 39: 1842−1851. 2009.

Wu MM, Xiao H, Liu G, Chen S, Tan B, Ren WK, Bazer FW, Wu GY and Yin YL. Glutamine promotes intestinal SIgA secretion through intestinal microbiota and IL-13. Molecular Nutrition & Food Research, 60, 7: 1637−1648.

Van der Wielen PW, Lipman LJ, Van Knapen F and Biesterveld S. Competitive exclusion of Salmonella enterica serovar enteritidis by Lactobacillus crispatus and Clostridium lactatifer mentans in a sequencing fed-batch culture. Applied and Environ-

mental Microbiology, 68: 555−559. 2002.
Vahabi-Asil O, Bouyeh M, Qotbi A, Kadim IT, Seidavi A, Centoducat G, Laudadio V and Tufarelli V. Effects of a prebiotic on growth performance, blood parameters and immunity response of turkeys fed low protein diets. European Poultry Science, 81: 1−12. 2017.
Yan F and Polk DB. Probiotics and immune health. Curr Opin Gastroenterol, 27: 496−501. 2011.
Yang Y, Choct M, Iji PA and Scott TA. Effect of dietary mannan oligosaccharide level on performance and gross morphology of digestive tract segments of broiler. Australian Poultry Science Symposium, 17: 72−75. 2005.
Zulkifli I, Abdullah N, Azrin NM and Ho YW. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. British Poultry Science, 41: 593−597. 2000.