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Effect of Biomin®IMBO on the humoral immune response of broiler chickens

Einfluss von Biomin®IMBO auf die humorale Immunantwort von Broilern

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Introduction

Probiotics are live microorganisms which administered in adequate amounts improve the balance of intestinal microbial populations thus having a beneficial impact on the host performance (Fuller, 1999). On the other hand, prebiotics are non-digestive food components which improve the health status of the host by selectively stimulating the populations of certain intestinal bacteria (Gibson and Roberfroid, 1995). The combination of these two additives (probiotics + prebiotics) in the form of synbiotics supplements may have synergistic effects (Roberfroid, 1998; Dhama et al., 2008). Nowad, the use of these types of additives in order to reduce drug and antibiotic inclusion into the feeds especially in poultry production has been widely practiced (Dhama et al., 2011; Tomar et al., 2011; Alloui et al., 2013; Smith, 2014).

Probiotics possess advantages over antibiotics in terms a reduction in harmful side effects such as toxic residues, withdrawal period before marketing, gaining antibiotic resistance, allergies and hypersensitivity and carcinogenic and mutagenic effects (Mousavi et al., 2015). The three major causes that result in imbalances in the microbial communities of birds are poor hygiene, antibiotic use and stress. In the wild, newly hatched chicks may ingest beneficial and protective microbiota through the faeces of the mother. Contrary to this the industrial hatching and rearing systems give poor chances to newly hatched chickens to acquire beneficial microbiota. Due to for example the sterile conditions of the incubators and hatcheries and the low microbial contamination of the egg shell there are strong effects on the selection of gastrointestinal microbiota. Thus, broilers in the early d of life may suffer a lack of beneficial microbiota and thereby may contract harmful microorganisms through the digestive system. In this sense, consumption of probiotics may be very beneficial (Kabir, 2009; Dhama et al., 2011).

The effects of feed additives on laying hens have been studied by several authors (Khan et al., 2012a; Pourhossein et al., 2015; Dhama et al., 2015). With the use of probiotics, Youssef et al. (2013) observed an improvement in egg quality (i.e., egg production, egg weight, shell thickness and yolk colour) and feed conversion on laying hens, while Moradi et al. (2010) found no effects of different levels of probiotics and organic acids on immunity of laying hens, antibody titre against sheep red blood cells (SRBC) nor the percentage of white blood cells.

Studies on probiotics as feed additives on growth performance demonstrated beneficial effects on weight gain, final body weight, uniformity of the carcass, feed efficiency and meat quality (Cumpănășoiu et al., 2009; Kabir, 2009; Dhama et al., 2011; Khan et al., 2012a, b; Chen et al., 2015; Dhama et al., 2015). The use of commercial probiotics was shown to have an effect on decreasing plasma cholesterol and improving antibodies titre against SRBC, while no differences were found in cutaneous hypersensitivity immune response, blood triglycerides and high density lipoprotein (HDL) (Khalaji et al., 2010). Further, several authors found an increase in antibodies titres in broilers given probiotics (Kabir et al., 2004; Haghighi et al., 2005; Khaksefidi and Ghourchi, 2006; Akbarian, 2008).

SPRING et al. (2000) proved that probiotics in the feed could increase the total number of leukocytes and erythrocytes, as well as the number of lymphocytes and monocytes. The probiotics may mediate their way by crossing the intestinal wall (lining) and modulating immune cells in livestock and poultry (SHOEIB et al., 1997; DHAMA et al., 2008).

Recently, MAHDAVI et al. (2010) observed an improvement of humoral immunity, growth parameters and production performance in broilers, with the use of several feed additives (i.e., probiotics, acid amplifier, growth-promoting antibiotics and prebiotics).

Regarding prebiotics, BAILEY et al. (1991) and VEGAD (2004) studied the effect of fructo-oligosaccharides, suggesting that they are effective in preventing harmful bacteria such as *Escherichia coli*, *Salmonella* and *Clostridium*, while increasing the populations and activity of beneficial *Lactobacillus* spp. Also, SAVAGE et al. (1996) indicated beneficial effects of mannan-oligosaccharides in turkeys, with increases of bile IgA entering from the bile duct into the intestine and the plasma IgG level.

The synbiotic Biomin® IMBO is a pilot material which includes a probiotic portion composed of *Enterococcus faecium* (5×10^{11} CFU/g) and a prebiotic portion, which consists of fructo-oligosaccharides. This material was developed to create stable normal microbial communities in the digestive tract with a rapid growth and to acidify the environment to prevent the growth of pathogenic microorganisms thereby acting as a barrier against disease-causing pathogens. The contents of cell wall and phycophytic components (extracted from seaweed) can stimulate the activity of macrophages and lymphocytes, having a direct effect on the immunity.

Therefore, the current experiment was performed in order to investigate the effect of different levels in the diet of the synbiotic Biomin® IMBO on humoral immunity of broiler chickens and to determine the effective inclusion level in broiler feed.

Materials and methods

Animals and housing

A total of 200 1-d-old male Ross 308 broiler chicks were used in this experiment. The chicks were randomly assigned to 5 treatments with 4 replicates (10 chicks/group). The chicks were housed in 20 pens (1.5 m^2 each) with similar environmental conditions. The pens were separated from each other with a metal mesh wall. All pens and equipment were cleaned, washed and disinfected properly before the chicks were placed. The experimental period lasted for 42 d, with a lighting program of 23L:1D (from 19:00 to 20:00). Procedures were conducted according to Council Directive 2010/63/EU guidelines on the protection of animals used for experimental and other scientific purposes.

Experimental design

Treatments were selected in order to determine different levels of the synbiotic Biomin®IMBO and their immune efficiency and economic benefits. Treatments were:

- Treatment 1 (Control): Commercial standard Ross diets;
- Treatment 2 (0.75 × Biomin® IMBO): Basal + synbiotics Biomin IMBO 25% less than the manufacturer recommendation levels (ML) (i.e., 0.075, 0.0375 and 0.01875% for starter, grower and finisher periods, respectively);
- Treatment 3 (1 × IMBO): Basal diets + the synbiotic Biomin®IMBO at manufacturer recommendation levels (ML; i.e., 0.1, 0.05 and 0.025% for starter, grower and finisher periods, respectively);
- Treatment 4 (1.25 × Biomin® IMBO): Basal + the synbiotic Biomin®IMBO 25% higher than the ML (i.e., 0.125, 0.0625 and 0.03125% for starter, grower and finisher periods, respectively).
- Treatment 5 (1.5 × Biomin® IMBO): Basal + synbiotics Biomin IMBO 50% higher than the ML (i.e., 0.15, 0.075 and 0.0375% for starter, grower and finisher periods, respectively).

Ingredients and nutritional composition of the diets used in this experiment are shown in Tables 1 and 2, respectively.

Table 1. Ingredient composition of basal diets

Zusammensetzung der Grundfutterrationen

Ingredients (%)	Starter	Grower	Finisher
Maize	46.09	50.91	48.88
Soybean meal	40.00	35.00	39.97
Oil	4.56	5.45	7.38
Fish meal	3.00	3.00	–
Meat meal	3.00	3.00	–
$Ca_{22\%} - P_{18\%}$	0.99	0.75	1.64
$CaCO_3$	0.98	0.76	1.00
Vitamin-Mineral Mixture	0.60	0.50	0.50
NaCl	0.37	0.37	0.45
DL-Methionine	0.29	0.23	0.17
Sodium Bicarbonate	0.05	0.03	–
L-Lysine	0.04	–	–
L-Threonine	0.03	–	–

Table 2. Chemical composition of basal diets

Nährstoffgehalt der Grundfutterrationen

Item	Starter	Grower	Finisher
Metabolisable energy (MJ/kg)	12.70	13.20	13.70
Crude protein (%)	24.9	23	22
Lysine (%)	1.41	1.26	1.22
Methionine (%)	0.67	0.59	0.50
Met+Cys (%)	1.05	0.94	0.85
Threonine (%)	1.98	0.87	0.85
Tryptophan (%)	0.30	0.27	0.28
Arginine (%)	1.68	1.54	1.51
Isoleucine (%)	1.04	0.95	0.94
Valine (%)	1.60	1.07	1.03
Leucine (%)	1.99	1.87	1.82
Calcium (%)	1.05	0.90	0.85
Available phosphorus (%)	0.50	0.45	0.42
Sodium (%)	0.23	0.23	0.20
Potassium (%)	1.00	0.90	0.93
Chlorine (%)	0.30	0.30	0.30
Choline (g/kg)	1.48	1.37	1.37
Linoleic acid (%)	1.21	1.27	1.24
Crude fibre (%)	3.78	3.52	3.73
Ether extract (%)	6.84	7.87	9.22

Vaccines

Three types of vaccines were used in the experiment: (a) Infectious Bronchitis virus (IBV) vaccine, administered at 1 and 16 d of age (DOA); (b) Newcastle Disease virus (NDV) vaccine, administered at 8 and 20 DOA and (c) Gumboro (Infectious Bursal Disease virus, IBDV) vaccine, administered at 14 and 23 DOA. All the sera samples for humoral immune responses were taken 7 d after the second administration of each vaccine (i.e., 23, 27 and 30 DOA).

All vaccines were administered orally with drinking water except for the first application of IBV, which was sprayed in distilled water. During vaccination the temperature was increased between 1 and 2°C. At the end of each vaccination, all remaining samples were collected and burned.

To evaluate the systemic antibody response, two chicks from each replicate were immunised intramuscularly with 0.5 ml of a mixture of 1 ml of phosphate buffered saline (PBS) and 10 ml of SRBC. These injections were applied on d

22 and 35; samples for the immune response were taken on d 28 and 42. For SRBC, injections were administered intramuscularly in the breast.

To assess the systemic antibody response, blood samples were collected from 1 chick per replicate via the wing vein on sampling d as described above. Blood samples were centrifuged at 4°C for 20 min at 1500 rpm, and serum was isolated and immediately analysed. To determine the antibody response to SRBC and NDV, a haemaglutination inhibition (HI) assay was used. Antibody responses to IBV and IBDV were measured by enzyme-linked immunosorbent assay (ELISA) (KEMENY, 1991).

Statistical analysis

The experiment was conducted in a randomised complete block design. Data were analysed by means of a one-way ANOVA with SPSS Statistical Software (1996). Significant differences between mean values were tested using the Tukey test. Differences among means with $P < 0.05$ were accepted as statistically significant. The model used for statistics was as follows: $X_{ij} = \mu + T_j + e_{ij}$

Before performing the statistical analysis of data, normality of all data was tested and, if necessary, an appropriate transformation was made. For antibody titre against SRBC and NDV, the data was transformed by log2, while for Gumboro and IBV the data was transformed by log10.

Results and discussion

The synbiotic Biomin®IMBO had different effects on the humoral immune response against SRBC injection, not only in the primary challenge (1st injection) but also in the 2nd challenge. The humoral immune response data are shown in Table 3. There was an increase in serum antibody titre over time in immunised groups for SRBC injections. In both samplings treatments at 1 × ML and 1.5 × ML had higher antibody titres compared to the Control ($P< 0.01$). Moreover, as reported in a recent study by MOUSAVI et al. (2015), the dietary supplementation with Biomin® IMBO improved also the growth performance of broiler chickens.

Table 3. Serum antibody titres (mean ± SEM) after vaccination and/or injection in broilers fed with different levels of the synbiotic Biomin®IMBO

Antikörpertiter (Mittelwert ± SEM) im Blutserum von Broilern nach Impfung bzw. Injektion mit unterschiedlichen Mengen des Synbiotikums Biomin® IMBO

Treatment	SRBC (1st injection)	SRBC (2nd injection)	IBDV vaccinated	IBV vaccinated	NDV vaccinated
	log_2 PFU	log_2 PFU	log_{10}	log_{10}	log_2
1 – (Control)	3.25^{cd} ± 0.026	4.47^{ce} ± 0.025	3,890 ± 1,350	290.0 ± 161.25	5.00 ± 0.060
2 – (0.75 × ML)	3.75^{bcd} ± 0.043	4.67^{bce} ± 0.170	2,492 ± 879	650.3 ± 109.12	4.25 ± 0.041
3 – (1 × ML)	4.75^{abd} ± 0.018	5.98^{abe} ± 0.030	4,879 ± 2,143	600.3 ± 301.16	3.25 ± 0.025
4 – (1.25 × ML)	3.52^{cd} ± 0.030	4.69^{bce} ± 0.037	2,147 ± 328	496.3 ± 92.72	5.10 ± 0.025
5 – (1.5 × ML)	5.25^{ad} ± 0.013	7.36^{ae} ± 0.090	4,652 ± 2,010	657.8 ± 224.50	4.50 ± 0.021
CV (%)	24.7	32.8	80.5	69.0	26.0

Values within the same column followed by different superscript letters differ significantly ($P < 0.05$).
SRBC, sheep red blood cells; IBDV, infectious bursal disease virus; IBV, infectious bronchitis virus; NDV, Newcastle disease virus; PFU, plaque-forming unit: ML, recommendation level; CV: coefficient of variation.

Our results are in agreement with those obtained by HAGHIGHI et al. (2005), where immunisation with SRBC resulted in the appearance of specific anti-SRBC antibody in serum, with higher antibody titres for birds immunised and probiotic-treated, although these authors found a gradual decrease in serum antibody titre over time.

In the present experiment, no differences were found between Controls and birds probiotic-treated with 0.75 × ML or 1.25 × ML, perhaps due to a small number of chicks used for blood evaluation. A high CV value for each tested vaccine (Table 3) indicates the need to increase the number of chicks analysed. Similar results were found by KHALAJI et al. (2010) and MORADI et al. (2010), where no increase in SRBC antibody titres were observed between non-probiotic and probiotic-treated chicks.

In the present experiment, no effects of the synbiotic Biomin®IMBO on antibody titres were observed for Gumboro, IBV and NDV vaccinations for any treatment ($P > 0.05$). There is a controversy in the literature on the effects of different additives. Several authors found an increase in humoral immunity with the use of additives (KABIR et al., 2004; KHAKSEFIDI and GHOURCHI, 2006; DASTAR et al., 2008), while some authors found no effect on antibody titres (BALEVI et al., 2009; KHALAJI et al., 2010; MORADI et al., 2010).

There are some factors affecting the stimulatory effect of probiotics on the immune system. One factor is that the competition of the established intestinal microbiota may create beneficial effects by elimination of the introduced pathogens (JIN et al., 1997; CHEN et al., 2005), reducing the effect on the humoral immune system. The competitive exclusion is mainly defined by the host age (BALEVI et al., 2009), explaining partly the lack of effects of probiotics on immune parameters obtained in this experiment. Another factor is related to the various species or strains of bacteria present in the probiotics, which may not stimulate the humoral immunity (BALEVI et al., 2009; MORADI et al., 2010).

Conclusions

Dietary supplementation of broiler feed with the synbiotic Biomin®IMBO at manufacturer's recommended level can improve humoral immunity against certain diseases. The antibody titres of broilers for NDV, IBV and IBDV vaccines were not modified by the inclusion of this additive to the diet. Increasing the number of chicks sampled may decrease the coefficient of variation of data thus increasing the power of the statistical analysis in detecting differences among treatments.

Summary

This experiment was performed to study the effect of the synbiotic Biomin® IMBO on the humoral immunity of Ross 308 strain broilers. Birds (n=200) were randomly assigned to 5 treatments (4 replicates of 10 birds each). Treatments were as follows: (1) Control; (2) 0.75 × Biomin® IMBO manufacturer's level (ML); (3); Biomin® IMBO at ML recommended (1 × Biomin® IMBO ML), (4) 1.25 × Biomin® IMBO ML; and (5) 1.5 × Biomin® IMBO ML. The birds received the synbiotic through the diet and were immunised with double doses of infectious bronchitis virus (IBV), Newcastle Disease virus (NDV) and Gumboro Disease (Infectious Bursal Disease virus, IBDV) vaccines and sheep red blood cells (SRBC) to evaluate humoral immunity by assessing antibody titres one week after the second immunisation, except for SRBC (one week after each immunisation).

All levels of synbiotic given resulted in similar antibody titres against IBV, NDV and IBDV compared to the control group. However, birds receiving 1.0 × and 1.5 × Biomin® IMBO ML had higher titres against SRBC in both primary and secondary antibody responses. The data obtained from the experiment indicated that supplementing the feed with the synbiotic Biomin®IMBO increased the humoral immunity response of broilers against sheep red blood cells.

Key words

Broiler, nutrition, symbiotic, immune response, humoral, IBV, NDV, IBDV, SRBC

Zusammenfassung

Einfluss von Biomin®IMBO auf die humorale Immunantwort von Broilern

Im Rahmen der Studie wurde der Einfluss eines Zusatzes des Synbiotikums Biomin®IMBO zum Futter auf die humorale Immunität von Broilern untersucht. Hierzu wurden 200 Ross 308 Broiler zufällig auf 5 Behandlungen mit 4 Wiederholungen a 10 Broiler verteilt. Folgende Behandlungen lagen vor: (1) unbehandelte Kontrolle, (2) Zusatz von Biomin®IMBO mit 0,75facher vom Hersteller empfohlener Dosierung, (3) Zusatz von Biomin®IMBO in der vom Hersteller empfohlenen Dosierung, (4) Zusatz von Biomin®IMBO mit 1,25facher vom Hersteller empfohlener Dosierung, (5) Zusatz von Biomin®IMBO mit 1,5facher vom Hersteller empfohlener Dosierung. Die Tiere erhielten des Synbiotikum über das Futter. Während des Versuchs wurden die Tiere zweimal über eine Impfung gegen Infektiöse Bronchitis (IBV), Newcastle Disease (NDV) und Gumboro Disease (IBDV) bzw. durch eine Injektion von Schaferythrozyten (SRBC) immunisiert. Zu Bestimmung der humoralen Immunität wurden eine Woche nach der zweiten Immunisierung die Antikörper-Titer ermittelt. Für die SRBC wurde dies nach jeder Immunisierung durchgeführt.

Alle Zulagen des Synbiotikums führten im Vergleich zur Kontrollgruppe zu ähnlichen Antikörper-Titern gegen IBV, NDV und IBDV. Allerdings waren die Titer gegen SRBC für die Zulagestufen 1,0 und 1,5fach Biomin®IMBO sowohl nach der ersten als auch nach der zweiten Immunisierung höher als in den anderen Behandlungsgruppen. Die Ergebnisse deuten darauf hin, dass der Zusatz des Synbiotikums Biomin®IMBO zu einer Erhöhung der humoralen Immunität der Broiler gegen Schaferythrozyten führt.

Stichworte

Broiler, Fütterung, Synbioticum, Immunantwort, humoral, IBV, NDV, IBDV, SRBC

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References

AKBARIAN, A., 2008: The use of probiotics and other feed additives in broiler chickens and its effect on the immune system. MSc Thesis. Mashhad Ferdowsi University, Mashhad, Iran.

ALLOUI, M.N., W. SZCZUREK, S. SWIATKIEWICZ, 2013: The usefulness of prebiotics and probiotics in modern poultry nutrition: A review. Ann. Anim. Sci. **13**, 17-32.

BAILEY, J.S., L.C. BLANKENSHIP, N.A. COX, 1991: Effect of fructo oligosaccharides on *salmonella* colonization of the chicken intestine. Poult. Sci. **70**, 2433-2438.

BALEVI, T., U.S. UCAN, B. COSKUN, V. KURTOGLU S. CETINGUL, 2009: Effect of dietary probiotics on performance and humoral immune response in layer hens. Archiv. Zootech. **12**, 14-23.

CHEN, Y.C., C. NAKTHONG, T.C. CHEN, 2005: Improvement of laying hen performance by dietary prebiotics chicory oligofructose and inulin. Int. J. Poult. Sci., **4**, 170-178.

CHEN, W.L., J.B. LIANG, M.F. JAHROMI, N. ABDULLAH, Y.W. HO, V. TUFARELLI, 2015: Enzyme treatment enhances release of prebiotic oligosaccharides from palm kernel expeller. BioResources **10**, 196-209.

CUMPĂNĂŞOIU, C., E. TÎRZIU, I. NICHITA, M. ŞEREŞ, 2009: Probiotics influence upon the main technico-economic parameters in broiler breeding. Zootech. Biotechnol. **42**, 341-344.

DASTAR, B., A. KHAKSEFIDI, E. MOSTAFALO, 2008: Effect of probiotics Tepax on broiler performance and protein levels. J. Agr. Sci. Nat. Res. **43**, 449-460.

DHAMA, K., M. MAHENDRAN, S. TOMAR, R.S. CHAUHAN, 2008: Beneficial effects of probiotics and prebiotics in livestock and poultry: the current perspectives. Intaas Polivet **9**, 1-13.

DHAMA, K., V. VERMA, P.M. SAWANT, R. TIWARI, R.K. VAID, R.S. CHAUHAN, 2011: Applications of probiotics in poultry: enhancing immunity and beneficial effects on production performances and health - A Review. J. Immunol. Immunopathol. **13**, 1-19.

DHAMA K., S.K. LATHEEF, S. MANI, H.A. SAMAD, K. KARTHIK, R. TIWARI, R.U. KHAN, M. ALAGAWANY, M.R. FARAG, G.M. ALAM, V. LAUDADIO, V. TUFARELLI, 2015: Multiple beneficial applications and modes of action of herbs in poultry health and production - A Review. Int. J. Pharmacol. **11**, 152-176.

FULLER, R., 1999: Probiotics for farm animals. In: Tannock, G.W. (Ed.), Probiotics: a critical review. Horizon Scientific Press: Wymondham, UK, p. 15-22.

GIBSON, G.R. M.B. ROBERFROID, 1995: Dietary modulation of the colonic microbial: introducing the concept of prebiotics. J. Nutr. **125**, 1401-1412.

HAGHIGHI, H.R., J. GONG, C.L. GYLES, M.A. HAYES, B. SANEI, P. PARVIZI, H. GISAVI, J.R. CHAMBERS, S. SHARIF, 2005: Modulation of antibody-mediated immune response by probiotics in chickens. Clin. Diagn. Lab. Immunol. **12**, 1387-1392.

JIN, L.Z., Y.W. HO, N. ABDULLAH, S. JALALUDIN, 1997: Probiotics in poultry: modes of action. World Poult. Sci. J. **53**, 351-368.

KABIR, S.M.L., M.M. RAHMAN, M.B. RAHMAN, S.U. AHMAD, 2004: The dynamics of probiotics on growth performance and immune in broiler. J. Poult. Sci. **3**, 61-64.

KABIR, S.M.L., 2009: Effect of probiotics on broiler meat quality. Afr. J. Biotechnol. **8**, 3623-3627.

KEMENY, D.M., 1991: A practical guide to ELISA. 1st ed. Wheaten Ltd: Pergamon Press, UK, p. 130.

KHAKSEFIDI, A., T. GHOURCHI, 2006: Effect of probiotics on performance and immunocompetence in broiler chicks. J. Poult. Sci. **43**, 296-300.

KHALAJI, S., M. ZAGHARI AND S. NEZAFATI, 2010: Health effects of probiotics on gastrointestinal technomouse, safety and performance of broilers. In: Proceedings of the IV Iranian Congress of Animal Science, Tehran, Iran.

KHAN, R.U., S. NAZ, Z. NIKOUSEFAT, V. TUFARELLI, V. LAUDADIO, 2012a: *Thymus vulgaris*: alternative to antibiotics in poultry feed. World Poult. Sci. J. **68**, 401-408.

KHAN, R.U., Z. NIKOUSEFAT, V. TUFARELLI, S. NAZ, M. JAVDANI, V. LAUDADIO, 2012b: Garlic (*Allium sativum*) supplementation in poultry diets: effect on production and physiology. World Poult. Sci. J. **68**, 417-424.

MAHDAVI, S., E. MEHMANNAVAZ, A. NOBAKHT, 2010: Comparative effects of probiotics, acid amplifier, antibiotics and prebiotics on the growth stimulating humoral and growth parameters and factors of production in broilers. In: Proceedings of the IV Iranian Congress of Animal Science, Tehran, Iran.

MORADI, S., S. GHAZI, A. MOZAFFARI, M.H. SELAJGHE, M. SALIMI E. MOHAMMADI, 2010: Effect of different levels of probiotics and organic acids on the immune system in laying hens. In Proceedings of the IV Iranian Congress of Animal Science, Tehran, Iran.

MOUSAVI, S.M.A., A. SEIDAVI, M. DADASHBEIKI, A. KILONZO-NTHENGE, S.N. NAHASHON, V. LAUDADIO V. TUFARELLI, 2015: Effect of a synbiotic (Biomin® IMBO) on growth performance traits of broiler chickens. Eur. Poult. Sci. 79: 1-15.

POURHOSSEIN, Z., A.A.A. QOTBI, A. SEIDAVI, V. LAUDADIO, G. CENTODUCATI V. TUFARELLI, 2015: Effect of different levels of dietary sweet orange (*Citrus sinensis*) peel extract on humoral immune system responses in broiler chickens. Anim. Sci. J., 86: 105-110.

ROBERFROID, M.B., 1998: Prebiotics and synbiotics: Concepts and nutritional properties. Brit. J. Nutr., 80: 197-202.

SAVAGE, T.F., P.F. COTTER E.I. ZAKRZEWSKA, 1996: The effect of feeding mannan oligosaccharide on immunoglobulins, plasma IgG and bile IgA of Wrolstad MW male turkeys. Poult. Sci., 75: 143-143.

SHOEIB, H.K.A.N. SAYED, S.A. SOTOHY S.K.A. HAFFAR, 1997: Response of broiler chicks to probiotics supplementation. Assiut Vet. Med. J., 36: 103-116.

SMITH, J.M., 2014: A review of avian probiotics. *J. Avian Med. Surg.* 28(2): 87-94.

SPRING, P.C., K.A. WENK, K. DAWSON K.E. NEWMAN, 2000: The effect of dietary mannan oligo-saccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella* challenged broiler chicks. J. Poult. Sci., 79: 250-211.

SPSS, 1996: SPSS Base 7.5 for Windows User's Guide. SPSS, Chicago, IL, USA.

TOMAR, S., V.K. SAXENA K. DHAMA, 2011: Effect of synbiotics on the performance of broilers. *Indian Vet J.*, 88(11): 27-28.

VEGAD, J.L., 2004: Prebiotics, probiotics, acidifiers and antibiotic growth promoters. In: Vegad, J.L. (Ed.). Poultry Diseases: A Guide for Farmers and Poultry Professional. International book distributing Co. India, pp. 339-346.

YOUSSEF, A.W., H.M.A. HASSAN, H.M. ALI M.A. MOHAMED, 2013: Effect of probiotics, prebiotics and organic acids on layer performance and egg quality. Asian J. Poult. Sci., 7: 65-74.

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