

INFLUENCE OF IN OVO INOCULATION OF BIFIDOBACTERIA ON PRODUCTIVE PERFORMANCE, ANTIOXIDANT AND IMMUNE STATUS AND GUT MICROFLORA OF BROILER CHICKENS

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SUMMARY

This study was conducted to assess the potential effect of *Bifidobacterium bifidum* ATTC 29521 (*B. bifidum*) and *Bifidobacterium longum* ATTC 15707 (*B. longum*) inoculated in yolk sac of developing embryo of broilers at 18 days of incubation on growth parameters, antioxidant and immunological status and gut microflora. Three hundred broiler breeder eggs (Cobb-500) obtained from maternal flock aged 49 weeks were individually weighed at day 18th of incubation then divided into six equal treatment groups as follow; C- (non-inoculated, negative control); C+ (inoculated with sterile distilled water (vehicle), positive control); *B. bifidum* H (inoculated with 5×10^9 cfu/ml); *B. bifidum* L (inoculated with 1×10^7 cfu/ml); *B. longum* H (inoculated with 2×10^9 cfu/ml); and *B. longum* L (inoculated with 7×10^7 cfu/ml). Results showed that, at 35 days of age live body weight (LBW), average daily weight gain (ADWG) and feed conversion ratio (FCR) were significantly improved in bacterial inoculation groups compared with control groups. Improvement in antioxidant status was noted at 35 days of age in bacterial injected groups. Serum GSH content was insignificantly increased while MDA content was significantly decreased in *B. bifidum* H and *B. longum* H groups. Moreover, SOD activity was significantly increased in *B. bifidum* H and *B. longum* L groups. Immunological parameters were also enhanced with inoculation of bifidobacteria strains. Serum concentration of total immunoglobulins, IgG and IgM were significantly increased with bifidobacteria inoculation compared with control groups. Furthermore, antibody titer against NDV was increased during the secondary response in injected groups than control groups. All examined microbiological parameters were also significantly affected by in ovo inoculation with bifidobacteria strains. A significant reduction was recorded for total bacteria, total coliform, fecal coliform and *Salmonella spp.* counts, however, bifidobacteria and total lactic acid bacteria were significantly increased in inoculation groups than controls. It is concluded that injection of *B. bifidum* and *B. longum* enhances growth, antioxidant and immunological status, activities of gut microflora and reducing the pathogenic bacteria of Cobb-500 broiler chickens. The recommended dose of in ovo inoculation is 5×10^9 cfu/ml of *B. bifidum* and 7×10^7 cfu/ml of *B. longum*.

Keywords: *Bifidobacterium bifidum*, *Bifidobacterium longum*, gut microflora, pathogenic bacteria, growth, antioxidant, immunological status and broiler.

INTRODUCTION

Nowadays, poultry production sector facing numerous challenges; the most important is health risks and diseases that caused via bacteria, fungi and viruses. Probiotics is defined as "live microbial feed supplements" which improve the intestinal balance (Fuller, 1989). Microorganisms that used for obtaining the probiotic preparations for animals are the species of *Bacillus*, *Enterococcus*, *Lactobacillus* and *Saccharomyces*. Probiotics are used to improve microbial balance within the intestine to enhance gut integrity and avoid enteric diseases. Therefore, the use of probiotics allows creating natural barriers against potential pathogens, and thus enhances immunity. Probiotics enhance the immune system by increasing production of immunoglobulins, increase the activity of macrophages and stimulate the production of γ -Interferon (Yang *et al.*, 2009). *Bifidobacterium* is one of the most promising probiotics through its active role in regenerating the normal intestinal microflora (Estrada *et al.*, 2001). Immunostimulation, competition with pathogenic bacteria for intestinal attachment sites and nutrients, and volatile fatty acids production, that supply the host with metabolic energy are the benefits of bifidobacteria which is associated with increased numbers of bifidobacteria in the intestine (Williams *et al.*, 1994). According to Erickson and Hubbard (2000) and Menten and Loddi (2003), the bacterium type

of probiotics that directly related to increase immunity is *bifidobacterium* and *Lactobacillus* especially when related to enteric diseases. Several strains of bifidobacteria have been used in modulating humans and animals microflora, including *Bifidobacterium bifidum* and *Bifidobacterium longum* (Gibson and Wang, 1994 and Abe *et al.*, 1995). These types of bifidobacteria produce lactic and acetic acids and antimicrobial substances. Increasing bifidobacteria population of the intestinal tract in broiler chickens is considered a remarkable mechanism for minimizing the numbers of pathogenic bacteria (Jin *et al.*, 1997).

In poultry, probiotics enhance performance, promote a microflora balance of the gut, and counteract the negative consequences of several enteric diseases. However, once the chicks have hatched, it will expose to the external environment and quickly begins to establish the microbial community in their intestine and this resident microflora, whether friendly or potentially harmful, may affect the colonization of the probiotic microorganisms. In ovo technology represents a means to take advantage of this crucial time and promote early colonization of probiotic to stimulate the development of intestine and immune system (Cox, 2013 and Song *et al.*, 2014).

The objective of the present study was to investigate the potential beneficial impact of in ovo inoculation with *Bifidobacterium bifidum* and *Bifidobacterium longum* in the yolk sac of developing broiler embryos at the 18th day of incubation on subsequent growth, antioxidant and immunological status, gut microflora and pathogenic bacteria.

MATERIALS AND METHODS

The present study was done at Poultry Production Farm, Poultry Production Dept., Fac. of Agri., Ain Shams Univ., from September to November 2015.

Incubation and in ovo administration:

A total of 300 eggs of broiler breeder (Cobb-500) obtained from a maternal flock aged 49 weeks of age were incubated at 37.8°C and 60% RH. At 18 d of incubation, eggs were candled and both unfertilized eggs and died embryos were discarded. Eggs that containing live embryos were randomly distributed into equal sex experimental groups (45 eggs in each) with approximately similar average egg weight as follow; C- (non-inoculated, negative control); C+ (inoculated with sterile distilled water (vehicle), positive control); *B. bifidum* H (inoculated with 5x10⁹ cfu/ml); *B. bifidum* L (inoculated with 1x10⁷ cfu/ml); *B. longum* H (inoculated with 2x10⁹ cfu/ml); and *B. longum* L (inoculated with 7x10⁷ cfu/ml).

Bacterial strains:

Bacterial strains of *Bifidobacterium bifidum* ATTC 29521 and *Bifidobacterium longum* ATTC 15707 were obtained from Microbiology Culture Collection, MIRCEN, Fac. of Agri., Ain Shams Univ., Cairo, Egypt.

Standard inoculums:

These bacterial strains standard inoculums were prepared by inoculation of conical flasks (100 ml in volume) containing 50 ml of MRS broth medium with a loop of tested strains. The inoculated flasks were incubated at 37°C for 72 hrs. One ml of this culture was contained about 1x10⁷ cfu as a low dose (L) and 5x10⁹ cfu as a high dose (H) of *Bifidobacterium bifidum* ATTC 29521 and 7x10⁷cfu as a low dose (L) and 2x10⁹cfu as a high dose (H) of *Bifidobacterium longum* ATTC 15707. The target site of injection was the yolk sac according to the procedure described by Bhanja *et al.* (2004).

This specific day (18th of incubation) was selected because, the embryo starts to ingest the amniotic fluid orally and absorbs the yolk sac largely before the injected bacteria may compete for the embryo for nutrients stored in egg yolk. Prior injection, both of the working bench and the eggs were disinfected with 70% ethanol and eggshell was punched at the blunt end of the egg to make a hole with a 21-gauge needle. Eggs were injected with a 23-ga needle. Before the eggs were returned to the incubator, the shell holes were sealed with adhesive tape.

At day 21 of incubation, hatched chicks within each treatment group were counted and individually weighed.

Housing and management:

Thirty hatched chicks from each treatment were randomly chosen, subdivided into 3 replicates per treatment (10 hatchlings/ rep.) and reared until 35 days of age. All chicks fed a commercial diet according to broiler performance and nutrition supplement of Cobb 500.

An artificial light source was used in order to provide 24 hours of light per day throughout the first week and then reduced to 22 h for the other experimental periods. Growth performance parameters; Live body weight (LBW), average daily weight gain (ADWG), average daily feed consumption (ADFC) and feed conversion ratio (FCR) were recorded during the experiment.

Vaccination and humoral immune response:

Vaccination against the Newcastle disease virus (NDV) was performed on 21 and 28 days of age using an eye dropper (Live Lasota strain; KBNP, Inc.; Hungnam, Korea). For humoral immune response assessment, blood samples obtained from the wing vein of the respective vaccinated chicks at 28 and 35 days of age. Blood samples were subsequently centrifuged at $2500 \times g$ for 10 min. at 4°C, and collected serum was stored at - 80°C. The anti-NDV titer was assessed by a haemagglutination inhibition test on sera obtained on days 28 and 35 of age (primary and secondary responses, resp.).

Serum IgG, IgM, IgA and total immunoglobulins (Ig) concentrations:

At 35 d of age, serum IgG, IgM, IgA and total immunoglobulins (Ig) concentrations were determined in appropriately diluted samples by a sandwich ELISA using microtiter plates and chicken-specific IgA, IgM, and IgG ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX). ELISA procedure was carried out according to the protocol of the manufacturer and absorbance was measured at 450 nm. The concentrations of IgG, IgM, and IgA were obtained using standard curves constructed from respective Ig standards run on the assay microtiter plate and were expressed as micrograms of IgG, IgM, or IgA per milliliter of serum. Summation of the respective serum IgG, IgM, and IgA concentrations were recorded as total serum Ig concentration (Mountzouris et al., 2010).

Antioxidant status:

Serum contents of Glutathione reduced (GSH), Malondialdehyde (MDA) and superoxide dismutase activity (SOD) were determined using commercial kits (Spinreact Co., Spain) by a colorimetric methods as described by Beutler *et al.* (1963), Nishikimi *et al.* (1972) and Ohkawa *et al.* (1979), respectively.

Microbial evaluation:

Samples from ileum and caecum contents were collected, pooled and microbiologically analyzed by aseptic transfer of 10 g. of homogenized sub-sample into 90 ml in sterile diluents. Serial dilutions were prepared in the same diluents. Different microbial groups were enumerated using the poured plate's technique (APHA, 1998). The microbiological parameters included densities of total bacteria, total lactic acid bacteria, *Bifidobacterium spp.*, *Salmonella spp.*, total and fecal coliform. The appropriate medium, incubation temperature and incubation period used to determine all microbiological parameters were shown in Table (1).

Table (1): Media and incubation conditions used for microbiological analysis of different samples.

Microbial analysis	Incubation condition		Growth medium
	Incubation Period	Temp.(°C)	
Total aerobic bacteria	24-72 hrs.	30	Nutrient agar (APHA, 1998)
Total lactic acid bacteria	24-72 hrs.	37	MRS agar (Difco 2006)
<i>Bifidobacterium spp.</i>	24-72 hrs.	37	<i>Bifidobacterium</i> agar (Atlas, 2004)
<i>Salmonella spp.</i>	24-48 hrs.	37	<i>Salmonella-Shigella</i> (SS) agar (Difco 2006)
Total and fecal coliform	24-48 hrs.	37&44	MacConkey agar (Oxoid 2010)

Statistical analysis:

Data were subjected to the analysis of variance by using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SPSS, 2010), according to the following model: $Y_{ij} = \mu + T_i + \epsilon_{ij}$ Where: Y_{ij} = observation. μ = overall mean. T_i = a fixed effect of injected treatment. ϵ_{ijk} = experimental error. Differences among treatment means were detected using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Data presented in Table (2) illustrated that both LBW and ADWG of broiler chicks which were in ovo injected with different doses of bifidobacteria strains. It is clear that LBW was improved significantly ($P<0.01$) in bifidobacterial injected groups at 35 days of age compared with that of the control groups. Moreover, the highest values of final LBW were recorded for *B. bifidum* H, *B. longum* L and *B. bifidum* L groups (1875.13, 1847.27 and 1819.20 respectively). Similar trend was observed for the values of ADWG which showed significant ($P<0.05$) increment among bacterial inoculation groups than those of the control groups. As expected, groups of *B. bifidum* H, *B. longum* L and *B. bifidum* L recorded the highest values of ADWG compared with other groups.

Table (2): Effect of in ovo injection of Bifidobacteria on productive performance of broiler chicks at 35 days of age.

Trait	Experimental groups						SEM	P value	Sig.
	C-	C+	<i>B. bifidum</i> H	<i>B. bifidum</i> L	<i>B. longum</i> H	<i>B. longum</i> L			
LBW 5 th w	1726.0 ^b	1786.1 ^{ab}	1875.13 ^a	1819.20 ^a	1798.17 ^{ab}	1847.27 ^a	12.17	0.008	**
ADWG 0-5wk	48.15 ^c	49.85 ^{bc}	52.41 ^a	50.79 ^{ab}	50.19 ^{abc}	51.59 ^{ab}	0.404	0.014	*
FC 0-5 wk	84.85	82.98	85.04	83.11	77.72	81.25	0.891	0.148	NS
FCR 0-5 wk	1.76 ^a	1.67 ^{ab}	1.62 ^b	1.64 ^b	1.55 ^b	1.58 ^b	0.021	0.019	*

SEM=Standard error of means.

Mean in the same row within each classification bearing different letters are significantly different, NS= non-significant, *($P\leq 0.05$), ** ($P\leq 0.01$).

This improvement in LBW and ADWG may be due to the intestinal tract status of chicks from the in ovo *Bifidobacterium* injected groups which resulted enhancement of microbial profile of the gut then create favorable conditions to enhance performance. A similar trend was also reported by Mountzouris *et al.* (2010) they noted that probiotic inclusion improved broiler body weight during growing and finishing periods. similar results were found by in ovo injection of probiotic (Pruszyńska-Oszmalek *et al.* (2015). Estrada *et al.* (2001) had observed growth improvement with providing 10^7 bacteria/ml *B. bifidum* by water supply for the first 10 days and 10^6 for the remaining 28 days. ADFC of broiler chickens was not significantly ($P>0.05$) changed as a result of in ovo injection with bifidobacteria strains.

Nevertheless, a different trend was observed for the values of feed conversion ratio (FCR) which showed significant ($P<0.05$) improvement in all bacterial inoculation groups during 0-5 weeks of age. The best values of overall FCR were recorded for groups *B. longum* H, *B. longum* L, *B. bifidum* H, and *B. bifidum* L (1.55, 1.58, 1.62 and 1.64, respectively).

Effects of in ovo supplementation of bifidobacteria strains on serum contents of antioxidant of broiler chicks are shown in (Table 3). Serum level of GSH showed insignificant differences among all bacterial treatment groups and control groups. Serum GSH level from chickens of *B. bifidum* L, *B. longum* H and *B. longum* L treatments were insignificantly increased while *B. bifidum* H and C+ were lower to those of the C- group.

Conversely, serum MDA contents were decreased significantly ($P<0.05$) in birds of *B. longum* H and *B. bifidum* H groups compared with those of other groups. The lowest value was observed in chicks of group *B. longum* H (1.905 μmol) followed by *B. bifidum* H (2.15 μmol) group. Furthermore, SOD activity was increased significantly ($P<0.01$) in chicks of *B. longum* L and *B. bifidum* L groups compared with those of other groups.

On the other hand, SOD activities in chicks of groups *B. longum* H and C+ were decreased significantly ($P<0.01$) in comparison with those of C-. The highest activity of SOD was observed in birds of group *B. longum* L (342 U/ml) while the lowest activity was recorded in birds of group *B. longum* H (177.33 U/ml). The potent antioxidant properties of probiotics have been mainly attributed to several factors, such as probiotic strain and challenge type.

The benefits of intestinal bacteria had been hypothesized by Lin and Yen (1999) who stated that probiotics produce certain agents that chelate free radicals, catching reactive oxygen species and inhibiting their cytotoxic activity.

Popović *et al.* (2015) concluded that serum MDA concentration of broilers fed a diet supplemented with symbiotic (1 g/kg of feed) during 15 to 42 days of age was significantly decreased compared to control.

Immunoglobulins (IgG, IgM, IgA and total Ig) and humoral immune responses against Newcastle disease virus (NDV):

It could be noticed from (Table 4) that, the immune response was enhanced with in ovo inoculation of bifidobacteria strains in comparison with control chicks. Serum levels of total immunoglobulins, IgM and IgG increased significantly in inoculated groups than control groups, while serum IgA concentrations did not significantly different among treatment groups. The highest levels of IgG, IgM and total Ig in the *Bifidobacterium* treated groups were observed in *B. longum* L followed by *B. longum* H. As well, results of haemagglutination inhibition test showed that non significant difference between all treated groups in total antibody titer during the primary response. While significant increase in total antibody titer during the secondary response was recorded in bacterial inoculation groups compared with controls. It is well known that the specific use of probiotics is modulating the immune responses to harmful antigens. So, understanding the responses of chickens to probiotics and modulate in the local immune responses is essential to enable the manipulation of the microbiota for improved intestinal health and performance. Torshizi *et al.* (2010) concluded that probiotic administration through drinking water significantly improved antibody production against SRBC than control and probiotic-fed groups. Dalloul *et al.* (2005) reported a positive effect of probiotic in enhancing some of the immune responses against *E. acervulina*, as investigated by early IFN- γ and IL-2 secretions. Several investigators demonstrated the influence of probiotic on immune modulation (Haghighi *et al.*, 2005, Mathivanan and Kalaiarasi, 2007;Nayebpor *et al.*, 2007and Apat, 2008).

Table (4): Effect of in ovo injection of Bifidobacteria on total immunoglobulins, IgG, IgM and IgA using ELISA technique at 35 days of age and on total antibody titer against NDV using haemagglutination inhibition test.

Age	Experimental groups						SEM	P value	Sig.	
	C-	C+	<i>B. bifidum</i> H	<i>B. bifidum</i> L	<i>B. longum</i> H	<i>B. longum</i> L				
ELISA Technique										
IgG		821.3 ^b	812. ^a	972.7 ^b	912.7 ^b	890.7 ^b	973.0 ^b	32.626	0.013	*
IgM		96.77 ^b	93.56 ^b	117.67 ^{ab}	116.90 ^{ab}	113.83 ^{ab}	150.00 ^a	11.080	<0.001	**
IgA		181.70	184.03	188.67	195.33	198.00	187.33	7.010	0.676	NS
Total Ig		1099.7 ^c	1089.6 ^c	1279.0 ^{ab}	1224.9 ^b	1202.5 ^b	1310.3 ^a	44.664	0.004	**
Haemagglutination Inhibition Test										
Total antibody titer (1 st response)		4	4.33	4	3.9	3.85	4	0.388	0.158	NS
Total antibody titer (2 nd response)		6.8 ^b	6.33 ^b	8.33 ^a	8 ^a	8.67 ^a	8.33 ^a	0.231	0.924	*

SEM=Standard error of means.

Mean in the same row within each classification bearing different letters are significantly different, *(P \leq 0.05), ***(P \leq 0.01)

Furthermore, Kabir *et al.* (2004) demonstrated that differences in the weights of spleen and bursa from probiotics treatment and conventionally fed broilers could be attributed to different levels of antibody production in response to SRBC.

In contrast, Mountzouris *et al.*(2010) concluded that probiotic inclusion levels had no effect on systemic humoral immune status of the broilers because the concentration of IgA, IgM, IgD and total Ig did not differ between the treated groups (10⁸, 10⁹ and 10¹⁰ cfu probiotic/kg of diet) and the control group.

Microbial evaluation:

Data illustrated by Figures (1, 2 & 3) revealed that all examined microbiological parameters were affected significantly by in ovo inoculation with *bifidobacteria* strains. Total counts of bacteria, coliform, fecal coliform and *Salmonella spp.* were significantly reduced. Conversely, *bifidobacteria* and total lactic acid bacteria counts were increased significantly in all treated groups. The lowest counts of total bacteria, total coliform and *Salmonella spp.* were recorded in samples of *B. longum* H chicks (21.135, 3.925 and 3.055 log cfu/g, respectively) followed by those of *B. bifidum* H chickens (20.205, 3.955 and 3.085 log cfu/g, respectively).

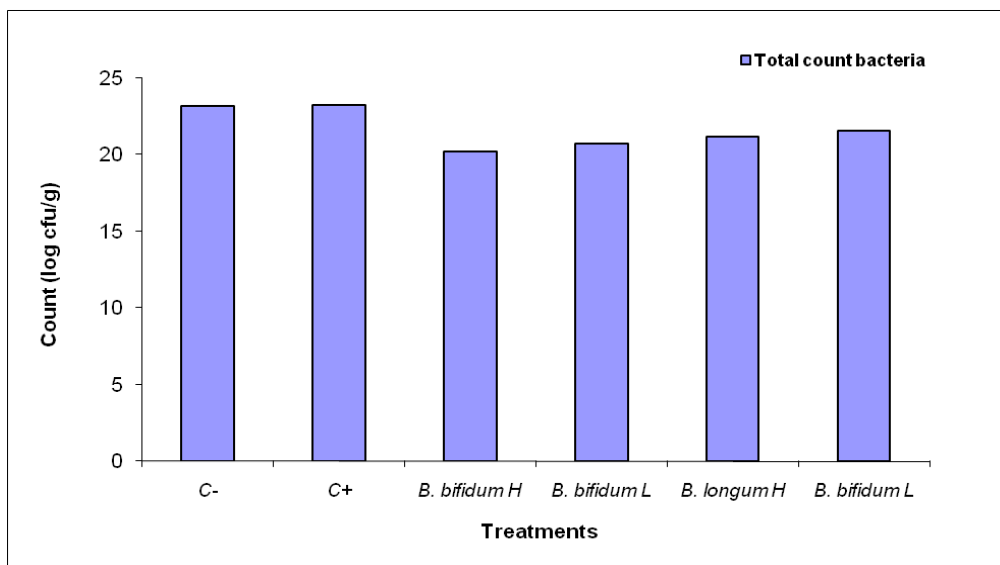


Fig. (1): Total bacteria count (log cfu / g) of broiler chickens in experimental groups.

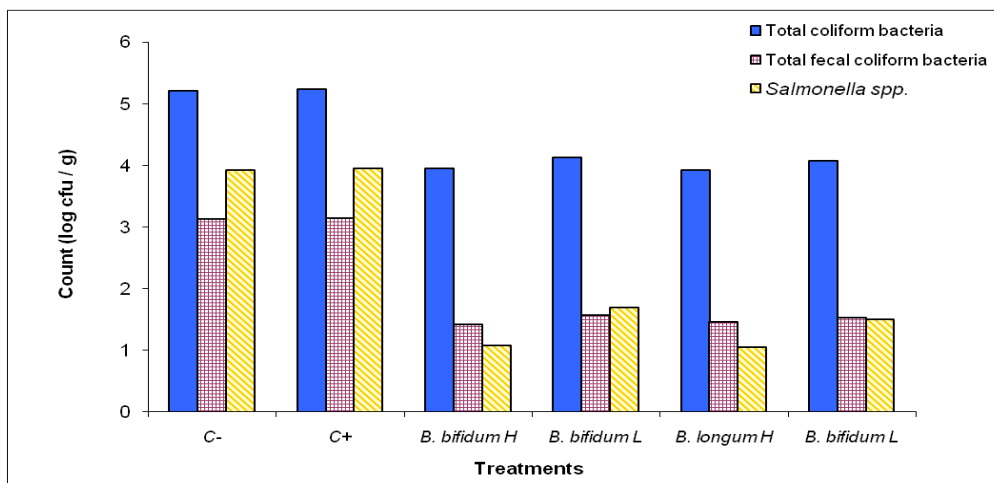


Fig. (2): Total coliform bacteria, Total fecal coliform bacteria and *Salmonella* spp. (log cfu / g) of broiler chickens in experimental groups.

As well, samples of *B. bifidum* H chickens recorded the lowest count of fecal coliform (2.425 log cfu/g) followed by those of *B. longum* H (2.455 log cfu/g). On the other hand, the highest counts of bifidobacteria and total lactic acid bacteria were recorded in samples of *B. longum* H chickens (9.465 and 11.785 log cfu/g, respectively) followed by those of *B. bifidum* H (9.415 and 11.755 log cfu/g, respectively). As expected in ovo feeding of *Bifidobacterium bifidum* and *Bifidobacterium longum* increased the bacterial counts of bifidobacteria and its bacterial family, (lactic acid bacteria). The increment percentages of these bacterial counts were in positive linear relation with inoculation doses of bifidobacteria.

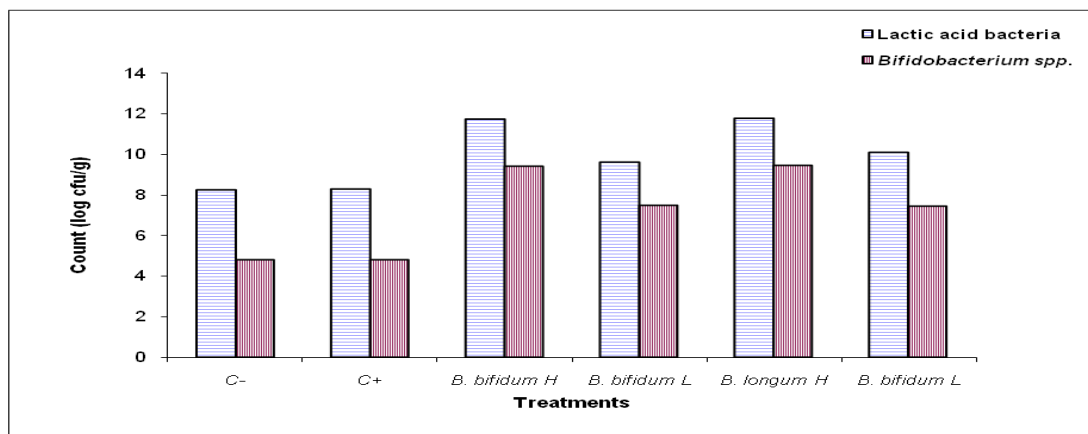


Fig. (3): Lactic acid bacteria and *Bifidobacterium spp.* (log cfu / g) of broiler chickens in experimental groups

Competitive exclusion mechanism explained the reduction in counts of total coliform, fecal coliform and *Salmonella spp.* which are considered pathogenic bacteria, this is illustrated by Fig. (4).

So, the increase of bifidobacterial dose associated with the decrease in total bacteria, total coliform, fecal coliform and *Salmonella spp.* counts. Scanlan (1997) proposed the important role of competitive exclusion in prevention of pathogenic bacteria in small intestine of chicks by microorganisms establish an enteric flora before exposure to these pathogens, the microorganisms from the inoculated flora and the beneficial micro-organisms produce volatile fatty acids that lower the intestinal pH.

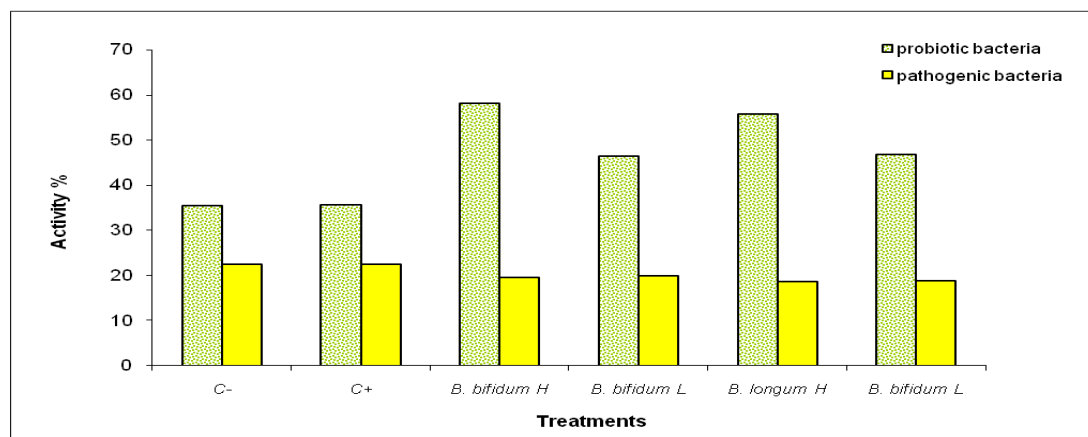


Fig. (4): Probiotic bacteria and pathogenic bacteria activity (%) of broiler chickens in experimental groups.

In agreement with our findings Leandro *et al.* (2010), revealed that the early use of probiotics using in ovo injection technique establishes a balance in microbial flora against pathogenic bacteria. Thus, using probiotic constituted by *Enterococcus faecium*, *Lactobacillus case*, *L. plantarum* inoculated at the dose of 10^6 cfu/g per egg has avoided the colonization of the gastrointestinal tract of broilers challenged with 0.1 mL aqueous solution containing 1.36×10^6 cfu *Salmonella enteritidis*, inoculated via crop. Moreover, Hashemzadeh *et al.* (2010), reported that probiotic administration was effective in preventing *Salmonella* colonization in neonatal broilers using in ovo injection, oral gavage, spray or vent lip applications.

Also, Mountzouris *et al.* (2010), found that the inclusion of 10^9 and 10^{10} cfu probiotic/kg feed provided benefit in the modulation of the composition of cecal microflora. Also, in agreement with the previous findings of the potential of probiotics to fortify the intestinal microflora of broiler chickens with beneficial bacteria and suppress potentially pathogenic bacteria are the results by Koenen *et al.*(2004), Teo and Tan(2007), Higgins *et al.*(2008), Vicente *et al.*(2008) and Oliveira *et al.* (2014).

CONCLUSION

In ovo injection of *B. bifidum* and *B. longum* with doses of 5×10^9 cfu/ml and 7×10^7 cfu/ml, respectively, in the yolk sac of broiler chickens at the 18th day of embryogenesis could be used as an effective tool for improving subsequent post hatch productive performance, antioxidant, Immunological status, the activities of gut microflora and reducing the pathogenic bacteria.

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تأثير حقن البيض ببكتريا البيفيدو علي الاداء الانتاجي وحالة مضادات الاكسدة والمناعة وميكروفلورا القناة الهضمية في دجاج التسمين

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أجريت هذه الدراسة في مزرعة إنتاج الدواجن- قسم إنتاج الدواجن - كلية الزراعة- جامعة عين شمس وذلك خلال الفترة من شهر سبتمبر حتي شهر نوفمبر 2015 م.

تم تصميم الدراسة لتقييم التأثير النافع المحتمل لحقن بيض التفريخ ببكتيريا من جنس البيفيدو في كيس الصفار وذلك في اليوم الثامن عشر من التفريخ و دراسة تأثير ذلك على الاداء الانتاجي، مستوى مضادات الأكسدة، الحمل الميكروبي في الجهاز الهضمي وكذلك بعض التغيرات المناعية في الكتاكتيف الفاقسة.

استخدم في هذه التجربة عدد 300 بيضة مخصصة من أمهات بداري التسمين (عمر 49 أسبوع) من سلالة الكوب-500. تم وزن البيض في اليوم الثامن عشر من التفريخ قبل الحقن وقد تم تقسيم هذا البيض الى ستة مجاميع تجريبية متساوية على النحو التالي: المجموعة الأولى كانت للمقارنة، المجموعة الثانية كانت المجموعة المحقونة بالمادة الحاملة (المذيب) للبكتيريا. المجموعتين الثالثة والرابعة تم حقنهما ببكتيريا *Bifidobacterium bifidum* بجرعتي 1×10^9 cfu/ml و 1×10^7 cfu/ml على الترتيب. بينما تم حقن المجموعتين الخامسة والسادسة ببكتيريا *Bifidobacterium longum* بجرعتي 2×10^9 cfu/ml و 7×10^7 cfu/ml على الترتيب

استغرقت فترة الدراسة خمسة أسابيع وكانت التغذية وكذلك مياه الشرب متاحة بحرية لجميع الطيور. في نهاية فترة الدراسة تم جمع عينات الدم لتقدير حالة مضادات الاكسدة والحالة المناعية. كما تم جمع عينات من محتويات اللفائفي وذلك لتقدير الحمل الميكروبي لبعض السلالات البكتيرية وأظهرت النتائج:

أن حقن البيض في كيس الصفار ببكتيريا *Bifidobacterium bifidum* و *Bifidobacterium longum* للجنة النامية في اليوم الثامن عشر من عمر التكوين الجنيني أدى الي تحسن في وزن الجسم والزيادة الوزنية لدجاج التسمين عند عمر 35 يوم وكذلك تحسن معامل التحويل الغذائي للمجاميع المحقونة بالبكتيريا تحسنت ايضا الحالة المناعية وارتفع مستوى سيرم الدم من الاجسام المضادة نتيجة الحقن بالبكتيريا وكذلك مقاييس مضادات التاكسد في سيرم الدم. كما سجلت المجاميع المحقونة بالبكتيريا مستويات عالية من البكتيريا النافعة وانخفاض في اعداد البكتيريا الضارة مقارنة بالكنترول.

من النتائج نستنتج ما يلي: ان حقن بيض دجاج التسمين في كيس الصفار ببكتيريا *Bifidobacterium bifidum* و *Bifidobacterium longum* للجنة النامية في اليوم الثامن عشر من عمر التكوين الجنيني يمكن ان يستخدم كوسيلة فعالة لتحسين الاداء الانتاجي بعد الفقس والحالة المناعية ومستوي مضادات الاكسدة في دم طيور بداري التسمين، بدون أى آثار سلبية على صفات الفقس وكذلك زيادة نشاط البكتيريا النافعة في الامعاء وتقليل أعداد الميكروبات المرضية .

وبناء على النتائج السابقة توصي الدراسة باستخدام بكتيريا *Bifidobacterium bifidum* و *Bifidobacterium longum* أو أحدهما بجرعات 5×10^9 cfu/ml و 7×10^7 cfu/ml على الترتيب