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EFFECT OF DIETARY MANNAN OLIGOSACCHARIDE AND LIGNIN ON POPULATION AND CHARACTERISTICS OF PROBIOTIC BACTERIA ISOLATED FROM JAPANES QUAILS

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ABSTRACT: The role of gut bacteria in animal health has became increasingly important, especially, with limitations use of antibiotics in animal feed due to consumer pressure and legislation. This study showed the relationships between gut bacteria and bird performance as a result of affecting by dietary supplementation of prebiotic such as (mannan oligosaccharide and lignin). A lower count of total aerobic bacteria was shown in mannan and lignin groups comparing with control during all experimental periods. Fortunately, count of the probiotic bacteria (*Bifidobacterium* spp. and *Lactobacillus* spp.) increased significantly ($P \leq 0.01$) in lignin and mannan groups compared to the control group at all three periods age, while the most increases were at 9 weeks. Determination of the susceptibility of isolated probiotics to antibiotics exhibited that the most effective antibiotic against the tested bacteria was ciprofloxacin (CIP), which reached up to 32 mm of inhibition zone, followed by vancomycin (VA) reaching up to 22 mm and finally tetracyclin (TE) which reached up to 20 mm. The antibacterial activity of the crude supernatant fluids obtained from lactic acid bacteria (LAB) culutures was generally greater against the Gram positive than the Gram negative tested bacteria. Sequencing of the 16S rRNA gene technique was considered to be a rapid and powerful method for identifying the probiotic isolates of *Bifidobacterium* spp. and *Lactobacillus* spp. to the species and at sub species level as *Bifidobacterium longum* subsp. *infantis, Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus acidophilus.*

Key words: *Bifidobacterium* spp., antibiotics, antimicrobial, probiotic, prebiotic, Japanese quail.

INTRODUCTION

Gut microbiology and its role in animal health has become increasingly important, particularly now, that the use of antibiotics in animal feeds to promote growth is facing restrictions due to legislation in some countries and consumer pressure. The microorganisms that colonise the gastrointestinal tract during the early post-hatch period form a synergistic relationship with their poultry host. Gastrointestinal microorganisms have a highly significant impacts on uptake and utilization of energy (Choct *et al*., 1996) and other nutrients (Steenfeldt *et al*., 1995; Smits *et al*., 1997) and on the response of poultry to anti-nutritional factors (such as non-starch polysaccharides), pre- and probiotic feed additives and feed enzymes (Apajalahti and Bedford, 2001). Microorganisms can also directly interact with the lining of the gastrointestinal tract (Van Leeuwen *et al*., 2004), which may alter the physiology of the tract and immunological status of the bird (Klasing *et al*., 1999).

Antibiotic substances have been added to livestock animal feeds as growth promoters since 1950, (Gibson and Roberfroid, 1995) because it was found that their use improved the performance and health of the animals. Nevertheless, in recent years, the public

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disapproval for antibiotic growth promoters, due to their residual effects, has created a growing interest in the identification and evaluation of alternative natural feed additives (Greko, 2001; Roe and Pillai, 2003). One such additive that is being tested as growth promoter is the mannan oligosaccharides (MOS) of the cell wall of the yeast *Saccharomyces cerevisiae.* When MOS are incorporated in the animal feed, they can adhere to pathogenic bacteria that have type-I fimbriae, and so, limit their ability to adhere to the mucosa of the digestive tract and multiply.

Prebiotics are non-digestible but fermentable food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or number of bacteria in the colon (Gibson and Roberfroid, 1995). The beneficial effects of prebiotics are known, and they are widely used as an alternative to antibiotics in poultry (Rehman *et al*., 2009), also they increase the number of bifidobacteria and lactobacilli, whereas the number of *Salmonella* spp. and *E.coli* in the gastrointestinal tract are reduced (Nelson *et al*., 1994). Alcell lignin (1.25% of DM) has been reported to improve growth performance of veal calves and to inhibit the growth of *Escherichia coli in vitro* (Phillip *et al*., 2000).

The objectives of this study were to determine the effects of dietary addition of lignin and a mannan oligosaccharide to Japanes quails diets on probiotic populations in the cecum and determination the susceptibility of *Bifidobacterium* as well as *Lactobacillus* bacteria isolated from the cecum of Japanes quail to several groups of antibiotics, Also, *Bifidobacterium* spp. and *Lactobacillus* spp. were screened of their antagonistic activity against some pathogenic bacteria.

MATERIALS AND METHODS

Bird Management and Experimental Design

Part of the present study was carried out at the laboratory belonging to the Poultry Physiology and Production Research Unit, Biological Applications Department, Nuclear Research Center, Atomic Energy Authority, Egypt. Ninety 1-day-old Japanes quail, were individually weighed and assigned randomly to 3 treatment groups with 30 birds each. The birds were housed in separate wire suspended cages until 64 days of age. All experimental birds were housed in galvanized metal cages equipped with an automatic nipple drinker and manual feeder. The birds were grown in these cages (100 × 60 × 50 cm; length × width × height), with *ad libitum* access to water and feed, at the same managerial, controlled, clean and hygienic environmental conditions.The composition and calculated chemical analyses of the basal diet are presented in Table 1.

The basal diet was given to control (group 1), while second group was fed diet with the addition of 1.25% lignin (L)/Kg diet (group 2). The Alcell lignin used was (Alcell Technologies Inc., Montreal, Quebec, Canada), and third group was fed basel diets supplemented with 0.2% g mannan oligosaccharide /kg diet (group 3). The MOS used was "MOS 500" (Ultra Bio-Logics Inc., Canada). The experimental basal diet was formulated to meet the recommendations of NRC (1994). The quails were allowed *add libitum* access to feed and water.

Vaccination against the Newcastle disease (ND) virus was performed on days 21 ,42 and 64 of age separately, using an eye dropper (Live Lasota strain; KBNP, Inc.; Hungnam, Korea). Artificial light source was used in order to give 24 hours of light per day throughout the experimental period.

Bacteriological Analyses

Bacterial populations of cecal digesta

Cecal contents of each bird were aseptically emptied into sterile plastic bag at 21, 42 and 64 day of age, and stored at -20°C for bacteriological analyses. Samples of the cecal contents were serially diluted in 0.85% sterile saline solution and used to assay lactobacilli, bifidobacteria, *E. coli* and total aerobic bacteria. All bacteriological analyses were performed in 3 replicates, and the average value of these determinations were used for statistical analysis. Lactobacilli were anaerobically assayed using lactobacilli MRS agar (Fisher Scientific, Ottawa, Ontario, Canada) by serial dilution (10^{-5} - 10^{-7}) and incubated at 37°C for 48 hr., (Baurhoo *et al.*, 2007_b). Enumeration of bifidobacteria was performed using Wilkins-Chalgren agar (Oxoid, Nepean, Ontario, Canada) by serial dilution (10^{-5}-10^{-7}). The Petri dishes were placed in anaerobic

Table 1. Ingredients and calculated chemical composition of the basal diet

Ingredient	(%)
Yellow corn	60.50
Soybean meal (44%)	26.00
Maize gluten meal (62%)	8.00
Vegetable oil	1.50
Limestone	1.12
Di-calcium phosphate	1.75
Premix*	0.30
NaCl (salt)	0.30
L-lysine	0.36
DL-Methionine	0.17
Total	100
Calculated composition**	
ME (kcal kg^{-1})	3050.00
Crude protein	22.06
Calcium	0.95
Non phytate phosphorus	0.45
Lysine	1.3
TSAA	0.95
Threonine	0.78
Tryptophan	0.22

*Provides each Kg of diet: Vitam. A: 12000 IU, Vitam. D_3: 5000 IU, Vitam. E: 130.0 mg, Vitam K_3: 3.605 mg, Vitam. B_1: 3.0 mg, Vitam. B_2: 8.0 mg, Vitam. B_6: 4.95 mg, Vitam. B_{12}: 0.17 mg, Niacin: 60.0 mg, Folic acid: 2.083 mg, D-Biotin: 200.0 mg, calcium D-Pantothenate: 18.333 mg, Copper: 80 mg, Iodine: 2.0 mg, Selenium: 150.0 mg, Iron: 80.0 mg, Manganese: 100.0 mg, Zinc: 80.0 mg, Cobalt 500.0 mg.

** Calculated according to NRC (1994).

jars, using anaeropacks (Oxoid), and incubated at 37°C for 5 days (Baurhoo *et al.*, 2007_b). *Escherichia coli* was assayed using Mac-Conkey agar -Difco, by serial dilution (10^{-3}-10^{-4}) and incubated aerobically at 37° for 24 hr (Baurhoo *et al.*, 2007_b). Total aerobic bacterial count was estimated on plate count agar (medium-Difco, using decimal dilution technique. Plates were incubated at 37°C for 48 hr., after which colony forming units (CFU) were counted by serial dilution (10^{-5}- 10^{-7}). Average results of the replicates are presented as $\log_{10}$ colony forming units (CFU)/g of the cecal content (Swanson *et al*., 2001).

Isolation of probiotic bacteria

During the enumeration of bifidobacteria and lactobacilli colonies in their specific media in Petri-dishes as recommended by (Baurhoo *et al.*, 2007_b), the isolation process was conducted during the time course of the experimental period at different stages of age and treatments to isolate probiotic bacteria from the highest dilutions (10^{-6}-10^{-7}) prepared from the cecal

contents. In each case, single colonies were picked up, grown, purified, chcked by microscopic examination, subculutred and preserved as recommended methods due to Swanson *et al.* (2001). Totality 27 lactobacilli isolates and 26 bifidobacteria isolates were obtained, and due to the growth characterization, only 6 isolates were selected for further study.

Antibiotic susceptibility test

The antibiotic susceptibility test was performed by the standard disc diffusion method (NCCLS, 2004). The following commercial antiobiotic discs were used: Gentamicin (CN 10 mcg), Neomycin (N 30 mcg), Amoxicillin (AX 25 mcg), Streptomycin (S 10 mcg), Erythromycin (E 15 mcg), Penicillin (G 10 mcg), Cefotaxime (CTX 30 mcg), Chloramphenicol (C 30mcg), Vancomycin (VA 30 mcg), Aminosidin (AN 60 mcg), Trimethoprim/Sulphamethoxazole (SXT 1.25 mcg), Tetracycline (TE 30 mcg), Kanamycin (K 30 mcg), Ciprofloxacin (CIP 5 mcg) and Ampicillin (AM 10 mcg). Pure cultures of *Bifidobacterium* and *Lactobacilllus* were enriched in LB broth at 37°C for 24 hr. Using a strile glass spreader, 100µl (2×10^8 CFU/ ml) from each bacterial culture were spread onto LB agar plates (Costa *et al*., 1998). The antibiotic discs were dispensed sufficiently separated from each other, so as to avoid overlapping of inhibition zones. After 30 min, the plates were inverted and incubated at 37°C for 18–24 hr. Results were recorded by measuring the diameter of the inhibition zones (mm) and compared with standards for antimicrobial disk susceptibility tests.

Antibacterial activity of the isolated *Lactobacillus* and *Bifidobacterium*

The isolated lactobacilli and *bifidobacterium* were grown in MRS broth at 37°C, for 2 days for lactobacilli, and for 4-5 days for *Bifidobacterium* were placed in anaerobic jars, using anaeropacks (Oxoid). Cultures were centrifuged at 12000g for 10 min at 4ºC, and the supernatant fluids were adjusted to pH 6.5 with 1 N NaOH, and filtered through a 0.45 µm membrane filter for obtaining the cell-free supernatants (CFSs). After the preparation of CFS of all lactobacilli and *Bifidobacterium* strains, antibacterial activity was determined by a disk diffusion assay (Herreros *et al.*, 2005). Ten milliliters of Brain heart infusion agar (1.5% *W/V*) was poured into a sterile plate and solidified. Brain heart infusion agar (0.7%, *W/V*) was first seeded with an indicator bacterial strain (36µl of overnight culture per 6 ml of agar, *i.e.* approximately 10^7 cells). Nine indicator bacterial strains namely (*Listeria monocytogenese, Salmonella enteric, E.coli 0157 : H7, Enterobacter cloacae subsp. dissolvens*, *Enterobacter aerogenes, Micrococcus caseolyticus*, *Aeromounas hedrophili*, *Staphylococcus aureus* and *Staphylococcus H*.) were kindly provided by the Dept. Agric. Microbiology Fac. Agric. Zagazig Univ., Egypt were used in this study. The inoculated soft agar was rapidly dispensed onto an agar plate. After solidification, the soft agar was dried for 30 min. under a laminar flow hood. Aliquot of 50 µl from each extract of CFSs were placed on the disk. Plates were held at room temperature for 3 hr., to allow the antibacterial agent to diffuse completely, and were incubated for 24 hr., at 37ºC. Antibacterial activity was expressed as the diameter of zone of inhibition (including the disc) which was recorded by measuring the diameter of the inhibition zones (mm).

Biochmical and molecular identification of bacterial isolates

Only, six bifidobacteria and lactobacilli bacteria isolated from cecum of japanes quailes were identified using biochemical methods according to Martin-Carnahan and Joseph (2005). The isolated bacteria in pure culture were grown on Luria Bertani (LB) agar plates and subjected to catalase test, oxidases, urease test, hydrogen sulfide production test, indole test, methyl red, citrate utilization, motility and Voges-Proskaür test. Also, pure bacterial isolates were grown in peptone water supplemented with 0.5% of the tested sugar *i.e.,* glucose, mannitol, sucrose, sorbitol, trehalose, raffinose, ribose, cellobiose, lactose, mannose, inositol and rhamnose after being sterilized by filtiration. Bromothymol blue and small inverted Durham`s tube were included in each tube. Acid production and gas production were detected according to Samelis *et al.* (1994). To confirm the biochemical results, only three isolates were identified in Sigma Scientific Services Company (El-Giza, Egypt) using 16S rDNA gene sequence. The DNA of the bacteria was isolated according to the protocol of Maniatis *et al*.

(1989), and the GeneJet genomic DNA purification Kit (Thermo K0721). Molecular sequencing of the DNA fragment containing the 16S intergenic spacer corresponding to the conserved region of 16S rDNA (Martinez-Murcia *et al.*, 1992) allowed for an unambiguous classification of the *Bifidobacterium* and *Lactobacillus* isolates. The primers designs to amplify 16S rDNA gene were: forward primer 5′AGAGTTTGATCATGG CTCAG-3 and reverse primer 5′-GGTTACCTT GTTACGACTT-3′ and the identification was performed according to Borrell *et al.* (1997).

Statistical Analysis

Data were analyzed as an one-way ANOVA using the GLM procedure. Treatment means were separated using Bonferroni's multiple comparison test. Statistical significance was declared at a probability of both $P \leq 0.05$ and $P \leq 0.01$. All bacteriological values were subject to base-10 logarithm transformation before analysis due to (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

Bacterial Loads in Cecum of Japanes Quail

Results presented in Table 2 show bacterial count of cecum of Japanes quail birds as affected by dietary supplementation of mannan oligosaccharide and lignin. The count of total aerobic bacteria was insignificantly affected in group 2 (lignin) and group 3 (mannan) compared to the control group during the experiments duration. A lower count of total aerobic bacteria was shown in mannan and lignin groups comparing with control group during all experimental periods. The effect of prebiotics supplementation on the native probiotics showed that, the count of *Bifidobacterium* spp. significantly increased ($P \leq 0.01$) in lignin and mannan groups compared to the control group at all three periods of age. Moreover, mannan was more potent for increasing *Bifidobacterium* compared to lignin group at all the tested bird age.

Counts of *Lactobacillus* spp. significantly increased ($P \leq 0.05$) in lignin and mannan groups during all expermintal periods compared to the control group. Also, the values were significantly increased ($P \leq 0.05$) in lignin group compared to mannan group at 3, 6 and 9 weeks of age. But, the results showed that total aerobic bacteria and *E. coli* were decreased with the advancing of age, whereas, count of *Lactobacillus* spp. and *Bifidobacterium* spp. increased with the advancing of age.

A similar trend was observed, as the count of *E.coli* non-significantly decreased in lignin and mannan groups during all the experimental periods compared to the control group. Supplementation induced a significant reduction in *E. coli* count than the antibiotic supplemented and control group. While, the antimicrobial growth parameter-supplemented group induced a significant reduction in the total aerobic count than the prebiotic and control groups.

On the other hand, pathogenic microbiota decreased in response to prebiotic supplementation. *Escherichia coli* was reported to decrease in response to prebiotic mannan oligosaccharide-supplementation (Gouveia *et al*., 2006; Swanson and Fahey, 2006; Middelbos *et al*., 2007). Also, other studies reported a reduction in *E.coli* count with mannan oligosaccharide supplementation (Xu *et al.,* 2003; Zdunczyk *et al*., 2005; Baurhoo *et al*., 2007a,b).

Lignin and Mannan oligosaccharides have been found to have beneficial effects on broiler. They have been shown to stimulate beneficial bacteria while also having a negative effect on pathogenic bacteria, *e.g. Escherichia coli* and *Salmonella* spp. in the broiler gut (Spring *et al*., 2000; Baurhoo *et al*., 2007a). However, the results of Yang *et al*. (2008) reported decrease in ilial and cecal lactobacilli with mannan oligosaccharide supplementation. Langlands *et al.* (2004) found that the prebiotics oligosaccharides changed bacterial species numbers in both the proximal and distal gut, significantly increasing bifidobacteria, lactobacilli, while decreasing clostridia in the distal gut (Langlands *et al*., 2004). A large number of authors observed changes in microbiota reported increased bifidobacteria (Swanson and Fahey, 2006 and Middelbos *et al.,* 2007). The reason for this might be that prebiotic could increase the population of

Table 2. Changes in bacterial counts (log cfu/ml) in cecum of Japanes quail as affected by dietary supplementation of mannan-oligosaccharides and lignin

Treated birds	Age (week)	T. aerobic bacteria	*E. coli*	*Bifidobacterium* Spp.	*Lactobacillus* Spp.
Group 1 (control)	3 weeks	6.60±0.07	5.49^{a}±0.42	7.39^{c}±0.07	5.92^{c}±0.24
Group 2 (lignin)		6.38±0.06	4.42^{ab}±0.18	8.67^{b}±0.11	7.24^{b}±0.22
Group 3 (mannan)		6.25±0.12	3.68^{b}±0.19	9.75^{a}±0.08	8.02^{a}±0.13
Sig.		NS	*	**	**
Group 1 (control)	6 weeks	7.33±0.16	5.44^{a}±0.26	7.51^{c}±0.14	7.07^{c}±0.09
Group 2 (lignin)		6.69±0.62	4.61^{b}±0.03	9.50^{b}±0.02	8.43^{b}±0.02
Group 3 (mannan)		6.35±0.48	3.47^{c}±0.15	10.86^{a}±0.14	9.48^{a}±0.02
Sig.		NS	**	**	**
Group 1 (control)	9 weeks	7.71±0.00	5.12^{a}±0.19	7.66^{b}±0.09	7.97^{c}±0.13
Group 2 (lignin)		7.07±0.80	4.45^{b}±0.10	9.74^{a}±0.03	9.16^{b}±0.02
Group 3 (mannan)		6.75±0.49	3.37^{c}±0.13	10.27^{a}±0.02	10.37^{a}±0.03
Sig.		NS	**	**	**

Means in the same column within each classification bearing different letters are significantly different.

NS = Not significant, * ($P \leq 0.05$) and ** ($P \leq 0.01$).

bifidobacteria and other beneficial microorganisms and they would occupy the adhesion sites in the intestine and inhibit the adhesion of pathogenic bacteria in the intestine through competitive expulsion (Guan *et al*., 2011).

Susceptibility of bifidobacteria and lactobacilli isolates to antimicrobial agents

The resistance patterns of the six isolates (Ma5, Mc22, Mb_{15}, $Lb_{20,}$ La_9 and Lc_{25}) against 15 antibiotics are shown in Table 3. Generally, there was a variation in the susceptibility of the bacterial isolates to antibiotics, as well as in the levels of strength of the tested antibiotics. Based on the average zone of inhibition for each antibiotic with all tested bacteria, Table 3 shows that ciprofloxacin (CIP) was the strongest one, reaching up to 32 mm followed by vancomycin (VA) reaching up to 22 mm. On the other hand, cefotaxime (CTX) was the weakest toward the tested bacteria reaching up to 09 mm, followed by kanamycin (K) and streptomycin (S) reaching up to 10 mm and erythromycin (E) reaching up to 11 mm. Regarding, the bacterial isolates (Ma_5) was the most sensitive to the tested antibiotics reaching an average inhibition of 18 mm, followed by (Lc_{25}) reaching up to 17 mm. While, La_9 was the most resistant bacteria to the tested antibiotics reaching up to 09 mm, followed by (Mb_{15}) and (Lb_{20}) reaching up to 14 mm. Despite the adverse effects of adding antibiotics to the broilers feed, it is stille a common practice in chicken rearing. Testing the antibiotic resistance of these isolated lactic acid bacteria in this study is of special significance, since the more resistant the bacterial strains, the more likely to persist in the colon of the chickens, and there by, impose their nowadays, its positive probiotic effect on the birds health.

Antibacterial Activity of the Isolated Probiotic Bacteria

Results in Table 4 show that antibacterial activity of the isolated bacteria from cecum of Japanes quails. All of the isolates were proved to be effective against all indicator pathogens used in this study. The antibacterial activity of (La_9) showed the highest inhibition zone against Gram

Table 3. Susceptibility of bifidobacteria and lactobacilli isolates to antimicrobial agents using the disc diffusion method

Tested bacteria	CN[1]	N	AX	S	E	G	CTX	C	VA	AN	SXT	TE	K	CIP	AM	Average of inhibition zone
Isolate (Lc_{25})	23	14	11	07	14	12	00	13	24	20	20	18	16	35	24	17
Isolate (La_{9})	02	00	00	21	08	14	00	00	33	02	00	19	00	34	00	09
Isolate (Lb_{20})	13	11	10	00	19	23	20	00	00	26	00	21	12	31	26	14
Isolate (Mb_{15})	12	07	40	20	14	06	16	14	19	12	00	18	00	32	00	14
Isolate (Mc_{22})	00	23	17	04	00	21	00	12	30	00	24	30	12	30	22	15
Isolate (Ma_{5})	21	15	12	12	13	14	19	33	24	20	25	19	17	30	00	18
Average antibiotics strength tested	12	12	15	10	11	15	09	12	22	13	12	20	10	32	12	

1: CN, Gentamicin; N, Neomycin; AX, Amoxicillin; S , Streptomycin; E, Erythromycin; G, Penicillin; CTX, Cefotaxime; C, Chloramphenicol; VA, Vancomycin; AN, Aminosidin; SXT, Trimethoprim/ Sulphamethoxazole; TE, Tetracycline ;K, Kanamycin; CIP, Ciprofloxacin ; AM, Ampicillin .

Table 4. Antibacterial activity of probiotic bacteria isolated from cecum of Japanes quail against some indicator pathogens

Indicator pathogen / Bacterial isolate	Isolate (Lc_{25})	Isolate (La_{9})	Isolate (Lb_{20})	Isolate (Mb_{15})	Isolate (Mc_{22})	Isolate (Ma_{5})	Average of inhibition zone
Listeria monocytogenese	1.7	1.5	1.4	1.6	1.7	1.7	1.6
Salmonella enteric	1.6	1.7	1.4	1.4	1.3	1.4	1.5
E.coli O157:H7	1.3	1.0	1.2	0.6	0.7	0.8	0.9
***Enterobacter cloacae* subsp. *dissolvens*.**	1.7	1.6	1.6	1.6	1.3	1.3	1.5
Enterobacter aerogenes	1.7	1.7	1.6	1.3	1.4	1.5	1.5
Maicrococcus caseolyticus	1.5	2.2	1.5	2.0	1.5	1.9	1.8
Aeromounas hedrophili	1.7	1.3	1.3	1.6	1.2	1.3	1.4
Staphylococcus aureus	1.8	1.5	1.6	1.0	1.3	1.1	1.4
Staphylococcus H	1.4	1.5	1.3	1.4	1.4	1.6	1.4
Average of bacteria isolates strength tested	1.6	1.5	1.4	1.3	1.3	1.4	

positive *Macrococcus caseolyticus* (22 mm), while showed the lowest inhibition zone against the gram negative bacteria *E.coli* (10 mm). Average inhibition zone aginst *Listeria monocytogenese* and *Macrococcus caseolyticus* reached to (1.6 mm) and (1.8 mm), respectively, whereas, at *Enterobacter aerogenes* reached to (1.5 mm). The antibacterial activity of the crude supernatant fluids obtained from LAB culutures was generally greater against the Gram positive than the Gram negative tested bacteria. Similar antibacterial results, were obtained by Zhu *et al*. (2012), who obtained that this effect could be explained on the bases of the difference in the chemical composition of the bacterial cell well. In Gram positive bacteria, as much as 90% of the cell wall consists of pepedoglycan, beside teichoic acids while in Gram negative only about 10% of the total cell wall consists of peptidoglycan. Instead, most of the gram negative cell wall is composed of the outer membrane containing polysaccharides linked to lipids to form a complex called lipopolysaccharide (Madigan *et al.*, 2012).

Mannan oligosaccharides are able to bind with certain intestinal microbiota due to the mannan component attachment of microbiota to the intestinal cells (Spring *et al*., 2000). Also, Mannan oligosaccharide reduces pathogen colonization by acting as an analogous receptor to type I fimbriae and by decreasing the number of binding sites in the intestin (Patterson and Burkholder, 2003).

Identification of Bacterial Isolates

The results for the identification of the aforementioned isolates based on the biochemical tests shown by Martin-Carnahan and Joseph (2005) are presented in Table 5. The isolates were identified as *Bifidobacterium longum* subspp.. *infantis* (La_9), *Bifidobacterium animalis* subspp. *lactis* (Mb_{15}) and *Lactobacillus acidophilus* (Lb_{20}). The identification was primarily based on the biochemical tests then the sequencing of the 16S rRNA gene was employed only with three isolates to confirm the identification process and these strains were identified as which as *Bifidobacterium longum* subspp. *infantis*, *Bifidobacterium animalis* subspp.. *Lactis* and *Lactobacillus acidophilus*. These strains were isolated from cecum of Japanes quails with mannan and lignin addition at 6 weeks of age.

16S rRNA Identification of the bacterial Isolates

Three of these isolates were selected as they were the most resistant for antibiotics and had higher ability for antibacterial activity against pathogenic bacteria. Generally, sequencing of the 16S rRNA gene has proven to be valuable in the identification of *bifidobacterium* spp. and *Lactobacillus* spp. Analysis of 16S rRNA gene was considered to be a rapid and powerful method for identifying isolates of *bifidobacterium* spp. and *Lactobacillus* spp. to the species level (Borrell *et al*., 1997). The amplified 16S rDNA gene products of representative isolates (n=6) from each identified group in 16S rDNA were sequenced using primer F:-AGA GTT TGA TCC TGG CTC AG, R:-GGT TAC CTT GTT ACG ACT T., from a commercial sequencing facility (Sigma Labs). The sequences were aligned independently and phylogenetically analysed using GATC Company by use ABI 3730xl DNA sequencer [Gene JET™] (Saitou and Nei, 1987). The sequencing of the 16S rDNA gene showed that the closest strain relatedness of *Bifidobacterium longum* subsp. *infantis* (La_9), *Bifidobacterium animalis* subsp. *lactis* (Mb_{15}) and *Lactobacillus acidophilus* (Lb_{20}) as showen in Table 6.

Conclusion

Supplementation of the diet with Mannanoligosaccharide (MOS at 0.2%) or Lignin (L at 1.25%) can enhance quail immunology by selectively stimulating the beneficial intestinal microflora. Specially adding mannan which can increase count of beneficial bacteria such as bifidobacterium and lactobacilli in the ceca. MOS also resulted in a major reduction in *E.coli* counts. In this investigation, selected characteristics of the physiological functionalities of *Lactobacillus* spp. and *Bifidobacterium* spp. were recorded *in vitro* tests of the above bacterial strains did not support their classification among probiotics, results of selected *in vitro* microbiological tests nevertheless suggested that the tested microorganisms show some abilites ascribed to probiotic microorganisms. The characteristics of the strains mentioned are assumed to be futher studied.

Table 5. Biochemical properties of the six isolated bacteria from cecum of Japanes quails at different stages of age

Characteristic	Bacterial isolates					
	Ma_5	La_9	Mb_{15}	Lb_{20}	Mc_{22}	Lc_{25}
Indole production	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+
Voges proskauer	-	-	-	-	-	-
Citrate utilization	+	+	+	+	-	-
H_2S production	+	+	+	+	+	-
Urea hydrolysis	-	-	-	-	-	-
Motility	-	-	-	-	-	+
Acid mannitol	+	+	+	+	+	+
Acid glucose	+	+	+	+	+	+
Gas glucose	+	+	+	+	+	-
Oxidases	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Inositol	+	+	+	+	+	-
Raffinose	-	-	-	-	-	-
Mannose	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+
Trehalose	+	+	+	-	-	+
Ribose	-	-	-	-	-	-
Rhuminose	-	-	-	-	-	-
Cellobiose	-	-	-	-	-	-
Catalase	+	+	+	+	+	+

Ma_5 : isolate from mannan group at 3 weeks of age. La_9 : isolate from lignin group at 3 weeks of age.
Mb_{15}: isolate from mannan group at 6 weeks of age. Lb_{20} : isolate from lignin group at 6 weeks of age.
Mc_{22}: isolate from mannan group at 9 weeks of age. Lc_{25} : isolate from lignin group at 9 weeks of age.

Table 6. Identification of three *Bifidobacterium and Lactobacillus* species

Isolate	Identification by biochemical and 16S rRNA	International bacterial strains
La_9	*Bifidobacterium longum* subsp. *infantis*	*Bifidobacterium longum* subsp. *Infantis* strain ATCC 15697 16S ribosomal RNA gene, partial sequence.
Mb_{15}	*Bifidobacterium animalis* subsp. *lactis*	*Bifidobacterium animalis* subsp. *lactis* strain YIT 4121 16S ribosomal RNA gene, partial sequence
Lb_{20}	*Lactobacillus acidophilus* NCFM strain NCFM	*Lactobacillus acidophilus* NCFM strain NCFM, 16S ribosomal RNA gene, complete sequence

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تأثير التغذية بالمنان عديد التسكر واللجنين على مجموعات وخصائص البكتيريا المساعدة الحيوية المعزولة من السمان الياباني

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أصبح للميكروبات المعوية أهمية ودور فعال في صحة الحيوان وذلك للحد من استخدام المضادات الحيوية لما لها من تأثير ضار، وهذه الدراسة تربط بين الميكروفلورا المعوية وأداء الطائر كنتيجة لإضافة البريبايوتيك (اللجنين والمنان) للعلائق، ولقد وجد أن إضافة البريبايوتيك أدى إلى أدني عدد للبكتريا الهوائية ظهر في المعاملة بالمنان واللجنين مقارنة بالكنترول وذلك خلال جميع مراحل التجربة، وبصفة عامة فان أعداد البفيدوبكتريا واللاكتوباسيلس تزداد زيادة معنوية في المعاملة باللجنين والمنان مقارنة بالكنترول في كل الفترات حيث كانت أعلى زيادة في الأسبوع التاسع بينما تكون الزيادة غير معنوية في معاملة اللجنين مقارنة بالمنان في نفس عمر الطائر وان كان استخدام المنان قد أعطي زيادة في كلا الميكروبين عند استخدام اللجنين في نفس عمر الطائر، وكان أكثر المضادات الحيوية تأثيراً على البكتريا المعزولة هو مضاد الثبروفلوكساسين حيث وصل قطر منطقه التثبيط إلى ٣٢مم يليه مضاد الفانكومايسين والذي وصل إلى ٢٢مم وأخيراً التتراسيكلين الذي وصل إلى ٢٠مم وكان تأثير البكتريا المعزولة علي البكتريا المرضية الموجبة لجرام أكثر وضوحا عنها في السالبة لجرام، وباستخدام تقنية ال 16s rRNA تم التعرف على ثلاثة من البكتريا المعزولة وكانت كالتالي: *Bifidobacterium longum* subsp. *infantis, Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus acidophilus*.

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