Effect of probiotics on some aspects of quail's health

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Abstract

The study was carried out to explained the effect of probiotic (protexin® and lactobacillus acidophilus), Saccharomyces cerevisiae (prebiotic) and immunmodulator (levamisole) in feed additive and in drinking water on immune response post vaccination and challenge with Newcastle disease virus. Three hundred and fifty quails were divided into five groups contained 50 chicks and as follows: Group 1: Received Saccharomyces cerevisiae in drinking water from day 1 to the day 45 in dose (107 CFU/ml). Group 2: Received Levamisol with dose (10 µg/kg b.w.) in drinking water from 1 day to the 45 days. Group 3: Received protxin® (Probiotic) in feed from 1 day to the 45 days in dose (0.5 g /kg feed). Group 4: Received lactobacilli isolated from quail in drinking water from day 1-45 of the experiment in dose (107 CFU/ml). Group5: Received lactobacilli isolated from quail in drinking water from day 1-3 of the experiment in dose (107 CFU/ml). Group 6: Control positive vaccinated not treated. Group6: Control negative not vaccinated and not treated. At age 10 and 20 day All quail groups were vaccinated except the control negative with NDV vaccine La Sota strain (Intervet-Holland). At age 42 day (sexual maturity), start collect the eggs products for hatching. ELISA in day 4 of the offspring will carry on estimating maternal immunity for ND. Isolate bacteria from yolk sac of the first chick offspring. Blood samples all groups, blood samples will be collected at day 4 to check the maternal Immunity for NDV and at day (20 and 30, 45, 75, 80 and 85) to check NDV titer by ELISA. Swabs will be taken from cloacae at (20 and 40) days in all groups to check Salmonella and E. coli. Challenge with NDV will take place on day 75. The obtained results referred Ability to use the lactic acid bacteria isolate as probiotics and its metabolites as prebiotics in quail chicks. High growth rates parameters in quail chicks. Significant enhancement the immune response against ND post vaccination and post challenge with field virulent isolate of NDV. Efficacy in improving the intestinal microbial balance. Challenge with $100LD_{50}$ NDV (10^6) at day 75 caused high morbidity and no mortality in all vaccinated group but high mortality and morbidity in control negative. (protexin®, sacchromyces cervisicea, Lactobacillus acidophilus and levamisole) did not protect the chicks from infection with virulent NDV but they reduced the severity of infection.

Key word: Newcastle disease, spray particles size, quail's, ELISA, viral load.

تأثير المعززات الحيوية على بعض الأوجه الصحية في السمان

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الخلاصة

أجريت الدراسة لتوضيح تأثير بروبيوتيك (protexin® والعصيات اللبنية)، خميرة الخبز والمنظم المناعي (الليفاميزول) كإضافات علفية وفي ماء الشرب على الاستجابة المناعية بعد التلقيح والتحدي مع فيروس مرض نيوكاسل، ثلاثمائة وخمسين من طير السمان تم تقسيمها إلى سبعة مجاميع تتضمن 50 فرخة لكل مجموعة وعلى النحو التالي: المجموعة 1: أعطت خميرة الخبز في مياه الشرب من 1 إلى 45 يوم بجرعة (10^7 كغم/ مل). المجموعة 2: أعطت الليفاميزول بجرعة (10 ميكروغرام/ كغ من وزن الجسم) في مياه الشرب من 1 إلى 45 يوم.

المجموعة 3: أعطت protxin® (بروبيوتيك) في العلف من 1 إلى 45 يوم في جرعة (0.5 جم/ كجم علف). المجموعة 4: أعطت العصيات اللبنية معزولة من السمان في مياه الشرب من 1− 45 يوم من التجربة في جرعة (10^7 CFU/ مل). المجموعة 5: أعطت العصيات اللبنية معزولة من السمان في مياه الشرب من 1 − 3 يوم من هذه التجربة بجرعة (10^7 CFU/ مل). المجموعة 6: اعتبرت سيطرة موجبة تلقيح بدون علاج. المجموعة 7: اعتبرت سيطرة موجبة لا تلقيح ولا علاج. في عمر 10 و20 كل المجاميع لقحت بلقاح ND (لقاح لاسوتا في الماء) باستثناء المجموعة 7. مجاميع السمان، لقحت باستثناء مجموعة السيطرة السالبة بلقاح NDV لا سوتا سلالة (إنترفيت − هولندا). وفي عمر 42 يوم (النضج الجنسي)، بدء جمع البيض للتفقيس. أخذت عينات من الدم من الأفراخ بعر 4 يوم لتقييم المناعة الأمية ضد مرض النيوكاسل بواسطة فحص الاليزا. عزلت البكتريا من كيس المح من الأفراخ الفاقسة حديثا. جمعت عينات الدم من جميع المجاميع بعمر 4 يوم لتقييم المناعة الأمية ضد النيوكاسل. أخذت مسحات من المخرجية في (20 و40) يوما في جميع المجاميع للتحقق من وجود السالمونيلا والإشريشيا القولونية. والتحدي مع فايروس NDV في 75 يوم. النتائج التي تم الحصول عليها تشير القدرة إلى استخدام بكتيريا حمض اللاكتيك المعزولة كالبروبيوتيك وعناصره كما البريبايوتكس في أفراخ السمان. معدلات نمو مرتفعة في أفراخ السمان. زيادة كبيرة في الاستجابة المناعية بعد التلقيح والتحدي بالعزلة المحلية من فيروس نيوكاسل. كفاءة عالية في تحسين التوازن الميكروبي في الأمعاء. وتعطي ارتفاع في معدلات الوزن وكفاءة التحويل الغذائي مع انخفاض معدلات التحويل الغذائي بالمقارنة مع مجموعات السيطرة. التحدي مع NDV $100ELD_{50}$ (10^6) في يوم 75 تسبب ارتفاع معدلات الإصابة وبدون هلاكات في كل المجاميع الملقحة ولكن على العكس من ذلك، ارتفاع معدل الهلاكات والإصابة في مجموعة السيطرة السالبة. كل المواد المضافة (protexin®، sacchromyces cervisicea، العصيات اللبنية والليفاميزول) لا يحمي الدجاج من الإصابة بفيروس الضاري NDV لكنها قللت من شدة العدوى.

الكلمات المفتاحية: مرض النيوكاسل، حجم ذرات الرذاذ، السمان، الاليزا، الحمل الفايروسي.

Introduction

Japanese quail had gained attention in poultry industry as they are resistant to pathogens and a good producer of organic egg and meat for healthy nutrition in human and is being used as beneficial animal model in researches (1). The global paradigm is shifting from an emphasis on productive efficiency to one of public concerns (2), as long term use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria, accumulation of drug residues in the body of the birds, imbalance of normal microflora and finally, harmful effects on human health (3). The use of feed additives has two objectives: first the control of pathogenic microorganisms and second to enhance the digestive microflora with beneficial microorganism. Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (4). The probiotics were improved feed conversion for the target species, reduced morbidity or mortality and benefits for the consumer through improved product quality (5). Probiotics were enhanced the growth of many domestic animals improved the efficacy of forage digestion and quantity and quality of milk, meat and eggs, Probiotics protected animals against pathogens, enhanced immune response, reduced antibiotic use and shows high index of safety (5). The use of probiotics for poultry is based on the knowledge that the gut flora is involved in resistance to enteric infections including *E. coli*, *Salmonella*, and *Campylobacter*. Feeding probiotics helps maintain beneficial intestinal micro flora and may modulate the mucosal immune system enhancing the host's resistance to enteric pathogens (5).

Saccharomyces cerevisiae fermentation product showed an increase in immune function of broilers when fed for 42 day (6) Levamisole (LMS) is an optic isomer of the phenylimidothiazole salts of tetramisole. It has been found to possess immune stimulating effects (7). LMS has also been shown with several species to be potent immune stimulants in modulation of leukocyte cytotoxic activity (8), phagocytosis (9), respiratory burst, antibody response and macrophage activating factor (10). The present study was conducted to investigate the effects of Protexin® and *Lactobacillus acidophilus* alone (probiotic), *Saccharomyces Cerevisiae* (prebiotic) and levamisol on immune status against Newcastle disease (ND) in Japanese quail's for commercial production and *Salmonella* and *E. coli* isolation.

Materials and Methods

Three hundred and fifty quails were brought in good condition from Agricultural Research Service at Abu Ghraib. The quails were weighed at hatching 7-9 gm, and divided randomly into 7 groups, each group contained 50 quails in cages distance 120 x 70 cm, and the ground mattress with Sawdust litter and supplemented all management requirements as poultry hygiene standardization. The chicks were fed on starter diet from 1 to 38 days at the beginning of the experiment with fine grinding. The composition of the ration is: Corn, Di Calcium, Protein (vegetable source), Soybean meal, Salt, Lipid, Limestone and Wheat. Then final feed was used from 39 days until the end of the experiment (day 75) with coarse grinding: Corn, Dicalcium, Protein (vegetable source), Soybean meal, Salt, Lipid, Limestone and Wheat. Three hundred and fifty quails were divided into seven groups contained 50 chicks and as follows: Group 1: Received *Saccharomyces cerevisiae*in drinking water from day 1 to the day 45 in dose (10^7 CFU/ml). Group 2: Received Levamisol with dose (10 µg /kg b.w.) in drinking water from 1 day to the 45 days. Group 3: Received protxin® (Probiotic) in drinking water from 1 day to the 45 days in dose (1 g/Liter). Group 4: Received *lactobacilli* isolated from quail in drinking water from day 1-45 of the experiment in dose (10^7 CFU/ml). Group5: Received *lactobacilli* isolated from quail in drinking water from day 1-3 of the experiment in dose (10^7 CFU/ml). Group 6: Control positive. Group7: Control negative. At age 10 and 20 day all groups were vaccinated with ND vaccine (*La Sota* strain Intervet - Holland vaccine in water) except group 7 by manual oral drench in 10 days old regarding to the ELISA Abs titer against NDV (maternal immunity), the second NDV vaccine applied at 20 days old by the same procedure and in both vaccinations the vaccine was dissolved in water. The single dose after vaccine dissolving is 1 ml/ bird contained $10^{6.5}$ EID_{50}. At age 42 day (sexual maturity), start collect the eggs products for hatching.

1. ELISA in day 4 of the offspring was carried out to estimate ND maternal immunity.
2. Swabs have been taken from cloacae at 20 and 40 days all groups (experiment birds) to check *Salmonella* and *E. coli.* Isolate bacteria from yolk sac of the first chick offspring.
3. Blood samples from all groups have been collected at day 4 to check the maternal Immunity for NDV and at day 20, 30, 45, 75 and 80 to check NDV titer by ELISA.
4. Challenge with NDV took place on day 75.

- **Characterization and Differentiation of Lactobacillus (LAB):** Cultures were grown for 24 h on De man Regosa Sharp agar medium (MRS) at 30°C, isolated *Lactobacilli* were presumptively identified by conventional methods and further identified through standard API-50 CHL system (Biomerieux, France), catalase activity was tested, production of acid and CO2 from glucose was tested in MRS broth containing Dirhams tube with citrate omitted (11). Production of Ammonia in MRS broth omitting glucose and meat extract while containing 0.3% Arginine and

0.2% Sodium. Citrate replacing ammonium citrate was monitored using Nesslers reagent, Ability to ferment various carbohydrates was evaluated using MRS broth supplemented with filter sterilized sugar solutions to final concentration of 1% w/v and 0.004% phenol red without glucose and meat extract (12). Identification of species was confirmed using a standard identification system, API-50 CHL according to the manufacturer's (biomerieux, France) instructions. The dose of *Lactobacillus acidophilus 3* was determined 1×10^7 CFU/ml, according to method of Reed, and Muench (13), it was given orally in three doses at (1, 10 and 15) days of age.

- **Quail excreta processing:** Ten grams of quail excreta were added to 90 ml of pre-enrichment medium (Buffered peptone water), and was incubated for 24 h at 37 Cº. Isolation of *Salmonella* from quail excreta was done according to **(**14). Procedure of bacterial counting was done according to (15).
- **Serological Tests:** Blood samples were taking from the jugular vein at1, 7, 14, 21, 28, 34 and 40 days to determine the antibody titer against NDV, All groups were challenged with a virulent local strain (stock) NDV (ELD_{50} $10^{8.5}$) at 28 days old.
- **ELISA Test:** was carried out according to the manufacture company ProFlock® NDV ELISA kit (Synbiotics- USA).
- **Challenge Virus:** Velogenic local field isolate of ND virus was determined (HA, mean death time, ICPI and titration of ELD_{50}) then; the virus was used as challenge virus by giving $100ELD_{50}$ method (13). All the challenged birds were observed daily for 6-10 days post challenge, morbidity (respiratory and nervous signs) and/or mortality were recorded (16).
- **Probiotic:** (Protoxin®) is a highly concentrated pre-mix containing seven strains of bacteria and two yeasts *(Lactobacillus plantarum* 1.89×10^{10} cfu/kg, *Lactobacillus delbrueckii* subsp. *Bulgaricus* 3.09×10^{10} cfu/kg, *Lactobacillus acidophilus* 3.09×10^{10} cfu/kg, *Lactobacillus rhamnosus* 3.09×10^{10} cfu/kg, *Bifidobacterium bifidum* 3.00×10^{10} cfu/kg, *Streptococcus salivarius* subsp. *Thermophilus* 6.15×10^{10} cfu/kg, *Enterococcus faecium* 8.85×10^{10} cfu/kg, *Aspergillus oryza* 7.98×10^9 cfu/kg, *Candida pintolopesii* 7.98×10^9 cfu/kg) (17).
- **Statistical Analysis:** Data were subjected to analysis of variance (ANOVA) and means compared for significance using least significant difference (L.S.D) for comparative of means on a computer program (18).

Results and Discussion

Thirty samples have been collected from intestine and crop of quails, 11 samples were from intestine and 19 were from crop. Two spp of *Lactobacilli* were isolated from intestine and three from crop. These were designated as L1, L2, L3,L4 and L5 (Tab. 1). Identification was done by using conventional methods as well as through standard API-50 CHL system (Tab. 1). Pure isolates were maintained in MRS broth at - 20°C with 10% (v/v) glycerol as well as on MRS agar slants at 4ºC. This is also reported by other authors (19, 20).

Table (1) Morphology and biochemical tests

Isolates	Colony Morphology	Microscopic Examination	Catalase test	Growth at 15ºC	Growth at 45ºC	Lactose fermentation	Indole Test	MR Test	VP Test	Citrate Test
L1	Rough, convex, grayish white colonies	G +ve, rods, 4.0-5.0 µ, straight, singly, non spore forming	-	-	+	+	-	-	-	-
L2	regular, smooth, entire margin, grayish	G +ve, rods, 3.0-4.0 µ, singly and tapering ends non spore forming	-	-	+	+	-	-	-	-
L3	regular, small, translucent, grayish colonies	G +ve, rods, 2.0-4.0 µ, straight, singly and chains, non spore forming	-	-	+	+	-	-	-	-
L4	regular shaped, convex colonies with sharp margins	G +ve, rods 2.0-3.0µ, short chains, non spore forming	-	-	+	+	-	-	-	-
L5	regular, smooth, translucent, grayish colonies	G +ve, rods, 3.0-4.0 µ, singly and tapering ends non spore forming	-	-	+	+	-	-	-	-

Table (2) *api web V5.1* (API- 50 CHL system) results

Isolate	Species identification	ID%	Remarks
L1	*Lactobacillus rhamnosus*	**68.8**	Low identification
L2	*Lactobacillus acidophilus 3*	**96.7**	Excellent identification
L3	*Lactobacillus salivarius*	**61.3**	Low identification
L4	*Lactobacillus acidophilus 1*	**74.6**	Good identification
L5	*Lactobacillus Casi*	**85.9**	Good identification

Biochemical and api 50 CH test indicated that the bacteria is *Lactobacilli* and also microscopic examination, and as confirmed by (21). It is well known that *Lactobacillus acidophilus* is catalase negative. Table 1 show that the isolated bacteria could ferment maltose, lactose, sucrose and glucose, but not sorbitol and arabinose. The results obtained coincided with *L. acidophilus* strain characteristic (22). The crop content is largely consisted of *Lactobacilli*, enterococci and yeast (19). Many *Lactobacillus* strains, isolated from various sources, are being used as probiotic (23). ND infectivity titer was $10^{8.5}$ ELD_{50}/0.1 ml, following the method of (13) while mean death time was 48 hours according to (24) and intra cerebral pathogenicity index was found to be 1.75. Maternal immunity against NDV was (2354±81.9) which mean good immune response which is in agreement with (25) who reported that the eggs have received a large amount of Mab from their own breeders, and this was represented in the hatched chicks. The results of the current study revealed the presence of significant differences at level ($P<0.05$) among all groups in Ab titer against ND at (20, 35, 45, 75, 80 and 85) days old chicks table (3). Generally, all groups showed an increase in antibody titer except the control negative group (seventh) which reflected undetectable antibody titers by ELISA test. It is thought that vaccination at 10 days give a better result than if it was done earlier (26). The increase in Ab titers in all groups of different age (20, 35, 45 and 75) days compared with control positive (sixth) group was due to the role of protexin® these finding are in agreement with (27, 28, 29), the positive effect of probiotic on the immune response indicated the enhancement of the lactic acid bacteria (LAB) to an acquired immune response exerted by T and B lymphocyte. The direct effect of probiotic related to stimulate the lymphatic tissue, whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract (5, 29) and (30). *Sacchromyces cereivices* in in drinking water lead to increase Abs titer against ND, this result is in agreement with Asli (31). Levamisole (LMS) may act as a multifunctional modulator after immunization to mediate the cell-mediated response of T cells, and at the same time promote activated B cells to produce antibody; this is another possible method of LMS to stimulate immune system. Higher functional antibody level (HI) in chickens which has also been seen in our study, completely proves mentioned notions (32). The increasing of Abs titer of ND in third group agree with (31) who noticed, that the dietary supplementation with S*acchromyces cereivices* improved the immune response of the birds. Lactic acid bacteria activate the innate immune defense effectors functions and support specific response against infectious agents by up regulation of IgA (32). In general, feeding probiotics could improve antibody titers against Newcastle disease; infectious bursal disease virus (33). The present study explains the role of probiotic (protoxin®, lactobacillus and *saccharomyces cereivices*) and immune modulator (levamisol) in enhance the immunity (cellular and humoral) against the vaccination and infection with NDV, these results in agreement with (34, 35) who demonstrated that probiotics, reduce the replication of the virus and decrease the infectivity. (36) found that Protexin® treated chicks have a higher titer against NDV and no mortality post challenge as compared to untreated chicks. The presence of significant differences at level ($P<0.05$) among all groups in Ab titer against ND at three days old chicks of the young compared with the control negative (seventh) group which did not record any immune response table (4).

Table (3) Results of antibody titer against Newcastle disease in different days by ELISA test

Groups	20 days	30 days	45 days	75 days*	80 days	85 days	LSD
G1	1713.8±130.7 BC e	3452.2±155.5 B d	4735.6±314.4 B c	5855.6±274.2 BC b	4591.2±155.5 B c	7731.2±315.5 C a	773.7
G2	2049±145.4 AB e	3953±159 A d	5565.2±214.7 A c	6585.2±214 AB b	5103.2±169.7 B c	9183.2±302.8 B a	676.06
G3	2352.4±177.3 A e	4154.2±114.8 A d	6209.4±147.8 A c	7289.4±163.2 A b	5839±152.7 A c	10859±334.2 A a	634.73
G4	1653.6±107.6 BC e	2922±119.6 C d	4455±287.2 B c	5155±421.3 CD b	3885.8±219.8 C c	7085.8±269.5 CD a	847.55
G5	1522±142.9 C e	2653.6±107.6 C d	4024.4±195.1 BC c	4946.4±160.4 D b	3099.2±193.9 D d	6499.2±318.6 DE a	644.11
G6	1323.8±134.09 C e	2523.8±181.7 C d	3399±359.4 C c	4319±384 D b	2436.6±183.1 E d	5699.6±271.3 E a	876.22
G7	0±0 D	0±0 D	0±0 D	0±0 E	0±0 F	All dead	
LSD	422.42	425.75	788.08	861.96	540.27	906.09	

Means having different big letters (in columns) and small letters (in rows) are significant difference. (P<0.05).
***challenge with virulent local isolate of Newcastle disease.**

Table (4) Antibody titer against Newcastle disease in quail progeny by ELISA test

Groups	3 days
G1	2154.4±169.6 C
G2	2826.2±209.9 B
G3	3530.4±172.9 A
G4	1815.6±72.2 CD
G5	1601.6±132.6 DE
G6	1323.2±96.3 E
G7	0±0 F
LSD	**448.31**

Means having different big letters (in columns) are significant difference.

Using Protexin® and levamisole improved (P<0.05) the transmission of maternal antibody titer from dams to progeny. This result agree with who found that polysaccharide stimulate production of antibodies after immunization with live ND vaccine Aamir Ghafoor (37) remarked that use of polysaccharide preparation resulted in a significantly higher antibody levels against NDV. (38) found that antibody responses against NDV were higher in the lipopolysaccharide group. Maternal antibody titers in progeny were also influenced by this supplementation. The results of the current study refer to the presence of significant differences (P<0.05) among all groups in means of bacterial colonies (*E. coli* and *Salmonella*) at 20 and 40 days old chicks as compared with the control groups (sixth and seventh) that revealed a significantly increase (P<0.05) in mean of bacterial (Table 4).

Table (4) Results of bacterial count

Days / Groups	E. coli count		Salmonella count	
	20 days	**40 days**	**20 days**	**40 days**
1	15.4±1.14 D	15.4±1.14 D	0.6±0.24 BC	3.8±0.5 DC
2	19.4±0.40 BC	19.4±0.40 BC	0.8±0.37 BC	7.4±0.68 B
3	11.6±0.51 E	11.6±0.51 D	0±0 D	1.4±0.40 D
4	18±0.83 C	18±0.83 C	1.2±0.58 C	5.6±0.81 BC
5	20.4±0.24 B	20.4±0.24 B	1.6±0.24 B	8.2±1.39 B
6	26.2±0.58 A	26.2±0.58 A	3.8±0.37 A	12.8±1.4 A
7	26.4±0.40 A	26.4±0.40 A	4.2±0.37 A	13.8±0.80 A
LSD	1.525	5.786	1.027	2.756

Means having different big letters (in columns) are significant difference.

β-glucan components of *saccharomyces cerivicea*in in the first group might stimulate the gut-associated immune system by acting as a non pathogenic microbial antigen, giving an adjuvant-like effect the importance of digestive microbial antigen stimulation on the development of lymphoid organ tissue (39). The enhancement of β-glucan can be explained in part by the improvement of intestinal function or gut health through the increase of villi height, uniformity and integrity. β-glucans have been shown to improve immune response and to block bacterial adhesion (especially enteric pathogens) to gut lining (40, 39). Moreover, it has been suggested that mannanoligosaccharide (MOS), *saccharomyce scerivicea*a yeast cell wall derivative, also lactobacillus bacteria might inhibit the colonization of bacteria to the intestine by binding to bacterial mannan binding lectin or could act as bio regulator of the intestinal microflora and reinforcing the host natural defenses through the sanitary effect by increasing the colonization resistance and stimulation of the immune response (41).

Competitive exclusion (42) is also a popular strategy for preventing poultry from intestinal infectious disease due to the effective inhibition of pathogenic bacteria (43).

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