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Effects of ProbChick® on the immunological response after new castle virus using LaSota stain vaccination in broiler

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Abstract

The vaccination process and immunological status of chickens during their life period have great importance in the poultry industry. We aimed in the current study to evaluate the effect of ProbChick® on the immunological status of broiler chickens after vaccination with Newcastle vaccination using the LaSota strain. A total of 200 one-day-old chicks were divided randomly into five groups. The first group is the control group, while the second group consumes ProbChick® with drinking water. The third group was vaccinated with the Newcastle vaccine (LaSota strain), and the fourth group was vaccinated with the Newcastle vaccine after 7 days. The ProbChick® was consumed. The fifth group was vaccinated with the Newcastle vaccine and consumed ProbChick® on the same day. The result showed that ProbChick® enhances the weight gain, food conversion ratio, and relative weight of the bursa of Fabricius and spleen. In addition, ProbChick® will enhance the antibody titer if it is added to drinking water on the same day of vaccination and give suitable antibody titer compared to control groups and in comparison, to a group where it is added to drinking water after 7 days of vaccination. We conclude that adding ProbChick® to the broiler at 1 g/litter of drinking water at the same vaccination by Newcastle vaccine using the LaSota strain will enhance the immune response during and after vaccination. This will improve the efficiency and titer of antibody production.

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Introduction

Probiotics are live bacteria, fungi, or yeasts that replenish the gastrointestinal tract's flora and aid in maintaining a healthy digestive system, which promotes bird growth and is an alternative to antibiotics; probiotics are becoming more widely used in poultry diets (1). Probiotic is derived from two Greek words, pro and biotic, which mean for life (2). In 1965 Lilly and Stillwell were the first to use the word (3); according to a joint FAO/WHO work group, Probiotics are live microorganisms that, when supplied in suitable proportions, impart a health benefit to the host (4). Elie Metchnikoff was the first scientist who discovered that the microbiota present in the intestine plays a vital role in maintaining a healthy body when he found that the Lactobacillus bacteria that produce lactic acid present in fermented milk products were able to increase the longevity of Bulgarian peasants (5). Primarily, live apathogenic bacterial strains in animals and poultry are generally considered probiotics: *Lactobacillus acidophilus*, *L. sporogenes*, *L. bulgaricus*, and *Streptococcus thermophilus*; *Bacillus subtilis*, and *Saccharomyces cerevisiae* (6). Probiotics have various action mechanisms, such as inhibiting all pathogens by producing organic acids and antibacterial substances such as hydrogen peroxide, bacteriocins, and defensins (7). Probiotics compete with pathogenic bacteria on intestinal epithelial binding sites and essential nutrients (8); they enhance the immune response by releasing regulatory T cells, effector T and B cells, and antigen-presenting cells (9). ProbChick® is a food additive for poultry with multiple beneficial bacteria: *Lactobacillus Plantarum*, *L. sporogenes*, *L. acidophilus*, *Streptococcus*

thermophilus, Bacillus subtilis, Bifidobacterium bifidum, and Saccharomyces cerevisiae. They also contain digestive enzymes, which maintain good gut flora, leading to a high rate of feed utilization, increasing weight gain of the broiler, inhibiting the growth of pathogenic bacteria in the gut, and enhancing the broiler's immune response (10). Probiotic supplementation improved the immune response to the Newcastle disease virus (11). Also, it can boost macrophage and natural killer cell activity, regulate cytokine and immunoglobulin release, and help maintain the integrity of the intestinal epithelial barrier (12). In addition, B lymphocytes will be activated, transforming into antibody-secreting plasma cells, which will then infiltrate the gut-draining lymphoid tissue (13). We aimed in the current study to investigate the effect of ProbChick® as an immune enhancer during the vaccination process in broilers.

Materials and methods

Experimental designs

A total of 200 one-day-old chicks were included in the current study and divided randomly into five groups (40 chicks in each group), the first group was considered the control group left without any treatment all over the experiment. The second group (positive control) ProbChick® administration in drinking water from day one at a dose of 1 gram/liter, and the third group vaccinated with live attenuated la sota virus vaccine in drinking water on day 7 of age at dose $2.5*10^{6}$ (EID). The fourth group was vaccinated with la sota virus at day 7 of age at a dose of $2.5*10^{6}$ (EID) and of ProbChick® at day 14 of age at a dose of 1 gram/liter. The fifth group (protective group) was vaccinated with la sota virus on day 7 at a dose of $2.5*10^{6}$ (EID) and administration of ProbChick® on the same day at a dose of 1 gram/liter. Ten birds from each group were euthanized at the end of each first, second, third, and fourth week of age. A thin blood smear was stained with Wright-Giemsa to calculate the stress index (14). Weekly body weight, weekly feed consumption, and food conversion ratio (15,16).

Newcastle vaccine

A live, lyophilized vaccine was used for Newcastle virus, La sota strain, containing 10^{6} virus/dose. The vaccine was used according to the manufacturer's instructions. A vaccine package containing 1000 doses was used. It was dissolved in ten liters of clean, cold water, and 2 g/liter of skimmed milk was added to stabilize and increase vaccine efficacy. Chicks were thirsty for 3 hours before vaccination (17).

Stress index

This test was used to identify the effect of *E. coli* infection and administration of probiotic ProbChick® on non-specialized cellular immunity in chicks. This index was calculated by staining blood slide smear with Wright-Giemsa stain. The number of lymphocytes and heterophils was recorded for each slide, and the stress index was calculated as follows: heterophils/lymphocytes (18).

Weight gain, FCR, and relative weight of spleen and bursa

The chicks' weight was measured and recorded on the first day of hatching using an electronic scale, and then the results of the weight gain of all chicks were recorded every week and throughout the experiment period to get the weight of the remaining food as compared to the consumed food (19). At the end of the week, the rate of feed consumption was calculated for each group, then the feed conversion ratio (FCR) measures an animal's efficiency in converting feed into increased body mass (19). Spleen and bursa of Fabricius were removed and cleaned of adherent tissues. The weight of each was measured and expressed as relative to final body weight by organs weight/body weight*100 (20).

Newcastle antibody titer

The titer of antibodies against Newcastle disease was measured using the indirect ELISA, where 5 µl of the whole blood was taken and placed in a dilution plate, 200 µl of the dilution solution was added, then 10 µl of the dilution dish was taken. It was placed inside the test plate, then 90 µl of the previous diluted solution was added to it to reach a dilution ratio of 1:500. The test plate was incubated for 30 minutes at a temperature of 22-27ºC. The plate was washed three times, and then 100 µl of the conjugating solution was added and incubated for 30 minutes at a temperature of 22-27ºC. After that, the plate was washed three times, and 100 µl of substrate solution was added and incubated for 15 minutes, and then 100 µl of stopping solution was added. The plate result was read with an e-reader at wavelength 405 (21).

ProbCkick®

ProbChick® contains 10 billion CFU/gram strains of beneficial bacteria like *Lactobacillus Plantarum*, *Lactobacillus sporogenes*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Bacillus subtilis*, *Bifidobacterium bifidum*, and *Saccharomyces cerevisiae*.

Statistical analysis

The means included in the current study were analyzed using one-way ANOVA, with Duncan's post-Hock test as $P<0.05$.

Results

Average body weight

The effects of the LaSota vaccine and probiotic ProbChick® are shown in table 1. On day one, no significant variation of $P<0.05$ among the BW means in all groups. On week 1 and week 2, the chicks in G2 (positive control) showed the highest BW means of 162.6 gm and 446.5 gm, respectively, which is a significantly higher $P<0.05$ among

all groups. On weeks 3 and 4, the higher BW means are shown in G2 (positive control) 997.7 gm, 1610 gm, and then G5 905.8 gm, 1526.6 gm, respectively P<0.05 among other groups.

Body weight gain and feed conversion rate

The average weekly weight gain and feed conversion rate FCR are summarized in table 2. The effects of LaSota vaccine and ProbChick® on weekly weight gain at week 1 no significant variation P<0.05 among all groups. On week 2 and week 3, the G2 (positive control) are significantly higher P<0.05 among all groups. On Week 4, the chick in G2, G4, and G5 showed higher weekly body weight gain with no significant between them at P<0.05. The FCR was higher in G2, G5, and G4, 1.34, 1.38, and 1.39, respectively.

Spleen relative weight

The effects LaSota vaccine and ProbChick® on spleen relative weight are shown in table 3. Week 1 showed no significant difference between groups at P<0.05. On week 2, G3 and G4 are significantly higher P<0.05 than other groups with no significant difference. On week 3, the G3, G4, and G5 are significantly higher P<0.05 among control groups, and there is no significant variation between them. On week 4, the G3 was significantly higher P<0.05 than other groups, and the chick in G2 showed less value of spleen relative weight among all groups.

Table 1: Effects of LaSota vaccine and probiotic on weekly average body weight

Groups	Age (Average Body weight [gm] ± SD)				
	Day one	Week 1	Week 2	Week 3	Week 4
G1	40.1±0.4^{a}	158.6±0.7^{b}	417.2±2.7^{b}	899.2±4.8^{bc}	1456.6±7.5^{c}
G2	41.6±0.4^{a}	162.6±1.1^{a}	446.5±6.9^{a}	997.7±4.9^{a}	1610.0±6.9^{a}
G3	40.6±0.8^{a}	156.5±1.0^{b}	405.8±1.7^{b}	877.6±5.6^{d}	1446.6±8.5^{c}
G4	42.6±0.6^{a}	156.4±1.2^{b}	408.4±2.1^{b}	884.1±4.1^{cd}	1501.6±4.4^{bc}
G5	41.2±0.7^{a}	157.2±1.1^{b}	410.7±2.3^{b}	905.8±8.1^{b}	1526.6±9.1^{b}

-Different letters within the same column mean statistically significant differences at P<0.05.

Table 2: Effects of LaSota vaccine and probiotic on weekly body weight gain and FCR

Groups	Weeks (Average body weight gain [gm] ± SD)				FCR
	Week 1	Week 2	Week 3	Week 4	
G1	118.5±0.7^{a}	258.6±1.8^{b}	472.0±5.6^{b}	567.4±7.6^{b}	1.49±0.15
G2	121.0±0.8^{a}	283.9±1.7^{a}	551.2±5.9^{a}	612.3±5.6^{a}	1.34±0.21
G3	115.8±0.7^{a}	249.3±1.1^{b}	471.8±1.8^{b}	569.0±9.3^{b}	1.46±0.13
G4	113.8±0.6^{a}	252.0±0.9^{b}	475.7±1.3^{b}	617.5±6.1^{a}	1.39±0.16
G5	116.3±0.9^{a}	253.5±1.3^{b}	495.1±5.3^{b}	620.8±9.1^{a}	1.38±0.11

-Different letters within the same column mean statistically significant differences at P<0.05.

Table 3: Effects of LaSota vaccine and probiotic on spleen relative weight

Groups	weeks (spleen relative weight[gm] ± SD)			
	Week 1	Week 2	Week 3	Week 4
G1	0.075±0.001^{a}	0.077±0.005^{b}	0.076±0.002^{b}	0.072±0.005^{b}
G2	0.084±0.008^{a}	0.072±0.001^{b}	0.062±0.007^{b}	0.058±0.001^{c}
G3	0.085±0.003^{a}	0.086±0.002^{a}	0.079±0.003^{a}	0.073±0.003^{a}
G4	0.087±0.004^{a}	0.087±0.002^{a}	0.083±0.002^{a}	0.069±0.002^{b}
G5	0.085±0.001^{a}	0.079±0.002^{b}	0.089±0.002^{a}	0.067±0.001^{b}

-Different letters within the same column mean statistically significant differences at P<0.05.

Bursa of Fabricius (FB) relative weight

The effects of the LaSota vaccine and ProbChick® on relative weight are shown in table 4. On weeks 1, 2, and 3, the G3, G4, and G5 are significantly higher P<0.05 than the positive and negative control, with no significant difference between them. On week 4, the G1, G3, G4, and G5 are significantly higher P<0.05 than G2 (positive control), with no significant difference between them.

Stress index

The effects LaSota vaccine and ProbChick® on stress index are shown in table 5. On day one, old chick, no significant variation P<0.05 among the stress index mean means in all groups. In Week 1, the G5 showed less value with a significant difference among all groups. On week 2, the G1, G2, and G3 are significantly higher P<0.05 than all other groups, followed by G4 and G5 with no significant difference. On week 3 and week 4, G1 is significantly higher, P<0.05, than all other groups, and the G5 of both weeks showed statistically less value among all groups.

New castle antibody titer

The effects of ProbChick® on the Newcastle antibody titer range are shown in table 6. Day one and week one showed no significant variation P<0.05 among the ND titer means in all groups. On week 2, the G3, G4, and G5 show a high titer range of antibodies with no significant difference. On week 3 and week 4, the highest antibody titer in G4 than the G5 and G3, respectively, with a significant difference between them at $p < 0.05$.

Table 4: Effects of LaSota vaccine and probiotic on Bursa of Fabricius relative weight

Groups	Weeks (FB relative weight[gm] ± SD)			
	Week 1	Week 2	Week 3	Week 4
G1	0.144±0.010[b]	0.139±0.019[b]	0.166±0.013[b]	0.122±0.012[a]
G2	0.157±0.018[b]	0.130±0.011[b]	0.114±0.017[c]	0.108±0.011[b]
G3	0.162±0.014[a]	0.167±0.012[a]	0.191±0.014[a]	0.128±0.020[a]
G4	0.164±0.017[a]	0.167±0.013[a]	0.190±0.015[a]	0.124±0.019[a]
G5	0.163±0.013[a]	0.169±0.010[a]	0.181±0.014[a]	0.119±0.014[a]

-Different letters within the same column mean statistically significant differences at P<0.05.

Table 5: Effects of LaSota vaccine and probiotic on relative kidney weight

Groups	Stress index mean ± SD				
	Day one	Week 1	Week 2	Week 3	Week 4
G1	0.77±0.014[a]	0.52±0.019[a]	0.59±0.016[a]	0.73±0.017[a]	0.76±0.018[a]
G2	0.72±0.012[a]	0.52±0.017[a]	0.58±0.014[a]	0.51±0.010[b]	0.52±0.015[b]
G3	0.79±0.021[a]	0.51±0.021[a]	0.58±0.013[a]	0.46±0.010[c]	0.50±0.015[b]
G4	0.77±0.016[a]	0.50±0.012[a]	0.46±0.019[b]	0.45±0.011[c]	0.50±0.012[b]
G5	0.71±0.012[a]	0.33±0.016[b]	0.35±0.011[c]	0.32±0.014[d]	0.33±0.017[c]

-Different letters within the same column mean statistically significant differences at P<0.05.

Table 6: Effects of probiotic on antibody titer for Newcastle virus using indirect ELISA test

Groups	ND antibody titer range mean ± SD				
	Day one	Week 1	Week 2	Week 3	Week 4
G1	4617±156[a]	1983±167[a]	1617±243[b]	330±15[d]	153±17[d]
G2	4364±164[a]	1971±170[a]	1591±187[b]	221±17[d]	199±12[d]
G3	4281±154[a]	1999±201[a]	8700±610[a]	3281±307[c]	1099±121[c]
G4	4678±155[a]	1926±198[a]	8984±646[a]	6522±478[a]	2562±120[a]
G5	4522±148[a]	1962±155[a]	8805±631[a]	5678±417[b]	1263±119[b]

-Different letters within the same column mean statistically significant differences at P<0.05.

Discussion

The Newcastle vaccine in broilers did not affect the average weekly weight, weight gain, and feed conversion ratio compared to the control group. The mechanism through which the probiotic works in improving the weight gain and, as a result, the feed conversion ratio is that giving the beneficial bacteria in poultry will increase in number in the small and large intestines, which helps significantly in improving the quality of the natural flora in the intestines of these birds and improving its ph in the middle of the intestine which is Close to neutral. These changes improve the food digestion and absorption from the intestine and its introduction into the blood circulation of poultry (22).

As for the relative weight of the spleen and the bursae of Fabricia, the results of the current study indicated that giving the probiotic has no effect on the relative weight of these two organs in the group given the probiotic only compared to the

control group. The groups that received the vaccine dose against Newcastle disease Using the Lasota strain alone or with the probiotic showed an increase in the relative weight of each of the spleen and bursae of Fabricia compared with the control group and the group given the probiotic alone. This result was in agreement with many other studies that indicated that the poultry vaccination process increases the organs' weight. Primary immunoglobulins such as the spleen, the bursae of Fabricia, and the thymus gland will be responsible for the immune response and the production of antibodies that help immunize the bird through vaccination (23,24).

The results of the current study indicated that giving the probiotic reduced the stress factor in the groups that were given the probiotic alone or on the same day of vaccination or seven days after it compared to the control group. The immune system will, in turn, cause the production of additional amounts of antibodies in the bird's body (25).

The results of the current study indicated that the level of antibodies in the group that was given the Newcastle vaccine and the probiotic on the seventh day caused an increase in the level of antibodies. They were observed in the second week, and then there was a gradual decrease in the following weeks, but the level of decrease in the concentration of antibodies in the blood was less than What was observed in the other groups. The natural flora in the intestines of birds plays an essential role in the formation and function of the immune system in poultry (26), as the commensal bacterial present in the intestine and adjacent to the intestinal cells have an essential role in stimulating the intestinal immune tissue (27). Moreover, it is also due to the oral administration of probiotics in poultry that activates the spleen's natural killer cells and stimulates these cells' phagocytic properties (28). In addition to the birds that were given probiotics containing yeast and lactobacilli bacteria whose serum contained a higher concentration of antibodies against sheep red blood cells (as an immune stimulus) than the birds that did not receive these probiotics (29), and the administration of probiotics had a significant effect on the response. Immunogenicity increases the antibody concentration when immunizing broiler chicks against Newcastle disease, infectious bronchitis, and bird flu (29).

Conclusions

We conclude that adding ProbChick® to the broiler at 1 g/litter of drinking water will enhance the immune response during and after vaccination. This will improve the efficiency and titer of antibody production.

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Conflict of interest

No conflict.

References

1- Jha R, Das R, Oak S, Mishra P. Probiotics in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. Anim. 2020;10(10):23-30. DOI: 10.3390/ani10101863

2- Gibson GR, Fulle, R. Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. J Nutrit. 2000;130(2):391S–395S. DOI: 10.1093/jn/130.2.391s

3- Popova T. Effect of probiotics in poultry for improving meat quality. Curr Opinion Food Sci. 2017;14:72-77. https://doi.org/10.1016/j.cofs.2017.01.008

4- FAO/WHO. Health and nutritional properties of probiotics in food, including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria;FAO/WHO:Amerian Córdoba Park Hotel, Córdoba, Argentina;2001. 1-34 p. [available at]

5- Abd El-Hack ME, El-Saadony MT, Shafi ME, Qattan SYA, Batiha GE, Khafaga AF, Abdel-Moneim AE, Alagawany M. Probiotics in poultry feed: A comprehensive review. J Anim Physiol Anim Nutr (Berl). 2020;104(6):1835-1850. https://doi.org/10.1111/jpn.13454

6- Dhama K, Verma V, Sawant PM, Tiwari R, Vaid RK, Chauhan RS. Applications of probiotics in poultry:Enhancing immunity and beneficial effects on production performances and health-A review. J Immunol Immunopathol. 2011;13(1):1-19. [available at]

7- Tiwari G, Tiwari R, Pandey S, Pandey P. Promising future of probiotics for human health: Current scenario. Chronicles Young Sci. 2012;3(1):34-44. DOI: 10.4103/2229-5186.94308

8- Alagawany M, Abd El-Hack ME, Farag MR, Sachan S, Karthik K, Dhama K. The use of probiotics as eco-friendly alternatives for antibiotics in poultry nutrition. Environ Sci Pollut Res. 2018;25(11):10611-10618. DOI: 10.1007/s11356-018-1687-x

9- Oelschlaeger TA. Mechanisms of probiotic actions – A review. Inter J Med Microbiol. 2010;300(1):57-62. DOI: 10.1016/j.ijmm.2009.08.005

10- Matsuzaki T, Yamazaki R, Hashimoto S, Yokokura T. The effects of oral feeding of Lactobacillus casei strain Shirota on immunoglobulin E production in mice. J Dairy Sci. 1998;81:48-53. DOI: 10.3168/jds.S0022-0302(98)75549-3

11- Khan SH, Rehman A, Sardar R, Khawaja T. The effect of probiotic supplementation on the growth performance, blood biochemistry and immune response of reciprocal F1 crossbred (Rhode Island Red× Fayoumi) cockerels. J Appl Anim Res. 2013;41(4):417-26. DOI: 10.1080/09712119.2013.792732

12- La Fata G, Weber P, Mohajeri MH. Probiotics and the gut immune system: Indirect regulation. Probiotics Antimicrob Proteins. 2018;10(1):11-21. DOI: 10.1007/s12602-017-9322-6

13- Park YH, Hamidon F, Rajangan C, Soh KP, Gan CY, Lim TS, Abdullah WN, Liong MT. Application of probiotics for the production of safe and high-quality poultry meat. Korean J Food Sci Anim Resources. 2016;36(5):567. DOI: 10.5851/kosfa.2016.36.5.567

14- Luna LG. Manual of histological staining methods of the Armed Forces Institute of Pathology. 3rd ed. New York: The Blakiston Division, McGraw- Hill Book Company;1968. 32-68 p.

15- Shewita RS, Ahmed HA. Influence of dietary phytase and multiple enzymes supplementations on growth performance, carcass characteristics, and immune response in Japanese quail. Am J Life Sci Res. 2015;3(1):112-127. [available at]

16- Al-Sabaawy HB, Rahawi AM, Al-Mahmood SS. Standard techniques for formalin-fixed paraffin-embedded tissue: A Pathologist's perspective. Iraqi J Vet Sci. 2021;35(III):127-135. DOI: 10.33899/ijvs.2021.131918.2023

تأثير المعزز الحياتي على الاستجابة المناعية بعد استخدام لقاح نيوكاسل عترة لاسوتا في فروج اللحم

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الخلاصة

إن عملية التلقيح والحالة المناعية للدجاج خلال فترة التربية لها أهمية كبيرة في صناعة الدواجن. إذ هدفت الدراسة الحالية تقييم تأثير المعزز الحيوي على الحالة المناعية في الدجاج المعد للتسمين بعد التلقيح بلقاح نيوكاسل باستخدام عترة لاسوتا. تم استخدام ٢٠٠ طائر بعمر يوم واحد وقسمت بشكل عشوائي إلى خمس مجاميع. عدت المجموعة الأولى مجموعة سيطرة والمجموعة الثانية استخدم المعزز الحيوي مع مياه الشرب، المجموعة الثالثة تم تلقيحها بلقاح نيوكاسل (عترة لاسوتا)، المجموعة الرابعة التي تم تلقيحها بلقاح نيوكاسل وبعد ٧ أيام تم استخدام المعزز الحيوي، أما المجموعة الخامسة تم تلقيحها بلقاح نيوكاسل مع المعزز الحيوي في نفس اليوم. أظهرت النتيجة أن المعزز الحيوي يعزز زيادة الوزن ومعامل التحويل الغذائي والوزن النسبي لجراب فابريشيا والطحال، بالإضافة إلى أن المعزز الحيوي عمل على زيادة معيارية الأجسام المضادة في المجموعة الملقحة والمعطاة المعزز الحيوي في نفس اليوم بالمقارنة مع مجموعة السيطرة والمجموعة الملقحة والمعطاة المعزز الحيوي بعد ٧ أيام من التلقيح. نستنتج أن إضافة المعزز الحيوي إلى دجاج اللحم بجرعة ١ جم / لتر من ماء الشرب مع التلقيح بلقاح نيوكاسل باستخدام عترة لاسوتا سيعزز من الاستجابة المناعية أثناء التلقيح وبعده، مما يؤدي ذلك إلى تحسين كفاءة ومعدل إنتاج الأجسام المضادة.

17- Khalifeh MS, Amawi MM, Abu-Basha EA, Yonis IB. Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poult Sci. 2009;88(10):2118-24. DOI: 10.3382/ps.2009-00215

18- Gross WB, Siegel PB, Dubose RT. Some effects of feeding corticosterone to chickens. Poult Sci. 1980;59:516-522. DOI: 10.3382/ps.0590516

19- Bansal GR, Singh VP, Sachan N. Effect of probiotic supplementation on the performance of broilers. Asian J Anim Sci. 2011;5(4):277-84. [available at]

20- Hossain MM, Begum M, Kim IH. Effect of *Bacillus subtilis*, *Clostridium butyricum*, and *Lactobacillus acidophilus* endospores on growth performance, nutrient digestibility, meat quality, relative organ weight, microbial shedding, and excreta noxious gas emission in broilers. Vet Med. 2015;60(2):77-86. [available at]

21- Bell JG, Lelenta M. An ELISA for the detection of antibodies against Newcastle disease virus in African village poultry. Inter Atomic Energy Agency. 2002;1:174-8. [available at]

22-Edens FW. An alternative for antibiotic se in poultry: Probiotics. Brazil J Poult Sci. 2003;5:75-97. DOI: 10.1016/j.psj.2022.101696

23- Sadeghi AA, Shawrang P, Shakorzadeh S. Immune response of Salmonella-challenge broiler chickens fed diets containing Gallipro®, a *Bacillus subtilis* probiotic. Probiotics Antimicrob Proteins. 2015;7(1):24-30. DOI: 10.1007/s12602-014-9175-1

24- Li RF, Liu SP, Yuan ZH, Yi JE, Tian YN, Wu J, Wen LX. Effects of induced stress from the live LaSota Newcastle disease vaccination on the growth performance and immune function in broiler chickens. Poult Sci. 2020;99(4):1896-1905. DOI: 10.1016/j.psj.2019.12.004

25- Mohammed A, Mahmoud M, Murugesan R, Cheng HW. Effect of a synbiotic supplement on fear response and memory assessment of broiler chickens subjected to heat stress. Anim. 2021;11(2):427. [available at]

26- Diarra SS, Kwari ID, Girgiri YA, Saleh B, Igwebuike JU. The use of sorrel (*Hibiscuss sabdariffa*) seed as a feed ingredient for poultry: A review. Res Opin Anim Vet Sci. 2011;1:573-577. [available at]

27- Haghigh HR, Gong J, Gyles CL, Hayes MA, Sanei B, Parvizi P, Sharif S. Modulation of antibody-mediated immune response by probiotics in chickens. Clin Vaccine Immunol. 2005;12(12):1387-1392. DOI: 10.1128/CDLI.12.12.1387-1392.2005

28- Haghighi HR, Gong J, Gyles CL, Hayes MA, Zhou H, Sanei B, Sharif S. Probiotics stimulate production of natural antibodies in chickens. Clin Vaccine Immunol. 2006;13(9):975-980. DOI: 10.1128/CVI.00161-06

29- Stavric S. Microbial colonization control of chicken intestine using defined cultures. Food Technol. 1978:23(4):12-20. [available at]