Yahya Sabah Abdulameer et al /J. Pharm. Sci. & Res. Vol. 10(2), 2018, 408-411

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Do Iodine and Colistin in broiler's drinking water affect immune competence and performance ?

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Abstract

A total of 150 unsexed chicks (Ross 308) , one day old were used in a completely randomized design to study the immune responses against Newcastle disease vaccine (NDV), infectious bronchitis vaccine (IBD), and performance of broiler supplemented with iodine and colistin. The chicks were divided into 6 treated groups with five replicated pens (5 birds per pen). The treatments consisted of the: G1: received Iodine (1 ppm) in drinking water during all week days of experimental period (35 days). G2: received Iodine (1ppm) in drinking water, added 3 days a week. G3: received Colistin (Colistin sulphate) (0.5 mg/liter) in drinking water during all week days. G4: received Colistin sulphate (0.5 mg/liter) in drinking water, added 3 days a week. G5: vaccinated with NDV+IBD but did not receive iodine or colistin as consider positive control. G6: Neither vaccination nor iodine or antibiotic as consider Negative control. The oral administration of colistin enhanced (P≤0.05) the antibody titers against NDV and IBD, growth performance (body weight gain, feed intake feed conversion ratio), and reduced the intestinal bacterial counts (P≤0.05) during all the experimental periods (35 days). Also Iodine caused a marked increase (P≤0.05) in the antibodies titers (Ab) against NDV and IBD during the maternal, primary, and secondary response and showed a moderate effect in reducing the intestinal bacteria counts (P≤0.05). In the present study, both colistin and iodine could improve the immune response and health status of broiler at all the experimental period. We can use colistin and iodine during outbreak periods of NDV and IBD at neighboring farms.

Key word: broiler, colistin, iodine, immune, vaccination.

INTRODUCTION

The improvement of the immune system and intestinal safety is associated with high production in a new strain of broilers (**1**), there for all methods that enhance these systems were studied deeply and we need to discover other strategies to create a balance between bird health and production. Although there are potential discussions about the use of some methods such as antibacterial substances due to public health concerns, but these substances continue to play an important role in animal production in developing countries so far (**2**). Colistin is an important antibiotic belonging to polymyxins group used widely in poultry production as growth promoter. Colistin is slowly absorbed by the gastrointestinal tract (**2**), so the body tissues are free of it under normal doses. Its effect on the cell membrane and make it useful against gram-negative bacteria (3). Additionally, the effect of disinfectants and sterilization on poultry production becomes a constant fact. Iodine is a common disinfectant used in the poultry industry. It has an effect on growth performance and immune response (**4**). The broiler chickens in tropical countries such as Iraq suffer from poor immune response to many vaccines especially IBV and NDV (**5**).Therefore, we need to give much more an attention on any substance that was shown the ability as an enhancer of immune responses and health status of broiler. The objective of this study was to assess the capacity of iodine and colistin on immune response against NDV, IBD, and also on health status with regard to intestinal bacteriology and growth performance.

MATERIALS AND METHODS:

Animal diet and Experimental Design

The experiment period was 35 days (20 November to 26 December 2016) at the farm of Al-Qasim Green University, Babylon, Iraq , a hundred and fifty one-day-old unsexed Ross 308 broilers was conducted in floor litter, during 35 days with initial weight 43g ± 2 were purchased from commercial hatchery. This study was conducted at the Poultry Research Centre at the University of Baghdad .The chicks were divided into 6 treatment groups with five replicated pens (5 birds per pen). Each pen was equipped with a single,tube feeder and adjustable nipple-type drinkers. The treatments consisted of the: G1: supplemented with Iodine (1ppm) in drinking water during all week days of the experimental period (35 days). G2: supplemented with Iodine (1ppm) in drinking water, added 3 days a week. G3: supplemented with Colistine sulphate6 (0.5 mg/liter) in drinking water during all week days. G4: supplemented with Colistin sulphate (0.5 mg/liter) in drinking water, added 3 days a week. G5: vaccinated with NDV (Newcastle disease (La Sota) (intervet, Italy) + IBD (infectious bronchitis disease vaccine, intermediate plus strain, Genera company, Croatia), but did not receive iodine or colistin as consider control positive. G6: Neither vaccination nor iodine or antibiotic as consider control Negative. The birds were raised in two stages consisting of a starter diet up to 21d of age and a finisher diet up to 35 d of age. Diets were prepared to meet the nutrients of broilers of this age according to the recommended laws of (**7**) (Table1). An iodine-based sterilizer containing 1.75% of tritic iodine (Premis Aqua, West Benetton, Montreal and Quebec) was mixed with water at 1% concentration in a 20 liter container before obtaining final concentration of 1 ppm iodine. The animals were kept in controlled floor pens (50x50x50 cm) for 5 weeks and the access to water and feed was free through the entire experimental period. The lighting program was 23 hours lightness and 1 hour of darkness. The pen temperature was gradually reduced from 33 C° in the first days to 23 C° on day 21.

Data collection:

Body weight gain (BWG) of broilers were recorded weekly during the experimental period. The final body weight (BW) and feed intake (FI) and feed conversion ratio (FCR) were also recorded. On day 10 and, the birds were vaccinated against NDV (by oral administration) and then followed by booster dose at 20 days of the age except the negative control group. Also the IBD (by oral administration) was offered to the birds on day 12 of age except the negative control group. Blood samples were collected randomly from the jugular vein of 5 chicks in each treatment to evaluate antibodies against ND and IBD were seen in days 12, 20 and 35 of the age using Eliza. In order to determine the total number of bacteria in feace, exetra was collected from 10 birds per treatment from the rectal area by a sterile loop and then mixed together and 10 grams of blended exetra from each treatment taken. The bacteria count were determined by using plate count

agar (Merk company, Germany), the microbiological process were achieved according to Yahya *etal*. (6), briefly approximately 1.2 ± 0.3 g of digesta sample was transferred to 15-ml plastic tubes containing 8 ml of phosphate buffered saline (PBS) to make 1:10 dilution (tube 10-1). The sample-buffer suspension was homogenized for 30 seconds and 0.9 ml of sterile saline solution (0.9 % NaCl) was added to each of six tubes. Decimal dilutions were prepared by adding 100 µl from tube 10^{-1} to the first tube and then 100 µl was transferred from this tube to the second tube and so on. 10 µl from each dilution tube was spread on plates count agar and the plates were incubated at 37 C° for 24hours. The result was recorded depending Log 10 colony forming unite per gram of exetra (log cfu/g).

The Statistical Analysis System- SAS (8) was used to clarify the effect of different factors in study parameters. LSD and Duncan (9) multiple range tests were used to compare between means in this study at level $p \leq 0.05$.

Table 1 Ingredient and nutrient composition of the basal diet

Ingredient, % (w/w) as fed basis	Starter	Grower
Corn	58.78	60.00
Soybean meal (44 g/kg protein)	34.75	32.3
Corbonate	-	1.30
Corn oil	3.50	3.50
DL-methionine	0.20	0.22
L-lysine	0.07	0.05
Dicalcium phosphate	2.00	2.00
Sodium chloride	0.20	0.20
Vitamin mixture*	0.25	0.25
Mineral mixture*	0.25	0.25
Total	100.00	100.00
Composition by calculation[1]		
ME kcal/kg	3019.80	2995.00
Crude protein, g/100g	20.46	19.39
Lysine, g/100g	1.15	0.96
Methionine, g/100g	0.50	0.48
Methionine + cyctine, g/100g	0.38	0.78
Threonine g/100g	0.79	0.71
Calcium g/100g	1.00	0.85
Available phosphorus g/100g	0.50-0.42	0.33

*****Vitamin and mineral supplied per kg of diet: vitamin A, 12,000 U; vitamin E, 10 mg; vitamin D, 2200 U; niacin, 35 mg; D-pantothenic acid, 12 mg; riboflavin, 3.63 mg; pyridoxine, 3.5 mg; thiamine, 2.4 mg; folic acid, 1.4 mg; biotin, 0.15 mg; vitamin B, 0.03 mg; manganese, 60mg; zinc, 40 mg; iron, 1280 mg; copper, 8 mg; iodine, 0.3 mg; selenium, 0.2 mg.

[1]Estimated from NRC (1994) composition table.

RESULTS AND DISCUSSION

Antibody titers againist ND and IBD vaccination:

The results are summarized in Table (2) showed that the addition of iodine and colistine had a significant effect (P<0.05) on antibody titers against NDV and IBD during the maternal, primary, and secondary immune response compared to the control group. The titers of maternal immunity against IBD are the highest when were compared with other titers of diseases like chicken anemia virus, infectious bronchitis virus, laryngotracheitis virus, Mycoplasma gallisepticum, Mycoplasma synoviae,Newcastle disease virus, and reovirus at 37 weeks of stocks breeder **(10).** On day 20 the result of Ab titer against IBD has revealed the presence of significant differences (P< 0.05) among all groups in Ab titer in which the highest mean value was observed in the third group that was 4102 followed by fourth and second groups which were 3854 and 3377.4 respectively, while the least means of antibody titer were recorded in the first, sixth and fifth groups which characterized by slightly homogeneous results 3160.8 and 2621 respectively, all treated groups were compared with control negative group 2028 , (Table 2). Also the results of antibody titter against IBD on day 35 was compared with the result of day 20 which showed the presence of significant differences (P < 0.05) among all groups in Ab titer in which the highest mean value was observed in the third group that raised up to 4664, followed by fourth and second groups which were 4298.8and 3984.8 respectively, the least means of antibody titer was recorded in first, sixth and fifth groups which were characterized by slightly homogeneous results 3667.4 and 3357.6 respectively, all treatment groups were compared with control negative that recorded nil result. (Table2). In general all vaccinated groups showed a significant increase (P < 0.05) in Ab titer against IBD at 35 days as compared with those at 10 and 20 days, while there was a sharp decrease in mean values of group five (control negative) in Ab titer at 20 and 35 days in comparison with those at 10 days. These findings are in agreements with Hamal *etal.* **(11)** who reported that the cumulative effect of Ab production resulted from several times of vaccination reach to a high titres. At 20 days the result showed presence of significant differences (P ≤ 0.05) among all groups in Ab titer against NDV in which the highest mean value observed in the third group was (3789.4), followed by (fourth, second and first) groups 3408, 3346.2 and 3043.4 respectively, while the least means of antibody titer was recorded in fifth group (2503.6), all treatment groups were compared with control negative group which recorded nil (Table 2). The findings of Ab titers against NDV at 35 days titers were compared with the result on day 20, they showed significant differences (P ≤ 0.05) among all groups in which the highest mean value was observed in the third group was 5089.4, followed by fourth, second and first groups which were 4690.2, 4038.4 and 3648.4 respectively, the least mean of antibody titer was recorded in group (five) which was (3263), all treatment groups were compared with control negative that recorded nil result. (Table 2). Cardoso , etal. **(12);** Bublot**, etal.(13)** have explained the role of live attenuated vaccine (La Sota) at 10 days, it leads to a multiplication of the virus (without interference with Mab that gradually decreased by time), thus this mechanism acts as stimulator of immune organs to produce more Abs. La Sota vaccine was given orally by drinking water, hence this route will stimulate the cell mediated immune response that requires longer time for replication of the virus in vivo, then stimulation of immune system will occur to furnish more Abs in the circulation **(14)**. It is known, the second immunization plays an important role in inducing immune organs to create more Ab titers (15). In general, all vaccinated groups showed a significant increase (P<0.05) in Ab titer at 35 days as compared with those at 20 and 10 days except the sixth group (control negative) which showed a significant decrease (P<0.05) in Ab titer (0-0) by ELISA at 35 day. The increase in antibody titer against the IBDV and NDV in third and fourth groups that were received colistin along the expermintal days are in accordance with Anne & Abrahamsen (16) who mentioned that polymyxin B stimulates monocytes to produce increased amounts of both complement factors and cytokines which are essential factors in local inflammatory response. The significant decrease in antibody titer level in first group returns to excess amount of iodine administration in drinking water, this agree with Song *etal*., (17) who explained the effect of iodine deficiency or in excess has an effect on thyroid hormone synthesis and, consequently, may influence the function of the animal's lymphocytes (**18**). This finding suggests that the iodine have an effect on the immune organs.

Table 2: Effect of iodine and colistin on immune response against NDV and IBD by ELISA test of broiler chickens[1] (1 to 35 d)

Groups[2] / Period	Vaccine[3]							
	Infectious bronchitis disease				Newcastle disease			
	12d	20d	30d	Significant	10d	20d	30d	Significant
G1	2621±63.2 D c	3160.8±26.9 D b	3667.4±32.2 D a	90.02	1892±25.5 D c	3043.4±23.4 D b	3648.4±39.2 D a	92.79
G2	2709.4±33.9 C c	3377.4±27.5 C b	3984.8±22.3 C a	87.07	2045.6±22.3 C c	3346.2±22.8 C b	4038.2±32.5 C a	80.99
G3	2939.8±26.5 A c	4102±39.2 A b	4664±25.7 A a	95.72	2630.4±27.8 A c	3789.4±30.2 A b	5089.4±37.8 A a	99.1
G4	2804.8±35.74 B c	3854±37 B b	4298.8±35 B a	110.5	2307.8±23.8 B c	3408±32.7 B b	4690.2±34.3 B a	94.27
G5	2475.6±27.6 E c	2621±39.8 D b	3357.6±28.14 D a	99.42	1458.2±26.7 E c	2503.6±33.8 E b	3263.4±35.8 E a	99.48
G6	2512.8±28.8 E a	202.8±23.9 E b	0±0 E c	66.49	1443.8±28.4 E a	0±0 F b	0±0 F b	50.52
Significant	88.37	96.25	77.26	90.02	75.34	95.74	77.26	

[A-E, a- c]. Means within a column or a row in each growth period with the same superscripts are not significantly different at $P \leq 0.05$.

[1]Mean five chicks per trearment

[2]Groups: G1: received Iodine (1ppm) in drinking water during all week days. G2: received Iodine (1ppm) in drinking water, added 3 days a week . G3: received Colistin (Colistin sulphate) (0.5 mg/liter)) in drinking water during all week days. G4: received Colistin sulphate (0.5 mg/liter) in drinking water in, added 3 days a week. G5: vaccinated with NDV+IBD but did not receive iodine or colistin as consider control positive. G6: Neither vaccination nor iodine or antibiotic as consider control Negative.

[3]Vaccine was offered by water

Table 3: Effect of iodine and colistin on feacal bacterial count of broiler chickens at 35 days old.[1]

Groups	Mean × 10^4 ±SE
1	35.8±1.77 F
2	69.6±3.25 D
3	51.8±2.93 E
4	97.2±2.27 C
5	202.2±2.73 B
6	263.8±2.71 A
LSD	7.73

[A-F] Means within a column with the same superscripts are not significantly different at $P \leq 0.05$.

[1]Mean of ten chicks per treatment

[2]Groups: G1: received Iodine (1ppm) in drinking water during all week days. G2: received Iodine (1ppm) in drinking water, added 3 days a week . G3: received Colistin sulphate (0.5 mg/liter) in drinking water during all week days. G4: received Colistin sulphate (0.5 mg/liter)) in drinking water in, added 3 days a week. G5: vaccinated with NDV+IBD but did not receive iodine or colistin as consider control positive. G6: Neither vaccination nor iodine or antibiotic as consider control Negative.

The results of the current study referred to the presence of significant reduce (P<0.05) in number of intestinal bacteria with iodine and colistine at 35 days. the means of bacterial colonies were (35.8) with first group then followed by the (third, second and fourth) groups (51.8, 69.6 and 97.2) respectively as compared with control groups (fifth and sixth) which were characterized by high number of bacteria as comparison to other groups (202.2 and 263.8) respectively, (Table3). In general, all vaccinated groups showed a significant decrease (P<0.05) in means of bacterial colonies at 35 days as compared with the sixth and fifth (control groups) which showed a significant increase (P<0.05) at 35 day. The decrease in bacteria in first group returns to the effect excess of iodine administration along the experiment and the role of iodine as excellent antimicrobial, this result is confirmed by Bloomfield **(19)** who stated that Iodine rapidly penetrates of microorganisms and attacks the free sulfur amino acids as like cysteine and methionine, **(20),** which culminates in cell. Iodine compounds are bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal **(21).**

Table 4. Effect of iodin and colistin on performance of broilers (1 to 35 d)[1]

Groups[2]	period	Group1	G2	G3	G4	G5	G6	Significant
Body Weight gain(g)	1-7 d	83.8±0.3 D	90.4±0.6 C	103.4±0.6 A	99.6±0.5 B	84.8±0.3 D	83.8±0.5 D	1.59
	7-14 d	283.8±0.3 F	289.66±0.9 D	312.4.8±0.7 A	298.8±0.5 B	286±0.3 E	291.6±0.5 C	25.32
	14-21d	251.8±0.9 E	294.8±0.3 C	327.2±0.3 A	315.14±0.3 B	255±0.3 E	269±0.7 D	1.89
	21-28d	650±1.5 F	710.2±3.9 C	721.7±3.9 A	749.2±0.8 B	673.4±1.4 D	682.4±0.5 E	5.8
	28-35	683.4±1.3 E	784.5±1.7 C	824.8±1.7 A	796.4±1.5 B	703±1.95 D	709.4±1.9 D	4.74
Final BW(g)	1-35d	1944.8±8.6 F	2169.4±3.4 C	2339.4±2.9 A	2279.2± 1.9 B	1999.4±2.5 E	2033.2±2.4 D	25.29
Feed consumption(g)	1-35d	3751.8±11.9 A	3704.2±7.7 B	3459.2±9.3 D	3663.6±7.46 C	3752.6±8.1 A	3680±6.8 B	36.13
RCR(g:g)	1-35d	1.921±0.005 A	1.7±0.004 D	1.47±0.004 F	1.57±0.007 E	1.87±0.004 B	1.8±0.004 C	0.0021

[a-c] Means within a row in each growth period with the same superscripts are not significantly different at $P \leq 0.05$.

[1]Means represent 5 pens.

[2]Groups: G1: received Iodine (0.1ppm) in drinking water during all week days. G2: received Iodine (0.1ppm) in drinking water, added 3 days a week . G3: received Colistin (Colistin sulphate) (0.5 mg/liter)) in drinking water during all week days. G4: received Colistin sulphate (0.5 mg/liter) in drinking water in, added 3 days a week. G5: vaccinated with NDV+IBD but did not receive iodine or colistin as consider control positive. G6: Neither vaccination nor iodine or antibiotic as consider control Negative.

Iodines function by denaturing proteins associated with the enzymatic systems of microorganisms (**22)**. Also the signification decrease in means of bacterial colonies in third group returns to the role of colistin in bacterial inhibition this result in accordance with *Barton etal.,* **(23)** who confirmed the possible mechanism of action of polymyxins is that they are surface active substances having dual action as lipophilic and lipophobic in a same molecule.

The mean body weight of all groups at day one was 42 gram, body weight gain result according to (Table 4) explained the presence of significant differences at level (P<0.05) among all groups in the average body gain weekly. Broilers were received colistin during all the week days with or without vaccination had significantly higher body weights compared to other treatments at all the experimental period (P<0.05). The iodine have affected significantly (P<0.05) on BWG only by intermediate administration (3 days per week). The oral administration of colistine as two like significantly improved the final BW during all the experimental period (P<0.05). Also, the administration of iodine at 3 days per week showed a significant effect as comparison with control. The FCR was showed a significant improve with colistine in two phases and iodine in intermediate phase (added 3 days per week). Feed intake increased significantly with the control group and iodine administration at 3 day per week (P<0.05). The progress has been evident in treated groups in significant differences at level (P<0.05) so the third group recorded more progress from the rest of treated groups weekly, but that progress in the weight gain of first groups is significant decrease at level (P<0.05) as compared with control groups (five and six). The results of current study registered significant difference at level (P<0.05) in feed conversation ratio among all groups, so the lowest means was recorded in the third group (1.47) followed by (fourth, second and sixth) were recorded (1.57, 1.7, 1.87 and 1.8) respectively, as compared with the first group that recorded high mean (1.92) (Table 3). The increase significant in growth performance in the third and fourth group was attributed to role of antibiotic as growth promoter besides the therapeutic applications, colistin is commonly used at lower concentrations as a growth promoter (prophylactic use) **(24)**. The added iodine has a positive effect on the growth and feed utilization by increasing metabolic rate as a reflection increase of the thyroid activity, but also the continuous dose of iodine has potential effect on intestinal microflora. These microflora are necessary to allow the increase of weight gain and feed efficiency (25). The iodine shows a more effect when the broiler chickens were reared un healthy condition (26).

In conclusion*,* our results confirm that iodine and colistine have positive effects on growth performance, immune competence, and intestinal bacteria. We can use colistine and iodine during outbreak periods of NDV and IBD at neighboring farms.

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