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Influence of Sodium Butyrate on Provoke of Immune Response in Vaccinated Broilers With Avian Influenza (H9N2) Vaccines

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THIS study aimed to evaluate the impact of sodium butyrate (SB) on immune response of broiler vaccinated with 2 different inactivated H9N2 vaccines. 150 day-old chicks were divided into 5 groups (every 30 chicks): groups A and B were vaccinated with classical inactivated avian influenza H9N2 vaccine at 1 day-old/ S.C. Group A left without treatment but group B was treated with SB with a dosage of 1g/L of drinking water daily till end of trail. Whereas groups C and D were vaccinated with a developed inactivated avian influenza H9N2P vaccine at the same age S.C. Group C was left without treatment but group D was treated with SB as group B, finally, group E was control group. The results showed no significant effect of SB on level interferon γ in treated and/or vaccinated groups. Significant effect on Abs level with SB supplementation was detected at 14 days in group D in comparison with other groups, then at 35 days SB and or developed H9N2 vaccine revealed an elevation in Abs titer in group B, C, and D respectively by ELISA and HI tests. The significant difference occurred in RW of bursa with group B in comparison with group D and E at 21 and 28 days. Finally, group B showed a significant increase in weight gain at 35 days followed by group D in comparison with other groups. We concluded that supplemented SB at 1gm/ L has a positive impact on both humoral immunity and weight gain of broiler.

Keywords: Sodium butyrate, Interferon γ, Avian influenza vaccine, Immune response.

Introduction

Improvement of health condition and growth performance of poultry is one of the crucial aspects to enhance the productivity of birds [1]. After ban supplementation of antibiotics as growth promoters in poultry diet in the last few decades, this decision has obliged nutritionists to discover other replacements for these antibiotics to enhance intestinal efficiency and productivity of broilers, thus short-chain fatty acids (SCFA) and their salts are one the promising replacements for antibiotics. Many tools were used to promote the dietary respect of new by-products or feed supplements by their action in modifying microbiota functions and induction of immune system [2]. Recently, butyrate one of the feed additives as an (SCFA), has obtained more interest as a good solution to improve the poultry production and their effect in triggering many systems including poultry GIT and immune response [3]. Nowadays sodium butyrate has obtained plentiful interest because of its helpful impact on growth performance, integrity, and immune function of GIT and its effect on the inhibition of harmful microbes [4]. The mechanism of action of sodium butyrate includes the penetrator effect of butyric acid to bacterial cell wall followed by alteration of intracellular pH, thus reducing the reproduction of these microorganisms [5].

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Different postulates about the butyrate effect in the modulating immune response including pro-inflammatory cytokines and humoral immune response, but many researchers explained their beneficial effects on body performance, immune response, intestinal morphology, and effects on characters of carcass [6], while in contrast [7] could not notice any impact of butyrate on body performance of broilers. Indeed, Butyrate decreases pro-inflammatory cytokines induction. In poultry, these pro-inflammatory cytokines induce a homeorhetic reaction that alters nutrients distribution during inflammation [8]. Moreover, inhibition of some pro-inflammatory cytokines stimulation leads to diminishing the quantity of reactive oxygen species and up-regulates the gene expression of the antioxidant system [9]. Furthermore, butyrate can diminish the inflammatory response and immune cells infiltration in peripheral tissues by affecting the chemotactic effect of inflammatory cells [10]. Unprotected butyrate supplementation with 1gm/kg to broilers lead to significant amplification of specific genes encoding to host defense peptides which participate powerfully in innate immune response [11] and dawn-regulate of tumor necrotic factor-α and interleukin-6 levels in serum at 21-day post-treatment [7]. Sodium butyrate (SB) as a feed additive has a significant improvement in the level of secretory IgA in the intestine in addition to its effect in stimulating the mucosal antibodies secretion in the GIT lumen, thus it enhances the immune responsiveness of the intestine [12]. Dietary supplementation of SB revealed also enhancement results in humoral immune response and hematological aspects in broilers [13].

Numerous outbreaks caused by H9N2 virus have appeared in many geographical provinces of Iraq and significant economic losses were documented in the poultry houses including broilers, layers, and breeders [14]. Different inactivated oil emulsion vaccines against the H9N2 virus and recently the H9N2P vaccine (p=pathogen-associated molecular pattern (PAMPs)) were used in broilers to reduce the occurrence and control these outbreaks [15]. Although massive vaccination programs and many attempts to restrict the disease occurrence have been applied to protect the poultry and reduce virus shedding, H9N2 viruses dissemination between poultry houses is still represented a major challenge for veterinary authorities, veterinarians as well as farmers [16]. Hence, our study was designed to evaluate humoral and cellular immune responses, lymphoid organs modulation, and weight gain associated with SB supplementation in broilers.

Materials and Methods

Experimental protocol

A total of one hundred fifty, day-old broilers were allocated into 5 groups in split pens (every 30 chicks, three replicates): group A and B were vaccinated with conventional avian influenza H9N2 oily inactivated vaccine (Intervet-Holland) at 1 day old/ 0.25 ml S/C. Group A left without other treatment but group B was treated with pure sodium butyrate (SB) powder (Biopoint company/ Poland) with a dosage of 1g/L of drinking water daily till the end of the trial. While group C and D were vaccinated with a developed avian influenza H9N2P oily inactivated vaccine (Intervet-Holland) at the same age and dosage S/C. Group C was left without other treatment but group D was treated with (SB) as group B. finally group E was left without any treatment and considered as a control group.

Serum samples

These samples were collected from the groups of the experiment at 14, 21, 28, and 35 days of age and then preserved in suitably labeled vials at -20C° for further processing.

ELISA test

Sera were examined for detection of the level of avian interferon γ and IgY antibodies against the H9N2 virus by Competitive and indirect ELISA test kits respectively for these two agents as indicative parameters of a cellular and humoral immune response. These tests were done according to the recommended procedure by the manufacturer (Bioassay technology laboratory / China) and (ID.Vet / France).

Hemagglutination inhibition (HI) test

Fifty microliters of each serum sample were diluted (two-fold dilution) in a 96-well microtiter plate with 50 µL PBS, then 50 µL of the H9N2 antigen (4HAU)(GD academy/Netherland) was added and mixed carefully. The microtiter plate was incubated at room temperature for 25 min, after that 50 µL of 1-2% chicken RBCs suspension was added and mixed thoroughly. The titer of antibodies by HI test was detected after incubation of the microtiter plate at room temperature for 40 min [17].

Lymphoid organs weight

Lymphoid organs of 4 chicks per group including bursa, thymus, and spleen were weights after

humanely sacrificed chicks by cervical displacement for recording the relative weight at 7, 14, 21, 28, and 35 days by the formula (organ weight/ bodyweight x 100).

Average weight gain (WG)

Chicks in the experiment were fed on a basal diet that was formulated according to the standard requirements of broiler [18]. The weight gain was calculated weekly to find out any differences between groups of experiment [19].

Statistical analysis

Data analysis was carried out using (SPSS. Version.21). The estimated values of avian interferon γ, IgY antibodies against the H9N2 virus, relative weight (RW) of lymphoid organs, and weight gain parameters were expressed as mean values ± Standard Error (SE) and competed using Duncan's test ($P \leq 0.05$) [20].

Results

The ELISA test was done to determine the level of interferon γ in groups of the experiment. The impact of SB at the 21 and 35-day post-treatment PT showed a decrease in the level of interferon γ in groups B and D but without significant differences ($P < 0.05$) between these groups and in comparison with groups A and C, while group A showed significant ($P < 0.05$) elevation in the level of interferon γ in compare with group E at the same age. At 14 and 28 days PT, no effect of SB on the level of interferon γ was detected between groups (Table 1).

The results of ELISA in table 2 explain the impact of SB and or developed vaccine (PAMP) on humoral immunity and this effect appeared early at 14 days PT in group D significantly, while at 21 days PT, groups C, and D showed a significant increase in antibodies (Abs) titer in comparing with other groups. Then in the last week of the experiment significant elevation of Abs titer was noticed in groups B, C, and D (treated with SB and/or vaccinated with H9N2P vaccine) in comparison with groups A and E.

The results of the HI test showed a significant increase in the level of Abs at 14 days of the experiment in group D followed by group C in comparison with other groups. At 21 days, a significant elevation of Abs titer was shown in groups C and D, then groups A and B in comparison with group E. Moreover, birds in groups B, C, and D at 28 and 35 days demonstrated an increment of Abs titer compared to group A, and then group C (Table 3).

The relative weight (RW) of lymphoid organs was varied in different intervals, but a significant variation occurred in RW of bursa with group B in comparison with group D and E at 21 and 28 days respectively, while the RW of this organ at 35 days decreased significantly ($P < 0.05$) in group C compared with group D. The RW of the thymus was increased significantly ($P < 0.05$) in groups C and D in compare with group E at 14 days, while groups A and C were shown a significant decrease in RW of the thymus at 35 days compared with group D. Finally the RW of spleen at 35 days was increased significantly ($P < 0.05$) in group D in comparison with groups B and E (Table 4).

Data in Table 5 showed many differences in weight gain (WG) between groups within intervals. At 7 days PT the significant difference ($P < 0.05$) occurred between groups A and B only, followed by the absence of the differences between groups at 14 days of age. At 21-day group D showed a significant decrease ($P < 0.05$) in WG

TABLE 1. Level of interferon γ (ng/L) at 14, 21, 28, and 35 days in groups of experiments (mean±SE).

	Groups				
Age/days	**A**	**B**	**C**	**D**	**E**
14	118.5±21.8^{a}	88.3±8.3^{a}	139.6±23.1^{a}	136±13.2^{a}	128.3±7.8^{a}
21	132.4±29.7^{ab}	116±25.1^{ab}	195.4±62.5^{a}	141.6±14.5^{ab}	71.5±14.5^{b}
28	210.8±43.8^{a}	126.8±15.5^{a}	173.4±52.1^{a}	214.9±52.1^{a}	89.7±17.8^{a}
35	473.6±159.5^{a}	217.5±46.6^{ab}	494.8±145^{a}	298.6±84.5^{ab}	113±33.5^{b}

* Values with different letter superscripts in the same row, mean significant difference ($P < 0.05$).

TABLE 2. Titer of antibodies (Abs) against H9N2 vaccines at 14, 21, 28, and 35 days in groups of the experiment by ELISA test (mean±SE).

	Groups				
Age/days	**A**			**B**	**C**
14	150.5±145.5^{b}	130±26.7^{b}	315.2±189.3^{b}	1046.2±465.1^{a}	198.7±67.9^{b}
21	3404±1239^{b}	2114±1901^{b}	23445±2628^{a}	18574±836^{a}	233±206^{c}
28	9745±1539^{ab}	11152±1071^{ab}	27036±913^{a}	15491±4611^{ab}	241±120^{c}
35	8110±990^{b}	10644±631^{a}	11518±569^{a}	12433±937^{a}	49±7^{c}

* Values with different letter superscripts in the same row, mean significant difference ($P < 0.05$).

TABLE 3. Titer of antibodies (Log2) against H9N2 vaccines at 14, 21, 28, and 35 days in groups of the experiment by HI test .

	Groups				
Age/days	**A**	**B**	**C**	**D**	**E**
14	2.2±0.25^{c}	2.5±0.28^{c}	4.5±0.86^{b}	8.5±0.28^{a}	3±0.4^{c}
21	5.5±0.86^{b}	5.5±0.95^{b}	8.7±0.47^{a}	9.2±0.47^{a}	2±0.4^{c}
28	5.7±0.85^{b}	8.2±0.62^{a}	8.7±4.7^{a}	9.2±0.25^{a}	1.7±0.47^{c}
35	6.2±0.85^{b}	8±0.4^{a}	8.5±0.64^{a}	9.2±0.25^{a}	1.5±0.5^{c}

* Values with different letter superscripts in the same row, mean significant difference ($P < 0.05$).

TABLE 4. Relative weight of bursa, thymus, and spleen at 7, 14, 21, 28, and 35 days in groups of the experiment (mean±SE).

Groups / **Age/days**	**Organ**	**A**	**B**	**C**	**D**	**E**
7	**Bursa**	0.244±0.27^{a}	0.221±0.12^{a}	0.213±0.42^{a}	0.246±0.40^{a}	0.25±0.21^{a}
	Thymus	0.223±0.06^{a}	0.316±0.02^{a}	0.291±0.06^{a}	0.293±0.05^{a}	0.296±0.03^{a}
	Spleen	0.082±0.12^{a}	0.084±0.18^{a}	0.069±0.06^{a}	0.06±0.07^{a}	0.064±0.08^{a}
14	**Bursa**	0.254±0.01^{a}	0.246±0.01^{a}	0.269±0.01^{a}	0.259±0.04^{a}	0.236±0.03^{a}
	Thymus	0.34±0.05^{ab}	0.419±0.03^{ab}	0.493±0.03^{a}	0.481±0.02^{a}	0.345±0.02^{b}
	Spleen	0.70±0.01^{a}	0.86±0.05^{a}	0.56±0.05^{a}	0.68±0.06^{a}	0.80±0.03^{a}
21	**Bursa**	0.244±0.01^{ab}	0.292±0.02^{a}	0.243±0.01^{ab}	0.228±0.007^{b}	0.236±0.004^{b}
	Thymus	0.250±0.02^{a}	0.290±0.02^{a}	0.302±0.02^{a}	0.244±0.01^{a}	0.260±0.03^{a}
	Spleen	0.085±0.007^{a}	0.075±0.01^{a}	0.068±0.006^{a}	0.095±0.008^{a}	0.091±0.009^{a}
28	**Bursa**	0.171±0.01^{ab}	0.233±0.03^{a}	0.185±0.01^{ab}	0.158±0.009^{b}	0.133±0.02^{b}
	Thymus	0.247±0.008^{a}	0.266±0.01^{a}	0.235±0.02^{a}	0.209±0.02^{a}	0.201±0.01^{a}
	Spleen	0.120±0.006^{a}	0.146±0.01^{a}	0.123±0.1^{a}	0.112±0.01^{a}	0.132±0.01^{a}
35	**Bursa**	0.146±0.03^{ab}	0.174±0.02^{ab}	0.126±0.02^{b}	0.215±0.01^{a}	0.181±0.007^{ab}
	Thymus	0.160±0.03^{b}	0.246±0.02^{ab}	0.172±0.01^{b}	0.309±0.03^{a}	0.225±0.02^{ab}
	Spleen	0.109±0.008^{ab}	0.080±0.006^{c}	0.101±0.009^{abc}	0.116±0.009^{a}	0.088±0.007^{bc}

* Values with different letter superscripts in the same row, mean significant difference ($P < 0.05$).

TABLE 5. Weight gain at 7, 14, 21, 28, and 35 days in groups of the experiment (mean±SE).

	Groups				
Age/days	**A**	**B**	**C**	**D**	**E**
7	170.4±8.6^{a}	141.2±11.5^{b}	153.1±4.1^{ab}	147±7.5^{ab}	158.3±8.9^{ab}
14	312.6±4.6^{a}	309.6±17.3^{a}	305.1±13.5^{a}	326.3±2.6^{a}	320.3±22.8^{a}
21	477.1±٢٣,١^{a}	450.6±24.5^{ab}	478.3±20.2^{a}	400.2±5.7^{b}	473.6±28.8^{a}
28	517.4±9.8^{ab}	478.3±28.8^{b}	490.2±34.6^{ab}	541.4±23.9^{ab}	538.2±28.8^{a}
35	446.9±17.6^{d}	751.2±23.6^{a}	610.3±17.3^{c}	702.2±11.5^{ab}	602.1±23.2^{bc}

* Values with different letter superscripts in the same row, mean significant difference ($P < 0.05$).

than other groups except for group B, then a significant difference appeared between groups B and E only at 28 days. Finally, at 35 days, group B showed a significant increase ($P < 0.05$) in WG followed by group D in comparison with other groups.

Discussion

Sodium butyrate has multiple beneficial effects on growth performance with a diminishing of pathogens existence in broilers, thus recently has been introduced in the poultry industry with feed supplements as an alternative agent to antibiotics depending on their many positive impacts including boosting of different arms of the immune system. The results of IFN-γ in our study were relatively agreed with Sedeik et al.[21] when they notified a significant increase ($P<0.05$) in the level of IFN-γ in the vaccinated groups than the non-vaccinated one against Newcastle disease vaccine, particularly in the group administered with an inactivated vaccine that contains the antigenic determinants for both Newcastle and avian influenza H5 subtypes. The relative agreement of results occurred with Astill et al.[22] that mentioned an elevation of IFN-γ expression in the vaccinated group with inactivated beta propiolactone H9N2 vaccine. Although no significant differences in IFN-γ level between vaccinated groups, sodium butyrate decreased this level in treated groups, these results explain the mechanism of action of butyrate by suppression of specific Anti-viral IFN-stimulated gene products, butyrate generally boost the expression of many cellular genes, but it powerfully inhibits 60% of interferon stimulating genes [23]. Another study referred to the role of Microcin C7 (antimicrobial peptide) as a significant peptide by reducing gene expression of pro-inflammatory including IFN-γ [24].

To explore the functional importance of SB in the induction of Abs production and parallel to our findings, [12] reported a high titer of Abs against Newcastle disease in treated chicks with SB by HI test, this reveals the modulatory effect of SB on B and T cells during processes of the antigenic expression, furthermore the supporting effect in the proliferation of T-helper-1 and T-helper-17 effector cells [25]. The significant increase of Abs titer in treated groups with SB and/or PAMP-associated vaccine is due to the effect of these two factors on the enhancement of humoral immunity by the improvement of Abs titers against infectious bursal disease and infectious bronchitis [26]. The result of SB on Abs titer is in agreement with [27] due to the modulatory effect of SB on the roles of B and T cells by reducing the expression of cytokines, such as IFN-γ.

The results of Table 4 agreed with El-Sayed et al. [28] when they referred to the effect of different inactivated vaccines against AI in the elevation of the RW of the spleen and bursa. However, due to the little obtainable knowledge in the literatures about the impact of AI inactivated vaccines on RW of thymus, spleen, and bursa no discussion was performed on these results, or our interpretation of bimodal significant differences in the RW of lymphoid organs that varies between intervals and groups, particularly in comparison with group E may be due to the processing of the inactivated antigen by the cells of the immune system which lead to induction of immune reaction in lymphoid organs ultimately increase of RW of these organs. The results of WG disagreed with Shahir et al.[29] when they mentioned no influence of SB on WG. While the significant differences ($P < 0.05$) in WG were most obviously shown at end of the experiment in the treated groups with SB. Therefore, the reason for the limited effect

of SB during an early stage of rearing is due to the usage of unprotected SB in our study, and these results agreed with Chamba et al.[30] who noticed the supplementation of partially protected SB improves the body performance including the converting of SB within the GIT that improved function of intestinal villi, thus nutrient absorption. The improvement effect of SB is first: suppling epithelial cells of the intestine with energy, hence patently stimulating the proliferation and differentiation of these cells. Secondly: improve the digestibility of feed ingredients. Thirdly: the effective performance of SB is due to the alteration of the GIT environment to the acidity that leads to creating the antimicrobial media which minimizes the pathogens' load [31]. Fourthly: SB acts to reduce the apoptotic processes of normal enteric cells, thus all of these beneficial effects maybe explain the causes why SB supplementation improved the WG, intestinal cell functions, and immune status [32].

Conclusions

Our results concluded that a supplementary diet with SB has a positive influence on the health status of chicks by strengthening the weapons of the immune system (humoral immunity) and body performance by enhancing weight gain and relative weight of immune organs, particularly at the finisher period of rearing in broiler chicks.

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Conflict of interest

There are no conflicts of interest declared by the authors.

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تأثير بيوتاريت الصوديوم على تحفيز الاستجابة المناعية لفروج اللحم الملقح بلقاحين لانفلونزا الطيور H9N2

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هدفت الدراسة إلى تقييم تاثير بيوتاريت الصوديوم (SB)على الاستجابة المناعية للفروج الملقح بنوعين مختلفين من اللقاحات المبطلة لانفلونزا الطيور H9N2 . تم استخدام ١٥٠ فروج بعمر يوم واحد قسمت الى ٥ مجاميع (٣٠ طائر لكل مجموعة): المجموعتين A و B تم تلقيحهم بلقاح انفلونزا الطيور التقليدي المبطل بعمر يوم واحد تحت الجلد وبعدها تركت مجموعة A بدون معاملة بينما تم اعطاء مادة SB للمجموعة B بجرعة ١ غم / لتر ماء الشرب طيلة التجربة. اما المجموعتين C و D فقد لقحت بلقاح انفلونزا الطيور المبطل والمطور بنفس العمر وطريقة الحقن ، وتركت المجموعة C بدون معاملة اما المجموعة D فقد اعطي لها SB كما في المجموعة B، اعتبرت المجموعة E كمجوعة سيطرة. اظهرت النتائج عدم وجود تاثيرمعنوي لل SB على مستوى الانترفيرون كاما في المجاميع المعاملة اوالملقحة . لقد كان هناك تاثير معنوي لل SB المعطى على مستوى الاضداد في افراخ المجموعة الرابعة D مقارنة مع المجاميع الاخرى عند عمر ١٤ يوم ، كما ولوحظ تاثير معنوي لل SB او اللقاح المطورH9N2P او كليهما معا في زيادة مستوى الاضداد في المجاميع B و C و D على التوالي وذلك بواسطة اختباري الاليزا وتثبيط التلازن. كما لوحظت زيادة معنوية في وزن غدة فابريشيا في المجموعة B مقارنة مع المجموعتين D و E بعمر ٢١ و٢٨ يوم ، واخيرا لوحظ وجود ارتفاع معنوي في معدل الزيادة الوزنية في المجموعة B ومن بعدها المجموعة D مقارنة مع المجاميع الاخرى. ومن هنا استنتجنا بان اعطاء SB بكمية ١غم / لتركان له تأثير ايجابي على كل من المناعة الخلطية والزيادة الوزنية في الفروج.

الكلمات المفتاحية : بيوتاريت الصوديوم ، انترفيرون كاما ، لقاح انفلونزا الطيور ، الاستجابة المناعية.