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Impact of sodium butyrate on stimulating of some host defense peptides and body performance in broiler vaccinated with different avian influenza (H9N2) vaccines

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Abstract

Our study is designated to determine the impact of SB in the induction HDPs, including AvBD-10 and CATH-B1, accompanied by two different inactivated H9N2 vaccines and their effect on body performance. One hundred fifty, day-old chicks were separated into five groups (30 chicks for each, three replicates): groups A and C were vaccinated with classical avian influenza H9N2 and developed H9N2P inactivated vaccines, respectively, but groups B and D were treated with sodium butyrate (SB) by a dosage of 1gm/liter of drinking water daily till the end of the trail, and these groups (B and D) received the same type of vaccines as they given to group A and C respectively, while group E is a control group. The results illustrated that SB improved the AvBD-10 level significantly in the treated group (B and D) at 14 days in comparison with groups A and E, but without significant with group C. Whereas at 35 days, this improvement occurred distinctly in treated groups B and D. The same improvement revealed with CATH-B1 at 35 days of experiments. Moreover, the supplementation of SB improved FCR in groups B and D at 35 days of the experiment, respectively, but no influence on WG between all groups at the same age. Thus, we concluded that supplemented SB enhanced innate immunity by stimulating the induction of AvBD-10 and CATH-B1. Also, these supplementations improved FCR but did not influence WG.

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Introduction

The gastrointestinal tract (GIT) is one of the central systems of poultry by its significant role in providing a beneficial effect for the digestion and absorption of the diet and, finally, the health status and body performance. Therefore, the maintenance of intestinal mucosa is the dynamic equilibrium among the epithelial cells, microbiome, and immune system in the GIT fractions (1). Sodium butyrate (SB) supplementation for poultry can enhance the GIT health status and body performance. The usage of SB in the poultry diet is well admitted because of its effect on reducing intestinal acidity (PH), thus reducing the harmful microorganism's settlement colonization in GIT (2). SB shows a crucial function in the diminution of GIT pH by limiting the establishment of pathogens and promoting the development of epithelial cells of the intestine, and lastly, supporting the growth performance of birds (3). Defensins and cathelicidins (CATHs) are a large group of a broad spectrum of host defense peptides (HDPs) or antimicrobial peptides (AMPs) invertebrates that perform a defense mechanism as the front line of native immunity with effective antimicrobial and immune-stimulant properties. Fourteen types of defensins known as avian β-defensin 1-14

(AvBD1-14) have been detected in poultry. These AvBDs were widely expressed in different chicken organs, including GIT, while four types of CATHs are recognized, known as fowlicidins 1, 2 and 3 and CATH-B1, and they are efficient in the destroying of a wide range of microorganisms (4). Achanta *et al*. (5) illustrated the application of Real-time PCR for the expression of these 4 CATHs in the GIT, respiratory, and urogenital tracts along with lymphoid organs of chickens. CATH-B1 was produced most plentifully in the bursa of Fabricius, and the production of fowlicidins 1 to 3 is correlated with the age of chicks, while all 4 CATHs were peaked in the bursa on day 4 of chick age, then gradually decreased by 28 days post-hatch. Many outbreaks due to the H9N2 virus were noted in several geographical regions of Iraq, and other economic losses were recorded in the poultry industry, including broilers, layers, and breeders (6). Vaccination with the different origins of inactivated oil emulsion vaccine of H9N2 and recently H9N2p (p = pathogen-associated molecular pattern (PAMPs)) was applied in the broiler (7). H9N2 viruses' circulation and transmission between poultry farms represent the main challenge for veterinary authorities, veterinarians, and farmers. However, the vaccination processes have been intensively used to protect the poultry flocks, may diminish the disease, and reduce virus shedding (8). Because of the absent and unavailable live attenuated vaccine against avian influenza virus AIV, improving immune response to inactivated vaccines with different techniques is the newest approach required to enhance the immune stimulation against AIV.

Thus, due to minor studies on innate immunity, particularly on HDP in correlation with the inactivated vaccine in broilers, this study pointed to determine the beneficial effect of SB in the induction of innate immunity by estimation of AvBD-10 and CATH-B1 accompanied by vaccination with two different types of inactivated H9N2 vaccines in addition to their effect on body performance of experimental groups.

Materials and methods

Experimental design

One hundred fifty-day-old broilers (Ross 308) were divided randomly into five groups in separated pens (supplies with a bed of wood shaving material (each group 30 chicks (three replicates)): groups A and B were vaccinated with classical avian influenza H9N2 oil inactivated vaccine (Intervet-Holland) at one day old (0.25 ml/bird/S.C). Group A left without other treatment, but group B was treated with pure SB powder (Biopoint company/Poland) with a 1gm/ liter of drinking water daily till the end of the experiment. At the same time, groups C and D were vaccinated with developed avian influenza $H9N2_P$ oil inactivated vaccine (Intervet-Holland) at the same age and dosage. 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 a control group.

Serum samples

About 2 ml of blood was collected from the wing vein of chicks at 14,21,28 and 35 days of age to get serum by centrifugation of these blood samples (1500 rpm/15 min) kept in suitably labeled vials at -20C° for ELISA test.

ELISA

Sera were tested for estimation of the level of AvBD-10 and CATH-B1 by sandwich ELISA test Kit for these two HDPs as indicative parameters of innate immunity according to the recommended procedure by the manufacturer (Bioassay technology laboratory /China).

Growth performance

Chicks of the experiment were fed on a basal diet formulated according to the standard requirements of the broiler (9). The primary and final body weight, entire feed intake/birds, and food conversion ratio (FCR) were assessed weekly to find out any dissimilarities between groups of the experiment (10,11).

Statistical analysis

Data analysis was carried out using (SPSS version 21.0). The estimated values of AvBD-10, CATH-B1, FCR, and weight gain parameters were expressed as mean values ± Standard Error (SE) and competed using Duncan's test ($P \leq 0.05$) (12).

Results

The ELISA test was performed to detect the level of AvBD-10 in groups of experiments. The influence of SB at the 14 days post-treatment (PT) improves the AvBD-10 in serum of treated group (B and D) but without significant in comparison with group C, while there is a significant difference in comparison with group A and E. At 21- and 28-days PT, there is no effect detected between groups. Whereas at 35 days, only groups A and E showed a significantly low level of AvBD-10 compared with other groups, with no significant data were detected between groups B, C, and D (Table 1).

As shown in table 2, there is no significant difference in the level of CATH-B1at 14 and 28 days of the experiment, while the CATH-B1 level was increased significantly in group B compared to group E, but no sign was detected with other groups at 21 days. In 35 days of the experiment, this level was incremented significantly in group B compared with groups A and E, whereas no differences appeared between groups B, C, and D.

The impact of dietary supplementation of SB on FCR as a parameter of body performance is shown in table 3. The FCR values were varied between groups, and the significant values of this factor were detected in groups E, D, and D at 7, 14, and 28 days, respectively. No significant influence was detected in the values of FCR at 21 days. Finally, at the end of the experiment at 35 days, significant differences were observed in treated groups with SB, including group B, followed by group D with high variances with other comparable groups.

The dietary treatment with SB on weight gain (WG) was varied, and group A (no treated with SB) showed a significant increment in weight gain at 7, 14, 21, 28 days in comparison with a group (B, C, D) (B) (D) (B) respectively. Lastly, no significant difference was detected between groups (Table 4).

Table 1: Level of avian beta defensing-10 at 14, 21, 28 and 35 days

Age (days)	Avian beta defensing-10 [mean ± SE (ng/ml)]				
	A	B	C	D	E
14	1.1±0.2 c	2.2±0.3 a	1.9±0.2 ab	1.5±0.2 abc	1.3±0.1 c
21	2.6±0.7 a	3.4±0.8 a	2.2±0.2 a	2.1±0.3 a	4.1±1.1 a
28	1.5±0.1 a	2.2±0.5 a	1.9±0.3 a	1.9±0.5 a	2.8±0.8 a
35	1.2±0.2 b	2.6±0.4 a	2.5±0.6 ab	1.9±0.2 ab	1.3±0.2 b

Values with different letter superscripts in the same row, mean significant difference at P<0.05.

Table 2: Level of avian CATH-B1 at 14, 21, 28, and 35 days

Age/days	Avian CATH-B1 [mean ± SE (ng/L)]				
	A	B	C	D	E
14	144.4±5.7 a	152.9±12.0 a	156.5±13.9 a	151.5±13.4 a	133.7±6.2 a
21	171.6±21.2 ab	315±83.1 a	173.9±9.4 ab	240.5±49.3 ab	159.7±16 c
28	180.4±16.8 a	376.4±97.8 a	245.9±56.8 a	313.9±74.3 a	177.7±17.2 a
35	168.8±18.7 b	543.1±114.5 a	347.6±126.3 ab	342±64.2 ab	175.2±16.2 b

Values with different letter superscripts in the same row, mean significant difference at P<0.05.

Table 3: Feed conversation ratio at 7, 14, 21, 28, and 35 days

Age/days	Feed conversation ratio [mean ± SE (gm feed/gm body weight)]				
	A	B	C	D	E
7	0.97±0.03 b	1.02±0.04 c	1.087±0.04 d	0.98±0.07 b	0.94±0.06 a
14	1.26±0.04 d	1.15±0.06 b	1.22±0.05 d	1.09±0.07 a	1.17±0.07 c
21	1.07±0.05 a	1.08±0.02 a	1.04±0.03 a	1.1±0.08 a	1.03±0.02 a
28	1.422±0.05 d	1.409±0.06 c	1.402±0.05 c	1.235±0.07 a	1.313±0.08 b
35	2.15±0.03 d	1.47±0.06 a	1.75±0.06 c	1.5±0.07 b	1.83±0.04 c

Values with different letter superscripts in the same row, mean significant difference at P<0.05.

Table 4: Weight gain at 7, 14, 21, 28, and 35 days

Age/days	Weight gain [mean ± SE (gm)]				
	A	B	C	D	E
7	206.5±3.3 a	179.4±5 c	193.3±3.3 b	186.8±3.8 bc	199.2±4.9 ab
14	521.8±7.7 a	490.5±7.7 b	499.5±12.4 ab	513±9.7 ab	517.6±8.4 ab
21	998.9±17.5 a	940.5±23.5 ab	977.5±25.3 ab	913.2±24.1 b	990.8±20.2 a
28	1516.8±40.6 ab	1418.5±53.4 b	1468.1±44.7 ab	1454.6±55.2 ab	1528.3±36.4 a
35	1964.6±121.7 a	2170.4±104.8 a	2079±126.5 a	2157.3±106.5 a	2130.5±53.3 a

Values with different letter superscripts in the same row, mean significant difference at P<0.05.

Discussion

Feed supplemented with sodium butyrate in high-producing broiler can improve chicken GIT performance and health status, including boosting immunity. As a result of recent investigations, SB exhibited their ability to augment HDPs as a particular agent of nonspecific immunity (13). Consequently, many attempts were proven to induce immune response with dietary SB, which enhanced the immune response to the Newcastle disease vaccine in vaccinated broilers (14).

The supplementation of SB to newly hatching offspring improves the immature immune system, including cellular and adaptive components of these chicks. Hence the results of the induction of AvBD-10 by SB in treated groups were fluctuated between week intervals and considered a time-dependent method of butyrate supplementation. Therefore, these results agreed with Sunkara *et al.* (15) that proposed different regulatory effects of HDPs by butyrate. Our results propose that SB is a forceful stimulant to HDPs in chickens. These results are supported by Bar-Shira and Friedman (16) as they mentioned that the enteric immune system showed elevation of expression of mRNAs beta-defensin on one-day post-hatching, later decreased in the first-week life of as they shown particularly in untreated (group A and E).

In contrast, its elevation in untreated (group C) is due to the presence of PAMPs as an immune enhancer in inactivated H9N2p vaccine (17). The constant level of AvBD-10 between groups in 2 intervals may be due to bacterial colonization in the intestine, which leads to an increase in the level of expression of beta-defensin and CATH genes in chickens as components of the innate immune system (18). The second phase of elevation of AvBD-10 level at 35 days of age occurs because of the induction of innate immune response by SB and/or PAMPs, which trigger the production of endogenous HDPs (15).

Unlike AvBD-10, the CATH-B1 level was elevated at 21 days instead of 14 days PT; the same was mentioned by Sunkara *et al.* (15) when observed upregulation of CATH-B1 in treated groups with SB and established that butyrate has a vital role in triggering many but not all chicken host defense peptides. The decline of CATH-B1 level in groups A and E at 35 days of the experiment is supported by the study of Achanta *et al.* (19) when they recorded differences in the level of CATH 1 to 3, which exhibited an age-dependent mode, while all 4 CATHs, including CATH-B1, were peaked in the bursa on day four after hatching, with a slow drop by day 28. Moreover, CATH-B1 demonstrate a distinctive expression model from other types of CATHs; thus, CATHs are competent in controlling the acquired immunity by the triggering of dendritic cell (20). therefore, feed supplementation with SB in our study will improve the CATH-B1 production in 35 days PT. The availability of SB and PAMPs like bacterial lipopolysaccharides (LPS) will shed light on this material in enhancing the expression of CATH-B1, which possesses a bactericidal effect against many invading bacteria (21). Another recent explanation for elevation of CATH-B1is may be due to the apparent anti-avian influenza action in comparison with other CATHs because the response of CATHs to vaccination with the H9N2 vaccine occur through the capturing of virus particles and aggregating of virions, then signaling the dendritic cells for promoting a cascade of immune reactions (22).

From the summary of table 3, the FCR was improved in treated groups with SB (groups B and D) from day14 of the experiment. However, this improvement varied at 21 and 28 days between groups. It also became significant at the final stage in groups B and D. Although these two groups were vaccinated with two different vaccines, the results are due to the promoting of epithelial cell lining of the intestine and reducing the population of pathogenic bacteria with an elevation the count of beneficial one by the effect of butyric acid in GIT (23). Then SB increases the growth rate of enterocytes and improves villi length, particularly in the jejunum and ileum (24). Contrary to these results, findings obtained by Pascual *et al.* (25), including SB, did not affect the gut's microbiome population or morphological parameters. The restricted effect of butyric acid in the first week may be due to the consumed material consumed by chicks is not more than 20%. Furthermore, the unacceptable taste and /or odor of SB by chicks tell to adaptation after 2 to 3 weeks later.

Finally, during the analysis of the impact of SB on WG, this study showed a negative effect of SB on WG in the first week, with restricted adverse effects occurring in groups B and D alternatively at 14,21 and 28 days of treatment. The same was reported by Lan *et al.* (14) when utilizing a high concentration of SB 1.2 gm/kg during 1-21 days of age led to a decrease of daily WG in comparison with a basal diet supplemented with 0.3 gm/kg or a basal diet supplemented with 0.6 g/kg of SB. Thus, the high concentration of SB has a negative effect during the early stage of rearing, which was diminished at 22-45 post-treatment. Another interpretation of these results includes the inadequacy of action of digestive enzymes in newly hatched chicks supplemented with SB, this material coated the fat in the GIT; thus, the digestion and absorption of feed ingredients are not entirely achieved primarily in the first week of life (26). These results agreed with Zhang *et al.* (27) when noticed that the application of coated SB at 1 gm/kg did not significantly upgrade WG and PH of GIT. The significant variation of WG between groups may occur due to the effect of different types of vaccines used. These results agreed with Essalah-Bennani *et al.* (28) when they showed significant differences in the vaccination with three types of AI-H9N2 inactivated vaccines; thus, the explanation of high values in WG at 28 and 35 days may be due to the limited number of chicks in each group, high quality of feed and standard condition of rearing. Our results

represent the actual data of the experiment and are close to the standard parameters of WG in the ROSS308 broilers guide. At 35 days of the experiment, no significant WG was detected, and these results are contrary to Shahir *et al*. (3), which referred to the positive effect of SB on WG.

Conclusions

This study indicates that SB dietary supplementation accompanied by the H9N2p vaccine improves innate immunity by stimulating HDPs, including AvBD-10 and CATH-B1. Furthermore, these supplementations did improve FCR but without influence on WG.

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Conflict of interest

Not applicable.

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تأثير بيوتاريت الصوديوم على تحفيز بعض الببتيدات الدفاعية وأداء النمو في فروج اللحم الملقح بلقاحين مختلفين لإنفلونزا الطيور H9N2

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الخلاصة

صممت التجربة لدراسة تأثير بيوتاريت الصوديوم في تحفيز الببتيد الدفاعي بيتا ديفينسين والكاثليسدين ب-١ في فروج اللحم مع استخدام نوعين من لقاحات انفلونزا الطيور المبطلة نوع H9N2 وبيان تأثيرهم على أداء النمو. تم استخدام ١٥٠ فروج لحم بعمر يوم، قسمت عشوائيا الى ٥ مجاميع بواقع ٣٠ فرخ / مجموعة (ثلاث مكررات). تم تلقيح المجموعتين أ و ج بلقاح إنفلونزا الطيور المبطل نوع H9N2 والمطور H9N2p على التوالي، في حين تم معاملة المجموعتين ب و د بمادة بيوتاريت الصوديوم بجرعة ١غم / لتر ماء الشرب يوميا وحتى نهاية التجربة، كما أن هاتين المجموعتين تم تلقيحهما بنفس اللقاحات التي أعطيت للمجوعتين أ و ج على التوالي، وتركت المجموعة هـ بدون معاملة، لقد أوضحت النتائج بان بيوتاريت الصوديوم احدث ارتفاع معنوي في الببتيد الدفاعي بيتا ديفينسين في المجموعتين ب و د بعمر ١٤ يوم مقارنة بالمجموعة أ و هـ ولكن بدون فرق معنوي مع المجموعة ج. بينما كان هذا الارتفاع بعمر ٣٥ يوم واضحا في المجموعتين المعاملتين ب و د. وكذلك نفس الارتفاع حدث مع الكاثليسدين ب-١ بعمر ٣٥ يوم، أن إعطاء بيوتاريت الصوديوم رفع من قيمة معامل التحويل الغذائي في المجموعتين ب و د بعمر ٣٥ يوم على التوالي، لكن إعطاء هذه المادة لم يكن لديه تأثير على معدل وزن الجسم في مجاميع التجربة عند نفس العمر. ومن هنا نستنتج بأن إعطاء بيوتاريت الصوديوم ساهم في تحسين الاستجابة المناعية الفطرية من خلال تحفيز إنتاج الببتيد الدفاعي بيتا ديفينسين والكاثليسدين ب-١، وان إعطائها رفع من قيمة معامل التحويل الغذائي دون التأثير على معدل أوزان الجسم.

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