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Effect of Antibiotic Growth Promoters Alternatives on Growth Performance, Hemato-biochemical and Immunological Profile of Healthy Broiler Chickens

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Abstract

The study was designed to explore the effect of Sanguinarine phytobiotic and sodium butyrate on growth performance, hemato-biochemical and immunological profile of healthy broiler chickens. Ninety, one-day old healthy broiler chicks were divided into three equal groups. Group 1 of healthy chicks was kept as a control group, group 2 in which the broilers received 2 mL/L sodium butyrate and group 3 in which the broilers received 1 g/10 L phytobiotic (Sangrovit)®. At the end of drug administration, two blood samples were collected from each group for hemato-biochemical and immunological parameters. The results revealed that, broiler chicks received sodium butyrate and Sanguinarine phytobiotic showed a significant increase in body weight (2250.64 and 2206.23 g versus 2005.43g in control), weight gain (966.19 and 945.66g versus 855.20g in control), feed consumption and feed conversion rate (1.72 and 1.75 versus 1.85 in control). Hemato-biochemical and immunological analysis revealed a significant increase in total erythrogram (RBCs 4.98 and 5.22 versus 4.37 in control) and leukogram (14.33 and 14.64 versus 11.26 in control), phagocytosis and phagocytic percent (61.23 and 62.05 versus 58.68 in control), nitric oxide (20.34 and 20.48 versus 19.05 in control), hemagglutination inhibition titer against Newcastle disease virus (3.20 and 3.25 versus 2.03 in control), total protein (6.23 and 6.35 versus 5.67 in control) and albumin (3.25 and 3.55 versus 2.95 in control), with non-significant increase in total globulin, α, β, γ globulins, albumin/globulin ratio, liver enzymes; aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase; uric acid and creatinine levels. In conclusion, sodium butyrate and Sanguinarine phytobiotic induced improvement in growth performance, hemato-biochemical and immunological profile in healthy broiler chickens. They could be safely used as alternatives to antibiotics growth promoters in the commercial broiler diet to lower the risk of antibiotic residues in meat and to avoid the antibiotic resistance to the consumers.

Keywords: Phytobiotic, Sodium butyrate, Sanguinarine, Broiler chickens.

Introduction

Antimicrobial growth promoters (AGPs) have been used for improvement of feed efficiency and decrease mortality rates in broilers [1]. The use of AGPs has been very useful for controlling some diseases; but acquired resistance and meat residues of these agents are their major hazards [2]. So, in many countries of the world, the use of AGPs has been banned [3]. The ban of the use of these antibiotics has led to spread of many diseases as necrotic enteritis (NE) which is caused by *Clostridium perfringens* and is considered as one of the most costly diseases in poultry production which causes high losses among affected broilers, reduction in body weight and increased therapy costs [4,5]. Consequently, search for alternatives to antibiotics is important to improve the broiler performance and to optimize the gut health [6].

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Phytobiotics are different substances, mainly plant material extracts of leaves, flowers, seeds, buds, fruits, twigs, root, bark, wood, or herbs [7]. The active materials had many various secondary plant metabolites with wide range of physiological effects [8]. The active compounds of these phytobiotics were mainly secondary plant constituents, such as treenails (monoterpenes, sesquiterpenes and steroids), phenolics (tannins), glycosides and alkaloids (present as alcohols, aldehydes, ketones, esters, ethers and lactones) [9]. Antimicrobial activity and immune enhancement are the two major properties belonging to phytobiotics which are essential for the health and well-being of the chickens [10]. Phytogenetic feed additives (PFA) have been reported for their positive effects as alternatives to antibiotics [11]. They stimulated the feed intake by the secretion of endogenous enzymes, in addition, their antibacterial and antioxidant effects resulting in increased absorption of nutrients from the gut [12].

Sodium butyrate was a salt of butyric acid which can be used in broiler as an alternative to antibiotic growth promoters to improve body performance, gut morphology, and immunity [13]. Sodium butyrate is transformed into butyric acid in the digestive tract of the birds so it improves the intestinal health through many mechanisms [14]. It is involved in the development of gut wall tissues and enhanced the growth of intestinal microflora [15]. In addition, it improves the immune response of broiler chickens [16].

Stemmed from the previous concept, this study was performed to spot the light on the effects of sodium butyrate and Sanguinarine phytobiotic on the growth performance, hematological, immunological as well as biochemical profiles of healthy boiler chickens.

Materials and Methods

Drugs

Sodium butyrate (Admix)® 30: It is a water soluble formulation obtained from Nutri-Ad International NV, Schietstandlaan 2, 2300 Turnhout-Belgium. It is produced by EGAVET Company, 106 Feisal St, Giza, Egypt, registration No: 1/9995 – 22/9/2014, at concentration of 45.00% sodium butyrate and 55.00% carrier water.

Sanguinarine (Sangrovit)® : It is a phytobiotics powder, natural herbal extract anti-Clostridial growth promoter, the active material is Sanguinarine 1% which extracted from Macleaya Cordata. It was made in Germany and obtained from Delta Vet Center, 185 El Orouba Road Heliopolis- Cairo, Egypt, Batch No: 1703a031.

Chickens

A total of 90, one-day old commercial mixed sex Cobb broiler chicks purshased from El-Kahera Poultry Company were used in this study and were kept in wire floor batteries under hygienic measures. All chicks were vaccinated with Newcastle disease virus vaccines (Hitchner B1 on 7 days and laSota on 18 days) and Infectious bursal disease virus vaccine on14 days and the ration used during our study was obtained from Feed Mix Company.

Vaccines

-Hitchner and LaSota live vaccines were obtained from Intervet Boxmeer Company**.**

-.Gumboro vaccine was obtained fromCEVAC® IBD L contains the Winterfield 2512 strain of Infectious Bursal Disease virus in live, freeze dried, France.

Experimental design

On the 19th day of age, all chickens were randomly divided into 3 equal groups (30 chicks each). The first group: healthy chicks (negative control); 2nd group: healthy chicks which received sodium butyrate (Admix) (2 mL/L); 3th group: healthy chicks that received phytobiotics (Sangrovit) (1 g/10 L). Administration of drugs in drinking water was continued for 5 successive days from 19th to 24th day of age. At first day post treatments, five birds from each group were weekly weighed till the end of experiment to determine BW, WG, FC and FCR at 1st, 7th and 14th days post treatments (corresponding to 25, 32 and 39 days of age). The experimental animals were managed according to the management standards. The

experimental study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Sampling

On the first day post administration to the last dose of the drugs, two blood samples from each bird were collected. 1st sample was taken and divided into 2 parts, the first part was put in a tube containing EDTA as anticoagulant, for the hematological studies to determine erythrogram and leukogram [17], packed cell volume [18] and hemoglobin content [19]. The second part was put in a tube containing heparin for determination of phagocytic activity (phagocytic percent and phagocytic index) [20]. The second sample was collected without anticoagulant to obtain clear serum for measuring transaminases (AST-ALT) [21], ALP [22], serum total protein [23], serum protein fractions using cellulose acetate electropheresis test [24], serum uric acid [25], creatinine [26], nitric oxide [27], and hemagglutination inhibition test (HI) for estimation titer of Newcastle virus [28].

Statistical analysis

The obtained data was analyzed by using computerized SPSS program version 21. Using one way analysis of variance (ANOVA). Duncan test was used for determining significance. Probability levels of less than 0.05 were considered significant [29].

Results and Discussion

This study was conducted to explore the effect of Sanguinarine phytobiotic and sodium butyrate on growth performance, hemato-biochemical and immunological profiles of healthy broiler chickens.

Table 1: Effect of Sodium butyrate and Sanguinarine phytobiotic on growth performance of healthy chickens

Groups	initial body Weight (19 day of age)	25th day of age				32nd day of age				39th day of age			
		Body weight	Weight gain	F.C	F.C.R	Body weight	Weight gain	F.C	F.C.R	Body weight	Weight gain	F.C	F.C.R
G1	581.23 ±1.35	768.26 ±1.23^{b}	187.03 ±1.11^{b}	282.40	1.51	1150.23 ±1.37^{b}	381.97 ±1.32^{b}	626.43	1.64	2005.43 ±2.37^{b}	855.20 ±2.40^{b}	1582.12	1.85
G2	585.37 ±1.25	832.32 ±1.48^{a}	246.95 ±1.13^{a}	343.26	1.39	1284.45 ±1.88^{a}	452.13 ±1.87^{a}	682.72	1.51	2250.64 ±1.99^{a}	966.19 ±1.86^{a}	1661.85	1.72
G3	579.42 ±1.20	815.52 ±1.60^{a}	236.10 ±1.14^{a}	335.26	1.42	1260.57 ±1.97^{a}	445.05 ±1.77^{a}	680.93	1.53	2206.23 ±1.87^{a}	945.66 ±1.68^{a}	1654.91	1.75

G1: control group; G2: treated with sodium butyrate; G3: treated with pyhtobiotic; F.C: feed consumption; F.C.R: feed conversion ratio. Means ± SD within the same column with different superscripts are significantly different ($P<0.05$**)**

Table 2: Effect of Sodium butyrate and Sanguinarine Phytobiotic on erythrogram and leukogram in healthy chickens

Groups	RBCs (106/mm^3)	Hb (g/dL)	PCV (%)	Total WBCs X10^3/µL	Deferential leukocytic count X10^3/µL				
					Heterophils	Lymphocyts	Monocytes	Eosinophis	Basophils
G1	4.37 ±0.22^b	10.08 ±0.35^b	31.15 ±0.30^b	11.26 ±0.42^b	2.67 ±0.15^b	3.61 ±0.29^b	1.38 ±0.15^b	1.73 ±0.13^b	1.87 ±0.07^b
G2	4.98 ±0.13^a	10.89 ±0.21^a	32.31 ±1.16^a	14.33 ±0.13^a	3.09 ±0.10^a	4.24 ±0.17^a	1.89 ±0.10^a	2.18 ±0.12^a	2.93 ±0.09^a
G3	5.22 ±0.23^a	11.21 ±0.2^a	33.94 ±1.63^a	14.64 ±0.74^a	3.21 ±0.15^a	4.40 ±0.15^a	1.96 ±0.09^a	2.08 ±0.10^a	2.99 ±0.04^a

G1: control group; G2: treated with sodium butyrate; G3: treated with pyhtobiotic; RBCs: Red blood cells; Hb: hemoglobin content; PCV: Packed Cell Volume; WBCs: White blood cells.
Means ±SD within the same column with different superscripts are significantly different (P<0.05)

The obtained results revealed that, healthy broiler chickens received sodium butyrate and Sanguinarine phytobiotic in the abovementioned doses showed a significant increase in body weight (BW) and weight gain (WG), feed consumption (FC) as well as improved feed conversion rate (FCR) compared with the control group as demonstrated in Table 1. These results were agreed with Owens et al. [30] who reported that, broilers received organic acid (formic acid) showed improved growth performance. They attributed its growth promoting effect to its positive effect on digestion which related to a slower passage of feed in the intestinal tract and more absorption of the necessary nutrients [31]. Also, Izat *et al*. [32] stated that, improvement in body weight gain and feed conversion rate was due to a decrease in pathogenic bacteria in the intestine without any effect on the intestinal pH. In addition, Brzóska *et al*. [33] stated that, improvement in weight gain was due to creation of acidic environment in the gut by sodium butyrate which minimized the load of pathogens. Sanguranine phytobiotic induced elevation in growth performance and health status of poultry [34-36]. The effects of phytobiotics might be due to the presence of essential oils which improved nutrient digestibility due to enhancement the activities of the digestive enzymes as trypsin and amylase [12]. The phytobiotic feed addidives decreasing the production of growth depressing microbial metabolites such as ammonia and biogenic amines and increasing nutrient availability to the host, this was evidenced by the higher numbers of Lactobacillus in the caeca of the phytogenic supplemented group which improved nutrients utilization and absorption [7]. In addition, sodium butyrate had antioxidant activity which lowered the intestinal pH, enhanced the protein digestion, and improved the growth performance in chickens under stress [37].

Table 3: Effect of sodium butyrate and Sanguinarine phytobiotic on phagocytosis, nitric oxide and HI titer in healthy chickens

Groups	Phagocytic percent	Phagocytic index	Nitric oxide	HI log^2 titer
G1	58.68±0.81^b	2.80±1.30^b	19.05±1.32^b	2.03±0.22^b
G2	61.23±0.30^a	3.65±0.32^a	20.34±0.14^a	3.20±0.11^a
G3	62.05±0.97^a	4.00±0.43^a	20.48±0.22^a	3.25±0.13^a

G1: control group; G2: treated with sodium butyrate; G3: treated with pyhtobiotic; HI: Hemagglutination Inhibition titers against Newcastle disease virus. Means±SD within the same column with different superscripts are significantly different (P<0.05)

Table 4: Effect of sodium butyrate and Sanguinarine phytobiotic on serum total protein, albumin and globulin fraction in healthy chickens

Groups	T.P	Alb	Globulin(g/dL)				
			α	β	γ	Total	A/G ratio
G1	5.67± 0.10[b]	2.95± 0.15[c]	0.75± 0.10[a]	0.93± 0.18[a]	1.04± 0.14[a]	2.72± 0.05[a]	1.08± 0.19[a]
G2	6.23± 0.16[a]	3.25± 0.13[b]	0.76± 0.14[a]	0.96± 0.19[a]	1.06± 0.16[a]	2.78± 0.19[a]	1.18± 0.18[a]
G3	6.35± 0.21[a]	3.55± 0.11[a]	0.77± 0.17[a]	0.97± 0.18[a]	1.04± 0.19[a]	2.78± 0.06[a]	1.28± 0.15[a]

G1: control group; G2: treated with sodium butyrate; G3: treated with pyhtobiotic; T.P: Total protein; Alb: Albumin; A/G ratio: Albumin/Globulin ratio

Means ±SD within the same column with different superscripts are significantly different (P<0.05)

Table 5: Effect of Sodium butyrate and Sanguinarine phytobiotic on liver enzymes and kidney Function in healthy chickens.

Groups	Liver enzymes			Kidney function	
	AST	ALT	ALP	Uric acid	creatinine
G1	39.35±0.74[a]	48.76±1.45[a]	235.32±1.3[a]	4.20±0.39[a]	1.04±0.11[a]
G2	39.57±0.86[a]	48.98±1.57[a]	238.42±1.68[a]	4.24±0.51[a]	1.11±0.1[a]
G3	40.39±076[a]	49.84±1.33[a]	238.80±1.59[a]	4.45±0.20[a]	1.22±0.1[a]

G1: control group; G2: treated with sodium butyrate; G3: treated with pyhtobiotic; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase. Means ±SD within the same column with different superscripts are significantly different (P<0.05)

In the current work, broiler chickens supplemented with Sanguinarine phytobiotic or sodium butyrate in the recommended doses showed a significant increase in total erythrocytic and leukocytic counts, hemoglobin content, packed cell volume, heterophils, lymphocytes, monocytes, eosinophils and basophiles (Table 2), with a significant increase in phagocytic activity, phagocytic index, nitric oxide and HI titer against ND virus compared with the control group (Table 3).

Herein, phytobiotics induced improvement in blood picture in broiler chickens that received phytogenic ration [38,39]. These results were in agreement with the results of various previous studies [40-42] who observed that, medicinal plants principles possess a strong improvement in erythrogram and leukogram**.** Additionally, phytobiotic induced an immune potentiating effect and increase phagocytosis and phagocytic index [43]. Similarly, Abd El-Ghany and Ismail [44] reported that, phytobiotic essential oil induced improvement in phagocytic activity.

The increase in total erythrocytic count might be attributed to that the sodium butyrate induced increase in the number of F-reticulocytes, an increase in the number of erythroid progenitors and the number of F-programmed progenitors [45].

In addition, Van Immerseel *et al.* [46] reported that**,** sodium butyrate induced a significant increase in the number of total leukocytic count due to the inhibitory action of sodium butyrate on the intestinal pH that improved the local immune status of the intestinal tract. This acidic pH improves growth and multiplication of beneficial bacteria which support the immune system and increases total leukocytic count production. The same observation was recorded by Abd El-Salam *et al.* [47] and Vinus et al. [48] who stated that; sodium butyrate improved both erythrogram and leukogram. Also, Belih *et al.* [37] observed that, sodium butyrate induced increase in phagocytic activity and phagocytic index in healthy broiler chicks.

Nitric oxide is well-known as antimicrobial chemicals produced by macrophages in response to infection [49]. Nitric oxide, is a key mediator of a great number of physiological and pathological processes, which produced by activated macrophages [50]. The macrophage stimulated by cytokines

(e.g., interferon γ) or microbial products (e.g., Lipopolysaccharides) result in activation of nitric oxide synthase that catalyzes the production of nitric oxide from L-arginine [51]. Our results agreed with a previous study [37] in which sodium butyrate increased nitric oxide in healthy broiler chicks. Also, the same observation was recorded by Chen *et al*. [52] and Erdog *et al.* [53] who demonstrated that, dietary supplementation of phytobiotic improved serum nitric oxide levels in broilers.

In the present work, sodium butyrate and Sanguinarine phytobiotic in the recommended doses showed a significant increase in HI titers against ND virus.

Our findings were in accordance with those recorded by Walter *et al.* [54] and Karimi *et al*. [55] who observed that, Sanguranine phytobiotic improved HI titers against ND virus. Also, healthy broiler chickens received sodium butyrate induced a significant elevation in HI titers against ND vaccine [56].

The current work revealed that, broiler chickens received phytobiotic or sodium butyrate in the recommended doses induced a significant increase in total protein, albumin and non-significant increase in α, β and γ globulins and total globulin compared with the control (Table 4). As documented previously, dietary supplementation of phytobiotic feed additives to the diet of broiler chickens induced increase total protein and albumin [57-60]. The improved protein picture in broilers fed phytobiotics might be due to the higher body weight, which associated with the higher protein mass of the body. In addition, the phytobiotics act on the intestinal walls, promoting the absorption of more nutrients and the secretion of digestive enzymes, which enhanced the nutrient digestibility, leading to improved protein profile [61].

Also, sodium butyrate improved protein picture in healthy broiler chicks [37]. This improvement may be due to the increase of digestibility of proteins, amino acids and the absorption of minerals on supplementation of organic acids [62]. The same results were supported by previous studies which recorded that, dietary organic acid induced significant increased total serum protein and albumin concentration in laying hens [47, 63- 64].

In another work, Tung and Pettigrew [65] stated that, chicks received sodium butyrate showed a significant increases in the total protein and albumin due to increase of the proteolytic enzymes activity and improvement of pancreatic secretions, stimulating the activity of digestive enzymes and increasing the nutrient digestibility. The increase in the absorption rate that provide more amino acids available for serum total protein and albumin synthesis.

On the other hand, broiler chickens received phytobiotic or sodium butyrate in the recommended doses showed non-significant elevation in, liver enzymes (AST, ALT and ALP), uric acid and creatinine (Table 5). Our results clearly reinforced by Therpour *et al*. [66] who concluded that, sodium butyrate induced non-significant elevation in liver enzymes, uric acid and creatinine levels. This may be attributed to the improvement of hepatic and kidney cell functions induced by sodium butyrate administration which related to the better utilization of protein and amino acid digestibility, as uric acid is the major end product of protein metabolism [67,68].

Conclusion

From the obtained results it could be concluded that, the use of sodium butyrate and Sanguinarine phytobiotic induced improvement in growth performance, hemato-biochemical and immunological profiles in healthy broiler chickens and they could be safely used as alternatives to antibiotics growth promoters (AGPs) in the commercial broiler diet to lower the risk of antibiotics` residues in the meat and to avoid the antibiotic resistance to the consumers.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربي

تأثير بدائل المضادات الحيوية المحفزة للنمو على معدل النمو وبعض الوظائف الهيماتوبيوكيميائية والمناعية في كتاكيت التسمين

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قد صممت هذه الدراسة لاكتشاف تأثير السانجيورانين فيتوبيوتك, بيوتيريك الصوديوم على معدل النمو وبعض الوظائف الهيماتوبيوكيميائية والمناعية.فى بدارى التسمين السليمة. إستخدم فى هذه الدراسة تسعون كتكوت تسمين عمر يوم بصحه جيدة و تم تقسيم الكتاكيت إلى ثلاث مجموعات متساوية.كتاكيت المجموعه الأولى وتشمل الكتاكيت السليمة وتعد المجموعه الضابطه . كتاكيت المجموعه الثانيه تم اعطائها الصوديوم بيوتيريك بجرعة ٢ملى / لتر من مياة الشرب , كتاكيت المجموعه الثالثه تم اعطائها الفيتوبيوتك بحرعة ١جم/ ١٠لتر من مياة الشرب. تم تجميع عينيتين دم من كل مجموعة عند نهاية اعطاء تلك المركبات وذلك لدراسه تاثيرها على الوظائف الهيماتوبيوكيميائية والمناعية . أوضحت النتائج أن الفيتوبيوتك, بيوتيريك الصوديوم التى تم اعطائها الى بدارى التسمين قد أدت إلى وجود زيادة معنوية فى وزن الجسم (٢٢٥٠.٦٤ و ٢٢٠٦.٢٣ جرام مقابل ٢٠٠٥.٤٣ جرام فى المجموعة الضابطة) ,الوزن المكتسب (٩٦٦.١٩ و ٩٤٥.٦٦ جرام مقابل ٨٥٥.٢٠ جرام فى المجموعة الضابطة) واستهلاك العلف ومعدل التحويل الغذائى (١.٧٢و ١.٧٥ مقابل ١.٨٥ فى المجموعة الضابطة). وأوضحت نتائج الوظائف الهيماتوبيوكيميائية والمناعية وجود زيادة معنوية فى العدد الكلى لكرات الدم الحمراء (٤.٩٨ و ٥.٢٢ مقابل ٤.٣٧ فى المجموعة الضابطة) ,البيضاء (١٤.٣٣ و ١٤.٦٤ مقابل ١١.٢٦ فى المجموعة الضابطة), نسبة الإلتهام (٦١.٢٣ و ٦٢.٠٥ مقابل ٥٨.٦٨ فى المجموعة الضابطة), اكسيد النيتريك (٢٠.٣٤ و ٢٠.٤٨ مقابل ١٩.٠٥ فى المجموعة الضابطة), التتر المناعى للنيوكاسل (٣.٢٠ و ٣.٢٥ مقابل ٢.٠٣ فى المجموعة الضابطة) ,البروتين الكلى (٦.٢٣ و ٦.٣٥ مقابل ٥.٦٧ فى المجموعة الضابطة) والزلال (٣.٢٥ و ٣.٥٥ مقابل ٢.٩٥ فى المجموعة الضابطة) بالاضافة الى زيادة غير معنوية فى الفا بيتا وجاما جلوبيولين ,الجلوبيولين الكلى ,الأسبرتيت امينوترانسفيراز,الالنين امينوترانسفيراز, الفوسفاتيز القاعدى ,حمض اليوريك والكرياتينين. نستخلص من هذه الدراسة أن اعطاء السانجيورانين فيتوبيوتك, بيوتيريك الصوديوم قد عملا على تحسين أداء النمو والوظائف الهيماتوبيوكيميائية والمناعية فى بدارى التسمين السليمة . ولذلك توصى الدراسة باستخدامهما كبدائل للمضادات الحيوية المحفزة للنمو فى النظام الغذائى التجارى لبدارى التسمين، وذلك للتقليل من مخاطر وجود بقايا المضادات الحيوية فى لحوم الدواجن المستهلكة آدميا.