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Immunopharmacological Evaluation of Synbiotics and Enramycin in Broilers

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Abstract

The present study was planned to evaluate the influence of synbiotic and enramycin on the broiler immunity and growth performance. In a complete randomized design, 90 unsexed day old Cobb broiler chicks were randomly assigned into three treatments with three replicated. The first control group fed basal diet only, the 2nd group consumed basal diet plus enramycin (0.5g/kg diet), and the 3rd group fed basal diet fortified with synbiotic (0.5g/kg diet) up to 42 days. The results revealed a significant (P<0.05) improvement of the growth performance considerations, phagocytic index, and phagocytic percentage in synbiotic fortified group in comparison with other groups. Oral supplementation with synbiotic resulted in up regulation of interleukin-4 (IL-4) and interferon-γ (IFN-γ) in cecal tonsils and spleens when compared with the control and enramycin groups. However, the antibody titers against Newcastle disease (ND), Avian Influenza (AI), and Infectious Bronchitis (IB) viruses were not obviously changed between the tested groups at both 28 and 42 days. Moreover, the enramycin caused a significant (P<0.05) adverse effect on the liver function enzymes as compared with other groups. In conclusion, the synbiotic can be considered as a potential feed additive alternative to antibiotic with desired effect as an enhancer for both cellular and gut immunity, as a growth promoter without adverse effect on the liver healthiness.

Keywords: Direct-Fed Microbiota, Enramycin, IFN-γ, IL-4, Synbiotics.

Introduction

Genetic selection of high performance poultry traits has been performed intensively to magnify its production, as poultry is one of the most important food suppliers for cheap protein source worldwide. But development of modern intensive farming and high stocking densities adversely affect the immune functions and the natural resistance of birds to pathogenic infections [1]. Therefore, the poultry producers widely used the antibiotics to magnify growth capacity and health condition of the birds [2].

Enramycin is a polypeptide antibiotic produced by *Streptomyces fungicides* [3]. Enramycin is one of the most common antibiotics that incorporated in the broilers feed for growth promotion purposes [4]. The misuse of antibiotic growth promoters (AGP) in the stockbreeding resulted in remaining of antibiotics in the animal-derived food [5]. Additionally, many countries have restricted or even banned the use of antibiotics as feed additives, due to increased concerns regarding the proliferation and the transmission of antibiotics resistant bacteria via the food chain [6]. Moreover, the excess quantity of the antibiotics can do great harm to human and environment. Although the strengthening the legislation, establishing a perfect detection methods, and setting up a strict management system, the antibiotics residue still a major problem [4]. Besides, development of vaccines and chemical drugs, including antibiotics was contributed in the control of various acute infectious diseases. Nevertheless, serious infections primarily attributed to spread of stress-linked immunosuppression which is a tough to treat with antibiotics [7]. Therefore, developing new substitutes of antibiotic can effectively solve the problems caused by antibiotics in animal-derived food.

From this aspect, the current trend in poultry production pointed to reduce use of

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AGP and increase the use of non-antibiotic feed additives such as direct-fed eco-friendly microbiota [5, 8]. The intestinal ecosystem contains a highly diverse microbial community which influences the equilibrium state of the intestinal community. Beneficial microflora not only prevents some specific intestinal pathogens, produces various nutrients, improves the chicken intestinal metabolome [9, 10], and enhance the general performances but also, improves the local and systemic immunity [11, 12]. The antibiotics alternatives used in the poultry field should have similar efficacy on the growth improving ability and proper production without the adverse effect of antibiotics [13] in sum, these substitutes lead to cheerful financial income with better production [14]. In addition, these natural supplements regulate the host immune system and provide a simple avenue for improving poultry health and production [15, 16].

Synbiotics are unique natural feed additives that have been used in poultry industry to avoid the side effects of antibiotics with valuable effects on the poultry manufacturing [17]. The synbiotics composed of a mixture of probiotics and prebiotics. They include prebiotics in order to overcome some possible difficulties in survival of probiotics in the intestinal tract and ensuring an appropriate environmental media for the probiotics [18]. The dietary inclusion of poultry synbiotic enhance the body weight and feed conversion rate [19]. Synbiotics have antimicrobial properties, other health-related benefits through maintenance of the intestinal biostructure and improve the immune system in poultry by enhancing the gut-associated lymphoid tissue (GALT) and generalized immunity [20]. The early administration of synbiotics to poultry has a profound effect on the production of cytokines and chemokines especially those involved in the regulation of specific and nonspecific immunity [21].

In this vein, the present study was intended to evaluate the growth-promoting activity of synbiotics and enramycin as feed supplementations in broilers, besides testing their effects on the cellular and humoral immunity and GALT.

Materials and methods

Feed supplements

Synbiotic used was Poultry Star® which is a poultry-specific synbiotic product (Biomin GmbH, Austria). The synbiotic composed of fructoligo-saccharides prebiotic 90% and 10% blend of bacteria which is a unique mixture of dried probiotic bacteria belonging to the genera Enterococcus, Pediococcus, Lactobacillu, and Bifidobacterium species with minimum of $5X10^{12}$ CFU/ kg. Enramycin HCl 40%, Enradin 40®, was purchased from MSD Animal health.

Birds and rearing condition

Adapted ninety one-day old Cobb broilers have been used. The chicks were housed on slatted floored pens system. Environmental conditions were adjusted at humidity 50±10% and temperature was ranged from (32±1 to 22°C) according to the age. Commercial ration purchased from El- Fajr Company, Alexandria, Egypt was used in this clinical trial as the following; from one day old to the 21^{st} day used commercial mash diet (23% protein and 2900 k. calory ME/kg). From the 21^{st} day to 42^{nd} day used commercial starter-grower mash diet (21% protein and 3050 k. calory ME/kg). These commercial diets were formed in accordance to the nutritional requests as recommended by the NRC [22]. The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University.

Experimental design and vaccination

Each group was placed in a specific slatted floor cage which was subdivided into three partitions (each contain 10 chicks). Control group fed on basal diet only, enramycin administered group fed on basal diet containing enramycin (Enradin® 0.5g/kg feed), and synbiotic-treated group consumed a synbiotic mixed basal diet (Poultry star® 0.5g/kg feed). All treatments continued for 42 consecutive days.

The birds were routinely immunizied against newcastle disease (ND), infectious bronchitis (IB), avian influenza (AI) (H9N2) and Infectious Bursal Disease (Gumboro, IBD). IB and ND vaccine (Live Hitchner B1 and IB (H120) vaccine (Izo S.p.A, Italy, Batch

No.: 5032058) was administered to the chicks at 7th day-old via ocular route.

Inactivated ND (clone30) and IB (H 120) vaccine (Intervet, Holland, Batch No: 5021CMR2) were applied at 8th day-old by subcutaneous injection. Inactivated subcutaneous injection of AI (H9N2) vaccine (Merial, Spain, Batch No: 33405821) was applied at 9th day-old. IBD (Nobilis®Gumboro - LiveD78 vaccine, Intervet, Holland, Batch No: 5034058) was applied at both 10th and 19th day-old by eye drop. Live attenuated Nobilis® of IB (MA5) and ND (Clone 30) vaccine (Intervet, Holland, Batch No: A261CMD1) were applied at 20th day-old through eye drops.

Growth performance parameters

The body weight, feed intake, total food conversion ratio (FCR) and feed efficiency were measured at the end of the experiment according to Awad *et al*. [23]. The feed intake was adjusted weekly and finally counted together at end of six weeks.

Sampling

Blood sampling and biochemical analysis

After 21th, 28th, 35th, and 42th days of the study, the blood samples (n=6) were collected from different replicates into heparinized and normal test tubes. The heparinized blood samples were used for assessing the Immunological parameters. The blood samples collected into the normal test tubes were left in room temperature then centrifuged at 3000 rpm for 5 minutes for sera collection. The separated sera were kept at -20 °C until estimation of some biochemical and immunological parameters. The sera collected at 21th and 42th days old were used for quantitative assessment of liver function enzymes using semi-automated spectrophotometer (Erbaa-Chemi7, Germany). Serum level of aspartate transferase (AST) and alanine transferase (ALT) were assessed. Serum levels of creatinine and uric acid were estimated in accordance to the methods of Donsbough *et al*. [24] and Caraway and Hald [25], respectively.

Tissue sampling

Cecal tonsils and spleens were quickly dissected out and rinsed with 0.9% NaCl. The tissues were snap frozen then stored at -80 until used for genes expression analysis.

Immunological studies

Cellular immunity

Heparinized blood samples that collected at 21th and 35th days-old were directed for Cellular immunity assessment. *Candida albicans* culture (50 µL) were added to one mL of heparinized blood and placed in water bath with shaker at 24-26°C for three up to five hours. Then, blood smears were taken and stained with Geimsa stain. The phagocytic activity was evaluated by calculating the numbers of phagocytes containing intracellular yeast cells up to 300 macrophages and stated as percentage of phagocytic activity (PA%) using this equation; Phagocytic activity= numbers of phagocytes containing *Candida* yeast/ number of Macrophages ×100. While, the phagocytic index (PI) can be determined by this equation; Phagocytic index= Number of cells phagocytized divided by the number of phagocytic cells [26].

Humoral immunity

At the age of four and six weeks of the experiment, six wing vein blood samples were collected (two samples per each replicate) for serum isolation. These serum samples were subjected to hemagglutination inhibition (HI) test against ND and AI (H9N2) antigens prepared in the Reference Laboratory for veterinary Quality Control on Poultry Production (RLQP), Dokki, Giza, Egypt. These ND and H9N2 antigens were accustomed to 4 haemagglutinating (HA) units. Controls on the antigen content in the HI test were created using serial two-fold dilutions starting at 1:2. The titers were represented by the maximum dilution viewing complete inhibition of HA and statistically analyzed to estimate the humoral antibody titers against ND and AI (H9N2) vaccines [27]. Antibody titers against the IB virus was estimated by ELISA test using commercial licensed ELISA kits (BioChek, Synbiotics, IDEXX) according the manufacturers instruction [28].

Gut immunity and quantitative RT-PCR analysis

Collected cecal tonsils and spleens from six birds per each group were used for evaluation of the immunity of the gut associated lymphoid tissue. Extraction of cecal tonsils and spleens RNA was performed using RNeasy Mini Kit for stabilization of RNA in harvested tissue and subsequent total RNA purification according to manufacturers' instructions (Qiagen GmbH, Düsseldorf, Germany), and used for cDNA synthesis. Real-time PCR was achieved using QuantiTect Probe RT-PCR Kit as one-step qRT-PCR using sequence-specific probes for gene expression estimation (Qiagen GmbH, Düsseldorf, Germany). The Cycler™ was programmed to 94°C for 10 min, 40 cycles at (94°C for 15 sec, 60°C for 1 min), and then a final extension at 72°C for 10 min followed by a melting curve program (55–95°C in increasing steps of 0.5°C). 28SrRNA gene was used as controls to normalize the qRT-PCR. The Primers and probes pair combinations used are 28SrRNA [Forward: 5' GGC GAA GCC AGA GGA AAC T 3', Reverse: 5' GAC GAC CGA TTT GCA CGT C 3' and Probe: 5' (FAM) AGG ACC GCT ACG GAC CTC CAC CA (TAMRA) 3'] [29]; interleukin-4 (IL-4) [Forward: 5' AAC ATG CGT CAG CTC CTG AAT 3', Reverse: 5' TCT GCT AGG AAC TTC TCC ATT GAA 3' and Probe: 5' (FAM) AGC AGC ACC TCC CTC AAG GCA CC (TAMRA) 3'] [29] and interferon-γ (IFN-γ) [Forward: 5' AAA CAA CCT TCC TGA TGG CGT 3', Reverse: 5'CCG TGA GAA ATA TGA TTC CTT GG 3' and Probe: 5' (FAM) TGA AAG ATA TCA TGG ACC TGG CCA AGC TC (TAMRA) 3'] [30].

Amplification curves and C_T values were evaluated by Stratagene MX3005P software. To detect the difference of genes expression on the RNA level of each groups, the C_T of each sample was matched with that of the control group agreeing with the "$^{\Delta\Delta}$Ct" method stated by Yuan *et al.*[31].

Statistical analysis

The obtained data were processed statistically by using the general linear model of Minitab 18. The variances between means were created by using Tukey honest significant difference (HSD) ($p \leq 0.05$). There was no significant effect of replicates on the measured parameters; therefore the data from all replicates for each group were combined.

Results and Discussion

The synbiotic fed group had significant higher body weight, body weight gain, and feed efficiency compared to the basal diet and enramycin containing diet fed groups, respectively as illustrated in Table 1.

Table 1: Effects of Enramycin and Synbiotic on the Total Body Performance of Broiler chickens

	Control	Enramycin	Synbiotic
Final body weight (g)	2170±22.5^{b}	2150.6±25.1^{b}	2419.4±30.9^{a}
Total feed intake (g)	4684.4±52^{a}	4739.3±57^{a}	4697.9±25.4^{a}
Total FCR	2.16±0.03^{a}	2.21±0.05^{a}	1.94±0.02^{b}
Total feed efficiency	0.46±0.006^{b}	0.45±0.009^{b}	0.52±0.006^{a}

All values are expressed as means ± standard error (SE).
Means at the same row with dissimilar superscripts are statistically different (P<0.05).

Oral supplementation with synbiotic feed additive resulted in a significant (*P<0.05*) improve in feed conversion ratio (FCR) in the control and enramycin received broilers (Table 1). However, feed intake did not significantly change between the experimental groups. The positive effect of synbiotic could be risen from synbiotic nature as a mixture of probiotic bacteria (*Enterococcus, Pediococcus, Lactobacillus* and *Bifidobacterium*). The probiotic portion of synbiotic possesses the competitive exclusion that deprives the harmful bacteria from attachment sites in the intestinal wall and in turn improve the survival and activity of beneficial bacteria [32]. The probiotic bacteria maximize the nutritive value of the normal diet. While, the prebiotic portion of synbiotic provide favorable intestinal condition that enhances the activity and metabolism of beneficial bacteria and stagnate the growth of hurtful bacteria [33].

Additionally, prebiotic (fructo-oligo-saccharides) could facilitate the colonization of beneficial bacteria. The summation of action of probiotics and prebiotics together create a cheerful ecosystem in the broilers gut resulting in increasing the host metabolic activity and decreasing the bacterial metabolic activity and ammonia production [34]. These results agreed with the outcomes of Sarangi *et al*. [35], Nikpiran *et al.* [36] who reported significant increase in broiler chickens performance after adding different types of bacteria and yeast to their diet. On the contrary, Lee *et al*. [37] reported no differences in body weight gain by direct-fed microbials in broiler chickens diet.

The liver can be affected by any chemical agents emitted from the intestine. Monitoring of serum enzymes is a useful marker of hepatocellular damage in chicken exposed to toxic substances in feed [38]. As, there is boundless relation between the liver healthiness and the body performance, higher serum hepatic enzymes (AST and ALT) is indicative of improper liver function causing deficient performance while, lower serum hepatic enzyme is indicative for proper liver function causing superior performance [39]. In the current study, the dietary incorporation of enramycin resulted in significant increase in the broiler serum level of ALT and AST at both 21 and 42 days of the experiment (Table 2).

Table 2: Effects of Enramycin and Synbiotic on the liver and kidney Function tests at both 21 and 42 days in Broiler Chickens

parameters	Time	Control	Enramycin	Synbiotic
ALT (U / L)	21 days	5 ± 0.19^{b}	8.75 ± 0.17^{a}	5.3 ± 0.16^{b}
	42 days	5.22 ± 0.18^{b}	8.65 ± 0.35^{a}	5.75 ± 0.26^{b}
AST (U / L)	21 days	197 ± 4.07^{b}	260 ± 7.03^{a}	204.83 ± 5.02^{b}
	42 days	204.17 ± 5^{b}	292.33 ± 6.18^{a}	203.17 ± 5.68^{b}
Creatinine (mg/dl)	21 days	0.54 ± 0.05^{a}	0.49 ± 0.04^{a}	0.5 ± 0.02^{a}
	42 days	0.54 ± 0.06^{a}	0.54 ± 0.04^{a}	0.5 ± 0.03^{a}
Uric acid (mg/dl)	21 days	6.12 ± 0.26^{b}	8.27 ± 0.33^{a}	5.75 ± 0.30^{b}
	42 days	7.12 ± 0.44^{b}	8.48 ± 0.60^{a}	7.07 ± 0.43^{b}

All values are expressed as means ± standard error (SE); n=6.
Means at the same row with dissimilar superscripts are statistically different (P<0.05).
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 3: Effect of Enramycin and Synbiotic on Phagocytic Index and Phagocytic Percent at 21 and 35 days in Broiler Chickens

Parameters		Control	Enramycin	Synbiotic
Phagocytic index	21 days	3.28 ± 0.08^{b}	3.15 ± 0.1^{b}	4.22 ± 0.15^{a}
	35 days	3.25 ± 0.13^{a}	3.2 ± 0.12^{a}	3.33 ± 0.14^{a}
Phagocytic percent	21 days	62.17 ± 1.4^{b}	62.17 ± 1.08^{b}	70.33 ± 1.36^{a}
	35 days	61.50 ± 1.59^{a}	61.33 ± 1.67^{a}	61.83 ± 2.21^{a}

Values are expressed as means ± standard error (SE); n=6.
Means within the same row with different superscripts are significantly different (P<0.05).

These results came fit with the results of Fayez *et al.* [40] who revealed that enramycin treatment had a negative effect on the liver that cause a distinctive elevation in the serum level of ALT and AST in broilers. The main threated effect of antibiotics on the liver is through its metabolism in the liver that mainly causes a great damage to the hepatic cells [41]. In the current study, the synbiotic treatment in this study, have no positive or negative effects on the liver and kidney healthiness. These results agreed with the observations of Das *et al*. [42] who elicited that, the synbiotic administration in broilers diet resulted in a pronounced enhancement in the growth without any dangerous effects on the liver.

The thymus contains the smaller lymphocytes which is responsible for cell mediated immunity (CMI), nevertheless, the bursa have the large lymphocytes, which transform into plasma cell in the tissue and play a significant role in humoral immunity

[43]. The current study examined the usefulness of enramycin and synbiotic on the gut immunity as a local site of action of these essences. Additionally, it inspected their effects on the systemic immunity as cellular and humoral immunity.

The measurement of cellular immunity in poultry can be carried out by determining the phagocyte activities. The phagocyte activities may be elucidated as, the bacterial cell activate immune response through a self-motivated interaction with specific Toll-like receptors on the surface of as Toll-like receptors dependent [44]. This interaction between host cells and pathogens or their structural components may play a fundamental role in the early innate immune response [44]. In this study, synbiotic administered group illustrated an obvious ($p<0.05$) increase in the phagocytic index (4.22) and activity (70.33%) at third week of the experimental trail than other studied groups as clarified in Table 3. However, at the fifth week of the experiment there were no substantial variances among varies tested chicks.These results in agreement with the results of Razek and Tony [45] and El-Sissi and Mohamed [46] who found that the dietary administration of synbiotic in broilers improve the phagocytic activities of cellular immunity through increase the phagocytic percent and phagocytic index. On the other hand, El-Shenway and Soltan [47] indicated that, the synbiotic had no distinctive effect on the phagocytic percent and phagocytic index.

However, the effects of enramycin and synbiotic on humoral immunity were demonstrated in Table 4. The humoral antibody titres against ND, H9N2 and IB at both 28^{th} and 42^{th} days were non-significantly differing among experimental groups.

Table 4: Effects of enramycin and synbiotic on the humoral antibody titers

parameters		Control	Enramycin	Synbiotic
ND	28 days	3.33±0.33 [a]	3.5±0.56[a]	4±0.97[a]
	42 days	5.17±0.79[a]	5±0.73[a]	5.83±0.6[a]
AI (H9N2)	28 days	2.5±0.43[a]	2.83±0.6[a]	3.17±0.31[a]
	42 days	3.5±0.43[a]	3±0.73[a]	3.83±0.54[a]
IB	28 days	3712.2±42.5[a]	3694.2±43.6[a]	3655.8±73.6[a]
	42 days	9579±218[a]	9345±149[a]	9111±112[a]

Values are expressed as means ± standard error (SE); n=6.
Means within the same row with different superscripts are significantly different (P<0.05).
ND: Newcastle disease, AI: Avian Influenza, IB: Infectious Bronchitis.

Table 5: Effects of Enramycin and Synbiotic on the Gene Expression of IL-4 and IFN-γ in both Cecal tonsils and Spleen at the age of 35 days of Broiler Chickens

Organ	Parameters	Control	Enramycin	Synbiotic
Cecal tonsil	IL4	1[b]	1.86±0.32 [b]	4.34±0.63 [a]
	IFN-γ	1[b]	1.42±0.47 [b]	3.97±0.41[a]
Spleen	IL4	1[bc]	2.84±0.37[b]	8.53±0.93[a]
	IFN-γ	1[b]	1.72±0.1[b]	7.37±0.33[a]

Values are expressed as means ± standard error (SE); n=6.
Means within the same column with different superscripts are significantly different (P<0.05).

In poultry, there are no lymph nodes, however, there is a lateral immune system which, comprises from spleen and gut associated lymphoid organs (GALT) as payer's patches and cecal tonsils [48]. The GALT is exposed to the microflora from feed and the environment. Thus, there was a close relation between the intestinal microflora and the GALT [49]. Where, the gastrointestinal community have evident effects on the gene expressions of the pro-inflammatory and anti-inflammatory cytokines as well as on the expressions of genes involved in immunity [50]. In the present study, we examined the effects of enramycin and synbiotic on the gene expression of pro-inflammatory cytokine IL-4 (cytokine of th2) and antiviral cytokine IFN-γ (cytokine of th1) in both cecal tonsils and

spleens at the age of 35 days of the study Table 5. The dietary inclusion of synbiotic resulted in a marked upregulation of gene expression of IL-4 and IFN-γ in both cecal tonsils and spleen at the age of 35 days compared to the control group and enramycin administrated group. These results come hand in hand with the results clarified by Yitbarek, *et al.* [20] who revealed that the synbiotics administration showed a significant upregulation in the immune-related cytokines in the intestinal immune organs. In contrast Płowiec *et al*. [51] reported that synbiotic treatment in broilers resulted in down regulation in the gene expressions of immune-related cytokines in both cecal tonsils and spleen. The upregulation of pro-inflammatory cytokine and antiviral cytokine in our study may be attributed to the proper mixture of probiotics and FOS in used synbiotic and also to the method and duration of administration.

The strength of the synbiotics on the broiler immunity is provoked from the combination of probiotics and prebiotics in the same product. Both probiotics and prebiotics are able to create a healthful environment inside the intestinal tract. Therefore, the beneficial intestinal ecosystem could result in a proper improvement in health, immunity and performance of the broilers.

Conclusion

The dietary incorporation of synbiotic in broilers feed resulted in a significant improvement in the broiler gut and cellular immunity without any noticeable effect on humoral immune response in addition to growth performance enhancement without any deleterious effects on the liver and kidney. However, the enramycin had an adverse effect on liver and kidney livability without any profitable effects on the growth performance and immunity. In the future, our lab aiming to study the effect of AGP and synbiotics of intestinal microbiome and metabolome.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربى

قياسات دوائية مناعيه على السينبيوتك والانراميسين في بداري التسمين

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اجريت هذه الدراسة لتقييم تأثير السينبيوتك والانراميسين على معدلات النمو و المناعة فى بدارى التسمين. تم تقسيم ٩٠ كتكوت كب عمر يوم عشوائيا الى ثلاث مجموعات متساويه (٣٠) بحيث ان كل مجموعة تحتوي على ثلاث مكررات. المجموعة الاولى (المجموعة الضابطة) ,المجموعة الثانية تم تغذية الكتاكيت على عليقة مضاف اليها الانراميسين (١/٢ جم لكل كجم علف) طوال ٤٢ يوم والمجموعة الثالثة: تم تغذية الكتاكيت على عليقه مضاف اليها السيتبيوتك (بولترى ستار) (١/٢ جم لكل كجم علف) طوال ٤٢ يوم. النتائج أظهرت زيادة معنوية في معدلات النمو ,في عمل وكفاءة الخلايا الاكوله في الدم وفى التعبير الجيني لانترلوكين ٤ وانترفيرون جاما في الطحال ولوزتي الاعورين في الطيور المغذاه على عليقة تحتوي على السينبيوتك عن المجموعتين الآخرتين. مع عدم وجود أي تحسن ملحوظ في مستوى الاجسام المناعية فى المصل ضد مرض النيوكاسل ,انفلونزا الطيور والالتهاب الشعبى ذلك عند عمر ٢٨ و٤٢ يوما بالترتيب بين الثلاث مجموعات. مع الاخذ في الاعتبار ان الانراميسين ادى الى تأثيرات سلبية في كفاءة وعمل الكبد والكليتين عن باقي المجموعات. فمن الدراسة والنتائج يتضح انه من الممكن استخدام السنبيوتك كبديل فعال وامن للمضادات الحيوية في تحفيز المناعة والنمو وبدون الاثار السلبية للمضادات الحيوية.