IMMUNOMODULATORY EFFECT OF VIR-CLEAR@ IN BROILER CHICKENS

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ABSTRACT

*A total of two hundreds and sixty day-old broiler chicks were used to investigate the immunomodulatory effect of **Vir-clear®** in broiler chickens. Chicks were grouped into four groups (each containing 60 chicks) and each group was sub-divided into three subgroups (each containing 20 chicks), while the remaining 20 chicks were handled as control group (the fifth group). Group A chickens were administered **Vir-clear®** as immunomodulator additives on the ration and respectively vaccinated with LaSota vaccine and AI-ND oil adjuvant vaccine via eye instillation and s/c injection at 10 days old and Gumboro disease vaccine at 15 days old via drinking water. Group B chickens were vaccinated with LaSota vaccine at 10 days old via eye instillation and fed on ration with immunomodulater additives (Vir-clear). Group C chickens were vaccinated with LaSota vaccine, AI-ND oil adjuvant vaccine via eye instillation and s/c injection at 10 days old and with Gumboro disease vaccine at 15 days old via drinking water with no immunomodulatory additives on the ration . Group D chickens were vaccinated with LaSota vaccine at 10 days old via eye instillation with no immunomodulatory additives on the ration. The remaining 20 chickens were left as a control non medicated & non vaccinated group (Group E). Blood samples were collected weekly for four successive times. Antibody titer for ND, AI and Gumboro?were estimated and body weights were recorded at weekly intervals during the experiment period. Antibody titer for ND was increased significantly ($p< 0.05$) in group treated with **Vir-clear®** than those of the non-treated groups. There was non signtficant increase in antibody titer of AI and Gumboro disease in comparison to non medicated groups. Also there was a non significant difference in body weights in treated groups and those of the non-treated ones.*

*Finally it could be concluded that the use of **Vir-clear®** as a feed additive in the broiler chicken ration has a beneficial effect on the immune status of the birds especially for ND vaccination. Therefore, it is recommended to use **Vir-clear®** as a feed additive in the broiler chickens especially those are under stress conditions.*

INTRODUCTION

The sequel of intensive poultry production is the insulation of chicken immune system that results in decreased resistance and increased susceptibility of birds to infectious agents and opportunistic pathogens. For many decades antibiotic growth promoters (AGPs) have been used in feeding farm animals to improve performance and minimizing incidence of diseases. The use of antibiotics as feed additives has been restricted and is now totally banned throughout the European Union, because of the general problem of increased resistance of bacteria and decreased acceptance of the consumers for this type of additive.

The development of antibiotic-resistant bacteria had led to a need for alternatives to antibiotics for growth promotion and discase prevention in poultry production. Helical polysaccharide beta-1,3/1,6-glucan is a derivative of Saccharomyces cervisiae cell wall and has immunomodulating activities (**Huff et al. 2006; Benites et al. 2008**). Differcnt substances, referred to as Natural Growth Promoters (NGP), had been identified as effective and safe alternatives to AGP to coup with consumer and animal welfare safety requirements.

NGP are supposed to achieve high consumer acceptance since they do not pose any risk in terms of bacterial rcsistance or residucs in animal products (**Recht, 2005**). At present, there is a large number of NGP available at the market, including probiotics, prebiotics and immuno-modulators. These products have the potential to influence thc intestinal tract in a positive way thus improving the health, well-being and performance of animals. Today probioties are widely used in modern animal nutrition because of their beneficial effect on the host (**Fuller, 1989**). Prebiotics are defined as "non-digestible feed ingredients" that beneficially affect the host by selectively stimulating the growth and / or activity of one or a limited number of bacteria in the colon, thus improving host health (**Gibson and Roberfroid,1995**). Through the combination of probiotics and prebiotics in so called synbiotics synergistic effects can be achieved (**Roberfroid, 1998**).

To the best of the author's knowledge, there are no pervious experimental evaluation for the clinical effects of **Vir-clear®** on poultry; therefore the objective of the present study was to evaluate the effect of **Vir-clear®** on the immune response against ND, AI, Gumboro and body weight.

MATERIALS AND METHODS

Experimental chicks :

Two hundred and sixty day-old broiler chicks were used. Experimental chicks were randomly divided into four groups(A,B,C&D) each containing 60 chicks. Each group was sub-classified into three sub groups(each containing 20 chicks). The remaining 20 chicks were left as non-medicated non-vaccinated controls(group E). Birds were fed ad lib. Body weights were recorded weekly during the experiment period.

Experimental design :

Group A fed **Vir-clear®**-medicated-ration from 2 days old till the end of the experiment. Also were vaccinated with inactivated ND-AI

via subcutaneous injection, LaSota vaccine via eye instillation at 10 days old and Gumboro-Intermediate Plus vaccine vat 15 days old via drinking water.

Group B chicks fed Vir-clear-medicated-ration from 2 days old till the end of the experiment. These chicks were only vaccinated with LaSota vaccine at 10 days old .

Group C chicks fed ration non medicated with Vir-clear, vaccinated with inactivated ND-AI vaccine via subcutaneous injection , LaSota via eye instillation at 10 days old and Gumboro-Intermediate Plus vaccine via drinking water at 15 days old .

Group D of chicks fed ration non medicated with Vir-clear and only vaccinated with LaSota vaccine via eye instillation at 10 days old .

Meanwhile group E chicks were remained as negative control (non-medicated, non-treated group) .

Serum samples

Fifteen serum samples were randomly collected from 15 experimental chicken at 10 days old just before vaccination. Fifteen serum samples were collected from each groups at 1,2,3, and 4 weeks post-vaccination. All collected sera were stored at -20C until use.

Haemoagglutination inhibition (HI) test for ND and AI:

Micro-HI test for both ND and AI were performed according to (**Terregino and Capua, 2009**).

Antibody titration for Gumboro:

Antibody titers for infectious bursal disease were estimated and calculating using Synbiotics IBD-ELISA test kits and the ProFILE 2.0 programs for windows. Plates were red using the Biotic ELX 800G plate reader.

Statistical analysis :

Statistical analysis was carried out using statistical software program (GMP for windows Version 5.1, SAS Institute, Cary, NC, USA). Treatment outcomes were assessed firstly by evaluating the homogeneity of experimental groups. The antibody titers on the day of the first examination were compared among groups by the Kruskal-Wallis nonparametric ANOVA test. Furthermore, data was subjected to repeated measures MANOVA to determine the main effects of drugs and time. The Walk's Lambda test was selected to evaluate within group interactions and evidence of time x treatment interactions. Where Walk's Lambda test indicated a statistically significant difference between treatment groups, one way ANOVA with Tukey-Kramer HSD post-hoc multiple comparison test was used to identify which group was statistically different from the rest. Differences between means at $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

The immunomodulation concept could be traced back to the observation that was recorded by workers more than a century ago (**Sedlacek et al. 1991**). Immune-modulator substances could produce either suppression or enhancement of immunologic activities of chickens (**Quinn 1990**). In the present study, it is the first report that study the immunomodulation effect of **Vir-clear®** in broiler

chickens. There was a significant increase of ND antibody titer but not AI or IBD antibody titer in group treated with **Vir-clear®** than those of the non-treated groups (Table 1, 2, 3). These results are in agreement with those reported by **Alison (1997)** who found that immunoadjuvants are used to increase efficacy of the vaccines. Immunostimulants are non specific in nature and are usually used to increase the resistance against infection.

Vir-clear showed non significant difference in body weights in all experimental chickens (table 4).

Finally, it could be concluded that the use of **Vir-clear®** product as a feed additive in the broiler chickens has a beneficial effect on the immune status of chickens especially for ND vaccination and increase the resistance of chickens exposed to different stress factors. Therefore, it is recommended to use Vir-**clear®** as feed additives in the broiler chickens especially those are under stress conditions. Further studies still needed to be done to evaluate the immunomodulatory effect of **Vir-clear®** in the breeders and layer and to clarify variable immunostimulating effects of Vir-clear on different vaccines.

Table 1 : Effect of **Vir-clear** on immune response [HI titers (log2)] to Newcastle disease vaccines (table 1).

Group	Weeks post vaccination				
	Zero	1	2	3	4
Group A	5.5	4.1±0.13a	6.1±0.23ab	8.0±0.35a	7.3±0.23a
Group B		4.2± 0.2a	5.3±0.26bc	4.5±0.37b	3.2±0.42c
Group C		4.7±0.15a	6.2±0.13a	7.3±0.44a	5.1±0.72b
Group D		5.1±0.37a	4.6±0.21cd	4.0±0.30b	2.3±0.23c
Group E		5.3±1.02a	3.7±0.18d	3.8±0.53b	1.0±0.00c

MANOVA fit, $p < 0.05$

Walk's Lambda test for within all interaction, $p < 0.05$

Walk's Lambda test for treatment x time interaction, $p < 0.05$

A,b,c,d: means with different superscript letter in the same column are significantly different at $p<0.05$

Table 2: Effects of **Vir-clear** on immune response [HI titers (log2)] to avian influenza H5N2 vaccine (table 2).

Group	Time post-vaccination (week)				
	Zero	1	2	3	4
Group A	4.0	1.7±0.37a	3.7±0.60a	5.2±0.55a	5.5±0.38a
Group C		2.3±0.42a	3.2±0.31ab	4.2±0.49a	5.5±0.41a
control		1.1±0.55a	1.7±0.52b	1.7±0.55b	0.2±0.25b

MANOVA fit, $p < 0.05$

Walk's Lambda test for within all interaction, $p < 0.05$

Walk's Lambda test for treatment x time interaction, $p < 0.05$

a,b,c,d: means with different superscript letter in the same column are significantly different at $p<0.05$

Table 3: Effect of **Vir-clear** on antibody response (ELISA, Synbiotic) to IBD vaccines (table 3).

Group	Weeks post vaccination				
	zero	1	2	3	4
Group A	15531	8936±658a	5591±795a	13998±754a	17850±706a
Group C		8936±658a	7363±2273a	12795±924a	17452±938a
Control		8936±658a	3230±594a	5089±1924a	15665±1167a

MANOVA fit, $p < 0.05$

Walk's Lambda test for within all interaction, $p < 0.05$

Walk's Lambda test for treatment x time interaction, $p < 0.05$

a,b,c,d: means with different superscript letter in the same column are significantly different at $p<0.05$

N.B.: Lateral transmission of live vaccines is assumed between different groups .

Table 4: Effect of feeding **Vir-clear** on body weights of broiler chickens (table 4).

Group	Weeks post **Vir-clear** administration				
	1	2	3	4	5
Group A	205.2±2.01a	388.9±3.4a	636.2±6.3a	915.7±8.4a	1245.6±12.3a
Group C	208.08±1.97a	396.4±3.1a	642.2±5.5a	936.8±8.1a	1257.6±17.8a

MANOVA fit, $p< 0.05$

N.B.: Birds were involuntary subjected to feed restriction between weeks 4&5.

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الملخص العربى

تأثير مركب الفيركلير على التحولات المناعية فى دجاج التسمين

كامل إبراهيم أبوالعزم محمد أحمد على يوسف
محمد أحمد على يوسف دينا العسيلى

فى هذه الدراسة تم إستخدامعدد ٢٦٠ كتكوت تسمين عمر يوم لبحث تأثير مستحضر الفيركلير كمحفز مناعى وتأثيره على أوزان دجاج التسمين، قسمت الكتاكيت عشوائياً لأربع مجموعات رئيسية (٦٠ مجموعة) كما تم تقسيم كل مجموعة رئيسية إلى ثلاث مجموعات فرعية (٢٠ مجموعة)، بينما تم معاملة الكتاكيت الباقية (٢٠ كتكوت) كمجموعة ضوابط (المجموعة الخامسة).

كتاكيت المجموعة (أ) تم تغذيتها على عليقة تحتوى على مستحضر الفير كلير كما تم تحصينهنا باستخدام لقاح لاسوتا (بالتقطير العينى)، لقاح النيوكاسل وانفلوانزا الطيور الثنائى الميت بالحقن تحت الجلد عند عمر ١٠ أيام لقاح الجمبورو الحى متوسط الضراوة عند عمر ١٥ يوم فى مياه الشرب.

كتاكيت المجموعة (ب) تم تغذيتها على عليقة تحتوى على مستحضر الفيركلير كما تم تحصينها باستخدام لقاح لاسوتا (بالتقطير العينى)، لقاح النيوكاسل وانفلونزا الطيور الثنائى الميت بالحقن تحت الجلد عند عمر ١٠ أيام لقاح الجمبورو الحى متوسط الضراوة عند عمر ١٥ يوم فى مياه الشرب.

كتاكيت المجموعة (ب) تم تغذيتها على عليقة تحتوى على مستحضر الفيركلير، كما تم تحصينها فقط باستخدام لقاح لاسوتا (بالتقطير العينى) عند عمر ١٠ أيام.

كتاكيت المجموعة (ج) تم تغذيتها على عليقة خالية من مستحضر الفيركلير وتم تحصينها باستخدام لقاح لاسوتا (بالتقطير العينى)، لقاح النيوكاسل وانفلونزا الطيور الثانى الميت بالحقن تحت الجلد عند عمر ١٠ أيام ولقاح الجمبورو الحى متوسط الضراوة عند عمر ١٥ يوم فى مياه الشرب.

كتاكيت المجموعة (د) تم تغذيتها على عليقة خالية من مستحضر الفيركلير، وتم تحصينها باستخدام لقاح لاسوتا (بالتقطير العينى) عند عمر ١٠ أيام.

بينما كتاكيت المجموعة (هـ) المكونة من ٢٠ كتكوت تصف كمجموعة ضابطة بدون تحصين وتغذت على عليقة بدون مستحضر الفيركلير.

تم تجميع عينات السيرم إسبوعياً لمدة أربعة أسابيع متتالية لقياس الأجسام المناعية لمرض النيوكاسل، انفلونزا الطيور والجمبورو كما تم قياس أوزان الطيور إسبوعياً.

فكانت النتيجة وجود إرتفاعاً معنوياً فى الأجسام المناعية لمرض النيوكاسل وعدم وجود إرتفاعاً معنوياً فى مرض إنفلونزا الطيور أو

مرض الجمبور فى المجموعات المعالجة بالمستحضر عن المجموعات الأخرى التى لم تتعاطى المستحضر، كما لوحظ عدم وجود إختلافاً معنوياً فى أوزان الطيور بين المجموعات المعالجة وتلك التى لم تأخذ العلاج.

الخلاصة :

إستخدام مستحضر الفيركلير كإضافة على العلف له تأثير فعال على الحالة المناعية للطيور وخاصة بعد تحصين النيوكاسل؛ لذا ينصح بإضافة فيركلير على العلف فى دجاج التسمين خاصة تلك التى تتعرض لحالات من الإجهاد.