



Research Article

Natural Herbs CLEANACTIV®; Immune-Modulator, Health Activator and Growth Promoter in Broiler Chickens

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ABSTRACT

Nowadays increasing attention to herbs as natural products used in the medicine exhibit a various and promising resource in poultry health. However, these products are often characterized for their chemical composition as well as mechanisms of action which improve the performance and immunity in chicken's flocks. The present study investigates the immune-modulatory, health activation and growth promotion effects of CLEANACTIV® which is a mixture of essential oil on broiler chickens. Four hundred one day old commercial broiler chickens were reared in two separated groups each one contains 200 chicks, 1st group "Drinking Water CLEANACTIV® Administrated", 2nd group "Control non CLEANACTIV® medicated group". Serum samples and organs as intestine collected for evaluation of immune status and epithelium healthiness at. Immunological Parameters as INF- γ , Phagocytic index and Mucosal IgA concentration were evaluated using manufactured kits accompanied by intestine histomorphology. The results proved that CLEANACTIV® Administrated stimulate immune response by increased level of measured immunological parameters and also increase immune cells infiltration in intestinal epithelium with high livability and low feed conversion rate.

Key words: CLEANACTIV®; Immue-modulator; Health Activator; Growth Promoter; Broiler chickens

INTRODUCTION

The immune system has evolved as a defence mechanism against infections as it comprises structural and cellular elements that are dispersed throughout the body, so inappropriate immune regulation lead to diseases which accompanied by inflammation (Calder *et al.*, 2009). Our study investigate health activation, growth promotion and immune-modulation of CLEANACTIV® essential oil (CEO), which is a mixture of volatile oils such as; cassia oil 1.2%, garlic oil 0.3%, oregano oil 0.5%, nettle extract 3.3%, quillaya extract 0.15%, carvacrol 0.3%, naringin 0.05 % and hesperidin 0.1%. At least there are 3000 essential oils derived from herbal plants considered as volatile plant secondary metabolites which found in glandular hairs or secretory cavities of plant-cell wall with small molecules exhibit excellent medicinal properties (Basri *et al.*, 2014). Hesperidin and naringin are members of flavonoids, is an inexpensive by-product of sweet orange and lemon (Bosi *et al.*, 2004). It has a similar effect as antibiotics and improves growth (Ying *et al.*,

2009) as it reduces oxidative DNA damage in blood cells, inflammatory response, and alleviates oxidative stresses (Morand *et al.*, 2011). Nettle herbal is one of the most valuable plants used in phytotherapy and recommended as supportive treatment for many illnesses and act as source of amino acids, vitamins as well as flavonoids, tanins, and lectins; so it has anti-inflammatory, antimicrobial, immunomodulatory and hepato-protective properties (Asgarpanah *et al.*, 2012). Oregano oil, thymol and carvacrol added to broilers' feed to promote growth, this oil act as an antioxidant and it can be maintained meat lipid oxidative stability (Symeon *et al.*, 2010). Many pathogens establish contact with host at mucosal surfaces at which adaptive immune defense is initiated by lymphocyte activation and the local secretion of IgA. One of the main functions of IgA is immune exclusion, where binding of IgA to antigen interferes with pathogen attachment and colonization. Saponin has stimulatory effects on the components of specific immunity and non-specific immune reactions such as inflammation (de Oliveira *et al.*, 2001) and monocyte proliferation (Yui *et*

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al., 2001). *Cinnamomum cassia* bark, commonly used as traditional Chinese medicine for treating inflammatory diseases as it contains several active components such as essential oils (cinnamic aldehyde, cinnamic alcohol, and cinnamic acid), and tannin (He *et al.*, 2005). Immunomodulation is one of the main targets for herbal extracts in broiler chickens. The use of herbal plants as health promoters is gaining increasing attention in both consumers and scientific immunomodulatory actions, like modulation of cytokine secretion; phagocytosis promotion and macrophage activation; immunoglobulin production; allergic reactions and lymphocyte proliferation. Recently, garlic has been suggested as a promising candidate for maintaining the homeostasis of the immune system (Mahima *et al.*, 2012), so in the current study CLEANACTIV® essential oil (CEO) evaluated as immunomodulatory agent by measuring immunological indices as INF- γ , Phagocytic index and Mucosal IgA also feed conversion rate, intestinal integrity and growth performance were recorded to illustrate its health activation and growth promotion effect.

MATERIALS AND METHODS

Broiler chickens

Four hundred commercial Hubbard broilers were used and All vaccination program including Infectious Bronchitis, Newcastle and Infectious Bursal Disease "Intermediate plus" were given, also they divided in two groups, First group (Gp1: CLEANACTIV® treated gp): consists of 200 chicks, anti-mycoplasma drug (Tylosin tartarate, 100 mg) was given in the first 3 days for 8 hours daily. On the other hand, CLEANACTIV® was given with the recommended dose for 16 hours daily. At days 8 and 9 of age, CLEANACTIV® was given for 2 successive days in drinking water continuously and repeated at days 20, 21 and 22 of age. Second group (Gp2, control group): consists of 200 chicks and given anti-mycoplasma in the first 3 days for 8 hours daily. Blood, serum and intestinal tissue samples were collected from both groups for measuring immunological parameters and histomorphology.

Mucosal IgA

Intestinal IgA measured according to Guillermo Tellez *et al.*, 2017 using chickens IgA ELISA kit for (Catalog No. E33-112, Bethyl Laboratories Inc., Montgomery, TX 77455) at 7, 15, 28 and 35 days of age from the two groups.

Phagocytosis assay

CytoSelect™ 96-Well Phagocytosis Assay (Red Blood Cell Substrate) Catalog Number: CBA-220 according to (Yu *et al.*, 2015) on blood samples collected at 7, 15, 28 and 35 days of age from the two groups.

INF- γ assay

Chicken INF- γ ELISA kits according to (Karakolev *et al.*, 2015) on serum samples that collected at 7, 15, 28 and 35 days of age from the two groups.

Health activation and growth performance

According to (Afsharmanesh and sadaghi, 2014), Feed intake and feed efficiency were calculated, while

Body weight gain also recorded according to (Abdel-Rahman *et al.*, 2013) during the period of experiment till 5th week of age.

Histomorphology

Intestinal specimens were collected from birds in each group at 15 and 35 days of age then were fixed in 10% buffered formalin (Bancroft and Layton, 2013)

Statistical analysis

The results were presented as means \pm SD. using the one way ANOVA (Start Soft INC.). Differences were considered significant at $P \leq 0.05$.

RESULTS

Results of SIgA conc.

Mucosal IgA conc. in CLEANACTIV® treated gp was 50 ng/ml at 7 days of age while at 15 day was 80 ng/ml and at 28 day was 150 ng/ml and increased to 270 ng/ml at 35 days of age, while in control non treated group the obtained results were 12 ng/ml at 7 days of age, 20 ng/ml at 15 days of age, 60 ng/ml at 28 days of age and 80 ng/ml at 35 days of age.

Results of phagocytosis assay

CLEANACTIV® treated gp showed representative phagocytosis resemble with number of RBCs inside phagocytic cells which is 2×10^3 at 7 day of age and increased to 4×10^3 , 3×10^4 , 3×10^5 at 15, 28, 35 day of age respectively, while in control group the RBCs number is 2×10^2 , 4×10^2 , 2×10^3 , 3×10^3 at 7, 15, 28, 35 day of age respectively.

Results of IFN- γ conc.

IFN- γ conc. in CLEANACTIV® treated gp were 80 pg/ml, 150 pg/ml-220 pg/ml and 420 pg/ml at 7, 15, 28 and 35 days of age respectively, in comparison with 40 pg/ml, 70 pg/ml, 110 pg/ml and 150 for the 2nd group at the same interval respectively.

Results of performance parameters and livability

The mean feed consumption in CLEANACTIV® treated gp was 3.2 kg/bird and the mean weight gain was 2.3 kg/bird with livability 96% while the mean feed consumption in control gp was 3.42 kg/bird and the mean weight gain was 1.95 kg/bird with livability 88%.

Results of histomorphology

Intestinal tissue histomorphology in CLEANACTIV® treated gp represented in Fig. 1 and Fig. 3 at 15 and 35 days of age respectively at which normal villous core and epithelium tissue appeared at 15 day of age with mild edema and heterophile infiltration at 35 day of age, in contrast in control group desquamated epithelium with crypts degeneration were recorded in Fig. 2 and Fig. 4.

DISCUSSION

Immuno-stimulants enhance the overall immunity of the host and present a non-specific response against the microbial pathogens. They also work to heighten humoral and cellular immune responses, by either enhancing

cytokines secretions, or by directly stimulating B or T lymphocytes To maintain immune homeostasis and its functions, the immune system requires building blocks and essential element for the production of signaling molecules, proliferation of cells, and synthesis of effector molecules (Puri *et al.*, 2000). Current research focus on the possibility of improving the functions of the immune system of by nutritional means, for example, some scientists are investigating the possibility that supplementation with certain nutrients, such as vitamins probiotics prebiotics and essential oils (Adams *et al.*, 2008). In the present study CLEANACTIV® which is a mixture of volatile and essential oils evaluated as assistant tools in improving broilers chickens growth , performance and immunity as the mean feed consumption in CLEANACTIV® treated gp was 3.2 kg/bird and the mean weight gain was 2.3 kg/bird compared with low value in control group. Gut immunity plays a crucial role in maintenance of the whole body immunity being the first and most important line of defense from various

pathogenic. CLEANACTIV® treated gp intestinal tissue histomorphology represented in fig 1 and fig 3 at 15 and 35 days of age respectively at which normal villous core and epithelium tissue appeared at 15 day of age with mild edema and heterophile infiltration at 35 day of age , in contrast in control group desquamated epithelium with crypts degeneration were recorded. The avian intestinal immune system can mount an effective antigen specific IgA antibody response to enteric pathogens (Muir *et al.*, 1998). Intestinal SIgA conc. in CLEANACTIV® treated gp is dramatically increase with age as at 7 days of age was 50 ng/ml while at 15 day was 80 ng/ml and at 28 day was 150 ng/ml and increased to 270 ng/ml at 35 days of age, while in control non treated group the obtained results were 12 ng/ml at 7 days of age , 20 ng/ml at 15days of age , 60 ng/ml at 28 days of age and 80 ng/ml at 35 days of age. Recently, polyphenols as hesperidin and naringin have gained attentions which are secondary metabolites of plants as it has antioxidant and immune-modulatory properties on the innate and acquired immune

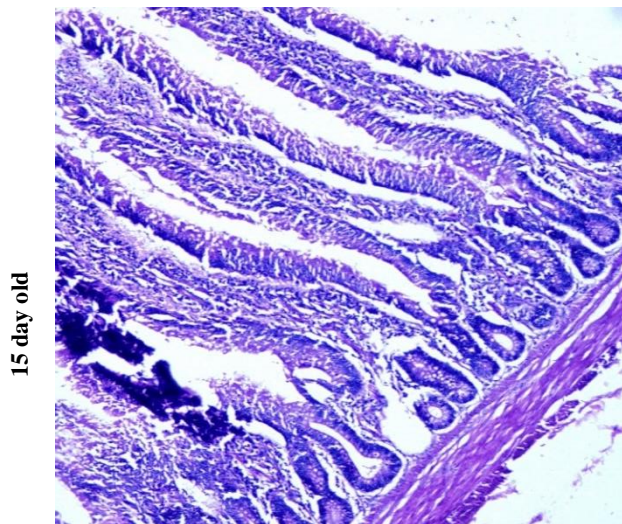


Fig. 1: Histograph of intestinal tissue of CLEANACTIV® treated gp showing the healthy villi and epithelium cells.

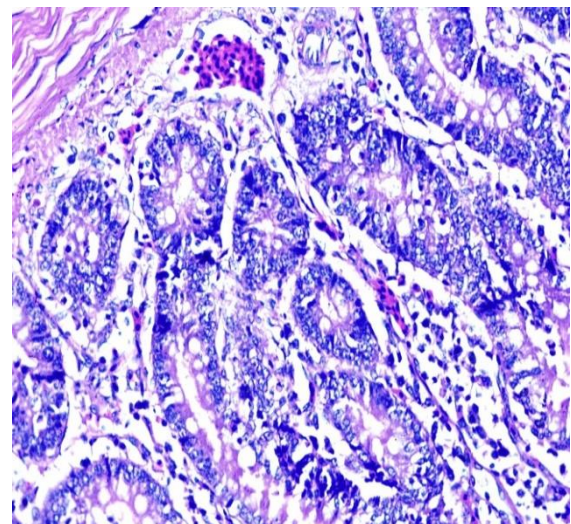


Fig. 2: Histograph of intestinal tissue of control gp showing desquamated epithelium cells with injured villous core.

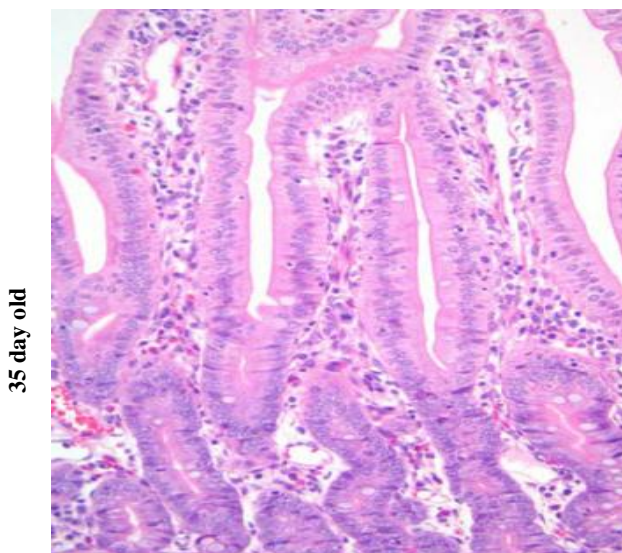


Fig. 3: Histograph of intestinal tissue of CLEANACTIV® treated gp showing mild edema infiltrated with numerous heterophile

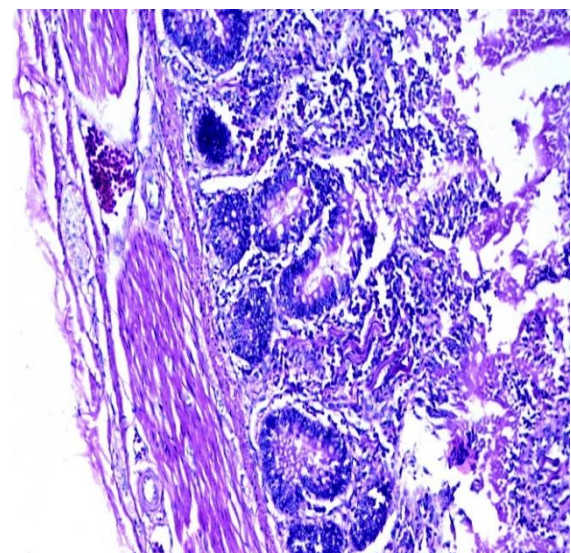


Fig. 4: Histograph of intestinal tissue of control gp showing hyperplasia with crypts degeneration.

response., also extract of *Urtica dioica* plant has shown significant antioxidant activity also an increase in the proportion of T-lymphocytes (Garg *et al.*, 2001; Ratnam *et al.*, 2006). Quillaya extract as saponin induce production of cytokines such as interleukins and interferons that might mediate their immune-stimulant effects, promoting cytotoxic T-lymphocyte (CTL) response (Sjölander *et al.*, 2001). IFN- γ conc. in CLEANACTIV® treated gp were 80 pg/ml, 150 pg/ml-220 pg/ml and 420 pg/ml at 7, 15, 28 and 35 days of age respectively compared with 40pg/ml, 70 pg/ml, 110 pg/ml and 150 for the 2nd group at the same interval respectively. The phagocytosis of pathogens is essential for fighting infections as it comprises a series of events, starting with the binding and recognition of particles by cell surface receptors, so The impairment of phagocytosis leads to severe infections and immune-deficiencies (Lei *et al.*, 2001), in the current study CLEANACTIV® treated gp showed high phagocytosis resemble with number of RBCs inside phagocytic cells which is 2×10^3 at 7 day of age and increased to 3×10^5 at 35 day of age respectively while in control group the RBCs number is 2×10^2 and 3×10^3 at 7 and 35 day of age respectively. In broilers chickens, the immune effect and production performance are essential items during rearing, and an unbalanced relationship between them lead to failure IFN- γ maintains regulation between production and immune function (van der *et al.*, 2011).

Conclusions

Health performance has significance for broilers chickens breeding in terms of economic issues. In commercial settings, improvements in growth rate, physiological and immunological function are always more profitable, in the present study CLEANACTIV® which is novel essential and volatile oils mixture has a reactive effect on health, growth, gut integrity and immunity of broiler chickens.

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