

## COMPARISON OF THE EFFECT OF PEPPERMINT, ALOE VERA AND VITAMIN E SUPPLEMENTATION ON BROILER IMMUNE RESPONSE

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### Abstract

Given the currently increased focus of researchers on applications of medicinal herbs as a natural additives to animal and poultry feed for enhanced performance of the immune system, the present study aims to examine effects of peppermint, Aloe vera, and Vitamin E on immune response in broilers. In this experiment, three hundred one-day old male broilers (Ross 308) were used on a completely randomized design in 4 groups with 5 replicates, each consisting of 15 broilers. The experimental groups included the control group (basal diet with no additive), a group that received 10 g/kg dry peppermint leaves (added to basal diet), a group given 10 g/kg Aloe vera gel (added to basal diet), and a group treated with 100 mg/kg Vitamin E. Evaluation of antibody titer against Newcastle disease virus indicated that on day 27, the highest level of antibody titer was observed in the Vitamin E group, showing a significant difference from other groups ( $P < 0.05$ ) except for the group that received the dry peppermint leaves. On day 37, the highest level of antibody titer against Newcastle disease virus was found in the Vitamin E group with a significant difference compared to the herb groups (the groups that received dry peppermint leaves and Aloe vera gel) as well as the control group ( $P < 0.05$ ). On the other hand, on days 27 and 37, the groups that received dry peppermint leaves and Aloe vera gel showed significant increase in their antibody titer against Newcastle disease virus compared to the control group ( $P < 0.05$ ). The results after injection of phytohemagglutinin-P on day 40 demonstrated that broilers in the Aloe vera gel group and those belonging to the dry peppermint leaves group significantly enhanced their response to phytohemagglutinin-P solution compared to the control group and to the broilers that were given Vitamin E ( $P < 0.05$ ). On day 42, a significant increase was observed in total white blood cell counts of broilers in the Aloe vera gel group and the dry peppermint leaves group compared to the control broilers, although the largest number of white blood cell was found in the broilers that received Vitamin E ( $p < 0.05$ ). In general, our results showed that supplementation of broiler feed with either dry peppermint leaves or Aloe vera gel enhances immune response.

**Key words:** Peppermint, Aloe vera, Vitamin E, Immune system, Broiler.

### INTRODUCTION

In the recent years many researchers have focused on improving broiler immune system and enhancing resistance against pathogens by using feed additives. Among these additives, medicinal herbs have received greater attention due to the ban on antibiotic growth promoters and research conducted in this area has shown that addition of herbs to feed can enhance responses of the immune system. In addition, herb supplements have been shown to improve growth performance or alter the intestinal microflora of broilers. Peppermint (*Mentha piperita* L.) has antioxidant, antitumor, anti-allergy, antiviral, antibacterial and fungicidal properties

(MacKay and Blumberg, 2006). Peppermint has been applied in veterinary practice for treatment of internal and external parasites as well as digestive diseases (Laudato and Capasso, 2013), and recently it has been used to supplement poultry feed to enhance growth performance and reinforce the immune system. Aloe vera (*Aloe barbadensis* Miller) has also antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory, antitumor, immunomodulatory, wound healing, and anti-diabetic properties (Christaki and Florou-Paneri, 2010). Furthermore, in the past few years, Aloe vera has been used as poultry feed additive to improve growth performance and the immune system and to treat coccidiosis. Vitamin E can also enhance humoral and

cellular immune response (Gore and Qureshi, 1997). Although previous studies have shown positive effects of Peppermint and Aloe vera on the immune system, further studies are needed to determine the extent to which these herbs can induce positive impacts. Therefore, the aim of this study was to evaluate the immunostimulatory effect of dry Peppermint leaves and Aloe vera gel supplementation on broilers. A secondary aim was to study whether using dry Peppermint leaves and Aloe vera gel as natural feed additives for broilers can positively affect the immune system in a similar mode to that of Vitamin E supplementation.

## MATERIALS AND METHODS

Three hundred male Ross 308 broilers were assigned randomly to four treatment groups with five replication of fifteen birds each. The treatment groups consisted of the control group (basal diet with no additive), a group receiving dried Peppermint leaves at 10 g/kg (mixed with broiler feed), a group receiving Aloe vera gel at 10 g/kg (mixed with broiler feed), and a group that received Vitamin E at 100 mg/kg (mixed with broiler feed in the form of alpha-tocopheryl acetate). Feed requirements for the broilers at the different growth stages, namely starter (day 0-10), grower (day 11-24), and finisher (day 25-42) were in accordance to the Ross 308 guidelines and the basal experimental diet based on corn-soybean meal was formulated using the UFFDA software. The amount of energy for starter, grower, and finisher periods was 3334, 3150, and 3200 kcal ME/kg, respectively with crude protein content of 25.12%, 22.10%, and 19.76%. During the experimental period, all chickens were given complete access to water and feed. In addition, management factors such as temperature, humidity, light, ventilation, and vaccination were the same for all groups.

Newcastle disease vaccine (LaSota) was administered with drinking water at 16 days of age. Blood samples from the right wing vein were collected 11 and 21 days after vaccination from three chickens from each replication. Antibody response was determined by using the hemagglutination inhibition (HI)

test and the titer obtained through this method was reported as  $\log_2$ .

On day 40 of the experiment period, three birds were randomly selected from each replication. First, 0.1 ml of phytohemagglutinin-P (PHA-P) solution was intradermally injected to the web of the third and the fourth digit of the right foot of each bird, and 0.1 ml of a phosphate buffer saline (PBS) as the control solution was injected to the web connecting the third and the fourth digit on the left foot. The web thickness was measured by a micrometer before and 24 hours after the injection. Broiler immune response to PHA-P was calculated with the following equation:

(response to PBS solution injected to the left foot) – (response to PHA-P solution injected to the right foot)

where:

- Response to PHA-P solution injected to the right foot = post-injection thickness of skin – pre-injection thickness of skin;

- Response to PBS solution injected to the left foot = post-injection thickness of skin – pre-injection thickness of skin.

White blood cell count was determined on blood samples from the right wing vein from three chickens from each replication that were randomly selected. Natt-Herrick method was used to count white blood cells.

Experimental data was analysed with one way ANOVA and post-hoc analysis was conducted the Duncan's multiple range test at  $P < 0.050$ . Statistical software package SAS version 9.2 (2009) for Windows (SAS Institute Inc., Cary, NC, USA) was used.

## RESULTS AND DISCUSSIONS

Antibody titer against Newcastle disease virus (NDV), response to PHA-P-injection, and total white blood cell count are presented to Table 1. Regarding antibody titer against NDV there were significant differences ( $P < 0.05$ ) between treatments and the highest values of antibody titer were observed in the Vitamin E group broilers in both examined periods (days 27 and 37). Furthermore, on day 27, antibody titer was higher ( $P < 0.05$ ) in the dry Peppermint leaves group in comparison to the Aloe vera gel group

whereas there was no difference between the two treatments on day 37.

There was a significant increase ( $P<0.05$ ) in the response to PHA-P injection for the dry Peppermint leaves and the Aloe vera gel groups when compared to the Vitamin E group whereas the smallest response to PHA-P was observed in the control group.

With regard to blood cell count, there was a significant increase ( $P<0.05$ ) in the Vitamin E group in comparison to the other groups. Furthermore, a significant increase was also observed in the dry Peppermint leaves and the Aloe vera gel groups compared to the control group.

Table 1. Effect of Peppermint, Aloe vera and Vitamin E supplementation on broiler immune system

Treatment	Antibody response to NDV ( $\log_2$ )		PHA-P (mm)	White blood cells count ( $\times 10^3/\mu\text{l}$ )
	27 days	37 days	40 days	42 days
Control	1.57 <sup>c</sup>	2.31 <sup>c</sup>	0.387 <sup>c</sup>	21.80 <sup>c</sup>
Peppermint	2.37 <sup>a</sup>	3.36 <sup>b</sup>	0.545 <sup>a</sup>	22.42 <sup>b</sup>
Aloe vera	2.21 <sup>b</sup>	3.32 <sup>b</sup>	0.547 <sup>a</sup>	22.47 <sup>b</sup>
Vitamin E	2.45 <sup>a</sup>	3.77 <sup>a</sup>	0.502 <sup>b</sup>	22.67 <sup>a</sup>
SEM	0.145	0.139	0.016	0.76

a, b, c Means with different superscripts within the same column are significantly different ( $P<0.05$ ).

In this experiment we used the medicinal herbs dry Peppermint leaves and Aloe vera gel as well as Vitamin E as immunostimulants, and as seen in the results, these additives (Peppermint, Aloe vera, and Vitamin E) improved antibody titer against NDV, response to PHA-P injection, and total white blood cell count of broilers compared to the control group. A limited number of studies have been conducted on the effect of Peppermint on broiler immune system. Emami et al. (2012) reported that essential oil of Peppermint (200 and 400 mg/kg) failed to enhance antibody titer against sheep red blood cell (SRBC) in male chickens. Moreover, Toghiani et al. (2010) did not observe a significant difference on day 42 in terms of total white blood cells in chickens that received dried Peppermint leaves (4 and 8 g/kg). On the other hand, Sabaghi-Darimiyan et al. (2014) examined the effect of Peppermint powder (1% and 2% of feed) on Japanese quail and

reported that Peppermint powder can enhance antibody titer against SRBC compared to the control group, with the highest level of antibody titer observed in a group that received 2% Peppermint powder. Dosti et al. (2012) reported improvements on the immune system capabilities in chickens that received Peppermint powder (10, 15, and 25 g/kg) which resulted in higher antibody titer against Gumboro and Bronchitis as well as larger total white blood cell count compared to the control group. The discrepancies in the results seem to have been stemmed from either the administration form or the dose level.

As shown in Table 1, dry Peppermint leaves increased antibody titer against NDV and total white blood cell count in broilers, particularly on day 40 when a significant increase was observed in the response to PHA-P injected compared to the Vitamin E group. The exact mechanism through which Peppermint affects the immune system is not well understood; however, this may be the result of strong antioxidant and antibacterial properties which indirectly affect this system. In fact, the menthol contained in Peppermint leaves can effectively stimulate appetite and help digestion while also act as an antiseptic (Kamel, 2000). Another study identified menthol as an effective agent that produces antimicrobial properties of Peppermint (Iskan et al., 2002).

Studies examining Aloe vera have revealed that chickens which received Aloe vera gel with their drinking water (Valle-Paraso et al., 2005), Aloe vera gel mixed with their feed (Darabighane et al., 2012), Aloe vera powder mixed with their feed (Alemi et al., 2012) presented increased antibody titer against NDV compared to the control groups (with no additive). Furthermore, broilers that received aquatic and ethanol extract of Aloe vera (Akhtar et al., 2012) and Aloe vera gel mixed with their feed (Darabighane et al., 2012) had enhanced cellular immune response to PHA-P injection in comparison to the control groups. Other studies reported increased number of white blood cell as a result of supplementing feed with Aloe vera gel powder (1%, 0.75%, and 0.5%) (Mahdavi et al., 2012) or addition of Aloe vera gel to drinking water (2%) (Valle-Paraso et al., 2005). Zhang and Tizard

(1996) attributed these positive impacts of Aloe vera on the immune system to acemannan (a polysaccharide contained in Aloe vera gel) that activates macrophages, produces cytokines, and releases nitric oxide. However, the enhancement of intestinal microflora and the subsequent improvement of the immune system response may be attributed to the antibacterial properties of Aloe vera similarly to the overall reported antibacterial properties of medicinal herbs.

## CONCLUSIONS

The results of our experiment indicated that supplementation of broiler diet with either dried Peppermint leaves or Aloe vera gel improved broiler immune system response. However, additional studies are required for the determination of optimum supplementation level as well as administration form.

## REFERENCES

- Akhtar M., Hai A., Awais M.M., Iqbal Z., Muhammad F., ul Haq A., Anwar M.I., 2012. Immunostimulatory and protective effects of Aloe vera against coccidiosis in industrial broiler chickens. *Veterinary parasitology*, 186(3): 170-177.
- Alemi F., Mahdavi A., Ghazvinian K., Ghaderi M., Darabighane B., 2012. The effects of different levels of Aloe vera gel powder on antibody titer against Newcastle disease virus and performance in broilers. *Proc. International Poultry Scientific Forum*. Georgia World Congress Center, Atlanta, Georgia, 47.
- Christaki E.V., Florou-Paneri P.C., 2010. Aloe vera: a plant for many uses. *Journal of Food, Agriculture and Environment*, 8(2): 245-249.
- Darabighane B., Zarei A., Shahneh A.Z., 2012. The effects of different levels of Aloe vera gel on ileum microflora population and immune response in broilers: a comparison to antibiotic effects. *Journal of Applied Animal Research*, 40(1): 31-36.
- Dosti A., Taherpour K., Nasr J., 2012. The effect of dietary Peppermint (*Mentha piperita*) supplementations on immune system of broiler chickens. *Proc. 5<sup>th</sup> Iranian Congress on Animal Science*, Isfahan, Iran.
- Emami N.K., Samie A., Rahmani H.R., Ruiz-Feria C.A., 2012. The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. *Animal Feed Science and Technology*, 175(1): 57-64.
- Gore A.B., Qureshi M.A., 1997. Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. *Poultry Science*, 76(7): 984-991.
- Iscan G., KIrimer N.E.S.E., Kùrkcüoglu M., Baser H.C., DEMIrci F., 2002. Antimicrobial screening of *Mentha piperita* essential oils. *Journal of Agricultural and Food Chemistry*, 50(14): 3943-3946.
- Kamel C., 2000. Natural plant extracts: Classical remedies bring modern animal production solutions. In 3rd Conference on Sow feed Manufacturing in the Mediterranean Region 31-8.
- Laudato M., Capasso R., 2013. Useful plants for animal therapy. *OA Alternative Medicine Italy*, 1(1): 1-6.
- Mahdavi A., Alemi F., Ghazvinian K., Ghaderi M., Darabighane B., 2012. Study of effects of different levels of Aloe vera gel powder on antibody titre against sheep red blood cells and other blood parameters in broilers. *Abstracts, British Poultry Abstracts* 8(1): 49-50.
- McKay D.L., Blumberg J.B., 2006. A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytotherapy Research*, 20(8) : 619-633.
- Sabaghi-Darimiyan V., Mehri M., Bagherzadeh-Kasmani F., Ghazaghi M., Vorassi-Ardakani H., Poortaheri M., 2014. The effect of different levels of Peppermint powder on humeral immune response of Japanese quail. *Proc. 6<sup>th</sup> Iranian Congress on Animal Science*, Tabriz, Iran.
- Toghyani M., Toghyani M., Gheisari A., Ghalamkari G., Mohammadrezaei M., 2010. Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigellasativa*) and peppermint (*Mentha piperita*). *Livestock Science*, 129(1) : 173-178.
- Valle-Paraso M., Vidamo P., Anunciado R., Lapitan A., 2005. Effects of Aloe vera (*Aloe barbadensis*) on the white blood cell count and antibody titre of broiler chickens vaccinated against Newcastle disease. *Philippine Journal of Veterinary Medicine*, 42(1) : 49-52.
- Zhang L., Tizard I.R., 1996. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from Aloe vera gel. *Immunopharmacology*, 35(2) : 119-128.