

## Effect of Aloe vera and vitamin E supplementation on the immune response of broilers<sup>□</sup>

*Efectos potenciales del Aloe vera y la vitamina E sobre la respuesta inmune en pollos de engorde*

*Efeitos potenciais da Aloe vera e vitamina E na resposta imunológica de frangos de corte*

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### Abstract

**Background:** herbs are among the feed supplements under investigation that could replace antibiotics to improve humoral and cellular immune response in birds. **Objective:** this study evaluated the potential of Aloe vera (*Aloe barbadensis* Miller) and vitamin E as immunostimulants on humoral and cellular immune responses in broilers. **Methods:** broilers were randomly assigned to three dietary treatments: a negative control (basal diet + with no additive), basal diet + 1% Aloe vera gel in drinking water, and basal diet + 100 mg/Kg vitamin E in the feed. Antibody titers against sheep red blood cells and Newcastle disease virus were used to examine the humoral immune response, whereas cellular immune response was evaluated using the phytohemagglutinin-P

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tests. **Results:** the highest level of antibody titer against sheep red blood cells on examination days 28 and 38, and the highest response to injection of phytohemagglutinin-P on day 38 was observed in the Aloe vera gel group ( $p < 0.05$ ). However, the response of broilers fed Aloe vera gel was not different from those receiving vitamin E ( $p > 0.05$ ). In addition, the greatest antibody level against Newcastle disease virus was obtained on days 25 and 35 in the vitamin E group, with no significant difference from the Aloe vera gel group ( $p > 0.05$ ). **Conclusion:** in general, our findings demonstrated that both Aloe vera gel and vitamin E can enhance humoral and cellular immune responses of broilers, while Aloe vera gel can be used as an immunostimulant in chickens.

**Keywords:** antibiotic, immune system, medicinal plant, poultry.

## Resumen

**Antecedentes:** en la industria avícola, es particularmente importante mejorar el rendimiento del sistema inmune con el objetivo de incrementar la resistencia contra patógenos. Las plantas están dentro de los suplementos alimenticios que podrían reemplazar a los antibióticos con el propósito de mejorar la respuesta inmune humoral y celular en aves. **Objetivo:** el presente estudio evaluó el potencial del Aloe vera (*Aloe barbadensis* Miller) y la vitamina E como inmunoestimulantes sobre la respuesta inmune humoral y celular en pollos de engorde. **Métodos:** los pollos de engorde fueron asignados aleatoriamente a tres tratamientos (dietas): un control negativo (dieta base sin aditivo), dieta base + 1% de gel de Aloe vera en el agua de bebida, y una dieta base + 100 mg/Kg de vitamina E en el alimento. Se usó la titulación de anticuerpos contra glóbulos rojos de oveja y contra la enfermedad viral Newcastle para examinar la respuesta inmune humoral. La respuesta inmune celular fue evaluada usando las pruebas de fitohemaglutinina-P. **Resultados:** el mayor nivel de titulación de anticuerpos contra glóbulos rojos de oveja en los días de experimentación 28 y 38, y la mayor respuesta a la inyección de fitohemaglutinina-P en el día 38 se observó en el grupo del gel de Aloe vera ( $p < 0,05$ ). Sin embargo, la respuesta de los pollos alimentados con gel de Aloe vera no fue diferente a la de los pollos que recibieron vitamina E ( $p > 0,05$ ). Además, el más alto nivel de anticuerpos contra la enfermedad viral Newcastle fue obtenida en los días 25 y 35 en el grupo que recibió vitamina E, sin diferencia significativa con respecto al grupo con gel de Aloe vera ( $p > 0,05$ ). **Conclusión:** en general, nuestros resultados demuestran que tanto el gel de Aloe vera como la vitamina E pueden mejorar la respuesta inmune humoral y celular en pollos de engorde, mientras que el gel de Aloe vera (agregado al agua) puede usarse como un inmunoestimulante en pollos de engorde.

**Palabras clave:** antibiótico, planta medicinal, producción avícola, sistema inmune.

## Resumo

**Antecedentes:** na indústria avícola, a melhoria nas respostas do sistema imunológico é importante, no sentido de aumentar a resistência contra patógenos. As ervas estão entre os suplementos que podem substituir os antibióticos na dieta com o objetivo de melhorar as respostas imunes humoral e celular. **Objetivo:** o presente estudo avaliou o uso potencial da Aloe vera (*Aloe barbadensis* Miller) e Vitamina E como imunoestimulantes sobre as respostas imunes humoral e celular em frangos de corte. **Métodos:** frangos de corte foram aleatoriamente distribuídos em 3 tratamentos, conforme a dieta: controle (dieta basal + sem aditivos), dieta basal + 1% de gel Aloe vera na água de bebida e dieta basal + 100 mg/Kg de vitamina E na dieta. Títulos de anticorpos contra células sanguíneas vermelhas de ovelha e o vírus da doença de Newcastle foram utilizados para avaliar a resposta imune humoral. A resposta imune celular foi avaliada através do teste da fitohemaglutinina-P. **Resultados:** o maior título de anticorpos contra células sanguíneas vermelhas de ovelha, aos 28 e 38 dias, e a maior resposta à injeção de fitohemaglutinina-P, aos 38 dias, foram observados no grupo recebendo o gel de Aloe vera ( $p < 0,05$ ). Entretanto, a resposta das aves que receberam o gel Aloe vera não foi diferente daqueles que receberam vitamina E ( $p > 0,05$ ). Além disso, o maior título de anticorpos contra o vírus da doença de Newcastle foi observado aos 25 e 35 dias no grupo que recebeu vitamina E sem diferença com o grupo que recebeu gel Aloe vera ( $p > 0,05$ ). **Conclusão:** em geral, nossos resultados indicam que tanto o gel Aloe vera quanto a vitamina E, podem ser utilizados para aumentar as respostas imune humoral e celular em frangos de corte, enquanto que o gel Aloe vera (adicionado à água de bebida) pode ser utilizado como um imunoestimulante em frangos.

**Palavras chave:** antibiótico, planta medicinal, produção avícola, sistema imune.

## Introduction

Nowadays, profitability in poultry production largely depends on the flock health status and enhanced resistance to pathogens as healthy flocks have improved performance, consistency, and lower mortality. The use of medicinal herbs as feed supplements is a strategy that has been adopted in the past few years by researchers and feed producers to improve broiler immunity against pathogens, (Dhama *et al.*, 2015). Among these herbs, Aloe vera (*Aloe barbadensis* Miller) has been identified as a promoter of immune system (Reynolds and Dweck, 1999; Choi and Chung, 2003). Aloe vera, which grows widely in tropical and sub-tropical areas (Boudreau and Beland, 2006), has a wide range of applications in traditional medicine (Rodriguez *et al.*, 2010). Its main part consists of Aloe vera gel, containing 98.5 - 99.5% water (Femenia *et al.*, 1999), and the remaining dry matter contains biologically active substances with antibacterial, antiviral, antifungal, immunomodulatory, antitumor, antioxidant, antidiabetic, and other healing properties (Choi and Chung, 2003).

Vitamin E, a fat-soluble vitamin, has also immunostimulant effects as well as antioxidant properties (Konjufca *et al.*, 2004; Khan *et al.*, 2012). Studies have shown that broiler feed supplemented with vitamin E can prevent losses due to infections by *Escherichia coli* (Tengerdy and Nockels, 1975), enhance phagocytosis activities of macrophages (Konjufca *et al.*, 2004), and improve cellular and humoral immune responses (Leshchinsky and Klasing, 2001).

The present study aimed to compare the immunomodulatory potentials of Aloe vera and vitamin E on humoral and cellular immune responses in broilers.

## Materials and methods

### *Ethical considerations*

The experiments were approved by the Scientific Board of the Semnan University and were conducted according to the International Guidelines for research involving animals (Directive 2010/63/EU).

### *Dietary treatments and animal management*

A total of 225 one-day old Ross 308 male chicks were randomly assigned to three treatments using a completely randomized design. Each treatment consisted of five replicates of 15 birds each. The birds within the control group were given the basal diet whereas birds in the Aloe vera group received the basal diet and 1% Aloe vera gel added to their drinking water and the vitamin E group received the basal diet supplemented with 100 mg alpha tocopheryl acetate/Kg of feed.

The trial lasted 42 days and in order to meet the nutrient requirements of the chickens over this period, a complete basal diet was formulated for each of the three stages of growth: starter (day 0-10), grower (day 11-24), and finisher (day 25-42) according to the guidelines for Ross 308 broilers feeding and management. Diets were formulated using the UFFDA (User-Friendly Feed Formulation) software. Metabolizable energy content for the starter, grower, and finisher diets was 2,850, 3,000, and 3,100 Kcal/Kg, respectively, while crude protein content was 22.18, 21.15 and 19.74% for each period, respectively. The broilers were kept on 2.2 x 1.2 m floor pens covered with straw. Over the entire feeding period, all environmental factors, e.g. temperature, humidity, light and ventilation, were the same for all groups. In addition, broilers were fed *ad libitum*. Aloe vera gel was obtained from Golazin Shahriar Company (Iran), pH was 4.50, and had negative microbial load.

### *Immune system assessment*

*Antibody against sheep red blood cells and Newcastle disease virus.* For the examination of antibody titer against sheep red blood cells (SRBC) on humoral immune response, 1 ml of 3% SRBC suspension was prepared in phosphate sterile solution and intravenously injected to the left wing of three chickens randomly selected from each group on days 21 and 31. On the seventh day following injection (i.e. days 28 and 38), 1 ml blood sample was taken from the right wing of the same chicken. In addition, antibody titer against Newcastle disease virus (NDV) was examined by the administration of Newcastle disease vaccine (LaSota) through drinking water on day 15. Then, 10 and 20 days following administration

of LaSota, three chickens were randomly selected from each group and blood samples were drawn from the brachial vein on their right wing. Micro-titer hemagglutination was used to determine antibody level against SRBC while antibody titer against NDV was identified using the hemagglutination inhibition (HI) test. Antibody titers against SRBC and NDV were reported as  $\log_2$ .

**Cellular immune response.** Cellular immune response was evaluated using phytohemagglutinin-P (PHA-P) tests on three birds randomly selected from each group on day 38. In this test, 0.1 ml PHA-P solution was intradermally injected to the web of the third and fourth digits on the right foot of each broiler. In order to adjust for this variable, 0.1 ml phosphate buffer saline (PBS) solution was also injected to the web joining the third and fourth digits on the left foot. A micrometer was used to measure thickness of the web before injection and 24 hours after PHA-P injection. Broiler response to PHA-P was calculated using the following equation:

$$\text{PHA-P injection} = (\text{response to PBS injected to the left foot}) - (\text{response to PHA-P injected to right foot}).$$

Where:

PHA-P response for the right foot = post-injection thickness of skin - pre-injection thickness of skin.

PBS response for the left foot = post-injection thickness of skin - pre-injection thickness of skin (Corrier and DeLoach, 1990).

#### Data analysis

Data were analyzed using the GLM procedure of SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA; 2002) based on a completely randomized design with three groups consisting of five replicates each. Mean values were compared at 0.05 significance level using Duncan's Multiple Range test.

## Results

The effect of Aloe vera and vitamin E supplementation on humoral and cellular immune

responses are presented in Tables 1 and 2, respectively. Evaluation of antibody titer against SRBC showed that on days 28 and 38, the Aloe vera gel group had the highest level of antibody titer, although there were no significant differences between broilers on the Aloe vera gel and vitamin E groups ( $p > 0.05$ ). However, mean SRBC, NDV and PHA-P responses of birds on the Aloe vera gel and vitamin E groups were significantly ( $p < 0.05$ ) higher in comparison to the control group. Furthermore, evaluation of antibody titer against NDV on day 25 and 35 showed that the broilers on the vitamin E group had the highest antibody titer, but there were no differences between birds on the vitamin E group and those on the Aloe vera gel group ( $p > 0.05$ ). A significant decrease ( $p < 0.05$ ) in antibody titer against NDV was observed in the control group in comparison to the Aloe vera gel and vitamin E groups. Analysis of broiler responses to PHA-P injection on day 38 showed that the control group had the lowest response, with significant decrease ( $p < 0.05$ ) compared to the other two groups. Although the highest response to PHA-P injection was observed in broilers that received Aloe vera gel, their response was not significantly different ( $p > 0.05$ ) from broilers supplemented with vitamin E.

**Table 1.** Humoral immune response of broilers fed Aloe vera gel and vitamin E.

Item	Day	Control	Aloe vera gel	Vitamin E	SEM
Antibody response to SRBC ( $\log_2$ )	28	3.28 <sup>b</sup>	4.26 <sup>a</sup>	4.07 <sup>a</sup>	0.132
	38	5.18 <sup>b</sup>	5.47 <sup>a</sup>	5.45 <sup>a</sup>	0.247
Antibody response to NDV ( $\log_2$ )	25	1.30 <sup>b</sup>	2.15 <sup>a</sup>	2.37 <sup>a</sup>	0.145
	35	2.10 <sup>b</sup>	3.25 <sup>a</sup>	3.26 <sup>a</sup>	0.197

Means with different superscript letters (<sup>a</sup>, <sup>b</sup>) within the same row are significantly different at  $p < 0.05$ . SEM: standard error of the mean. SRBC: sheep red blood cells. NDV: Newcastle disease virus.

**Table 2.** Cellular immune response of broilers fed Aloe vera gel and vitamin E.

Item	Control	Aloe vera gel	Vitamin E	SEM
PHA-P (mm)	0.385 <sup>b</sup>	0.525 <sup>a</sup>	0.512 <sup>a</sup>	0.019

Means with different superscript letters (<sup>a</sup>, <sup>b</sup>) within the same row are significantly different at  $p < 0.05$ . SEM: standard error of the mean. PHA-P: phytohemagglutinin-P.

## Discussion

In the present study, broilers on the Aloe vera gel group exhibited improved antibody titers against SRBC and NDV, as well as enhanced response to PHA-P injection, compared to broilers in the control group. Previous studies have also shown that broilers receiving feed supplemented with either 1.5, 2, or 2.5% Aloe vera gel had increased antibody titers against SRBC and NDV in comparison to control broilers (Darabighane *et al.*, 2012). Moreover, the addition of Aloe vera powder to broiler feed at levels of 0.75 and 1% increased antibody titers against SRBC in comparison to broilers in the control group (Mahdavi *et al.*, 2012). Akhtar *et al.* (2012) reported that ethanolic and aqueous extracts of Aloe vera improved antibody titers against SRBC, whereas addition of Aloe vera to drinking water at a level of 2% improved antibody titers against NDV (Valle-Paraso *et al.*, 2014). Furthermore, Akhtar *et al.* (2012) and Darabighane *et al.* (2012) reported similar positive effects on cellular immunity. The improvement in the immune system performance may be attributed to acemannan, a polysaccharide contained in Aloe vera. Acemannan is a  $\beta$  (1-4)-linked acetylated mannan, which can activate macrophages in addition to increase cytokines production, release of nitric oxide, or expression of surface molecule (Zhang and Tizard, 1996). Similarly, polysaccharides in Aloe vera increased interleukin 2, interleukin 4, and interferon-gamma in weaned piglets (Qiao *et al.*, 2013).

In our study, vitamin E resulted in better responses (enhanced antibody titers against SRBC and NDV, as well as enhanced response to PHA-P) compared to the control group. This is in agreement with studies by Leshchinsky and Klasing (2001), Konjufca *et al.* (2004), Niu *et al.* (2009), and da Silva *et al.* (2009). Leshchinsky and Klasing (2001) reported also increased antibody titers against SRBC when using 50 IU/Kg vitamin E in broilers. In addition, Niu *et al.* (2009) reported increased antibody titers against SRBC in broilers supplemented with 100 and 200 mg/Kg vitamin E under heat stress conditions. As previously noted with regard to antibody titers against NDV, in our experiment the highest antibody titer was observed in broilers that received vitamin E, in agreement with the study of da Silva *et al.* (2009).

In the present study, broilers in both Aloe vera gel and vitamin E groups had noticeably higher response than that by broilers in the control group. However, there was no significant difference between the Aloe vera gel and vitamin E groups for antibody titers against SRBC and NDV or responses to PHA-P injection. A number of studies have compared the effect of medicinal herbs and other immunostimulant agents on immune response. Miran *et al.* (2010) found no significant difference between broilers that received either *Echinacea angustifolia*, Levamisole and vitamin E immunostimulants for antibody titers against NDV and avian influenza on days 21 and 42, and against SRBC on day 21. However, on day 42, the greatest antibody titer against SRBC was observed for the Levamisole group. Moreover, vitamin E and *E. angustifolia* groups showed no significant difference in their responses to PHA-P injection. In another study, Bahrami *et al.* (2011) reported better performance of the immune system in laying hens whose diets were supplemented with thyme extract compared to chickens supplemented with vitamin E. In general, these studies have demonstrated positive effects of herbs on the immune system, probably because of the presence of polysaccharides, saponins, flavonoids, alkaloids, and phenolic compounds (Tan and Vanitha, 2004). Guo *et al.* (2004) and Chen *et al.* (2003) also reported enhanced immune performance due to dietary supplementation with herbs and fungi. However, is important to note that the effect of medicinal plants depends on the form of the substance (ethanolic or aqueous extract, powder) and route of administration (drinking water, feed additive), which can modify the impact on animals.

In conclusion, supplementation of broiler diets with either Aloe vera gel or vitamin E can enhance cellular and humoral immunity.

## Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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