Efficacy of *Echinacea purpurea* and protexin on systemic and mucosal immune response to Newcastle diseases virus vaccination (VG/GA strain) in commercial turkey poults

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Abstract:

BACKGROUND: It is important to understand the efficacy of immunoregulatory materials, herbal remedies or probiotics, in different parts of immune system following vaccination with different tropism. **OBJECTIVES:** Aim of this study was to evaluate the effect of *Echinacea purpurea* and a probiotic (protexin) on systemic and mucosal immune response in turkey. **METHODS:** A total of 288 1-day-old male turkey poults were randomized into 6 groups as follow: Group T1: Turkeys received *Echinacea purpurea* at the rate of 1 ml /1 liter water and Newcastle disease virus (NDV) vaccine, Group T2: Turkeys received probiotic at the rate of 1 g /1 liter water and NDV vaccine, Group T3: Positive control, turkey received NDV vaccine without any additives. Group T4: Turkeys received *Echinacea purpurea* at the rate of 1 ml /1 liter water without NDV vaccine. Group T5: Turkeys received probiotic at the rate of 1 g /1 liter water without NDV vaccine, Group T6: Negative control group, neither vaccinated against NDV vaccine nor given additives. At age of 10 and 20 days, poults were vaccinated with Villegas-Glisson/University of Georgia (VG/GA) strain of Newcastle disease vaccine by eye dropper method. For systemic and mucosal antibody analyses, blood samples and tracheal lavages were collected at different ages. The titers of antibody against NDV were measured using ELISA and HI tests. **RESULTS:** Addition of Echinacea to the water increased the systemic IgG, IgA and HI compared to the positive control group. Protexin supplementation to the water of T2 turkeys increased serum IgG and both total and specific IgA compared to the T3 group turkeys. Generally, turkeys that were supplemented with probiotic had higher specific and total tracheal IgA antibody levels than the other vaccinated groups. Among vaccinated turkeys only T1 group showed significantly higher HI antibody titers on day 42. **CONCLUSIONS:** Results indicated that systemic and mucosal immunity of turkeys following vaccination against Newcastle disease (ND) could be improved by supplementation of Echinacea and probiotic. The effect of *Echinacea purpurea* on systemic immunity of turkeys seemed more pronounced than on mucosal immunity; further, the effect of probiotic on mucosal immunity was more obvious.

Introduction

Nowadays, turkeys are raised under intensive production systems in densely populated colonies or flocks to achieve high levels of economic efficiency. In this intensive rearing process, turkeys may get stress from various factors such as overcrowding, unfavorable ambient temperature, feed-intake and vaccination. The application of herbal supplements such as *Echinacea purpurea* (EP) and probiotic could boost immunological reactivity, which contributes to better health and minimizes the stresses in turkeys.

Echinacea purpurea is one of the most important medical herbs. Its root and subterranean stem are widely used around the world to treat common cold and other infectious disorders with the claim of having para immunity-inducing and non-specific immune responses stimulating effects. All kinds of EP contain similar main ingredients including caffeic acid derivatives, alkamides, flavonoids, essential oils, and polyacetylenes (Manayi et al., 2015). The EP has been proven to show good immunoregulation, anti-inflammation and antioxidant capacity (Lee et al., 2009, 2012) with no hypersensitivity or other side effects (Saunders et al., 2007). A recent review showed that Echinacea treatment resulted in an increase of various cytokines, lymphocytes, and phagocytosis activity (Bauer, 2002; Fonseca et al., 2015). Studies with Echinacea to improve animal husbandry are rare. Kuhn et al. (2005) reported an immune stimulating effect in sows by a repeated 5 days of application of Echinacea juice.

Probiotics are defined as feed additives containing live microorganisms. Administration of probiotics has improved growth performance and feed efficiency (Getachew, 2016) and also stimulated the production of natural antibodies in chickens (Wondmeneh, et al., 2015). Actually, probiotics act by different means including adherence to the binding sites of the intestinal epithelium, competition with pathogenic bacteria and stimulus to the immune system (Menten, 2002). Balanced microbiota and a concomitant modulation of the host immune system are potential health-promoting probiotic effects. The use of protective probiotic cultures in poultry farming may serve as a useful strategy to improve food product safety right from the beginning of the food chain and thus may serve to protect consumer health (Seifert et al., 2011).

The importance of antibodies in the defense mechanism against viral infection has been emphasized by many studies (Scott, 2004). It appears that local immunity acts as a barrier at surfaces where primary viral infections occur, thereby interfering with further spread of the virus (Jayawardane and Spradbrow, 1995). Birds have a well-developed mucosal immune system and its characteristics include local production and secretion of IgA antibodies and traffic of IgA producing plasma cells (Al-Garib et al., 2003). The IgA class predominates and is detectable in tears, saliva, tracheal, intestinal washings and bile. The intranasal or eye drop vaccination with NDV induces antigen specific- IgA which can be detected in tears and tracheal washings (Ganapathy et al., 2005).

In the present study, the tropism of vaccine virus in turkey poults was used with the follow up of the local and systemic immunity measurements. To this aim, tracheal lavages were obtained in order to measure the level of IgA titer induced by specific

VG/GA strain vaccination.

The VG/GA strain of NDV isolated from the intestine of healthy turkeys is proposed to replicate both in the respiratory and intestinal tract, with preference for the intestine (Perozo et al., 2008). Most of the commercially available lentogenic vaccines are able to induce antibodies against NDV, however, systemic humoral immune response measured as the presence of specific NDV antibodies in serum is not enough for protection (Kapczynski et al., 2013; Reynolds and Maraqa, 2000). It has been established that the mucosal immunity represented by immunoglobulin A (IgA) production plays an important role in the development of protection in chickens vaccinated against NDV (Reynolds and Maraqa, 2000; Scott, 2004). We believe that it could be the same in turkeys. Therefore, in the present study, the mucosal and systemic humoral immune responses of male turkey poults were measured when they were vaccinated by VG/GA strain and also treated with *Echinacea purpurea* and probiotic to possibly enhance immune response of turkeys following NDV vaccination.

Materials and Methods

Experimental design and birds: A total of 288 1-day-old turkey poults of premium strain were obtained from a commercial hatchery (ZarinJooje) and kept for 42 days in the experimental rooms, in the Research Center, Faculty of Veterinary Medicine, University of Tehran, located in Karaj.

The poults were raised in concrete floor pens covered with 8 cm of clean pine wood shavings, and each pen was equipped with one tube feeder and one automatic drinker. Throughout the study, the turkeys were grown following standard temperature regimens, which were gradually decreased from 38 to 23ºC. Turkeys were maintained on a 16L: 8D schedule and allowed to consume feed and water ad libitum. Air temperature was controlled according to Aviagen recommendations. A completely randomized experimental design was used, and poults were randomized into six groups, with four replicates per treatment and 12 poults (tom) per replicate as follows: Group T1: Turkeys received *Echinacea purpurea* at the rate of 1 ml/1 liter water and Newcastle disease virus (NDV) vaccine, Group T2: Turkeys received probiotic at the rate of 1 g/1 liter water and NDV vaccine, Group T3: Positive control, turkeys received NDV vaccine without any additives. Group T4: Turkeys received *Echinacea purpurea* at the rate of 1 ml/1 liter water without NDV vaccine. Group T5: Turkeys received probiotic at the rate of 1 g/1 liter water without NDV vaccine, Group T6: Negative control group, neither vaccinated against NDV vaccine nor given additives. Diets were fed in pellet form. The basal control diet was formulated and compounded to meet the nutritional requirements of commercial turkey, according to Aviagen manual. The juice of *Echinacea purpurea* (EP) and Protexin were treated intermittently which was supplied on days 1-3 of age and repeated three times on days 8-9, 18-19 and finally days 30-31 of the rearing period.

In this study a commercial probiotic (Protexin®) was used. Protexin is a highly concentrated pre-mix containing seven strains of bacteria (*Lactobacillus plantarum*, *Lactobacillus delbrueckii* subsp. Bulgaricus, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus salivarius* subsp. Thermophilus,

Enterococcus faecium) and two yeasts (Aspergillus oryza, Candida pintolopesii). The juice of EP which was used in this trial contained caffeic acid derivatives, alkamides, flavonoids, polyacetylenes, mannos, glucomannan, arachidonic acid, and acetylated glucomannan. For preparation of EP juice, briefly, aloe vera leaves were obtained from Pars Immen Darou Co. They were washed with distilled water and sterilized with 70% ethyl alcohol. The gel was drained out. Then, leaf gel was dried at 80ºC for 48 h and was powdered. 30 g of the powder was soaked in 200 ml of ethanol for 24 h, filtered and evaporated to dry. The obtained extract was dissolved in distilled water.

Vaccination and serology: Newcastle disease vaccine used in this study was a freeze-dried live vaccine against Newcastle disease produced by Merial Animal Health Limited (Lyon, France). The vaccine contained ND viruses of the VG/GA strain (lineage IIvirus), a lentogenic and naturally occurring strain in turkeys. Each dose (1 m) was determined via viral titration to contain 106.5 EID50 (50 percent Embryo Infectious Dose). At the age of 10 and 20 days, all poults were vaccinated with Avinew® Newcastle disease vaccine by eye dropper except for negative control group according to the recommendation of the manufacturer.

Systemic humoral immune response: On 10, 20, 28, 35 and 42 days of age, twelve turkeys from each experimental group were bled. Serum samples were taken, frozen and used for titration of antibody against NDV by Haemagglutination-inhibition (HI) test. Measurement of serum IgA and IgG was performed by Elisa method using a commercial ND Elisa kit (Flock check; IDEXX, Maine, USA).

Total and NDV-specific IgA detections: On 21, 22 and 23 days post hatch, six turkeys per group were selected to obtain tracheal lavages. Briefly, turkeys were killed humanely using a carbon dioxide chamber, the trachea was dissected out immediately after death for the collection of tracheal secretion. They were washed internally by flushing through 1.5 ml PBS 10 times (tracheal washings) and cut longitudinally, then transversely to give 4 pieces, each of which was gently scraped on its internal surface with a scalpel (tracheal scrapings). The scrapings were collected into a total of 2 ml PBS. At all stages great care was taken to avoid contamination with blood. Samples were placed in sterile containers and processed fresh. NDV-specific IgA levels in tracheal lavages were assayed two times using an indirect ELISA as described previously (Raj and Jones, 1996), except that the coating antigen used was the VG/GA strain of NDV and the chicken IgA binding to the coating antigen was detected with horse radish peroxidase (HRP)-labeled goat anti-chicken IgG (AbDSerotec, Oxford, UK).

To estimate the total (unspecific) IgA production in the respiratory mucosa, direct total IgA ELISA was performed. Microtiter plates were coated with 100 µl of serially diluted tracheal lavages. Overnight plates were washed with PBST and blocked with 100 µl of PBS-Blotto for 2 h at room temperature. Plates were washed three times with PBST before adding 100 µl of (1:10000) diluted conjugate and HRP-labeled goat anti-chicken IgA (AbDSerotec, Oxford, UK). Absorbance at 450 nm was measured using a microplate reader (Stat FAX 2000, Awareness Technology, Inc., USA).

Statistical analysis: Statistical analysis was performed using the ‘‘General lin-

ear model procedure'' (SAS Institute Inc., 2008). If a significant overall effect ($p<0.05$) was found, treatment means were compared using the Scheffe test.

Results

Serum antibody response: The effects of *Echinacea purpurea* and probiotic treatments on specific serum IgG, IgA and HI antibodies production against NDV in turkeys are presented in Figs. 1-3. In the present study, vaccinated turkeys showed significantly ($p<0.0001$) higher titers of serum IgG, IgA and HI against NDV when compared to those of the non-vaccinated turkeys in all ages, except on day 20 that T3 group (positive control) turkeys showed significantly lower specific serum IgA titer (Fig. 2).

Addition of *Echinacea purpurea* to the water of T1 turkeys increased the IgG antibody titer production compared to the positive control turkeys from day 20 to day 42. EP increased the specific serum IgA titer of T1 turkeys compared to that of the positive control (T3), however, the difference was significant on 20, 28 and 35 days of age ($p<0.01$).

Among the vaccinated turkeys, addition of *Echinacea purpurea* to the water of T1 group turkeys influenced HI antibody titers compared to the T3 turkeys, however, the difference was only significant on day 42 of age ($p<0.01$) (Fig. 3).

Overall, Protexin supplementation to the water of T2 turkeys increased serum IgG antibody against NDV compared to the T3 group, however, the difference was only significant on day 28 ($p<0.05$) (Fig. 1). Protexin treated turkeys showed significantly higher specific serum IgA antibody

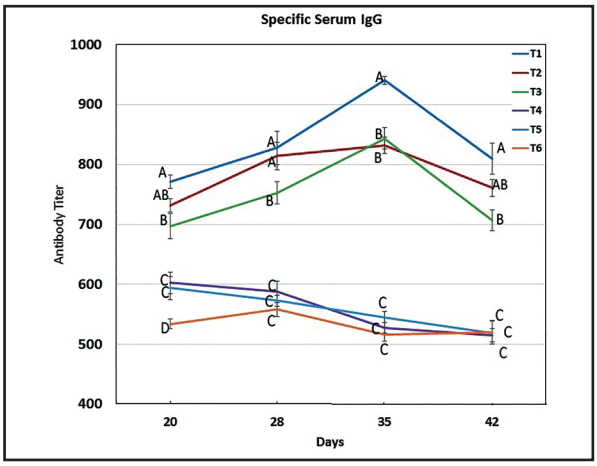

Figure 1. Effect of *Echinacea purpurea* and Protexin on specific serum IgG antibody titers against NDV vaccination turkey poults (12 samples/group) from 20 to 42 days of age. Values within an age with no common letters differ significantly. T1: Turkeys received NDV vaccine and *Echinacea purpurea*; T2: Turkeys received NDV vaccine and probiotic; T3: Turkeys received NDV vaccine without additives; T4: Turkeys received *Echinacea purpurea* without vaccination; T5: Turkeys received probiotic without vaccination; T6: Turkeys received no vaccine and no additives.

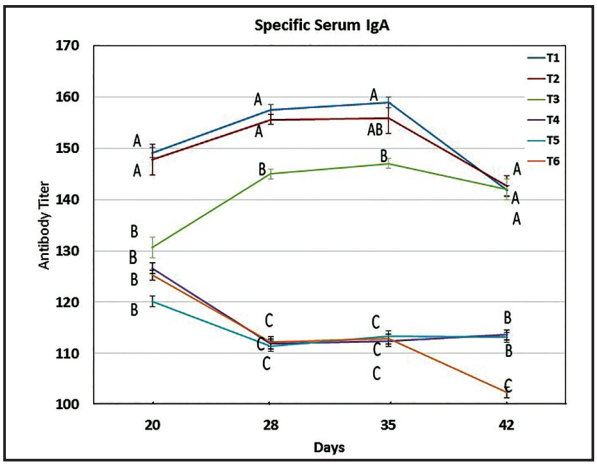

Figure 2. Effect of *Echinacea purpurea* and Protexin on specific serum IgA antibody titers against NDV vaccination turkey poults (12 samples/group) from 20 to 42 days of age. Values within an age with no common letters differ significantly. T1: Turkeys received NDV vaccine and *Echinacea purpurea*; T2: Turkeys received NDV vaccine and probiotic; T3: Turkeys received NDV vaccine without additives; T4: Turkeys received *Echinacea purpurea* without vaccination; T5: Turkeys received probiotic without vaccination; T6: Turkeys received no vaccine and no additives.

titers (Fig. 2) against NDV compared to the T3 group turkeys on days 20, 28 and 35 of

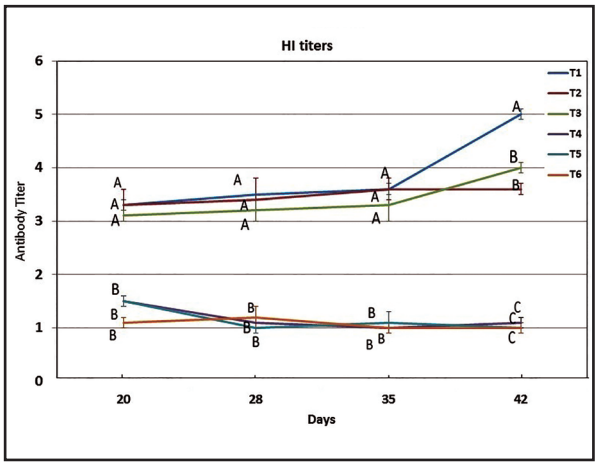

Figure 3. Effect of *Echinacea purpurea* and Protexin on HI antibody titers against NDV vaccination turkey poults (12 samples/group) from 20 to 42 days of age. Values within an age with no common letters differ significantly. T1: Turkeys received NDV vaccine and *Echinacea purpurea*; T2: Turkeys received NDV vaccine and probiotic; T3: Turkeys received NDV vaccine without additives; T4: Turkeys received *Echinacea purpurea* without vaccination; T5: Turkeys received probiotic without vaccination; T6: Turkeys received no vaccine and no additives.

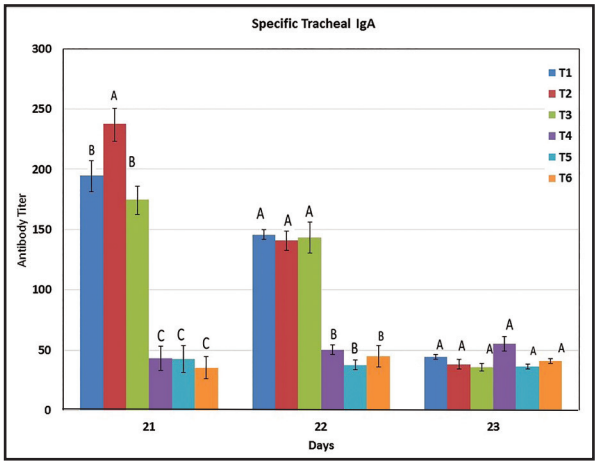

Figure 4. Effect of *Echinacea purpurea* and Protexin on specific tracheal IgA antibody titers against NDV vaccination turkey poults (6 samples/group) from 20 to 42 days of age. Values within an age with no common letters differ significantly. T1: Turkeys received NDV vaccine and *Echinacea purpurea*; T2: Turkeys received NDV vaccine and probiotic; T3: Turkeys received NDV vaccine without additives; T4: Turkeys received *Echinacea purpurea* without vaccination; T5: Turkeys received probiotic without vaccination; T6: Turkeys received no vaccine and no additives.

age ($p<0.001$). Between two groups of vaccinated (T2 and T3) turkeys, Protexin supplementation had no significant effect on HI titers ($p>0.05$) (Fig. 3).

Throughout the experimental period T1 group turkeys that received EP showed higher serum IgG titers against NDV than Protexin treated turkeys (T2), however, the difference was only significant on day 35 of age ($p<0.005$) (Fig. 1). No significant differences on serum IgA titer was found between the EP (T1) and the Protexin (T2) treated turkeys during the whole experimental period (Fig. 3). Comparing two vaccinated group turkeys (T1 and T2) it could be seen that HI titer of EP treated turkeys was only significantly higher than the Protexin supplemented on day 42 of age ($p<0.005$) (Fig. 3).

Mucosal antibody response: The results of specific and total tracheal IgA titers against NDV in turkeys are shown in Figs. 4 and 5. Specific tracheal IgA titers against NDV were significantly increased in vaccinated turkeys compared to those of the non-vaccinated group turkeys on days 21 and 22 of age ($p<0.0001$), however, it was not observed on day 23. On day 21, specific tracheal IgA titer tended to be significant ($p<0.06$) which was higher in protexin treated turkeys (T2) compared to the EP treated (T1) and even with the positive control (T3) group (Fig. 4). EP or Protexin did not show significant effect on specific tracheal IgA titers on days 22 and 23 of age ($p>0.05$). On day 23 of age, almost the same specific IgA titers were observed between vaccinated and non-vaccinated turkeys.

Total tracheal IgA titers against NDV were significantly higher in three groups of vaccinated turkeys in comparison with those of the non-vaccinated turkeys on days 21 and 22 of age ($p<0.0001$) (Fig. 5). However, on day 23 the picture was different

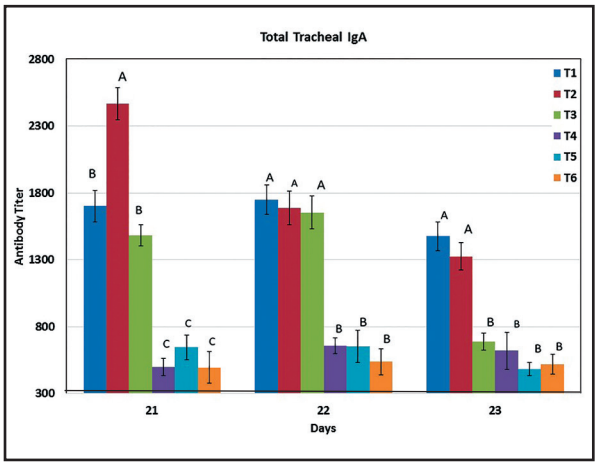

Figure 5. Effect of *Echinacea purpurea* and Protexin on total tracheal IgA antibody titers against NDV vaccination turkey poults (6 samples/group) from 20 to 42 days of age. Values within an age with no common letters differ significantly. T1: Turkeys received NDV vaccine and *Echinacea purpurea*; T2: Turkeys received NDV vaccine and probiotic; T3: Turkeys received NDV vaccine without additives; T4: Turkeys received *Echinacea purpurea* without vaccination; T5: Turkeys received probiotic without vaccination; T6: Turkeys received no vaccine and no additives.

among the three vaccinated groups of turkeys. In this age, EP (T1) and protexin (T2) treated turkeys presented significantly higher total tracheal IgA titers compared to the positive control (T3) group ($p<0.01$) (Fig. 5). On day 21, the turkeys supplemented with Protexin (T2) had significantly higher total tracheal IgA levels compared to those of the T1 and T3 groups ($p<0.0001$).

Discussion

The current study was the first report on the effect of EP and a probiotic like Protexin on systemic and local immunity of turkey poults, when birds received the VG/GA strain vaccine against NDV. In the present study, the vaccinated turkeys which consumed EP showed higher serum and mucosal antibody responses. However, regarding our results, it seems that efficiency of EP was more pronounced on the serum specific IgG and IgA titers than probiotic. The effect of probiotic on specific and total tracheal IgA was much better than EP.

It has been shown that Echinacea treatment has resulted in an increase of various cytokines, lymphocytes, and phagocytosis activity (Bauer, 2002; Fonseca et al., 2015). This effect might be related to alkamids in EP. The role of alkamides as an active principle of Echinacea has already been mentioned by Cruz et al. (2014). Authors argued that alkamides were considered as a class of cannabinomimetics and therefore a possible mode of action to modulate immune functions. Additionally, Cundell et al. (2003) found a significant increase in lymphocytes in rats when they were fed with dried Echinacea preparations for one week. Habibian Dehkordi et al. (2011) found a stimulation of the phagocytic response in chickens provided with ground root preparations of Echinacea. Also, the effect of Echinacea on the immune system is suggested by various substances such as derivate of caffeic acid (cichoric acid) and polysaccharides. The phagocytic cells of the innate system act within the context of antibody- and B cell-mediated immunity (Davison et al., 2008). These mechanisms were supported by many researchers who reported that increase of lymphocytes and phagocytic activities were a characteristic reaction associated with efficacy of Echinacea (Habibian Dehkordi et al., 2011; Barrett, 2003, Cundell et al., 2003). Moreover, Park et al, (2016) reported that stimulation of both humoral and cell mediated immunity through the enhanced production of natural interferons/cytokines increased macrophage, lymphocyte and natural killer (NK) cell activity, up-regulated oxidative burst in heterophils and also increased IgG, IgM and IgA. Based on this information it could be suggested

that the improvement of systemic and mucosal antibodies observed in the present study could also be related to increase in activity of different kinds of immune cells by consumption of EP in turkeys.

It has been well documented that intermittent application of EP in humans has a significant effect on immunity, because immunity over stimulation was eliminated. Furthermore, the interval between applications was also recommended two to three times during the application (Jurcic et al., 1989). In the present study, the juice of EP was supplemented intermittently for a limited period of time on days 1-3, 8-9, 18-19 and 30-31 of the rearing period. This might have been potentiated to increase the humoral immune response of turkeys, especially serum IgG concentration. The benefits of such model treatment have already been studied in some other animals, e.g. layers, sows and piglets (Schranner et al., 1989; Kuhn et al., 2005; Abdel-Fattah et al., 2008; Bohmer et al., 2008).

According to our data, probiotic supplementation showed a slight increase in specific serum IgG, however, it significantly affected specific serum IgA when compared to the results of the control positive (T3) group turkeys. Furthermore, the effect of probiotic supplementation on mucosal antibodies was clearer, especially on day 21 of age, when compared to the effect of EP treated group.

Talazadeh et al. (2016) pointed that the direct effect of probiotic on increase in immune activity might be related to stimulation of the lymphatic tissue, whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract. Mehdizadeh Taklimi, et al. (2012) pointed out that the bursa of probiotic-treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Similarly, increase in the weight of the thymus might be due to the effect of probiotic bacteria on functional activities of the immune system responses; this could result in increase in the number of lymphocytes in the primary lymphoid organs, as feeding of broilers with probiotic increased relative weights of spleen (Guimarães et al., 2014). Brisbin et al., (2011) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria.

Ao et al. (2013) indicated that defense cells in the gut-associated lymphoid tissue (GALT) could detect the presence of microbes by recognizing molecules unique to microorganisms then could increase the number of different lymphocytes and/or leukocytes in the GALT and in peripheral blood. In agreement with all of these researchers we could conclude that stimulating of such different defense cells, e.g. B and T lymphocytes or tissues like bursa or thymus, could directly modulate systemic and mucosal immune responses (Toloei et al., 2010; Ghahri et al., 2010).

The main site of the mucosal immune system in the respiratory tract is referred to as bronchus-associated lymphoid tissue (BALT), with the immune-associated cells including mast cells, goblet cells and secretory IgA (sIgA)-positive cells that are involved in many processes to prevent pathogen invasion (De Geus et al., 2012). Active replication in the mucosa induces virus protein production and local antigen presentation through MHC class I and II molecules stimulating a T-dependent B-cell response at the site of infection in the form of IgA-producing plasma cells in the respi-

ratory trachea (Al-Garib et al., 2003).

Our results showed that the production of total and specific tracheal IgA increased in the treated groups which received vaccine in comparison with the control groups. The level of both total and specific tracheal IgA responses increased from 24 to 72 hours in the vaccinated turkeys and the highest level of antibody was observed at 24 and 48 hours after vaccination. These findings are very important because specific IgA plays a major role in protecting mucosal surfaces against colonization and possible invasion of pathogenic microorganisms (Corthesy and Kraehenbuhl, 1999).

Also, our results demonstrated that the level of the total and specific tracheal IgA following vaccination decreased shortly in few days. This might be because of fast clearance of VG/GA from the respiratory tract after vaccination (Perozo et al., 2008). Based on how fast the VG/GA is cleared from respiratory tract after priming the mucosal immune response, the mucosal IgA production in the respiratory and the levels of protection afforded by single or multiple doses of the vaccine, initial vaccination with the VG/GA strain may be advantageous for the integrity of the respiratory mucosa in young chickens when multiple vaccination and field exposure are expected (Perozo et al., 2008).

It is concluded that the systemic and mucosal immunity of turkeys following vaccination against NDV could be improved by supplementation of *Echinacea purpurea* and probiotic. It seems the effect of *Echinacea purpurea* on systemic immunity of turkeys is much better than on mucosal immunity. The effect of probiotic on mucosal immunity of tracheal was observed much clearer. So far, there are no studies available on the exact mode of action on activating the specific immune systems by Echinacea components or even probiotic. It is possible that the presence of pathogen associated molecular patterns, which are recognized by toll-like receptors and other pattern recognition receptors, could contribute to affect the specific immune system. This effect of Echinacea to stimulate adaptive immune function needs further researches.

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مطالعه مقایسه‌ای تأثیر عصاره گیاه سرخارگل و پروتکسین بر روی پاسخ ایمنی در گردش و مخاطی متعاقب واکسیناسیون علیه ویروس بیماری نیوکاسل (سویه VG/GA) در جوجه بوقلمون‌های گوشتی تجاری

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چکیده

زمینه مطالعه: استفاده از داروهای گیاهی و پروبیوتیک‌ها در غذای انسان و حیوان به دلیل تأثیرات طبیعی تحریک سیستم ایمنی که دارند پیشنهاد می‌شود. **هدف:** تأثیر عصاره گیاه سرخارگل و پروتکسین بر روی پاسخ ایمنی در گردش و مخاطی در جوجه‌های بوقلمون تجاری مورد ارزیابی قرار می‌گیرد. **روش کار:** ۲۸۸ جوجه نر بوقلمون تجاری به ۶ گروه با ۴ تکرار تقسیم و تا ۴۲ روزگی نگهداری شدند. گروه ۱: پرندگان در برابر ویروس بیماری نیوکاسل واکسینه شده عصاره گیاه سرخارگل را دریافت کردند. گروه۲: پرندگان در برابر ویروس بیماری نیوکاسل واکسینه شدند و پروبیوتیک دریافت کردند. گروه۳: گروه کنترل مثبت، فقط در برابر ویروس بیماری نیوکاسل واکسینه شدند. گروه۴: پرندگان عصاره سرخارگل را دریافت کردند بدون اینکه واکسینه شوند. گروه ۵: پرندگان پروبیوتیک دریافت کردند بدون اینکه واکسینه شوند. گروه ۶: گروه کنترل منفی که واکسینه نشدند و هیچ ماده در آب آن‌ها اضافه نشد. در روزهای ۱۰ و ۲۰ بر علیه ویروس بیماری نیوکاسل با روش قطره چشمی واکسیناسیون انجام شد. برای بررسی پاسخ ایمنی در گردش و مخاطی، نمونه خون و نمونه محتویات شستشو شده نای تهیه گردید. و تیتر آنتی بادی توسط آزمون Elisa و HI بررسی گردید. **نتایج:** عصاره گیاه سرخارگل منجر به افزایش معنی‌دار تولید آنتی بادی IgA ، IgG و HI نسبت به گروه کنترل مثبت گردید. پروتکسین (گروه ۲) در مقایسه با گروه کنترل مثبت باعث افزایش معنی‌دار تیتر سرمی IgG و نیز IgA مخاطی (اختصاصی و کل) شد در حالیکه این افزایش در مورد تیتر آنتی بادی HI معنی‌دار نبود. در بین جوجه‌های واکسینه، آنهایی که پروتکسین دریافت کردند نسبت به گروهی که عصاره سرخارگل مصرف کردند تیتر آنتی بادی IgA مخاطی (اختصاصی و کل) بهتری از خود نشان دادند. **نتیجه‌گیری نهایی:** استفاده از عصاره گیاه سرخارگل و پروتکسین می تواند باعث بهبود پاسخ ایمنی در گردش و مخاطی در بوقلمون‌ها شود. همچنین نتایج ثابت کرد که تأثیر عصاره گیاه سرخارگل برپاسخ ایمنی در گردش بوقلمون بیشتر از ایمنی مخاطی است. در حالی که تأثیر پروبیوتیک مورد استفاده در این مطالعه بر ایمنی مخاطی بوقلمون بیشتر بوده است.

واژه های کلیدی: سرخارگل، پارامترهای ایمنی شناسی، پروبیوتیک، بوقلمون، سویه VG/GA واکسن نیوکاسل

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