Effect of dried Citrus sinensis peel on gastrointestinal microbiota and immune system traits of broiler chickens

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Abstract

Two hundred broiler chickens (Ross-308) were used in a completely randomised study to evaluate the effects of supplementing the feed with different levels of dried Citrus sinensis peel (DCSP) on the gastrointestinal microbiotal population and immune system traits. Feed was supplemented with different DCSP amounts: 0.25% w/w (DCSP-0.25), 0.5% w/w (DCSP-0.50), 0.75% w/w (DCSP-0.75), and 1% w/w (DCSP-1). Control diet (DCSP-0), with no feed addition was used as reference. The study involved five treatments in a time frame of six weeks (four replicates per treatment and each replicate had 10 chickens). Data analysis was performed using SAS software and mean comparison was performed using the Duncan test. The results allowed to observe that the mean of Escherichia coli in caecum on day 42 was significantly different (P<0.05) but did not affect other gastrointestinal microbial population traits (P>0.05). The mean of total sheep red blood cells and immunoglobulin G and M (IgG and IgM) on day 28 (P>0.05) were also determined. Total sheep red blood cells on day 42 were significantly different (P<0.05). The IgG and IgM mean titers on days 28 and 42 was of no significant difference (P>0.05). Supplementing the feed with Citrus sinensis had no significant effect on Newcastle disease on day 42 (P>0.05). The mean value for hemagglutination inhibition on day 42 was significantly different (P<0.05). It can be then concluded that DCSP feed supplementation ameliorated the gastrointestinal microbiota and immune system traits.

Introduction

Management and nutrition are nowadays considered the most important issues in the poultry industry. Management and optimal nutrition reduce costs and economise production, and simultaneously allow the market to offer higher quality products to the consumers (Pope and Emmert, 2001).

The effect of plant extracts on performance, immune status and gut microflora of chickens is currently a topic of great scientific and practical importance, due to the trend in reducing the use of antibiotics in feed as well as in therapy (Yang et al., 2009). Studies on the plant products efficacy have given conflicting results (Huysgebaert et al., 2011), and this is a stimulus to implement research activities in this direction.

Citrus sinensis – belonging to the Rutaceae family (Vivek Kumar et al., 2010) – is one of the oldest plants whose fruits have been used by mankind. It constitutes about 60% of the total citrus world production, which has been estimated in about 122.09 million tons in 2008 (FAO, 2010). In 2012, 4.6 million tons of citrus fruits were produced in Iran, and, among these, about 2.7 million tons of oranges. Citrus sinensis peel ingredients are interesting from a nutritional point of view since they are a source of vitamin C, phenolic compounds, ascorbic acid, coumarin, volatile oils, nobiletin (Fernandez-Lopez et al., 2005), flavonoids (Chang, 1990), pectin (May, 1990) and bioflavonoids including hesperidin (Parhiz et al., 2014), naringin (Shafeqhat, 2010) and hesperetin (Harats et al., 1998). The activity of Citrus sinensis as an anti-microbial agent against Escherichia coli O157:H7 (Nannapaneni et al., 2008) and Salmonella typhimurium (O’Bryan et al., 2008), and its ability to reduce harmful microorganism growth have been observed. Moreover, several types of Citrus sinensis essential oil derivatives and their ability to inhibit the growth of bacteria such as Campylobacter and Arcobacter spp. have been studied (Nannapaneni et al., 2009).

Dried Citrus sinensis peel (DCSP) can significantly improve the immune system activities, and this action has been attributed to their antioxidant properties (Chen et al., 2012). Moreover, it has been demonstrated that herbal extracts can increase the antibody production, especially immunoglobulin G (IgG), indirectly improving the immune system activity with their anti-virus and anti-bacteria action (Catala-Gregori et al., 2007). Due to the great interest on beneficial properties of DCSP, Gallus gallus domesticus – a gallinaceous domesticated fowl, bred and raised specifically for meat production – has been selected to verify the effect of feed supplementation on the gastrointestinal microbial population and the immune system.

Materials and methods

Animals and dietary treatments

Two hundred broiler chickens (Ross-308) from Rasht, one of the main cities in Iranian province of Guilan, were used in a completely randomised design adopting five diet supplemented feed regimen. The procedures have been approved by the Iranian Author’s Institution Ethic Committee (Protocol number is: 03-16-5-3065), and maximum care was taken to minimise the number of animals used. Experiments were performed under controlled and standard conditions as recommended by Aviagen (Aviagen, 2007). Land cages were used. The study involved four replicates (10 chickens per replicate) and lasted 42 days (six weeks). Each treatment used different amounts of DCSP, namely DCSP-0.25% w/w,
DCSP-0.50% w/w, DCSP-0.75% w/w and DCSP-1.00% w/w in the feed. As control diet, feed without peel addition (DCSP-0%) was used.

Vaccination programme was conducted based on farm veterinarian; vaccination schedule is shown in Table 1. Vaccines were administered via drinking water and, in order to ensure optimal use of the vaccine on all chickens, drinking water was removed from the chickens for 1-2 hours before the administration of the vaccine to ensure chickens to be thirsty.

In addition, and with the aim of reducing the stress caused by vaccination, a multi-electrolyte solution diluted in a ratio 1:1000 was added to the drinking water, 24 hours before and after vaccination. For sanitation, all drinkers were daily regularly washed twice with fresh clean water and refilled to prevent the water from being contaminated with faeces and being exposed to microbial and/or viral contamination.

Tables 2 and 3 report some relevant composition data and energetic value of the starter and grower diets fed to broilers during the 42 days growing period.

As reference, basal tables based on Nutrition Requirements of Poultry (National Reserarch Council, 1994) were used. Dried Citrus sinensis peel was supplied from a local juice factory (Khazarnoush Co., Chaboksaar, Iran). After fine milling, dried peel was mixed with the other ingredients and the composition was determined according to the AOAC official methods (AOAC, 1990).

Microbiota traits measurement

For measuring the microbial population on day 42, one chicken was randomly selected from each experimental unit and slaughtered. Each experimental unit (group) included 4 replicates with 10 animals each. The contents of ileum and caecum sections collected for microbiological cultures were collected in dischargeable containers used also for waste microbial culture.

To evaluate microbial population, the colony forming unit (CFU) method was used. Collection tubes were labeled and treated and number of iterations were determined. Then they were weighed individually and the weight recorded. Collecting tubes were wrapped into aluminum sheet and were autoclaved for sterilising. As culture media, MRS agar (Man Rogosa Sharpe Agar, 1.10660.500) to culture Lactobacilli, Eosin Metilan Blou (EMB, 1.01347.0500) to culture Escherichia coli, and MacConkey agar (105465.0500) to culture coliforms, were used. Culture media were prepared 24 hours before collecting samples and poured into Petri dishes. Samples were transferred to the laboratory using collection tubes, weighed again and their weights were recorded. The amount of sample in each tube was calculated from the difference between these two values. Tubes were shaken for half an hour. The action was performed for bacteria isolated from gastrointestinal contents and preparation of suspension. One mL was removed from the prepared suspension and was added into 9 mL buffer phosphate saline (PBS) in the other tube. Suspensions were prepared by dilutions 10^{-2} and serial dilutions were done (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}). One hundred μL were removed from 10^{-4}, 10^{-5} and 10^{-6} dilutions and poured into the Petri dish already prepared and containing the medium. Lactobacilli bacteria incubation was performed at 37°C in anaerobic conditions for 72 hours. Anaerobic jar was used to create anaerobic condition. Enterobacteriaceae and total aerobic bacteria were incubated at 37°C in aerobic conditions for 48 hours. Bacteria counting on Petri dishes was done by counting colonies counter and the result adjusted to 1 g sample content.

Immunity traits measurements

Blood samples were taken from one broiler chicken randomly chosen on day 42 according to culture Lactobacilli, Eosin Metilan Blou agar (Man Rogosa Sharpe Agar, 1.10660.500) to culture Escherichia coli, and MacConkey agar (105465.0500) to culture coliforms, which were prepared 24 hours before collecting samples. Bacillus cereus strains were transferred to the laboratory using collection tubes, weighed again and their weights were recorded. The amount of sample in each tube was calculated from the difference between these two values. Tubes were shaken for half an hour. The action was performed for bacteria isolated from gastrointestinal contents and preparation of suspension. One mL was removed from the prepared suspension and was added into 9 mL buffer phosphate saline (PBS) in the other tube. Suspensions were prepared by dilutions 10^{-2} and serial dilutions were done (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}). One hundred μL were removed from 10^{-4}, 10^{-5} and 10^{-6} dilutions and poured into the Petri dish already prepared and containing the medium. Lactobacilli bacteria incubation was performed at 37°C in anaerobic conditions for 72 hours. Anaerobic jar was used to create anaerobic condition. Enterobacteriaceae and total aerobic bacteria were incubated at 37°C in aerobic conditions for 48 hours. Bacteria counting on Petri dishes was done by counting colonies counter and the result adjusted to 1 g sample content.

Table 1. Vaccination schedule.

| Type of vaccine | Days of vaccine | Method of vaccination |
|-----------------|----------------|----------------------|
| AI              | 1              | Spray                |
| IBV             | 1              | Spray                |
| ND              | 8              | Oral                 |
| IBD             | 14             | Oral                 |
| ND-Clon 30      | 20             | Oral                 |

AI, avian influenza; IBV, infectious bronchitis virus; IBD, infectious bursal disease; ND-Clon 30, nobilis Newcastle disease Clone 30.

Table 2. Composition of basal starter and grower diets fed to chicken broilers.

| Ingredient, % (w/w) as fed-basis | Starter | Grower |
|----------------------------------|---------|--------|
| Corn                             | 58.78   | 60.00  |
| Soybean meal                     | 34.73   | 32.73  |
| Carbonate                        |         | 1.30   |
| Corn oil                         | 3.50    | 3.50   |
| DL-methionine                    | 0.20    | 0.22   |
| L-lysine                         | 0.07    | 0.05   |
| Dicalcium phosphate              | 2.00    | 1.50   |
| Sodium chloride                  | 0.20    | 0.20   |
| Vitamin mixture                  | 0.25    | 0.25   |
| Mineral mixture                  | 0.25    | 0.25   |
| Total                            | 100.00  | 100.00 |

Vitamin and mineral supplied per kg of diet: vitamin A, 12,000 U; vitamin E, 10 mg; vitamin D, 2200 U; niacin, 35 mg; D-pantothenic acid, 12 mg; riboflavin, 3.63 mg; pterodoxine, 3.5 mg; thiamine, 2.4 mg; folic acid, 1.4 mg; biotin, 0.15 mg; vitamin B, 0.03 mg; manganese, 60 mg; zinc, 40 mg; iron, 1200 mg; copper, 8 mg; iodine, 0.3 mg; selenium, 0.2 mg.

Table 3. Some relevant data and energetic values of the starter and grower diets fed to broilers.

| Ingredient                | Starter | Grower |
|---------------------------|---------|--------|
| ME, kcal/kg               | 3019.80 | 2995.00|
| Crude protein, %          | 20.48   | 19.39  |
| SID, %                    |         |        |
| Lysine                    | 1.15    | 0.96   |
| Methionine                | 0.50    | 0.48   |
| Met+Cys                   | 0.83    | 0.78   |
| Threonine                 | 0.79    | 0.71   |
| Calcium, %                | 1.00    | 0.85   |
| Available phosphorus, %   | 0.50    | 0.42   |
| DCAB, mEq/kg              | 236.00  | 202.00 |

ME, metabolisable energy; SID, standardized ileal digestible amino acids; Met, methionine; Cys, cysteine; DCAB, electrolyte balance.
to the same procedure adopted elsewhere (Poorghasemi et al., 2015; Pourhossein et al., 2015). Hemagglutination inhibition test was used to determine vaccine titers of Newcastle disease (ND) and avian influenza (AI). This test was described by Hurst (1942) for the first time, and it is based on the virus or bacteria ability to cause red blood cells agglutination. In our experiment, the antigen located in the presence of the studied serum and red blood cells is neutralised and loses the ability of agglutination red blood cells in presence of antibodies and antigen binding.

Newcastle disease and AI antibody titer was measured by the hemagglutination test. At days 21 and 35, chickens from each replicate were injected 0.1 mL per kg of body weight with 0.5% sheep red blood cells into the wing vein. On days 28 and 42, blood samples were taken and analysed. First, after serum separation and decomponention at 36°C, the passive hemagglutination tests were performed. Twenty-five μL serum and 25 μL PBS added into the first 96 well plates (8×12) and the plates were incubated at 37°C for half an hour. After half an hour to rest 25 μL wells of PBS were added and then dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 were prepared. After preparing this dilution, 25 μL sheep red blood cells (SRBC) (1% solution) were added to each well, then the plates were incubated for 45 minutes at 37°C, and the number of first slipping cell was recorded. Titers were reported based on log2.

It was thus suggested that the different results of the trials are due to: i) differences in the composition of phytochemical preparations, ii) different methods used to extract the essential oils (EO) form the herbs, iii) level of the application in feed (Cross et al., 2007). Antimicrobial activities (anti-bacterial, anti-fungal and anti-yeast activity) have been reported for EO from various plants or fruits (Debeaufort, 2007). Essential oils function mainly as antimicrobials and antioxidants; their antimicrobial ability may modulate the gut ecosystem to affect fat digestibility (Lee et al., 2004a). Essential oils function mainly as antimicrobials and antioxidants; their antimicrobial ability may modulate the gut ecosystem to affect fat digestibility (Lee et al., 2004a). A commercial preparation of essential oil components reduced faecal C. perfringens counts of chickens in a field study (Mitsch et al., 2002). In addition, dietary supplementation of EO reduced the intestinal populations of E. coli (Jang et al., 2007) and increased digestive enzymes in either pancreas and/or intestinal mucosa (Jang et al., 2007); however intestinal mucosal morphology was not affected by EO supplementation (Garcia et al., 2007).

The effects of various herbs and oils on broiler performance, allowed assessing that the quality as well as the quantity of active chemicals in plant extract determine the bird response (Cross et al., 2007). In addition, the efficacy of dietary EO can be affected by intrinsic and extrinsic factors such as nutritional status of animals, infection, environment, and diet composition (Giannenas et al., 2003; Lee et al., 2004b). It is known that feeding rye to chickens increases the number of bacteria in the intestine (Feighner and Dashkevich, 1987). The microbial over-population by rye feeding is attributed to its pentosan content that raises intestinal viscosity.

It is suggested that intestinal viscosity, caused by ingestion of soluble fibre, impairs the normal digestion process so that more undigested materials travel to distal parts where they can be used as substrates by the microflora. Increased microbial populations are also apparent in the upper part of the small intestine (Smits et al., 1998) where digestion occurs. The yellow coloured Citrus sinensis essential oil (CEO) obtained from citrus peel antibacterial activities is well known, and it has been observed that an injured orange releases a much greater amount of terpene peel-oil constituents than healthy fruits (Norman et al., 1967; McCallet and Torres-Griñol, 1992). This might be the source of antibacterial activity found in CEO. Various components of CEO may act synergistically and several compounds might have stimulating actions on fungal spore germination (French, 1985). There are several reports on the antimicrobial action of CEO (Murdock and Allen, 1960; Subba et al., 1967). These reports demonstrated that the fungi are more resistant than yeasts and bacteria. Flora includes Clostridium, Lactobacillus Peptostreptococcus, Bifidobacterium, Faubacterium, Escherichia, Streptococcus, and Bacteroides (Feighner and Dashkevich, 1987).

Gut microflora has significant effects on host nutrition, health, and growth performance (Barron, 1992) by interacting with nutrient utilisation and the development of gut system of the host. This interaction is very complex and, depending on the composition and activity of the gut microflora, it can have either positive or negative effects on the health and growth of birds. For example, when pathogens attach to the mucosa, gut integrity and function are severely affected (Droleskey et al., 1994) and immune system threatened (Neish, 2002).

Statistical analysis

Data recorded for broilers’ gastrointestinal microbial population and immune system were statistically analysed using the one-way variance analysis (ANOVA). Statistics was carried out using SAS v8 (SAS Institute Inc., Cary, NC, USA). Duncan’s multiple range test was applied to compare the differences between the means (Steel et al., 1997).

**Results and discussion**

Natural medicinal products originating from herbs and spices have been used as feed additives for farm animals in for long time. To differentiate from the plant products used for veterinary purposes (prophylaxis and therapy of diagnosed health problems), phytophobiotics were redefined by Windisch and Kroisamayr (2006) as plant-derived products added to the feed in order to improve performance of agricultural livestock.

Many phytochemicals in phytophobiotics are well known to have antimicrobial ability (Cowan, 1999). Polysaccharides also are considered to be the most important immunoactive components (Xue and Meng, 1996). Increased feed intake and consequent increased digestive secretions have been observed in animals offered phytophobic-supplemented feed (Windisch and Kroisamayr, 2006). The same observation holds true for the addition of phytopogenic additives in broiler feed. Many experiments investigating the effects of herbs, plant extracts and essential oils on broiler performance gave however contradicting results. Some authors reported a significant positive effect of phytochemicals on broiler performance (Cross et al., 2007), while other trials using different phytophobic additives and essential oils did not affect body weight gain, feed intake or feed efficiency in broilers (Ocak et al., 2008).

It has been suggested that the different results of the trials are due to: i) differences in the composition of phytopogenic preparations, ii) different methods used to extract the essential oils (EO) form the herbs, iii) level of the application in feed (Cross et al., 2007). Antimicrobial activities (anti-bacterial, anti-fungal and anti-yeast activity) have been reported for EO from various plants or fruits (Debeaufort, 2007). Essential oils function mainly as antimicrobials and antioxidants; their antimicrobial ability may modulate the gut ecosystem to affect fat digestibility (Lee et al., 2004a). A commercial preparation of essential oil components reduced faecal C. perfringens counts of chickens in a field study (Mitsch et al., 2002). In addition, dietary supplementation of EO reduced the intestinal populations of E. coli (Jang et al., 2007) and increased digestive enzymes in either pancreas and/or intestinal mucosa (Jang et al., 2007); however intestinal mucosal morphology was not affected by EO supplementation (Garcia et al., 2007).
proposed that structural properties are responsible for the antibacterial activity (Bowles and Miller, 1993).

It is thought that membrane perforation or binding is the main mode of action (Shapiro and Gugenheim, 1995; Stiles et al., 1995), leading to an increase of permeability and leakage of vital intracellular constituents (Juven et al., 1994).

Microbiota traits measurements

All animals used for this study were healthy and survived until the end of the study.

Table 4 shows the effect of different levels of DCSP on broilers intestinal microbial population at the end of the treatment with different feed supplemented diets.

Supplementation of *Citrus sinensis* had no significant effect on *Lactobacillus* sp. (ileum and caecum), on coliforms (ileum and caecum) and *Escherichia coli* (ileum) on day 42 (P>0.05), but supplementation with *Citrus sinensis* had significant effect on *Escherichia coli* (caecum) on day 42 (P<0.05). The highest average number of log CFU•g⁻¹ *Lactobacillus* sp. (ileum) was observed in the experimental group feed with the DCSP-0.5.

Compared with the DCSP-0 treatment, lower number of *Lactobacillus* sp. was observed. The highest average number of logs CFU•g⁻¹ *Lactobacillus* sp. (caecum) was observed in the experimental group DCSP-1, and, compared with the DCSP-0 treatment, we observed lower number of *Lactobacillus* sp. The highest average number of log CFU•g⁻¹ coliforms (ileum and caecum) was in the experimental DCSP-0 treatment. Compared with the DCSP-1 treatment, we observed lower numbers of coliforms. The highest average number of log CFU•g⁻¹ *Escherichia coli* (ileum) was in the experimental DCSP-0 treatment. Compared with the DCSP-0.25, we observed lower numbers of *Escherichia coli*. The highest average number of log CFU•g⁻¹ *Escherichia coli* (caecum) was in the experimental DCSP-0 treatment. Compared with the DCSP-1, we observed lower numbers of *Escherichia coli*.

Table 4. Effect of different levels of dried *Citrus sinensis* peel on broilers' intestinal microbial population (log₁₀).

| Treatment   | Lactobacilli | Coliforms | *Escherichia coli* |
|-------------|--------------|-----------|--------------------|
|             | Ileum | Caecum | Ileum | Caecum | Ileum | Caecum |
| DCSP-0      | 7.52  | 8.19  | 9.45  | 9.64  | 8.46  | 9.19b |
| DCSP-0.25   | 7.90  | 9.58  | 8.25  | 8.85  | 7.54  | 8.44ab |
| DCSP-0.50   | 9.46  | 9.06  | 8.89  | 9.16  | 8.14  | 8.88ab |
| DCSP-0.75   | 8.30  | 9.31  | 7.85  | 8.88  | 7.77  | 7.87ab |
| DCSP-1.00   | 9.01  | 9.58  | 7.21  | 8.02  | 8.30  | 7.69ab |
| SEM         | 1.37  | 0.57  | 0.87  | 0.58  | 0.37  | 0.58  |
| P           | 0.18  | 0.00  | 0.09  | 0.45  | 0.55  | 0.01  |

DCSP; dried *Citrus sinensis* peel; DCSP-0, control diet without feed addition; DCSP-0.25, diet with 0.25% DCSP w/w feed addition; DCSP-0.50, diet with 0.50% DCSP w/w feed addition; DCSP-0.75, diet with 0.75% DCSP w/w feed addition; DCSP-1.00, diet with 1% DCSP w/w feed addition. *Means within a column with different superscript letters are significantly different (P<0.05).

Table 5. Effect of different levels of dried *Citrus sinensis* peel on broilers’ immune system (log₂).

| Treatment | 28th day | 42nd day |
|-----------|----------|----------|
|            | TSRBC    | IgG      | IgM      | TSRBC    | IgG      | IgM      |
| DCSP-0     | 3.33     | 1.00     | 2.33     | 6.33b    | 3.33     | 3.00     |
| DCSP-0.25  | 6.66     | 2.66     | 4.00     | 7.66ab   | 5.33     | 2.33     |
| DCSP-0.50  | 6.00     | 2.66     | 3.33     | 7.66ab   | 4.00     | 3.55     |
| DCSP-0.75  | 6.00     | 2.66     | 3.33     | 8.66b    | 5.33     | 3.33     |
| DCSP-1.00  | 4.66     | 1.33     | 3.33     | 8.00ab   | 4.33     | 3.66     |
| SEM        | 1.33     | 0.82     | 0.59     | 0.84     | 0.86     | 0.55     |
| P          | 0.07     | 0.12     | 0.52     | 0.05     | 0.05     | 0.04     |

TSRBC, sheep red blood cell; IgG, Immunoglobulin G; IgM, Immunoglobulin M; DCSP, dried *Citrus sinensis* peel; DCSP-0, control diet without feed addition; DCSP-0.25, diet with 0.25% DCSP w/w feed addition; DCSP-0.50, diet with 0.50% DCSP w/w feed addition; DCSP-0.75, diet with 0.75% DCSP w/w feed addition; DCSP-1.00, diet with 1% DCSP w/w feed addition. *Means within a column with different superscript letters are significantly different (P<0.05).

Table 6. Effect of different levels of dried *Citrus sinensis* peel on Newcastle disease and avian influenza (log₂).

| Treatment | ND (42nd day) | DCSP-0 | DCSP-0.25 | DCSP-0.50 | DCSP-0.75 | DCSP-1.00 | SEM | P |
|-----------|--------------|--------|-----------|-----------|-----------|-----------|-----|---|
| ND        | 4.00         | 5.66   | 7.66      | 6.66      | 5.66      | 5.00      | 0.98 | 0.15 |
| Al (42nd day) | 2.66b | 2.66b | 4.33a | 4.33a | 3.00ab | 0.69 | 0.01 |

ND, Newcastle disease; Al, avian influenza; DCSP, dried *Citrus sinensis* peel; DCSP-0, control diet without feed addition; DCSP-0.25, diet with 0.25% DCSP w/w feed addition; DCSP-0.50, diet with 0.50% DCSP w/w feed addition; DCSP-0.75, diet with 0.75% DCSP w/w feed addition; DCSP-1.00, diet with 1% DCSP w/w feed addition. *Means within a column with different superscript letters are significantly different (P<0.05).
significantly different (P<0.05). According to the results of this study on day 28, the lowest titer of total anti-SRBC was related to DCSP-0 treatment and the highest rate was related to DCSP-0.25 treatment. On day 42, the lowest titer of total anti-SRBC was related to DCSP-0 treatment and the highest rate was related to DCSP-0.75 treatment. In Table 5 the means of IgM and IgG titers on days 28 and 42 are also shown. According to the results of this study, they were no significantly different (P>0.05). On day 28, the lowest titer of IgG was related to DCSP-0 treatment and the highest rate was related to DCSP-0.25, DCSP-0.5 and DCSP-0.75 treatments. On day 42, the lowest titer of IgM was related to DCSP-0 treatment and the highest rate was related to DCSP-0.25 and DCSP-0.75 treatments. On day 42, the lowest titer of IgG was related to DCSP-0.5 treatment and the highest rate was related to DCSP-0.25 treatment. According to the results of this study on day 42, the lowest titer of IgG was related to DCSP-0 treatment and the highest rate was related to DCSP-0.25 and DCSP-0.75 treatments. On day 42, the lowest titer of IgM was related to DCSP-0.25 treatment and the highest rate was related to DCSP-1 treatment.

Newcastle disease and avian influenza titer

Table 6 shows the mean ND and AI titer on day 42. According to the results shown, the mean of ND titers on day 28 was not significantly different (P>0.05). On day 42, the lowest titer of ND was related to DCSP-0 treatment and the highest rate was related to DCSP-0.5 treatment. According to the results of this study, for the mean of AI titers on day 42, no significant difference was observed (P>0.05). On day 42, the lowest titer of AI was related to DCSP-0 treatment and DCSP-0.25 treatment and the highest rate was related to DCSP-0.5 treatment.

Conclusions

Dried Citrus sinensis peel feed supplement of broiler chickens resulted in ameliorating the gastrointestinal microbiota and immune system traits. The mean of Escherichia coli in caecum on the 42nd day improved while no other gastrointestinal microbial population trait was affected. Feed supplementation with Citrus sinensis dried peel had no significant effect on ND at the 42nd day while the mean value for hemagglutination inhibition on the 42nd day was significantly different. The results suggest a possible use of DCSP as feed supplement for broiler chickens.

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