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Investigation on effects of dried *citrus sinensis* peel on broilers intestinal pathogens

Zohreh Pourhossein, Ali Ahmad Alaw Qotbi, Alireza Seidavi

Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

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ABSTRACT

The aim of this experiment was to study the effects of dried citrus sinensis on intestinal pathogens of broilers. Studied treatments were included treatment 1: Control treatment included standard diet without additive aterials; treatment 2: standard diet + 1.5% dried citrus sinensis peel during 1-21st days; treatment 3: standard diet + 1.5% dried citrus sinensis peel during 1-42nd days; treatment 4: standard diet + 3.0% dried citrus sinensis peel during 1-21st days; treatment 5: standard diet + 3.0% dried citrus sinensis peel during 1-42nd days. The results from the mean of Escherichia coli in ileum in the day 42 indicated that the mean of treatment was significantly different ($p<0.05$). The lowest mean was related to 1.5 % treatment up to end of the rearing period and the highest rate was related to control treatment. The results from the mean of Escherichia coli in cecum in the day 42 indicated that all of treatment was significantly different with control ($p<0.05$). The lowest mean was related to 1.5 % treatment up to end of the rearing period and the highest rate was related to control treatment. The results from the mean comparison of coliforms in ileum in the day 42 indicated that experimental treatment was significantly different with control ($p<0.05$). The highest mean was concerned to control treatment and the lowest one was related to treatment which consumed 3% (DCSP) up to the end of rearing period. The results from the mean comparison of coliforms in cecum in the day 42 indicated that experimental treatment was significantly different with control ($p<0.05$). The highest mean was concerned to control treatment and the lowest one was related to treatment which consumed 3% (DCSP) up to the end of rearing period and 1.5%(DCSP) to day 21.

Keywords: Chick, Dried *citrus sinensis* Peel, Pathogen, Intestine, Salmonella.

INTRODUCTION

Protein is the most important components that constituents of human diet, which plays an important role in maintaining health. At least 25% of needed protein should be provided from animal sources because different types of amino acids found in animal protein is more complete and better than amino acids found in plant protein. Proteins of approximately 33% essential amino acid and about 66% and non essential amino acid are called complete proteins and proteins which are about 25% essential amino acid are called incomplete. All animal protein, excluding gelatin that lacks two essential amino acid, tryptophan and lysine are a complete protein [1].

Study of gastrointestinal microflora of chickens goes back to 1901 [2]. The investigation continued until the 1940s [3], but did not perform comprehensive investigations in 1970s [4]. Renewed attention to microorganism was in the late 1980s and early 1990s [5].

Gastrointestinal microbial population described with different words and opposites by Dobus et al (1965) which the indigenous microbial population was divided into two categories [6]. Native organisms such as Lactobacilli and Bacteroid that eventually create a symbiotic and evolving relationship interact with the host and non-native

organisms such as *Escherichia coli* and Clostridium that are potentially pathogenic factors. Normal microbial population is composed of above microbes and non-intestinal organisms with environmental origin [7].
Gastrointestinal microflora in adult chickens is including more than 400 species that are often located in the specific environment of jejunum and cecum. The number of anaerobic bacteria and aerobic in the duodenum and ileum is similar [8].

Ramadan et al [9] indicated that *citrus sinensis* peel extract citrus is found in the peel including D-limonen and D-syclic aldehid which have antimicrobial properties and also other materials including linalool and terpene Dl- neol and a number of flavonoids (anti-cancer effects) naming neohesperidin, hesperidin, naringin, tanzhrtyn, orantin, noubilitin, vitamin E, qoumarin, carotenoids and pectin [9]. *Citrus Sinensis* is including oils, which contain harmful bacteria is lethal [10, 11].

The aim of this experiment was to study the effects of dried *citrus sinensis* on intestinal pathogens of broilers.

MATERIALS AND METHODS

400 one-day-old chicks of Ross 308 were purchased and transferred to the experiment place. The average weight of broilers was 43.5 g and breeders were 38 weeks of age. The temperature of each breeding farm was supplied by three gasoline rocket heater and was controlled by three thermostats that were installed in different parts of the farm building. In order to provide moisture, the water spray to floor was used, so that moisture was retained during this period between 50 to 60 percent.

Lighting in the farm on the first day was 24 hours and by starting the second day became permanent and 23 hours which ensure that lighting, in addition to windows, used the typical 26-watt bulbs and fluorescent in three rows with a distance of approximately 3 m from each other and were installed at a height of 2 m from the floor. In order to air conditioning in every farm, three fans with a diameter of 60 cm which had proper discharge power and were installed on the south side and three fans with impeller diameter of 140 cm at the end of the hall which was installed for tunnel ventilation, was used. During the first two weeks of rearing, one plastic trays feeding per each cage were used. Starting the third week, all the trays feeding were collected and were replaced by proper feeding.

For sanitation, all drinkers daily regularly washed twice with fresh clean water and were filled until is prevented water from being contaminated with feces and thus microbial and viral contamination. Vaccination program was conducted based on farm veterinarian, vaccines were used to drinking practices which in order to ensure optimal use of the vaccine on all chicken and 2 hours before giving the vaccine to chickens was thirsty. Also to reduce the stress caused by vaccination 24 hours before and after vaccination, of multi-electrolyte solution compared to 1 in 1000 was used in drinking water.

Studied treatments were included:
Treatment 1: Control treatment included standard diet without additive aterials.
Treatment 2: Standard diet + 1.5% dried *citrus sinensis* peel during 1-21st days.
Treatment 3: Standard diet + 1.5% dried *citrus sinensis* peel during 1-42nd days.
Treatment 4: Standard diet + 3.0% dried *citrus sinensis* peel during 1-21st days.
Treatment 5: Standard diet + 3.0% dried *citrus sinensis* peel during 1-42nd days.

Collected samples for microbial culture
For measuring the microbial population in the days 14 and 42, a chicken was selected from each experimental unit and slaughtered. The contents of ileum and cecum sections collected for microbial cultures in discharged containers and passing microbial culture.

Measurment of microbial population
In this study, colony forming unit (CFU) method was used. At first, the collection tubes were labeled. Treatment and the number of iterations were determined. Then they were weighed individually and their weights were recorded. Collecting tubes wrapped into aluminum sheet and were autoclaved for steriling. The culture mediums were prepared and 24 hours before collecting samples were poured into the petri dish.

Eosin Metilan Blou (EMB, 1.01347.0500) to cultuer *Escherichia coli*, and maccanky agar (105465.0500) to culture Coliform was used. To find Salmonella ss agar (Salmonella-shigelal.10660.500), Hi chrom agar (M14660500G) and XLD (zylose Lysine Deoxycholate Modified Agar (18403) were used.

Statistical design and data analysis

This study was conducted in a completely randomized design with five treatments and four replicates and twenty observations at each of replications. For data analysis related to the immune system and intestinal microorganisms, SAS software, using the GLM procedure and Duncan test at 5% level of statistical comparison was used.
The mathematical model was as follows.

$$X_{ij} = \mu + T_i + e_{ij}$$

x_{ij}= Value observed in each experimental unit
μ =Mean population
T_i= The effect of each treatment
e_{ij}= The effect of experimental errors

RESULTS

Gastrointestinal bacteria counts at day 14

Table 1 shows the average number of gastrointestinal bacteria of experimental treatment in the day 14. The results from the comparison of *Escherichia coli* mean in ileum in the day 14 showed significantly difference ($p<0.05$). The lowest mean was related to 3% (DCSP) treatment up to day 21 and the highest rate was related to control treatment. The results from the comparison of *Escherichia coli* mean in cecum in the day 14 showed no significantly difference ($p>0.05$). The highest mean was related to control treatment and the lowest rate was related to 3% (DCSP) treatment up to day 21.

The results from the comparison of Coliforms mean in ileum in the day 14 showed no significantly difference ($p>0.05$). The results from the comparison of Coliforms mean in ileum in the day 14 showed significantly difference ($p<0.05$). The lowest mean was related to 3% (DCSP) treatment up to the end of the rearing perioda and the highest rate was related to control treatment.

Table 1. Bacterial populations (log_{10} CFU/g) of cecum and ileum contents at 14th day

Treatment	Coliforms (Ileum)	Coliforms (Cecum)	*Escherichia coli* (Ileum)	*Escherichia coli* (Cecum)	Salmonella (Ileum)	Salmonella (Cecum)
CONTROL	$7.78^{a}\pm0.23$	$8.31^{a}\pm0.14$	$8.01^{a}\pm0.12$	$8.03^{a}\pm0.17$	Neg	Neg
[A]DCSP(1.5%), 1st- 21st day	$7.44^{a}\pm0.23$	$7.88^{b}\pm0.14$	$7.47^{b}\pm0.12$	$7.79^{a}\pm0.17$	Neg	Neg
DCSP(1.5%), 1st- 42nd day	$7.57^{a}\pm0.23$	$7.87^{b}\pm0.14$	$7.41^{b}\pm0.12$	$7.77^{a}\pm0.17$	Neg	Neg
DCSP(3.0%), 1st- 21st day	$7.37^{a}\pm0.23$	$7.81^{b}\pm0.14$	$7.30^{b}\pm0.12$	$7.68^{a}\pm0.17$	Neg	Neg
DCSP(3.0%), 1st- 42nd day	$7.25^{a}\pm0.23$	$7.76^{b}\pm0.14$	$7.48^{b}\pm0.12$	$7.75^{a}\pm0.17$	Neg	Neg

[A]DCSP = Dried Citrus Sinensis Peel
Means with the same letter are not significantly different (P<0.05).

Gastrointestinal bacteria counts at day 42

Table 2 shows the average number of gastrointestinal bacteria of experimental treatment in the day 42.The results from the comparison of *Escherichia coli* mean in ileum in the day 42 showed significantly difference ($p<0.05$). The lowest mean was related to the treatment which consumed 1.5% (DCSP) up to the end of the rearing period and the highest rate was related to control treatment. The results from the comparison of *Escherichia coli* mean in cecum in the day 42 showed significantly difference ($p<0.05$). The lowest mean was related to the treatment which consumed 1.5% (DCSP) up to the end of the rearing period and the highest rate was related to control treatment. The results from the comparison of Coliforms mean in ileum in the day 42 showed significantly difference between experimental treatment and control ($p<0.05$). The highest rate was related to control treatment and the lowest mean was related to 3% (DCSP) treatment up to the end of the rearing period. The results from the comparison of Coliforms mean in cecum in the day 42 showed significantly difference between experimental treatment and control ($p<0.05$). The highest rate was related to control treatment and the lowest mean was related to 3% (DCSP) treatment up to the end of the rearing period and 1.5 % (DCSP) treatment up to day 21.

Salmonella in the gastrointestinal tract by the days 14 and 42

Table 1 and 2 a show the Salmonella search of all treatment in gastrointestinal tract by the days 14 and 42. According to the results of this study, all treatment in this search was negative.

Table 2. Bacterial populations (log_{10} CFU/g) of cecum and ileum contents at 42nd day

Treatment	Coliforms (Ileum)	Coliforms (Cecum)	*Escherichia coli* (Ileum)	*Escherichia coli* (Cecum)	Salmonella (Ileum)	Salmonella (Cecum)
CONTROL	8.40^{a}±0.19	8.60^{a}±0.07	8.23^{a}±0.19	8.38^{a}±0.16	Neg	Neg
DCSP(1.5%), 1st- 21st day	7.65^{b}±0.19	7.94^{b}±0.07	7.66^{ab}±0.19	7.72^{b}±0.16	Neg	Neg
DCSP(1.5%), 1st- 42nd day	7.81^{ab}±0.19	7.86^{b}±0.07	7.56^{b}±0.19	7.70^{b}±0.16	Neg	Neg
DCSP(3.0%), 1st- 21st day	7.73^{b}±0.19	7.86^{b}±0.07	7.58^{b}±0.19	7.85^{b}±0.16	Neg	Neg
DCSP(3.0%), 1st- 42nd day	7.62^{b}±0.19	7.94^{b}±0.07	7.69^{ab}±0.19	7.79^{b}±0.16	Neg	Neg

Means with the same letter are not significantly different (P<0.05).

DISCUSSION

The results from the comparison of *Escherichia coli* mean in ileum in the day 14 showed significantly difference (p<0.05). The results from the comparison of *Escherichia coli* mean in cecum in the day 14 showed no significantly difference (p>0.05).

The results from the comparison of Coliforms mean in ileum in the day 14 showed no significantly difference (p>0.05). The results from the comparison of Coliform**s** mean in cecum in the day 14 showed significantly difference (p<0.05). The results from the comparison of *Escherichia coli* mean in ileum in the day 42 showed significantly difference (p<0.05). The results from the comparison of *Escherichia coli* mean in cecum in the day 42 showed all treatment are significantly difference with control (p<0.05). The results from the comparison of Coliforms mean in ileum in the day 42 showed experimental treatment are significantly difference with control (p<0.05). The results from the comparison of Coliforms mean in cecum in the day 42 showed experimental treatment are significantly difference with control (p<0.05). In this study, all treatments were negative for Salmonella.

Antimicrobial properties of extract are primarily related to its phenol compounds. Whatever phenol substances in the extract is much higher, their antimicrobial properties is more [10]. Nannapaneni et al (2008) showed that natural compounds of *citrus sinensis* peel have inhibitory effects in different strains of *Escherichia coli*, Salmonella and some food pathogenic bacteria [12]. *Citrus sinensis* peel extracts has appropriate antimicrobial effects on gram positive and gram negative bacteria [13]. The present study result is quite consistent with a researcher [14].

Charis [15] in a research showed that different citrus have different effects on different bacteria. Antimicrobial properties of oils extracted from the citrus peel it is directly affected by its constituents [15]. Essential fatty acids and alcaloeid, lacton, polyacetilen are compounds effective on different bacteria. The antimicrobial effects of citrus is depends to soil separation, harvest season, the plant physiologist, and the extraction process and the type of bacteria.

Citrus sinensis had antimicrobial properties against *Escherichia coli*, Staphylococcus and *Bacillus antrasis*. Interestingly, the ethyl acetate extract of *citrus sinensis* without acid (*Citrus paradisi*) and (*Citrus pomolo*) has inhibitory properties against *Aspergillus's fumigates*.

Gulluce et al [16] reported that the herb Oregano extract has inhibitory effect on the bacteria *Salmonella interitidis* [16]. Sadeghzadeh et al. [17] in a research showed that Thyme has remarkable inhibitory and germicidal effects on the bacteria *Salmonella tayphi A* and *salmonella para tayphi B* [17].

Ziziphora clinopodioides has inhibitory and germicidal effect on gram negative bacteria, including *Klebsiella pneumonia*, *Escherichia coli*, Introbacter and *Salmonella interitidis* [18]. The results of this study is consistent with above findings and has been inconsistent with findings of Sagdıc et al [19]. They reported that cumin has no effect on inhibition of and *Salmonella interitidis* and *Salmonella tayphimurium bacteria.*

CONCLUSION

Based on the results of present study, using dried *citrus sinensis* peel was reduced *Escherichia coli* and coliforms count.

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