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Full Length Research Paper

Investigation on the effects of different levels of *Citrus sinensis* peel extract on gastrointestinal microbial population in commercial broilers

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The experiment was conducted to evaluate the effects of different levels of *Citrus sinensis* peel extract (CSPE) on gastrointestinal microbial population of broilers. Four hundred Ross 308 one-day broilers in a completely randomized design with five treatments (four replicates per treatment and each replicate had 20 chicks) were categorized. Each treatment used regulatory diet including either 1000 or 1250 ppm (CSPE) in the drinking water and in two periods of 1st to 21st day and 1st to 42nd days or based diet without any additive for six weeks. Data analysis was performed using SAS software and mean comparison was conducted by Duncan method. The results determined that the mean of Lactobacilli in ileum on day 42 showed that the means of the treatments were significantly different ($p < 0.05$). The highest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the lowest mean to the control treatment. The results determined that the mean of Lactobacilli in cecum on day 42 showed that the means of the treatments were significantly different ($p < 0.05$). The results determined that the mean of *Escherichia coli* in ileum on day 42 showed that the means of the treatments were significantly different ($p < 0.05$). The lowest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the highest mean to the control treatment. The results determined that the mean of coliforms in ileum on day 42 showed that all the experimental treatments were significantly different from the control treatment ($p < 0.05$). The highest mean was related to the control treatment and the lowest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period.

Key words: Broilers, *Citrus sinensis* peel extract, microbial population, *Salmonella*.

INTRODUCTION

Among the animal proteins, no doubt, poultry meat has a special place from consumers' point of view. Poultry meat is a good source of protein, iron and phosphorus for human nutrition, and in addition, it contains a large amount of vitamin D. The lower price of this protein source compared to other sources of animal proteins is the key to success in this industry. Also, from viewpoint of consumers who take care of their health (in terms of lower fat), poultry meat is highly desirable. According to poultry industry owners' point of view, poultry farming because of short period of poultry breeding time and lower feed conversion ratio compared to that of livestock, is economical Hence, less capital immobility. On the other hand, about 35% of the total protein requirements are met by poultry products. These issues have been considered by agricultural planners in recent years (Zohari, 2005).

Nutrition has an important impact on strengthening the immune system in response to pathogens, as pathogens have negative effect on nutrition. Nutritional effects on the immune system can be specific or nonspecific. Some substances such as herbs exert an indirect and stimulating effect on the immune system. A huge portion of food in Iran and other developing countries is wasted

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because of various reasons like the absence of a proper procedure, lack of adequate transportation systems, etc. Hence, equivalent to what is wasted must be imported from abroad and this means reliance. We should therefore seek ways to reduce waste and thus increase the efficiency of our food industry and this is not feasible except in light of further research and study. One of the ways to reduce food waste is recycling the waste materials from food plants. In this way not only can we avoid some imported materials required in some industries, but also we can consider them as non-oil exports. Thus, it will help to develop non-oil economy policy or non-oil –based exports. Iran, the world's major citrus producer, produces large amounts of citrus per annum. Citrus is mainly consumed by human or part of it used in producing fruit juice (Economides, 1974).

As Oluremi et al. (2007) stated, "the phytochemical screening of the citrus fruit peel meal samples revealed the presence of limonene, tannin, saponin, phytate, oxalate and flavonoid in all the citrus fruit peel meals". They stated that "flavonoids are detected in citrus fruits peel meals with phytochemical". Flavonoids have been reported to function as pigments and antioxidants (Kumar, 1991; Oluremi et al., 2007) and can inhibit enzymes in mammals (Hollman, 1997; Oluremi et al., 2007).

Composition and activities of gastrointestinal microbes can be changed by manipulating dietary compounds, using feed additive and antibiotics (Coates et al., 1981). Gastrointestinal microflora is influenced by different factors such as environmental stresses, the type of diet, drugs, chemical toxins and weather conditions. But, the main factor in maintaining the balance of gastrointestinal microflora is bacterial interaction effects. The microbial population of the different parts of the intestine is different due to a disorder in acidity and food passage rate (Wang et al., 1998).

One day after hatching, significant changes have been reported in microbial population in different parts of the gastrointestinal tract (Naqui et al., 1970). Suitable microflora replacement, consisting of useful bacteria species guarantees gastrointestinal health. Many anaerobic bacteria were replaced 3 to 6 h after leaving the egg in the chicken cecum. During the first 2 to 4 days after leaving the egg, Streptococcus and Entrobacter were located in the small intestine and cecum. Lactobacilli in the colon are predominant after 7 days. Mainly, in the cecum, anaerobic bacteria (Bacteroides) are replaced by fewer aerobic bacteriaum. Generally, microflora in adult bird is naturally located at 2 weeks in the small intestine. Cecum flora development in adult birds may take 30 days, and finally Bifidobacteriaum and Bacteroides are predominant.

Calabro et al. (2004) showed that *Citrus sinensis* has antimicrobial activity against *Escherichia coli* O157: H7 and *Salmonella typhimurium*, and reduces harmful microorganism growth. Nannapaneni et al. (2008) studied several types of *C. sinensis* oil derivatives capable of inhibiting the growth of some bacteriaum such as Campylobacter and Acrumobacter. This study showed that the *C. sinensis* oil acts as a natural anti-microbial against *Campylobacter jejuni* and *Campylobacter coli* and different strains of Acrumobacter. Nannapaneni et al. (2008) examined the antimicrobial activity of natural *C. sinensis* extract against *E. coli* O157: H7 and mutant strains. The results showed that natural *C. sinensis* peel compounds have different inhibiting effects on different strains of *E. coli*.

The aim of this project was to investigate *C. sinensis* peel extract effect on the gastrointestinal microbial population of broilers.

MATERIALS AND METHODS

The experiment location was in Some'esara, one of the cities of Guilan province (Iran). The experiment was conducted for 42 days in 2011. Using scaffoldings, cages with dimensions 2 × 1 m and height of 1 m were installed, and each cage was assigned to a repeat.

The first stage of preparation was evacuating the fertilizer from the previous period. Then, the farm buildings were thoroughly cleaned and rinsed with water pressure. Completely dried, the floor was scorched by a flame thrower. By bringing the temperature to 32℃ using 1% formalin solution, the farm buildings were disinfected.

The farm building walls were sprayed as high as 1 m with lime solution. After lime spraying, Hydro Care solution was used (1 L/100 L) as a spray. Then, 750 g Flomajon powder mixed in 500 L was sprayed with a strong push to the floor and walls. After half an hour, the rinse was repeated. Fogging involved three stages. First, the empty farm buildings were sprayed with pure formalin and by turning on the heaters, the material on the floor evaporated. It was done three days before the main fogging. Following disinfection, the main fogging was carried out using Azomit. After flattening the roll and placing drinkers and feeders, the hall was gasified with Azomit 24 h before entering the broilers.

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 the breeders were 38 weeks of age. The temperature of each breeding farm was supplied with three gasoline rocket heaters and was controlled by three thermostats that were installed in different parts of the farm building. In order to provide moisture, water spray was applied to the floor, so that moisture was retained throughout the rearing period between 50 and 60%.

Lighting was provided for 24 h on the first day and this diminished to the permanent 23 h a day from the second day on, to ensure adequate lighting, in addition to windows, by the typical 26-watt bulbs and fluorescent lights in three rows at a distance of approximately 3 m from each other installed at a height of 2 m from the floor.

In order for air conditioning in every farm, three fans with an impeller of 60 cm diameter, with proper discharge power were installed on the south side and three fans with an impeller of 140 cm diameter at the end of the hall for tunnel ventilation. During the first two weeks of rearing, one plastic trays feeder was used per cage. Starting from the third week, all the trays feeders were collected and were replaced with starry feeders.

To observe sanitary requirements, all drinkers were regularly washed twice daily with fresh clean water and were filled with the same, hence preventing water from being contaminated with feces and thus microbial and viral contamination. Vaccination program was implemented by farm veterinarian in accordance with Table 1;

Table 1. Vaccination program.

Type of vaccine	Age of vaccine	Method of vaccination
Inactive IBV[1], AI[2], ND[3]	0	Spray
ND, IBV	7	Oral
IBD	16	Oral
ND Clon 30	20	Oral
ND Clon 30	30	Oral

1- Infectious Bronchitis Virus (IBV), 2- Avian Influenza (AI), 3- Newcastle Disease (ND).

the vaccines were applied via oral usage. In order to ensure optimal consumption of the vaccine, all chickens were denied water for 2 h before vaccination. To reduce the stress caused by vaccination, multi-electrolyte solution was used by 1 in 1000 drinking water 24 h before and after vaccination.

Studied treatments were included:

Treatment 1: Control treatment included standard diet without additive materials.
Treatment 2: Standard diet + 1000 ppm *C. sinensis* peel extract during 1 - 21st days.
Treatment 3: Standard diet + 1000 ppm *C. sinensis* peel extract during 1 - 42nd days.
Treatment 4: Standard diet + 1250 ppm *C. sinensis* peel extract during 1 - 21st days.
Treatment 5: Standard diet + 1250 ppm *C. sinensis* peel extract during 1 - 42nd days.

The compositions of the based diet and its nutrients in the starting and growing periods are shown in Tables 2 and 3. The based diet was formulated according to NRC (1994).

According to Li et al. (2000), 40 g of *C. sinensis* peel were mixed in 320 ml of 72% ethanol. It was then treated in water both 50℃ for 3 h. The aquired suspension was centrifuged in 3000 rpm for 10 min. The upper liquid was filtered by the filter paper whatman No. 42 and concentrated by a rotary evaporator. The concentrate was dried in room temperature utilizing the lab cabinet.

Table 2. Diets used during experimental periods.

Ingredient	Starter (%)	Grower (%)
Corn	54.32	58.69
Soybean meal	39.43	31.87
Oyster shell	0.90	0.79
Corn oil	2.16	5.83
DL-methionine	0.20	0.22
L-lysine	0.07	0.05
Di-calcium phosphate	2.05	1.68
Salt	0.37	0.37
Vitamin mixture	0.25	0.25
Mineral mixture	0.25	0.25
Total	100	100

Table 3. Nutrients Analysis of used diets during experimental periods.

Ingredient	Starter	Grower
Energy (ME) (kcal/kg)	2900.00	3200.00
Crude protein (%)	22.16	19.20
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

Collected samples for microbial culture

In order to measure the microbial population on days 14 and 42, one chicken was selected inadverantly from each experimental unit and slaughtered. The contents of the ileum and cecum sections were collected in discharge containers to grow microbial cultures from.

Measuring microbial population

In this study, Colony Forming Unit (CFU) method was used. At first, the collecting tubes were labeled. Treatment and the number of iterations were determined. Then, they were weighed individually. And, their weights were recorded. The collecting tubes were wrapped in aluminum sheet and autoclaved to be steriled. The culture mediums were prepared and poured into the petri dishes 24 h before collecting the samples. MRS agar (Man Rogosa Sharpe agar, 1.10660.500), Eosin Metilan Blou (EMB, 1.01347.0500) and maccanky agar (105465.0500) were used to culture Lactobacilli; *E. coli* and Coliforms were used respectively. To find *Salmonella*, SS agar (*Salmonella shigelal*. 10660.500), Hi chrom agar (M14660500G) and XLD (zylose Lysine Deoxycholate Modified Agar (18403) were used. To culture Enterococci and total aerobic bacteria counts, Slantez and Bartley agar (450430) and Nutrient agar (1.05450.0500) were used respectively.The samples transferred to the laboratory in the listed tubes were weighed again and they were all recorded. The amount of sample in each tube was calculated from the difference between these two values. The tubes were shaken for approximately half an hour to isolate the gastrointestinal contents from the bacteriaum and prepare the suspension. An amount of 1 ml was removed from the prepared suspension and was added to 9 ml buffer phosphate saline (pbs) in another tube. From dilutions 10^{-1}, the concerned suspension was prepared and in repeating the same process serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were obtained from which an amount of 100 µl was removed from 10^{-4}, 10^{-5} and 10^{-6} dilutions and poured into the already prepared petri dishes containing the medium and

Table 4. Bacterial populations (log $_{10}$ CFU/g) of cecum and ileum contents at 14th day of broilers fed with different *Citrus sinensis* peel extract sources.

Treatment	Lactobacilli (Ileum)	Lactobacilli (Cecum)	Coliforms (Ileum)	Coliforms (Cecum)	*Escherichia coli* (Ileum)	*Escherichia coli* (Cecum)
Control	7.49 ± 0.12^{b}	7.88 ± 0.1^{c}	7.78 ± 0.216^{a}	8.31 ± 0.116^{a}	8.01±0.150^{a}	8.03 ± 0.14^{a}
A CSPE (1000 ppm), 1st- 21st day	8.08 ± 0.12^{a}	8.07 ± 0.13^{bc}	7.50 ± 0.216^{a}	7.62 ± 0.116^{b}	7.28 ± 0.150^{b}	7. 54 ± 0.14^{b}
CSPE (1000 ppm), 1st- 42nd day	8.15 ± 0.12^{a}	8.38 ± 0.13^{ab}	7.39 ± 0.216^{a}	7.64 ± 0.116^{b}	7.02 ± 0.150^{b}	7.55 ± 0.14^{b}
CSPE (1250 ppm), 1st- 21st day	8.21 ± 0.12^{a}	8.48 ± 0.13^{ab}	7.37 ± 0.216^{a}	7.63 ± 0.116^{b}	7.29 ± 0.150^{b}	7.48 ± 0.14^{b}
CSPE (1250 ppm), 1st- 42nd day	8.19 ± 0.12^{a}	8.55 ±0.13^{a}	7.34 ± 0.216^{a}	7.58 ± 0.116^{b}	7.27 ± 0.150^{b}	7.48 ± 0.14^{b}

[A] CSPE= *Citrus sinensis* peel extract. Means with the same letter are not significantly different (P < 0.05).

distributed evenly to all parts of the medium. The bacteria were incubated in certain conditions due to growing reasons. Enterococci and Lactobacilli bacteria were incubated at 37°C in anaerobic conditions within 72 h. Anaerobic jar was used to create an anaerobic condition. Introbactriaccea and Total aerobic bacteria counts incubated at 37°C in aerobic conditions and took 48 h. Colony counter was used to count the bacteria. Finally, the number of the bacteria for 1 g of sample was determined.

***Salmonella* finding**

Samples were first placed in selenit for 24 h, then was transferred into SS, Hi chrom agar, XLD, and incubated for 72 h.

Statistical design and data analysis

This study was conducted in a completely randomized design with five treatments and four replicates along with twenty observations for each of the replicates. For data analysis related to the intestinal microorganisms, SAS software, utilizing the GLM procedure and Duncan test at 5% level of statistical probability, were 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 on day 14

Table 4 shows the average number of gastrointestinal bacteria of experimental treatment on day 14. According the results of this study, the average number of bacteria in the gastrointestinal tract was significantly different ($p < 0.05$). The results of the mean comparison of lactobacilli in ileum on day 14 showed a significant difference ($p < 0.05$). The lowest mean was related to the control treatment and the highest rate to 1250 ppm (CSPE) treatment up to day 21. The results of the mean comparison of Lactobacilli in cecum on day 14 showed a significant difference ($p < 0.05$). The lowest mean was related to the control treatment and the highest rate to 1250 ppm (CSPE) treatment up to the end of the rearing period. The results of the mean comparison of *E. coli* in ileum on day 14 showed a significant difference ($p < 0.05$). The lowest mean was related to 1000 ppm (CSPE) treatment up to the end of the rearing period and the highest rate to the control treatment. The results of the mean comparison of *E. coli* in cecum on day 14 showed a significant difference ($p < 0.05$). The lowest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and 1250 ppm (CSPE) treatment up to day 21 and the highest rate to the control treatment.

The results of the mean comparison of Coliforms in ileum on day 14 showed no significant difference ($p > 0.05$). The results of the mean comparison of Coliforms in cecum on day 14 showed a significant difference ($p < 0.05$). The lowest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the highest rate to thecontrol treatment.

Table 6 shows the mean comparison of Entereocci in ileum on day 14 which was not significantly different ($p > 0.05$). The lowest mean was related to the control treatment and the highest rate to 1250 ppm (CSPE) treatment up to day 21. The results of the mean comparison of Entereocci in cecum on day 14 were not significantly different ($p > 0.05$). The lowest mean was related to the control treatment and the highest rate to 1250 ppm (CSPE) treatment up to the end of the rearing period.

Table 6 shows the mean comparison of the total aerobic bacteriaum in ileum on day 14 which was not significantly different ($p > 0.05$). The highest mean was related to the control treatment and the lowest rate was

Table 5. Bacterial populations (log_{10} CFU/g) of cecum and ileum contents at 42nd day of broilers fed with different *Citrus sinensis* peel extract sources.

Treatment	Lactobacilli (Ileum)	Lactobacilli (Cecum)	Coliforms (Ileum)	Coliforms (Cecum)	*Escherichia coli* (Ileum)	*Escherichia coli* (Cecum)
Control	7.58 ± 0.12^{c}	8.05 ± 0.12^{b}	8.40 ± 0.20^{a}	8.61 ± 0.12^{a}	8.23 ± 0.19^{a}	8.38 ± 0.17^{a}
CSPE (1000 ppm), 1st - 21st day	8.25 ± 0.12^{b}	8.43 ± 0.12^{ab}	7.67± 0.20^{b}	7.86 ± 0.12^{b}	7.53 ± 0.19^{b}	7.63 ± 0.17^{b}
CSPE (1000 ppm), 1st - 42nd day	8.55 ± 0.12^{ab}	8.69 ± 0.12^{a}	7.50 ± 0.20^{b}	7.61 ± 0.12^{b}	7.40 ± 0.19^{b}	7.55 ± 0.17^{b}
CSPE (1250 ppm), 1st - 21st day	8.33 ± 0.12^{ab}	8.52 ± 0.12^{a}	7.53 ± 0.20^{b}	7.90 ± 0.12^{b}	7.55 ± 0.19^{b}	7.74 ± 0.17^{b}
CSPE (1250 ppm), 1st - 42nd day	8.69 ± 0.12^{a}	8.84 ± 0.12^{a}	7.49 ± 0.20^{b}	7.66 ± 0.12^{b}	7.30 ± 0.19^{b}	7.51 ± 0.17^{b}

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

Table 6. Bacterial populations (log_{10} CFU/g) of cecum and ileum contents at 14th day of broilers fed with different *Citrus sinensis* peel extract sources.

Treatment	Enterococci (Ileum)	Enterococci (Cecum)	Total aerobic bacteria (Ileum)	Total aerobic bacteria (Cecum)	*Salmonella* (Ileum)	*Salmonella* (Cecum)
Control	7.80 ± 0.18^{a}	8.03 ± 0.15^{a}	7.86 ± 0.21^{a}	8.12 ± 0.13^{a}	Negative	Negative
CSPE (1000 ppm), 1st - 21st day	7.99 ± 0.18^{a}	8.31 ± 0.15^{a}	7.70 ± 0.21^{a}	8.01 ± 0.13^{a}	Negative	Negative
CSPE (1000 ppm), 1st - 42nd day	8.15 ± 0.18^{a}	8.30 ± 0.15^{a}	7.61 ± 0.21^{a}	7.97 ± 0.13^{a}	Negative	Negative
CSPE (1250 ppm), 1st - 21st day	8.22 ± 0.18^{a}	8.42 ± 0.15^{a}	7.53 ± 0.21^{a}	8.04 ± 0.13^{a}	Negative	Negative
CSPE (1250 ppm), 1st - 42nd day	8.10 ± 0.18^{a}	8.51 ± 0.15^{a}	7.26 ± 0.2^{a}	7.87 ± 0.13^{a}	Negative	Negative

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

related to 1250 ppm (CSPE) treatment up to the end of the rearing period. The result of the mean comparison of the total aerobic bacteria in cecum on day 14 was not significantly different (p > 0.05). The highest mean was related to the control treatment and the lowest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period.

Gastrointestinal bacteria counts on day 42

Table 5 shows mean comparison of Lactobacilli in ileum on day 42 which was significantly different (p < 0.05). The highest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the lowest mean to the control treatment. The result of the mean comparison of Lactobacilli in cecum on day 42 was significantly different (p < 0.05). The highest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the lowest mean to the control treatment.

The result of the mean comparison of *E. coli* in ileum at day 42 showed a significant difference (p < 0.05). The lowest mean of *E. coli* was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the highest rate was related to the control treatment. The results of the mean comparison of *E. coli* in cecum on day 42 were significantly different to the control treament (p < 0.05). The lowest mean of *E. coli* was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the highest rate to the control treatment. The results of the mean comparison of Coliforms in ileum on day 42 were significantly different between all experimental treatments and the control treatment (p < 0.05). The highest rate was related to the control treatment and the lowest of Coliforms to 1250 ppm

Table 7. Bacterial populations (log $_{10}$ CFU/g) of cecum and ileum contents at 42nd day of broilers fed with different *Citrus sinensis* peel extract sources.

Treatment	Enterococci (Ileum)	Enterococci (Cecum)	Total aerobic bacteria (Ileum)	Total aerobic bacteria (Cecum)	*Salmonella* (Ileum)	*Salmonella* (Caecum)
CONTROL	7.63 ± 0.25^{a}	7.86 ± 0.13^{b}	7.98 ± 0.21^{a}	8.23 ± 0.18^{a}	Negative	Negative
CSPE (1000 ppm), 1st - 21st day	8.11 ± 0.25^{a}	8.26 ± 0.13^{ab}	7.83 ± 0.21^{a}	8.05 ± 0.18^{a}	Negative	Negative
CSPE (1000 ppm), 1st - 42nd day	8.33 ± 0.25^{a}	8.56 ± 0.13^{a}	7.83 ± 0.21^{a}	7.69 ± 0.18^{a}	Negative	Negative
CSPE (1250 ppm), 1st - 21st day	8.22 ± 0.25^{a}	8.41 ± 0.13^{a}	7.75 ± 0.21^{a}	8.07 ± 0.18^{a}	Negative	Negative
CSPE (1250 ppm), 1st - 42nd day	8.06 ± 0.25^{a}	8.60 ± 0.13^{a}	7.66 ± 0.21^{a}	7.89 ± 0.18^{a}	Negative	Negative

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

(CSPE) treatment up to the end of the rearing period. The results of the mean comparison of Coliforms in cecum on day 42 showed a significant difference between all experimental treatments and the control treatment ($p < 0.05$). The highest mean was related to the control treatment and the lowest of Coliforms to 1000 ppm (CSPE) treatment up to the end of the rearing period.

Table 7 shows that the mean comparison of Entereocci in ileum on day 42 was not significantly different ($p > 0.05$). The lowest mean was related to the control treatment and the highest rate to 1000 ppm (CSPE) treatment up to the end of the rearing period. The results of the mean comparison of Entereocci in cecum on day 42 were not significantly different ($p > 0.05$). The lowest mean was related to the control treatment and the highest rate to 1250 ppm (CSPE) treatment up to the end of the rearing period.

Table 7 shows that the mean comparison of the total aerobic bacteriaum in ileum on day 42 was not significantly different ($p > 0.05$). The highest mean was related to the control treatment and the lowest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period. The results of the mean comparison of the total aerobic bacteriaum in cecum on day 42 were not significantly different ($p > 0.05$). The highest mean was related to the control treatment and the lowest mean was related to 1000 ppm (CSPE) treatment up to the end of the rearing period.

Salmonella search in the gastrointestinal tract on days 14 and 42

Tables 6 and 7, show *Salmonella* searching in all treatments in gastrointestinal tract on days 14 and 42. According to the results of this study, all treatments in this search were negative.

DISCUSSION

Lactobacilli count

The results of the mean comparison of Lactobacilli in ileum on day 14 showed a significant difference. The lowest mean was related to control treatment and the highest rate to 1250 ppm (CSPE) treatment up to day 21. The results of the mean comparison of Lactobacilli in cecum on day 14 showed significant difference. The lowest mean was related to thecontrol treatment and the highest rate to 1250 ppm (CSPE) treatment up to the end of the rearing period.

The results of the mean comparison of Lactobacilli in ileum on day 42 showed significant difference between the mean of treatments. The highest rate was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the lowest mean to the control treatment. The results of the mean comparison of Lactobacilli in cecum on day 42 showed significant difference. The highest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the lowest mean to the control treatment.

Hesselman and Aman (1986) reported that viscosity of gastrointestinal contents with the presence of insoluble nonstarch polysaccharides dissolved in water will be increased. This reduces the amount of nutrients passing through and changes gastrointestinal microbial balance by reducing the small intestine oxygen, and thus creates

a suitable environment for the microorganism fermentation. Thus, the polysacarid extract consumption causes greater viscosity and slower passing rate of nutrients in the small intestine (ileum) and large intestine (cecum and rectum). And, it means that gastrointestinal bacteriaum will have enough time to increase the microbial population. Fungi and herbs extract used in the diet greatly reduced the species of Bacteroid, Entrococcus and *Escherichia coli* and also increased the number of Bifidobacterum and Lactobacilli.

The extract improves endogenous enzyme secretion, increases appetite, aids digestion and absorption of nutrients, improves the balance of gastrointestinal microflora, reduces the population of *E. coli* and *Clostridium perfringens* and stimulates Lactobacillus. It also proliferates the gastrointestinal villi, protects them, and stimulates the immune system (Jamroz et al., 2005).

Essential oil due to its natural potential is used in the lower values in animal feed in small amounts and can positively influence gastrointestinal microflora. Also, it has antiseptic, antifungi and also antioxidant properties. Hence, being a good alternative to antibiotics. The use of essential oil in poultry diets improves the balance of gastrointestinal microflora, reduces harmful microbial population and stimulates the growth of useful bacteria (Gauthier, 2007).

Essential oil has antiseptic (Hammer et al., 1999), antifungi (Apisariyakul et al., 1995), antioxidant (Aeschbach et al, 1994), which stimulates feed intake (Petit et al., 1993) and digestive enzymes (Patel and Srinivasan, 1996). Dietary essential oil influences growth, thereby affecting gastrointestinal microflora. This positive effect on gastrointestinal microbial status influences animal performance (Lee et al., 2004). The derivatives of isoprene, flavonoeid, glcosinolate and other herbal metabolite may affect biochemical and physiological intestinal performance (Bakhiet and Adam, 1995).

Huyghebaert (2000) examined the effect of a mixture of essential oil obtained from herbs on the performance of digestive enzymes and intestinal anti-microbial activities in the developing broilers and did not observed considerable reduction in the number of *E. coli* bacteria comparison with the control group. The Lactobacilli population was not affected by the diets which is inconsistent with the results of this study.

Escherichia coli and coliforms counts

The results of the mean comparison of *E. coli* in ileum on day 14 showed significant difference. The lowest mean of *E. coli* was related to 1000 ppm (CSPE) treatment up to the end of the rearing period and the highest rate to the control treatment. The results of the mean comparison of *E. coli* in cecum on day 14 showed a significant difference. The lowest mean of *E. coli* was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and to 1250 ppm (CSPE) treatment up to day 21 and the highest rate to the control treatment.

The results of the mean comparison of Coliforms in ileum on day 14 showed no significant difference between the treatments. The results of the mean comparison of Coliforms in cecum on day 14 showed a significant difference between the treatments. The lowest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the highest rate to the control treatment.

The results of the mean comparison of *E. coli* in ileum on day 42 showed significant difference. The lowest mean of *E. coli* was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the highest rate to the control treatment. The results of the mean comparison of *E. coli* in cecum on day 42 showed a significant difference between all treatments and the control treament. The lowest mean was related to 1250 ppm (CSPE) treatment up to the end of the rearing period and the highest rate to the control treatment.

The results of the mean comparison of Coliforms in ileum on day 42 showed significant difference between all treatments and the control treatment. The highest mean was related to control treatment and lowest mean to 1250 ppm (CSPE) treatment up to the end of the rearing period. The results of the mean comparison of Coliforms in cecum on day 14 showed significant difference between other treatments and the control treatment. The highest mean was related to the control treatment and the lowest mean to 1000 ppm (CSPE) treatment up to the end of the rearing period.

C. sinensis peel oil is a complex mixture containing 400 combinations, and it can vary according to the variety, extraction and separation methods. Thus, it can show different antibacterial effects on particular bacteriaum (Nostro et al., 2000). Essential oil is the most important ingredients in *C. sinensis* peel that acts as a natural antimicrobial agent. Essential oil exerts bacteriacidal effects on the cell walls; it also increases the permeability of the cell membranes which damages the bacterial enzyme system and creates a potentially high antimicrobial activity (Alcicek et al., 2004). Although, dried in temperature less than 55°C, all this extra ct which was taken from the dried and fresh peel had antimicrobial properties against Staphylococcus, those which were prepared from fresh peel showed better inhibitory properties. Lemon extract had a controlling effect on *E. coli*; while Lemon dried peel extract had no such effect. Antimicrobial properties of essential oil obtained by distillation or extraction utilizing ethyl acetate of fresh citrus peel showed that the extract drived through ethyl acetate had stronger antimicrobial properties than liquid extract (Chanthaphon et al., 2008). The extract is effective on a wide range of gram positive and gram negative bacteria. However, gram negative bacteria are somehow more resistant due to lipopolysaccharide in their outer membranes (Singh et al., 2002).

In a study where 0.1% thyme was used in the diet of laying hens, essential oil significantly reduced the *E. coli* populations of the ileum and cecum (Canan et al., 2006). Cross et al. (2002) showed that thyme reduces the Coliforms number in the feces of chickens. The increased bacteria counts in lactic acid in the treatment of thyme and purple cone flower can be attributed to the antibacterial effect of these two plants. Herbal extract stimulates useful gastrointestinal flora growth and in turn reduces the presence of gram negative bacteria such as *E. coli* (Taymourizadeh et al., 2010).

Kivanc and Akguel (1986) showed that the resulting mixture of clove, thyme, mint and lemon oils reduces the production of coccidia oocytes and *C. perfringens* bacterium in broilers. Since most of the essential oil contains a mixture of hydrocarbons and oxygenated compounds such as alcohols, esters, aldehydes and ketones and a small percentage of non-volatile compounds such as paraffin and beeswax, there is evidence that suggests some of these compounds have antimicrobial and appetite- stimulating properties that may improve birds performance (Botsoglou et al., 2002).

Salmonella finding

In this study, all treatments were negative for *Salmonella*. Akhond et al. (2004) in a research on Shirazi thyme showed that log percent chance of *Salmonella typhimurium* growth was significantly influenced by different concentrations of essential oils.

Chebil et al. (2006) in an experiment showed that yarrow extract has germicidal effect on pathogenic microbes of Staphylococcus, *Salmonella tayphi*, *Shigella flexeneri* and *E. coli*.

The results of this study observed that *C. sinensis* peel extract reduced *E. coli* and coliforms counts and increased the number of Lactobacilli in all treatments which used the extract.

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