Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (9):4474-4479
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Does different levels of dried *citrus sinensis* peel affect on broilers gastrointestinal microbial population?

Zohreh Pourhossein, Ali Ahmad Alaw Qotbi, Alireza Seidavi

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

---

ABSTRACT

The experiment was conducted to evaluate the effects of different levels of dried citrus sinensis peel (DCSP) on gastrointestinal microbial population of Ross broilers. Four-handred 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 1.5 and 3 percent (DCSP) in the Base diet and in two periods of 1^{st}-21^{st} day and 1^{st}-42^{nd} days and base 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 Lactobacilli in ileum on day 42 was not significantly different ($p>0.05$). The highest rate was concerned to 3% (DCSP) treatment up to the end of rearing period and the lowest one was related to 1.5% (DCSP) treatment up to the end of rearing period. The results from the mean of lactobacilli in cecum in the day 42 indicated that the mean of treatment was not significantly different ($p>0.05$).The highest rate was concerned to 3% (DCSP) treatment up to the end of rearing period and the lowest one was related to 1.5% (DCSP) treatment up to day 21.

Keywords: Broiler, Dried *citrus sinensis* Peel, Microbial, Cecum, Ileum

---

INTRODUCTION

Poultry meat is supplier of the best food sources of protein needed for human. In recent years, herbal supplements as a natural additive that having a variety of active ingredients (such as insoluble nonstarch polysaccharides and essential oil) could possibly involve improving digestive and reducing the number of some bacteria in the colon and body immune system stimulants. And be considered as an effective potential alternative and without side effects [1]-[2]. Limited use of antibiotics in poultry and anti bacterial and anti toxic properties of some herbs and their extract are the main motivation to use of herbal supplements in poultry feed [3, 4, 5]. The herbs combinations can help to improve microflor balance by influencing on gut microbes [6].

Citrus extract due to having water-soluble vitamins, especially vitamin C has an important role in health and immune system. Feeding by-product and processed residues to feed livestock is common historically. In recent years, many factories have been built in order to extract citrus extract. After extraction of citrus extract, large remnants including external shell, the internal parts and seeds will remain. Dried citrus is a mixture of various citrus fruits rich in pectin, which is as a rich source of energy and calcium [7].

Anaerobic obligate bacteria including Eubacterium, Fusobacterium Propionibacterium, Clostridium and anaerobic facultative bacteria (Staphylococcus, Streptococcus, Lactobacilli and Bacteroid) has also been identified in the small intestine [8]. Approximately 70% of the sequences ileum is associated with Lactobacilli [9].

The aim of this project was to study the effects of dried *citrus sinensis* on gastrointestinal microbial population of broilers.

MATERIALS AND METHODS

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

The first stage of preparation was evacuated fertilizer related to previous period. After unloading manure, the farm buildings thoroughly cleaned and rinsed with water pressure completely. After drying, the floor was burned. After bringing the temperature to 32°C with 1% formalin solution, the farm buildings were disinfected. The farm buildings walls were sprayed as high as 1 meter with lime solution. After lime spraying, Hydro Care solution was used(1lit/100lit) as a spray. The farm building was washed with water pressure. After washing, 750 g Flomajon powder mixed in 500 liters and was sprayed with a strong push to the floor and walls. After half an hour the rinse was repeated. Fogging involves three stages. First, the empty the farm buildings sprayed with pure formalin and by turn on heaters, the material will evaporate on the floor. It was done three days before the main fogging. The main fogging was carried out after disinfection and using Azomit. Azomit is two separate cans which are mixed together. They are derived from a combination of gas which is sufficient for 1000 cubic meters. After flatten the roll and put drinkers and feeding and 24 hours before entering the broilers, the hall was gasified with Azomit.

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.
Basal and its nutrient in the starter and grower periods are shown in Tables 1 and 2. Basal diet based on NRC (1994) was formulated. The chemical composition of orange peel using AOAC (1990) has been measured separately and given in Table 3 [11, 12].

Table 1. Used diets 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 (DCP)	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 2. 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

Table 3. *Citrus sinensis* peel analysis

Protein	Moisture	Dry Matter	Calcium	Phosphorous	Ash	Carbohydrate	Ether extract	Fibre
5.46	12.00	88.00	1.10	0.05	7.00	63.54	2.00	10.00

Measurment of microbial population

In this study, colony forming unit (CFU) method was used.MRS agar (Man Rogosa Sharpe agar, 1.10660.500) to cultuer Lactobacilli was used. Slantez and bartley agar (450430) and Nutrient agar (1.05450.0500) were used to culture Enterococci and total aerobic bacteria counts, respectively.

Samples were transferred to the laboratory in the listed tubes and and again weighed 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 approximately half an hour. The action was performed for bacteria isolated from gastrointestinal contents and preparation of suspension. 1 ml was removed from the prepared suspension and was added into 9 ml buffer phosphate saline (pbs) in the other tube. So the concerned suspension was prepared from dilutions $10^{-1}$and serial dilution were done (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}). 100µ l was removed from (10^{-4}, 10^{-5} and 10^{-6}) dilutions and had been poured into the petri dish that had already been prepared and containing the medium and completely distributed to all parts of the medium. Under certain conditions, incubation was performed for growth of bacteria. Enterococci and Lactobacilli bacteria incubation at 37 $^{\circ}$ C in anaerobic conditions within 72 hours. Anaerobic jar was used to create anaerobic condition. Introbactriaccea and total aerobic bacteria counts incubated at 37 $^{\circ}$C in aerobic conditions and took 48 hours. Counting bacteria in petri dishes was done by colony counter. Calculate the number of bacteria was adjusted to 1 g sample.

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 4 shows the average number of gastrointestinal bacteria of experimental treatment in the day 14. According to the results of this study, the mean of gastrointestinal bacteria counts was significantly difference ($p<0.05$). The results from the comparison of Lactobacilli mean in ileum in the day 14 showed significantly difference ($p<0.05$). The lowest mean was related to control treatment and the highest rate was related to 1.5% (DCSP) treatment up to the end of the rearing period. The results from the comparison of Lactobacilli mean in cecum in the day 14 showed significantly difference ($p<0.05$). The lowest mean was related to control treatment and the highest rate was related to 3% (DCSP) treatment up to the end of the rearing period.

Table 4, shows the average number of Entercocci in ileum in the day 14 that showed no significantly difference ($p>0.05$). The lowest mean was related to 1.5% (DCSP) treatment up to the end of the rearing period and the highest rate was related to 3% (DCSP) treatment up to day 21. The results from the comparison of Entercocci mean in cecum in the day 14 showed no significantly difference ($p>0.05$). The lowest mean was related to 1.5% (DCSP) treatment up to day 21 and the highest rate was related to 3% (DCSP) treatment up to the end of the rearing period.

Table 4, shows the average number of total aerobic bacteria in ileum in the day 14 that showed no 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 1.5% (DCSP) treatment up to day 21. The results from the comparison of total aerobic bacteria mean in cecum in the day 14 showed no significantly difference ($p>0.05$). The lowest mean was related to 1.5% (DCSP) treatment up to the end of the rearing period and the highest rate was related to 3% (DCSP) treatment up to the end of the rearing period.

Table 4. Bacterial populations (log_{10} CFU/g) of cecum and ileum contents at 14th day

Treatment	Lactobacilli (Ileum)	Lactobacilli (Cecum)	Enterococci (Ileum)	Enterococci (Cecum)	Total aerobic bactria (Ileum)	Total aerobic bactria (Cecum)
CONTROL	7.49^{b}±0.08	7.88^{b}±0.08	7.80^{a}±0.22	8.03^{a}±0.19	7.86^{a}±0.16	8.12^{a}±0.19
[A]DCSP(1.5%), 1st- 21st day	7.83^{a}±0.08	7.98^{ab}±0.08	7.69^{a}±0.22	8.02^{a}±0.19	8.01^{a}±0.16	8.03^{a}±0.19
DCSP(1.5%), 1st- 42nd day	7.87^{a}±0.08	8.11^{ab}±0.08	7.64^{a}±0.22	8.19^{a} ±0.19	7.92^{a}±0.16	7.99^{a}±0.19
DCSP(3.0%), 1st- 21st day	7.78^{a}±0.08	8.13^{ab}±0.08	8.09^{a}±0.22	8.19^{a} ±0.19	7.71^{a}±0.16	8.02^{a}±0.19
DCSP(3.0%), 1st- 42nd day	7.80^{a}±0.08	8.18^{a}±0.08	8.00^{a}±0.22	8.20^{a}±0.19	7.98^{a}±0.16	8.14^{a}±0.19

[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 5, shows the average number of Lactobacilli in ileum in the day 42 that showed no significantly difference ($p>0.05$). The highest rate was related to 3% (DCSP) treatment up to the end of the rearing period and the lowest mean was related to 1.5% (DCSP) treatment up to the end of the rearing period. The results from the comparison of Lactobacilli mean in cecum in the day 42 showed no significantly difference ($p>0.05$). The highest rate was related to 3% (DCSP) treatment up to the end of the rearing period and the lowest mean was related to 1.5% (DCSP) treatment up to the day 21.

Table 5, shows the average number of Enterococci in ileum in the day 42 that showed no significantly difference ($p>0.05$). The lowest mean was related to 1.5% (DCSP) treatment up to day 21 and the highest rate was related to 3% (DCSP) treatment up to the end of the rearing period. The results from the comparison of Enterococci mean in cecum in the day 42 showed no significantly difference ($p>0.05$). The lowest mean was related to 1.5% (DCSP) treatment up to day 21 and the highest rate was related to 3% (DCSP) treatment up to the end of the rearing period.

Table 5, shows the average number of total aerobic bacteria in ileum in the day 42 that showed no 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 1.5% (DCSP) treatment up to the end of the rearing period. The results from the comparison of total aerobic bacteria mean in cecum in the day 42 showed no significantly difference ($p>0.05$). The lowest mean was related to 1.5 % (DCSP) treatment up to day 21 and the highest rate was related to control treatment.

Table 5. Bacterial populations (log_{10} CFU/g) of cecum and ileum contents at 42nd day

Treatment	Lactobacilli (Ileum)	Lactobacilli (Cecum)	Enterococci (Ileum)	Enterococci (Cecum)	Total aerobic bactria (Ileum)	Total aerobic bactria (Cecum)
CONTROL	$7.58^a \pm 0.20$	$8.05^a \pm 0.20$	$7.63^a \pm 0.27$	$7.86^a \pm 0.20$	$7.98^a \pm 0.20$	$8.23^a \pm 0.18$
DCSP(1.5%), 1st- 21st day	$7.84^a \pm 0.20$	$7.94^a \pm 0.20$	$7.51^a \pm 0.27$	$7.65^a \pm 0.20$	$7.90^a \pm 0.20$	$7.79^a \pm 0.18$
DCSP(1.5%), 1st- 42nd day	$7.55^a \pm 0.20$	$8.18^a \pm 0.20$	$7.55^a \pm 0.27$	$8.07^a \pm 0.20$	$8.01^a \pm 0.20$	$8.10^a \pm 0.18$
DCSP(3.0%), 1st- 21st day	$7.99^a \pm 0.20$	$8.06^a \pm 0.20$	$7.65^a \pm 0.27$	$8.04^a \pm 0.20$	$7.76^a \pm 0.20$	$8.13^a \pm 0.18$
DCSP(3.0%), 1st- 42nd day	$8.11^a \pm 0.20$	$8.31^a \pm 0.20$	$7.69^a \pm 0.27$	$8.06^a \pm 0.20$	$7.96^a \pm 0.20$	$8.17^a \pm 0.18$

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

DISCUSSION

Citrus sinensis peel is a major source of pectin that is non-digestible carbohydrates that stimulate the growth of probiotic bacteria in the colon. These bacteria are prevented from the growth of pathogenic. *Citrus sinensis* peel is one of the largest natural sources of vitamin C and pectin, which is an antioxidant compound. As a dietary supplement, *citrus sinensis* peel can enhance the immune system and decrease the risk of contamination of food with pathogenic bacteria [12].

According to the results from this study, the average number of gastrointestinal bacteria showed significantly difference ($p<0.05$). The results from the comparison of Lactobacilli mean in ileum and cecum in the day 14 showed significantly difference ($p<0.05$). The results from the comparison of Lactobacilli mean in ileum and cecum in the day 42 showed no significantly difference ($p>0.05$). The average number of Entercocci in ileum in the days 14 and 42 that showed no significantly difference ($p>0.05$). The average number of Entercocci in cecum in the days 14 and 42 that showed no significantly difference ($p>0.05$). The average number of total aerobic bacteria in ileum in the days 14 and 42 that showed no significantly difference ($p>0.05$). The average number of total aerobic bacteria in cecum in the days 14 and 42 that showed no significantly difference ($p>0.05$).

In research that comprises a mixture of herb extract including Capsaicin (the active ingredient in pepper), cinnamaldhyde (active ingredient of cinnamon) and carvacrol (active ingredient thyme) to 100 mg / kg in broiler diets based on corn and wheat were used, Lactobacilli number increased in the broiler fed with the mixture of herb extract. This action was done probably through the antioxidant activity [13].

Tschirch (2000) reported that use of carvacrol (active ingredient thyme) stimulates growth and proliferation of Lactobacilli [14]. Therefore in this study increased Lactobacilli counts could be due antibacterial effect of *citrus sinensis* peel extract. This can be attributed to flavonoids with antioxidant properties and essential oils in extracts.

Some of the bacteria in the colon, act more specifically to hydrolysis of large molecules of carbohydrates such as oligosaccharides and polysaccharides and it has converted the to smaller molecular weight carbohydrates. They are then fermented and resulting to increase in the number of bacteria. Fermentation end products such as short-chain fatty acid lower the intestinal pH and cause damage to the gastrointestinal harmful bacteria and stimulate beneficial bacteria [15, 16]. Thus the present study is consistent with all research that mentioned above.

Anti-nutritional effects of insoluble nonstarch polysaccharides *citrus sinensis* peel in poultry known for years that increased intestinal viscosity and increases the microflora and are effective in digestion and absorption of nutrients [17]. Increased gastrointestinal contents viscosity reduces the influence of substrate with digestive enzymes and prevents them from being effective responses. To cope with these changes, the gastrointestinal secretary mechanisms become more active and increase the growth of the digestive organs. The increased size of the digestive system is actually a response to the increasing need for enzymes [18, 19]. Fermentation of fibers and vitamins synthetic has been proven that has a positive impact on the microflora and stimulates the immune system. Since the fibers are not used by the host cells are used as food for feeding microflora [20]. If banding terpene is added to the diet against to bacteria, release compounds that decrease the pH and prevents growth a series bacteria. Since different plants have different combinations, so, the use of a plant matter in diets for poultry, including suitable and selected terpene is the best choice.

Most additives that claim to be alternative antibiotics are directly or indirectly have effects on the microflora [21]. The gastrointestinal microflora cans hydrolysis the conjugated bile salts which is limited fat digest. It is clear that control of micro flora can have a positive impact on bird performance and that nutrition supplement with antibacterial activity is suitable alternatives for antibiotics [22].

Generally, essential oil are inhibited and proliferation bacteria through four ways: effect on cell wall with removing phospholipids membrane and the obstruction ions passive passing; The effect on cell membranes through non-passive passing obstruction the active ions, and inhibition of ATP synthesis; Effect on cytoplasm by destruction of the bacteria cytoplasm structure through to cytoplasm proteins; and The effect on mitochondria inhibiting the synthesis of energy in the mitochondria [23].

Acknowledgments
This manuscript is obtained from MSc. thesis of Zohreh Pourhossein at Islamic Azad University, Rasht Branch, Rasht, Iran. We are grateful to the Islamic Azad University, Rasht Branch, Rasht, Iran and Mr Abbas Ebrahimi for support.

REFERENCES

[1] Ammar, N.M., S. Y. Al-okbi and D. A. Mohamed. **1997**. *Medical Journal of Islamic Academy of Sciences*, **10**: 127-130.
[2] Yeganeh Parast, M., M, Torabi. **2006**. Qom Center of Agriaulture and Natural Resources.
[3] Chen, H. L., H. Zhu, H. T. Chen and H. F. Zhu. **1995**. *Chinese Journal of Veterinary Medicine*, **21**: 21-24.
[4] Bourke, C.A. **1997**. *Plant protection Quarterly*, **12** (2), P. 91 –92.
[5] Bombik, T., E. Bombik, H. Bis –wencel and L.saba. **2002**. *Rocniki Naukowe zootechniki* **29** (1), 2002 P. 155 – 165.
[6] Hernandez, F., J. Madrid, V. Garcia, J. Orengo, and M.D. Megias. **2004**. *Poultry Science*, **83**: 169-174.
[7] Economides, S. **1974**. Technical paper No.7, Agriculture Research Institute, Nicosia, Cyprus.
[8] Salanitor, J.P., I. G. Blake, P.A. Muirhead, M. Maglio, and J. R. Goodman. **1978**. *Applied and Environmental Microbiology*. **35**: 782-790.
[9] Gong, J., R.J. Forster, h. YU, J.R. Chambers, R. Wheatcroft, P.M. Sabour, and S. Chen. **2002**. *FEMS Microbiology Ecology*. **41**:171-179.
[10] NRC. **1994**. Nutrition Requirements of Poultry, 9th revised ed. National Research Council, Washington. P.47-1.
[11] AOAC. **1990**. Official methods of analysis. 15 Th ed. Association of Official Analytical Chemists. Washington D.C .USA.
[12] Chanthaphon, S., S. Chanthachum, T. Hongpattarakere. **2008**. *Songklanakarin J. Sci. Technol*. **30** (Suppl.1), 125-131.
[13] Jamroz, D. **2005**. Food *Chemistry Toxicology*, **4**: 207-219.
[14] Tschirch, H. **2000**. Zeszyty Naukowe Akademii Rolniczej Wroclaw, Zootechnika, XXV(**376**): 25-39.
[15] Langhout, D. J. **1999**. Pages 203–212 in Proceedings of the 12th European Symposium on Poultry Nutrition. WPSADutch Branch. Spelderholt, The Netherlands.
[16] Sunvold, G.D., H.S. Hussein, G.C. Fahey, Jr., N.R. Merchen,and G.A. Reinhart. **1995**. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculumfrom cats, dogs, horses, humans, and pigs and fuminal fluid from cattle. J. Anim. Sci. 73:3639–3648.
[17] Choct, M., R.J. Hughes, J. Wang, M.R. Bedford, A.J. Morgan, & G. Annison. **1996**. *British Poultry Science*, **37**: 609–621.
[18] Edwards, C.A., I.T. Johnson, & N.W. Read. **1988**. *European Journal of Clinical Nutrition*,**42**: 302–306.
[19] Bedford, M.R. and H.L. CLASSEN. **1992**. *Journal of Nutrition*, **122**: 560–569.
[20] Klasing, K.C. **2005**. (Plymouth, Science Publishers).
[21] Taylor, D. J. **2001**. *British Poultry Science*, **42**(1): 67-68.

[22] Lee, K. W., Everts, H., Kappert, H. J., Wouterse, H., Frehner, M. and Beynen, A. C. **2004**. *International Journal of Poultry Science*, **3**(9): 608-612.
[23] Alcicek, A., Bozkurt, M. and Cabuk, M. **2004**. *South African Journal of Animal Science*, **34**(4): 217-222.