Evaluation of dietary fish oil plus green tea supplementation on the gizzard, ileum and cecum microflora in broiler chickens

Seidavi, A.[1] and Simões, J.[2]

[1]Department of Animal Science. Rasht Branch. Islamic Azad University. Rasht. Iran.
[2]Department of Veterinary Science. University of Trás-os-Montes e Alto Douro. Vila Real. Portugal.

Additional keywords

Cecum.
Fish oil.
Gizzard.
Green tea.
Gut microflora.
Ileum.

SUMMARY

In order to evaluate the effect of soybean oil substitution by fish oil (FO) plus green tea (*Camellia sinensis* L.; GT) powder at low levels on digestive tract microflora, 270 one-day old male chicks of Ross 308 strain were randomly assigned into 9 groups with 3 replicates and 10 birds each. All starter, grower, and finisher diets included a combination of 0, 1.5 or 2.0% of FO, substituting a part of soybean oil, with 0, 1.0 or 1.5% of GT powder. At day 42, the gizzard, ileal and cecal contents were independently collected, from one euthanized bird of each replicate, for microbial cultures and colony counts. The mean number of aerobic (7.396±0.931; least-squares mean log10 CFU/g±SEM), lactic acid-producing (8.165±0.900), coliforms (7.033±0.892) or intestinal negative lactose (6.559±0.823) bacteria of cecal contents from birds fed with 2% FO were similar to all other groups. No significant differences between groups were also observed from gizzard and ileal contents. These results suggest that the FO until 2.0% plus GT until 1.5% supplementation on diet don't affect negatively or even can preserve the gizzard, ileum and cecum microflora balance in apparent healthy broilers. Soybean oil may be substituted by FO until 2.0% level without apparent adverse effect on gut microflora.

Palabras clave adicionales

Aceite de pescado.
Microflora instestinal.
Ciego.
Íleon.
Molleja.
Té verde.

Evaluación de los efectos de la suplementación de aceite de pescado y té verde en la microflora de la molleja, íleon y ciego en pollos de engorde

RESUMEN

Con el fin de evaluar los efectos de la sustitución del aceite de soja por aceite de pescado (AP) y del té verde (*Camellia sinensis* L.; GT) en polvo, a bajos niveles, sobre la microflora del tracto digestivo, 270 pollitos machos de la cepa Ross 308 y un dia de edad fueron asignados aleatoriamente en 9 grupos con 3 repeticiones y 10 aves cada uno. Las dietas de los períodos de inicio, crecimiento, y acabado incluían la sustitución de una parte el aceite de soja por 0, 1,5 o 2,0% de AP y 0; 1,0 o 1,5% de polvo de GT. En el día 42, se recogieron los contenidos de la molleja, íleon y ciego de un ave sacrificada en cada réplica, para cultivos microbianos y recuento de colonias. El número medio (mínimos cuadrados medios log10 UFC/g±SEM) de bacterias aeróbicas (7,396 ± 0,931), bacterias productoras de ácido láctico (8,165±0,900), coliformes (7,033±0,892) y bactérias intestinales lactosa negativas (6,559±0,823), provenientes del contenido cecal de las aves alimentadas con 2% AP fueron similares a todos los demás grupos. Tampoco se observaron diferencias significativas entre los grupos para el contenido de la molleja y del íleon. Estos resultados sugieren que la suplementación en la dieta del AP hasta 2,0% más GT hasta el 1,5% no afectan negativamente o incluso puede preservar el equilibrio de la microflora de la molleja, el íleon y el ciego de pollos aparentemente sanos. El aceite de soja se puede sustituir parcialmente por AP hasta el nivel 2,0% sin efectos adversos aparentes sobre la microflora intestinal.

Información

Cronología del artículo.
Recibido/Received: 15.7.2014
Aceptado/Accepted: 5.10.2015
On-line: 10.12.2015
Correspondencia a los autores/Contact e-mail:
alirezaseidavi@iaurasht.ac.ir

Introduction

In past, feed additive substances, such sub-therapeutic antibiotics, were amply used in order to modulate the intestinal microflora and consequently improving the zootechnical performance and protect the health status of poultries (Dibaji *et al.*, 2014). Due to the consumer's pressure in whole world, including the prohibition of antibiotic usage as growth promoters in European Union from 2006 (regulation EC 1831/2003),

the research of alternative natural substances for diet incorporation was enhanced in last years (Gérard-Champod *et al.,* 2010; Alloui *et al.,* 2013; Ayasan, 2013; Khan, 2014) in order to improve performances, health and the meat quality from livestock and poultry industry.

In general, probiotic microorganisms, prebiotic substrates or symbiotic combinations of them are serious candidates to attain the above described objectives (Abdel-Raheem *et al.,* 2012). However, phytogenic feed additives (see the review of Wallace *et al.,* 2010), e.g. phytobiotics such as green tea (*Camellia sinensis* L.; GT), also received increased attention. A large amount of bioactive compounds, including major substances such as polyphenols (catechins) and alkaloids (caffeine, theophylline, threobromine), was identified in this herbal (Karori *et al.,* 2007). There are strong evidences that botanical maintains intestinal microflora balance (Hara *et al.,* 1995; Ishihara *et al.,* 2001; Lee *et al.,* 2006), exhibits antimicrobial effects against pathogenic bacteria (Hara-Kudo *et al.,* 2005; Erener *et al.,* 2011) or anticoccidial (*Eimeria* spp.) effects (Jang *et al.,* 2007) and reduces mortality (Cao *et al.,* 2005). The improvement of feed efficiency and body weight gain (Biswas and Wakita, 2001; Sarker *et al.,* 2010), maintenance of the oxidative stability of meat in broilers (Yang *et al.,* 2003) also were observed.

The fish oil (FO), derived from the tissues of oily fish, is a commonly and economic fishery sub-product, and can partially replace the soybean oil in broiler diets. The FO contains n-3 polyunsaturated fatty (n-3 PUFA), such omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid (Hulan *et al.,* 1988). These n-3 PUFA are precursors of Eicosanoids, which can mediate the inflammation process (Korver and Klasing, 1997; He *et al.,* 2007; Yang *et al.,* 2008; Cherian, 2011; Liu *et al.,* 2014). Imbalanced eicosanoid production can affect poultry health, promoting cardiac problems and sudden death (Squires and Summers, 1993; Ajuyah *et al.,* 2003; Bautista-Ortega *et al.,* 2009; Saki and Hemati Matin, 2011). These n-3 PUFA also can be improved in poultry meat by the inclusion of FO in their diets (Hulan *et al.,* 1989) whit obvious advantages for human feed. Other than potential animal and human health benefits, the inclusion of FO in poultry diets can also improve meat quality of broilers, reducing their abdominal fat or modifying the characteristics of processed products like the observed by observed by Yang *et al.* (2010).

The FO can replace the soybean oil in diets only at low levels in order to minimize its latent negative impact on the sensory attributes of meat broiler (López-Ferrer *et al.,* 2001; Bou *et al.,* 2004) or on the broiler immune function (Al-Khalifa *et al.,* 2012). However, the sensory alterations or detrimental immune function were not evident at 2-4% (Jeun-Horng *et al.,* 2002) or 3% (Al-Khalifa *et al.,* 2012) FO supplementation in broiler diets, respectively. Alparslan and Özdogan (2006) suggested to use 2% of FO in order to improve the feed cost efficiency and preserve the performance and health of broilers.

Although dietary supplementation of GT plus FO can present significant advantages in broilers production, little research exists evaluating microbial populations of intestinal segments of commercial broilers fed diets differing in FO and/or GT powder supplementation.

The main objective of the present study was to determine the effect of different dietary levels of FO plus GT powder to broiler chicks on bacteria communities of cecum, ileum and gizzard. The final purpose was to evaluate the viability of partial soybean meal substitution by FO plus GT powder at low levels supplementation in diet regarding the potential microflora unbalance of digestive tract in apparent healthy broilers.

MATERIAL AND METHODS

Broiler farm management

The experiment was conducted for 42 days in 2013 at Islamic Azad University facilities (Rasht Branch), in Abkenar, one of the cities in Guilan province, Iran. Using iron scaffoldings, cages with dimensions 1.5×1 meters and a height of 1 meter were installed and a cage was assigned to each of the replicates (total 27 cages).

The house and cages have been thoroughly cleaned and disinfected.

The temperature of the building was maintained by the use of gasoline rocket heaters. Temperature was controlled by three thermostats that were installed in different parts of the 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 building 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 condition the rooms, some fans with a diameter of 60 cm which had proper discharge power and were installed on the south side and some fans with impeller diameter of 140 cm at the end of the building which was installed for tunnel ventilation.

During the first two weeks of rearing, one plastic feeding tray per cage was used. Starting the third week, all the feeding trays were collected and replaced by appropriate feeders. For sanitation, all drinkers were washed twice daily with fresh clean water.

Vaccination program was conducted based on the advice of the farm veterinarian. The vaccines were given in drinking water and water was withheld from all birds 2 hours before giving the vaccine to ensure that chickens were thirsty. To reduce the stress caused by vaccination, a multi-electrolyte solution was added to drinking water (in a ratio of 1:1000) 24 hours before and after vaccination.

All procedures described in the present study have been approved by Islamic Azad University Ethics Committee (Protocol number 17-16-4-16987), and care was taken to minimize the number of animals used.

Animals, treatments and diets

Two hundred and seventy one-day-old male chicks of Ross 308 strain were purchased and brought to the experimental building. The average weight of the broilers was 44.5±0.6 g (±SEM).

The experiment included nine treatments, three replicates per treatment with ten animals replicate. One-day old chickens were randomly assigned to each of the selected nine treatment groups. All starter, grower, and finisher diets included a combination of 0, 1.0 or 1.5% of GT powder with 0, 1.5 or 2.0% of FO. Broilers fed with diets contain GT powder 0% and FO 0% were include in the group control.

The composition of nutrients in the feed during the starter, grower and finisher periods is shown in **table I**. Diets were formulated according the Ross 308 strain breeding manual (Aviagen, 2007) and the UFFDA software program was used.

Microbial sampling and cultivation

At the end of the trial, a chicken from each replicate was slaughtered and cecum, ileum, and gizzard were removed. Agar plates were streaked and samples sent to the laboratory along with intact intestinal segments for further culture. To determinate bacterial growth and colony counts, the agar plates streaked on the site were used.

Collecting tubes were weighted, wrapped into aluminium sheet and autoclaved. The culture mediums were prepared and 24 hours before collecting samples were poured into the petri dish. MRS agar (Man Rogosa Sharpe agar, 1.10660.500) to culture lactic acid-producing bacteria, MacConkey agar (105465.0500) to culture coliforms and intestinal negative lactose bacteria was used. Also Nutrient agar (1.05450.0500) was used to culture total aerobic bacteria counts, respectively (Dibaji *et al.*, 2014; Jahanpour *et al.*, 2014).

Like to the reported by Abbasi *et al.* (2015), samples were transferred to the laboratory in the listed tubes and again weighed. The amount of sample in each tube was calculated from the difference between these two values. Tubes were shaken for approximately 30 minutes. 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. So the concerned suspension was prepared from dilutions 10-1and serial dilution were done (10-2, 10-3, 10-4, 10-5 and 10-6). 100 µl was removed from (10-4, 10-5 and 10-6) dilutions and had been poured into the petri dish that had already been prepared containing the medium and were distributed to all parts of the medium. Incubation was performed for growth of bacteria. Anaerobic jar was used to create anaerobic condition. Total aerobic bacteria counts incubated at 37°C in aerobic conditions and took 48 hours. Counting bacteria in petri dishes was done by colony counter. Bacterial counts were reported as log 10 bacteria number per 1 gram sample.

Table I. Composition of basal diets during starter, grower and finisher periods (Composición de las dietas basales, durante los periodos de iniciación, crecimiento y terminación).

Ingredient (g/kg)	Periods[1]		
	Starter	Grower	Finisher
Corn	583.1	628.1	656.2
Soybean meal (CP: 44%)	377.7	333.6	302.7
Soybean oil	12.6[2]	11.7[3]	14.5[4]
DL-methionine	1.8	1.8	1.8
Lysine-hydro-chloride	0.8	0.8	0.8
Mineral mixture[5]	3	3	3
Vitamin mixture[6]	3	3	3
$CaCO_3$	10	10	10
Phytase enzyme	0.5	0.5	0.5
Multi-enzyme	0.5	0.5	0.5
Ca%22 P%18	7	0.5	7
Total	1000	1000	1000
Calculated nutrient analysis			
Energy (kcal/kg)	2969	3005	3050
Protein (%)	22.13	20.54	19.39
Crude fiber (%)	3.340	3.063	2.866
Calcium (%)	0.654	0.640	0.630
Tryptophan SID (%)	0.420	0.403	0.390
Available Phosphorus (%)	0.246	0.244	0.242
Linoleic Acid (%)	1.768	1.790	1.954
Lysine SID (%)	1.331	1.213	1.129
Methionine SID (%)	0.567	0.543	0.525
Cysteine (%)	0.681	0.635	0.602
Sodium (%)	0.045	0.045	0.045

[1]On each period, 0.0%, 1.0% or 2.0% of green tea were added on remaining groups. [2]Only 3.0 g/kg and 0.0 g/kg of soybean oil were added for 1.5% and 2.0% fish oil diet of starter period, respectively. 0.0%, 1.0% or 2.0% of green tea was added in remainin groups. [3]Only 2.0 g/kg and 0.0 g/kg of soybean oil were added for 1.5% and 2.0% fish oil diet of grower period, respectively. [4]Only 5.0 g/Kg and 2.8 g/kg of soybean oil were added for 1.5% and 2.0% fish oil diet of finisher period, respectively. [5]Cu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g. [6]Vitamin A: 5000 IU/g; Vitamin D3: 500 IU/g; Vitamin E: 3 mg/g; Vitamin K3: 1.5 mg/g; Vitamin B6: 13 mg/g; Vitamin B2: 1 mg/g; Calciumpantothenate: 4 mg/g; Niacin: 15 mg/g SID (standardized ileal digestible).

Statistical analysis

Data were analyzed by analysis of variance using a 3×3 factorial design with three FO treatments (0, 1.5, and 2.0% in diet) and three GT powder treatments (0, 1.0, and 1.5% in diet), using a two-way ANOVA procedure. Data were analysed by SPSS (1997) statistical software and GLM procedure was used. The means were compared by using least significant difference (LSD) for significance at 0.05 level.

RESULTS AND DISCUSSION

No significant differences ($p>0.05$) of the bacterial counts (total aerobic, lactic acid-producing, coliforms and intestinal negative lactose bacteria) between different FO or GT levels, or between all nine groups

Table II. Microflora mean (±SEM) of gizzard at 42nd day of age in Ross 308 broilers fed diets containing the different levels of fish oil and green tea (least-squares mean log10 CFU/g; p>0.05) (Microflora (media±SEM) de la molleja a 42 días de edad en broilers Ross 308 alimentados con dietas conteniendo diferentes niveles de aceite de pescado y te verde (media cuadrática, log10 CFU/g; p>0.05)).

Treatment		Trait			
		Aerobic bacteria total	Lactic acid-producing bacteria	Coliform bacteria	Intestinal negative lactose bacteria
Fish oil (% in diet)	0	5.210	5.730	5.583	4.228
	1.5	7.554 (±0.989)	7.342 (±0.839)	7.171 (±0.797)	6.323 (±0.924)
	2.0	6.811	6.606	6.114	4.773
Green tea powder (% in diet)	0	6.770	6.471	6.219	5.423
	1.0	5.873 (±0.989)	6.436 (±0.839)	6.297 (±0.797)	4.810 (±0.924)
	1.5	6.932	6.771	6.352	5.090

Table III. Microflora mean (±SEM) of ileum at 42nd day of age in Ross 308 broilers fed diets containing the different levels of fish oil and green tea (least-squares mean log10 CFU/g; p>0.05) (Medias de la microflora de íleon (±SEM) al día 42 de edad en pollos de engorde Ross 308 alimentados con dietas que contienen los diferentes niveles de aceite de pescado y té verde (media cuadrática, log10 CFU/g; p>0.05)).

Treatment		Trait			
		Aerobic bacteria total	Lactic acid-producing bacteria	Coliform bacteria	Intestinal negative lactose bacteria
Fish oil (% in diet)	0	6.370	6.205	6.082	5.550
	1.5	8.311 (±0.928)	8.181 (±0.903)	7.885 (±0.901)	7.049 (±0.814)
	2.0	7.424	7.332	7.163	6.290
Green tea powder (% in diet)	0	7.414	7.257	7.117	6.494
	1.0	7.348 (±0.928)	7.214 (±0.903)	6.959 (±0.901)	6.212 (±0.814)
	1.5	7.343	7.247	7.053	6.183

Table IV. Microflora mean (±SEM) of cecum at 42nd day of age in Ross 308 broilers fed diets containing the different levels of fish oil and green tea (least-squares mean log10 CFU/g; p>0.05). Medias de la microflora de ciego (±SEM) al día 42 de edad en pollos de engorde Ross 308 alimentados con dietas que contienen los diferentes niveles de aceite de pescado y té verde (media cuadrática, log10 CFU/g; p>0.05)).

Treatment		Trait			
		Aerobic bacteria total	Lactic acid-producing bacteria	Coliform bacteria	Intestinal negative lactose bacteria
Fish oil (% in diet)	0	6.507	6.364	6.236	4.803
	1.5	8.326 (±0.931)	7.288 (±0.900)	7.908 (±0.892)	7.043 (±0.823)
	2.0	7.396	8.165	7.033	6.559
Green tea powder (% in diet)	0	7.493	7.336	7.226	5.801
	1.0	6.487 (±0.931)	7.263 (±0.900)	6.114 (±0.892)	5.661 (±0.823)
	1.5	8.248	7.218	7.838	6.944

were observed on gizzard, ileum or cecum microflora. Obtained results are reported in **tables II-IV**.

Our results suggested that the feed supplementation with FO and GT powder, combined at different low levels, don't affect or at least can preserve the intestinal microflora balance until 42nd old day's unapparent healthy broilers. In fact, the maintenance and/or increase of non-pathogenic intestinal bacteria can contribute to inhibit the pathogens development, incentives optimal growth and performance and reduce mortality such the reported by Guo *et al.* (2004) and Torok *et al.* (2011). Recently, Saraee *et al.* (2015) observed positive effects on broiler performances using similar levels of GT (up to 1.5%) and FO (up to 2.0%) on diet suggesting their use in broiler production. A maintenance of carcass quality of broilers fed with similar diets was also reported (Saraee *et al.*, 2014).

A decrease of the number of total bacteria and bacteroidaceae in cecum was observed by Terada *et al.* (1993) 24 days after fed broilers with GT polyphenols supplementation (2 g/kg). On 56th day after supplementation, these researchers also observed a lactobacilli bacteria counts increase and enterobacteriaceae counts decrease, including *Proteus* genus, suggesting that the intestinal flora may modulate a decrease in putrefactive bacteria due to a cecal lower pH ambiance.

An overall and non-selective decrease on counts of cecal microflora (bifidobacteria, bacteroidaceae, peptococcaceae, lactobacilli, eubacteria and lecithinase-positive bacteria such as clostridia, streptococci, staphylococci and bacilli) was observed by Cao *et al.* (2005) in broilers, 28 to 42 days old, after tea polyphenols supplementation.

More recently, Thomas *et al.* (2010) observed an increase of beneficial bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.) and decrease of pathogenic bacteria (*Clostridium* spp. and *Bacteroide*s spp.) in cecum after 35 days of Chinese GT supplementation in broilers. A decrease of faecal bacterial ß-glucuronidase activity, mainly produced by mainly by *Escherichia coli*, *Bacteroides* spp. and *Clostridium* spp., and the faecal pH were also observed by these researchers. Contrarily,

the ß-glucosidaseenzime activity mainly produced by lactobacilli and bifidobacteria was increased. These effects on cecal microflora were more marked in Chinese GT with selenium high content. Similar results were observed by Molan *et al.* (2010) in the cecum of rats. Moreover, higher antioxidant and prebiotic in vitro activities had already been observed by Molan *et al.* (2009) in selenium-containing GT than normal GT.

A limitation of the present study is the fact that the major active GT compounds were not identified and quantified. A natural variability of these active compounds can occurs when dried herbs from different origin were used, and the quantity of anti-oxidant compounds may not have been sufficient in order to modulate the gut microflora. However, the GT herbs are widely disseminated and our study simulated this condition.

The results of our study also suggested that the partial substitution of soybean oil, by FO, until 2.0% level, don't had negative effects on the bacterial population studied of the broiler digestive tract. Geier *et al.* (2009) using diets containing FO also observed that overall gut microbial communities were not altered in broilers. However, there are several evidences in vivo and in vitro of microbiota modulation by FO or PUFAs (Kankaanpää *et al.,* 2001; Hekmatdoost *et al.,* 2008). Consequently, different sources of PUFAs, dietary composition (Geier *et al.,* 2009) and biological variations of the animal species or even breeds should be considered for gut microbiota modulation.

These aspects are very important, because FO has potential to be used in order to modulate the inflammatory and immune responses under ambient stress conditions or diseases.

Recently, Liu *et al.* (2014) demonstrated that the FO decreased the levels of serum total cholesterol, triglyceride, high and low density lipoprotein-cholesterol, low density lipoprotein cholesterol and reduced proinflammatory eicosanoid contents by inhibiting phospholipase A2 production in broilers (at 42 days old) when compared with corn oil. There are also some evidences that the fish oil can module the broiler immunity.

As preliminary results, MacAlintal *et al.* (2013) observed an immunoglobulin M titer improvement, following 1 mL of 7% sheep red blood cells intravenous administration, when microalgae strain Schizochytrium containing 70%, with a fatty acid profile similar to FO, was used in broiler diet. Maroufyan *et al.* (2012) also observed an enhancement of proinflammatory cytokines using a balanced FO (at 2.5% level) and methionine supplementation feed in Infectious Bursal Disease Challenged broilers. However, is necessary to emphasize that the detrimental effects on immune function of broilers can occur when high levels of FO (50-60 g/kg) are supplemented until slaughter, like the observed by Al-Khalifa *et al.* (2012).

Although the sample population used in the present study was small, our results suggest that the feed supplementation with FO until 2.0% and GT powders until 1.5% levels, or their combinations, don't affect negatively, or even can preserved the cecum, ileum and gizzard microflora in apparent healthy broilers.

The soybean oil can be partially replaced by FO without adverse effect in gut microflora.

Further researches are needs in order to determine the effect of these feed additives combinations on digestive tract microflora under environmental stress and disease conditions in commercial broilers.

ACKNOWLEDGEMENTS

This manuscript is obtained from research project at Islamic Azad University, Rasht Branch, Rasht, Iran. We are grateful to the Islamic Azad University, Rasht Branch, Rasht, Iran for support. We also thank to Dr Leila Asadpour and Dr Mohammad Dadashbeiki (Department of Veterinary Science, Rasht Branch, Islamic Azad University, Rasht, Iran) for their technical assistances.

BIBLIOGRAPHY

Abbasi, H.; Seidavi, A.; Liu, W. and Asadpour, L. 2015. Investigation on the effect of different levels of dried sweet orange *(Citrus sinensis)* pulp on performance, carcass characteristics and physiological and biochemical parameters in broiler chicken. *Saudi J Biol Sci,* 22: 139-146.

Aviagen. 2007. Ross 308 broiler: Nutrition Specification. http://www.natchix.co.za/pdf/nutrition_specifications.pdf (2/12/2014).

Abdel-Raheem, S.M.; Abd-Allah, S.M.S. and Hassanein K.M.A. 2012. The effects of prebiotic, probiotic and synbiotic supplementation on intestinal microbial ecology and histomorphology of broiler chickens. *Int J Agro-Vet Med Sci*, 6: 277-289.

Ajuyah, A.O.; Cherian G.; Wang Y.; Sunwood, H. and Sim, J.S. 2003. Maternal dietary FA modulate the long-chain n-3 PUFA status of chick cardiac tissue. *Lipids,* 38: 1257-1261.

Al-Khalifa, H.; Givens, D.; Rymer, C. and Yaqoob, P. 2012. Effect of n-3 fatty acids on immune function in broiler chickens. *Poult Sci,* 91: 74-88.

Alloui, M.N.; Szczurek, W. and Świątkiewicz, S. 2013. The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. *Ann Anim Sci,* 13: 17-32.

Alparslan, G. and Özdogan, M. 2006. The effects of diet containing fish oil on some blood parameters and the performance values of broilers and cost efficiency. *Int J Poult Sci,* 5: 415-419.

Ayasan, T. 2013. Effects of dietary inclusion of protexin (probiotic) on hatchability of Japanese quails. *Indian J Anim Sci,* 83: 78-81.

Bautista-Ortega, J.; Goeger, D.E. and Cherian, G. 2009. Egg yolk omega-6 and omega-3 fatty acids modify tissue lipid components, antioxidant status, and *ex vivo* eicosanoid production in chick cardiac tissue. *Poult Sci,* 88: 1167-1175.

Biswas, A.H. and Wakita, M. 2001. Effect of dietary Japanese green tea powder supplementation on feed utilization and carcass profiles in broilers. *J Poult Sci,* 38: 50-57.

Bou, R.; Guardiola, F.; Tres, A.; Barroeta, A.C. and Codony, R. 2004. Effect of dietary fish oil, alpha-tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. *Poult Sci,* 83: 282-292.

Cao, B.H.; Karasawa, Y. and Guo, Y.M. 2005. Effects of green tea polyphenols and fructo-oligosaccharides in semi-purified diets on broilers' performance and caecal microflora and their metabolites. *Asian Australas J Anim Sci,* 18: 85-89.

Cherian, G. 2011. Essential fatty acids and early life programming in meat type birds. *Worlds Poult Sci J,* 67: 599-614.

Dibaji, S.M.; Seidavi, A.; Asadpour, L. and Moreira da Silva, F. 2014. Effect of a synbiotic on the intestinal microflora of chickens. *J Appl Poult Res,* 23: 1-6.

Erener, G.; Ocak, N.; Altop, A.; Cankaya, S.; Aksoy, H.M. and Ozturk, E. 2011. Growth performance, meat quality and caecal coliform bacteria count of broiler chicks fed diet with green tea extract. *Asian Australas J Anim Sci,* 24: 1128-1135.

Geier, M.S.; Torok, V.A.; Allison, G.E.; Ophel-Keller, K.; Gibson, R.A.; Munday, C. and Hughes, R.J. 2009. Dietary omega-3 polyunsaturated fatty acid does not influence the intestinal microbial communities of broiler chickens. *Poult Sci,* 88: 2399-2405.

Gérard-Champod, M.; Blanquet-Diot, S.; Cardot, J.M.; Bravo, D. and Alric, M. 2010. Development and validation of a continuous *in vitro* system reproducing some biotic and abiotic factors of the veal calf intestine. *Appl Environ Microbiol, 76*: 5592-5600.

Guo, F.C.; Williams, B.A.; Kwakkel, R.P.; Li, H.S.; Li, X.P.; Luo, J.Y.; Li, W.K. and Verstegen, M.W.A. 2004. Effects of mushroom and herb polysaccharides as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poult Sci,* 83: 175-182.

Hara, H.; Orita, N.; Hatano, S.; Ichikawa, H.; Hara, Y.; Matsumoto, N.; Kimura, Y.; Terada, A. and Mitsuoka, T. 1995. Effect of tea polyphenols on fecal flora and fecal metabolic products of pigs. *J Vet Med Sci, 57*: 45-49.

He, X.; Yang, X. and Guo, Y. 2007. Effects of different dietary oil sources on immune function in cyclophosphamide immunosuppressed chickens. *Anim Feed Sci Tech,* 139: 186-200.

Hekmatdoost, A.; Feizabadi, M.M.; Djazayery, A.; Mirshafiey, A.; Eshraghian, M.R.; Yeganeh, S.M.; Sedaghat, R. and Jacobson, K. 2008. The effect of dietary oils on cecal microflora in experimental colitis in mice. *Indian J Gastroenterol,* 27:186-189.

Hulan, H.W.; Ackman, R.G.; Ratnayake, W.M. and Proudfoot, F.G. 1989. Omega-3 fatty acid levels and general performance of commercial broilers fed practical levels of redfish meal. *Poult Sci,* 68: 153-162.

Hulan, H.W.; Ackman, R.G.; Ratnayake, W.M.N. and Proudfoot, F.G. 1988. Omega-3 fatty acid levels and performance of broiler chickens fed redfish meal or redfish oil. *Can J Anim Sci,* 68: 533-547.

Ishihara, N.; Chu, D.C.; Akachi, S. and Juneja, L.R., 2001. Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. *Livest Prod Sci,* 68: 217-229.

Jahanpour, H.; Seidavi, A.R.; Qotbi, A.A.A.; Delgado, F. and Gamboa, S. 2014. Effect of intensity and duration of quantitative feed restriction on broiler caecum microbiota. *Indian J Anim Sci,* 84: 554-558.

Jang, S.I.; Jun, M.H.; Lillehoj, H.S.; Dalloul, R.A.; Kong, I.K.; Kim, S. and Min, W. 2007. Anticoccidial effect of green tea-based diets against *Eimeria maxima. Vet Parasitol,* 144: 172-175.

Jeun-Horng, L.; Yuan-Hui, L. and Chun-Chin, K. 2002. Effect of dietary fish oil on fatty acid composition, lipid oxidation and sensory property of chicken frankfurters during storage. *Meat Sci,* 60: 161-167.

Karori, S.M.; Wachira, F.N.; Wanyoko, J.K. and Ngure, R.M. 2007. Antioxidant capacity of different types of tea products. *Afr J Biotechnol,* 6: 2287-2296.

Khan, S.H. 2014. The use of green tea *(Camellia sinensis)* as a phytogenic substance in poultry diets. *Onderstepoort J Vet,* 81: 1-8.

Kankaanpaa P.E.; Salminen S.J.; Isolauri E. and Lee, Y.K. 2001. The influence of polyunsaturated fatty acids on probiotic growth and adhesion. *FEMS Microbiol Lett,* 194: 149-153.

Korver, D.R. and Klasing, K.C. 1997. Dietary fish oil alters specific and inflammatory immune responses in chicks. *J Nutr,* 127: 2039-2046.

Lee, H.C.; Jenner, A.M.; Low, C.S. and Lee, Y.K. 2006. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Res Microbiol,* 157: 876-884.

Liu, G.; An, S.; Yuan, J.; Guo Y.; Liu, D.; Chen, H. and Huang, R. 2014. Dietary fish oil and zinc reduced plasma prostaglandin E2 content by inhibiting phospholipase A2 production in broilers. *J Poult Sci,* 51: 66-70.

López-Ferrer, S.; Baucells, M.D.; Barroeta, A.C. and Grashorn, M.A. 2001. n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. *Poult Sci,* 80: 741-752.

Macalintal, L.M.; Ao, T.; Pescatore, A.J.; Cantor, A.H.; Timmons, B.; Conn, C.; Ford, M.J. and Dawson, K.A. 2014. Effects of dietary supplementation of microalgae on growth performance, immunity and fatty acid profile of broiler chicks. *Poult Sci,* 92: 121-122.

Maroufyan, E.; Kasim, A.; Ebrahimi, M.; Loh, T.C.; Hair-Bejo, M. and Soleimani, A.F. 2012. Dietary methionine and n-6/n-3 polyunsaturated fatty acid ratio reduce adverse effects of infectious bursal disease in broilers. *Poult Sci,* 91: 2173-2182.

Molan, A.L.; Flanagan, J.; Wei, W. and Moughan, P.J. 2009. Selenium containing green tea has higher antioxidant and prebiotic activities than regular green tea. *Food Chem,* 114: 829-835.

Molan, A.L.; Liu, Z. and Tiwari, R. 2010. The ability of green tea to positively modulate key markers of gastrointestinal function in rats. *Phytother Res,* 24: 1614-1619.

Saki, A.A. and Hemati Matin, H.R. 2011. Does nutrition help to alleviate sudden death syndrome in broiler chicken? An Overview. *Global Vet,* 6: 262-268.

Saraee, M.H.A.; Seidavi, A.; Dadashbeiki, M.; Laudadio, V. and Tufarelli, V. 2014. Effect of dietary supplementation with different levels of green tea powder and fish oil or their combination on carcass characteristics in broiler chickens. *Pak J Zool,* 46: 1767-1773.

Saraee, M.H.A.; Seidavi, A.; Dadashbeiki, M.; Laudadio, V. and Tufarelli, V. 2015. Supplementing fish oil and green tea *(Camellia sinensis)* powder in broiler diet: Effects on productive performance. *Res Opin Anim Vet Sci, 5*: 99-104.

Sarker, M.; Kim, G. and Yang, C. 2010. Effect of green tea and biotite on performance, meat quality and organ development in Ross broiler. *Egypt Poult Sci,* 30: 77-88.

SPSS. 1997. SPSS Base 7.5 for Windows users guide/Publication. SPSS Inc. Chicago, IL.

Squires, E.J. and Summers, J.D. 1993. A consideration of comparative metabolic aspects of the aetiology of sudden death syndrome and ascites in broilers. *Brit Vet J,* 149: 285-294.

Terada, A.; Hara, H.; Nakajyo, S.; Ichikawa, H.; Hara, Y.; Fukai, K.; Kobayashi, Y. and Mitsuoka, T. 1993. Effect of supplements of tea polyphenols on the caecal flora and caecal metabolites of chicks. *Microb Ecol Health D,* 6: 3-9.

Thomas, D.V.; Molan, A.L. and Ravindran, V. 2010. The ability of green tea to positively modify the gut microflora in broiler chickens. 21st Annual Australian Poultry Science Symposium Sydney. New South Wales. February 1-3. pp. 203-206.

Torok, V.A.; Allison, G.E.; Percy, N.J.; Ophel-Keller, K. and Hughes, R.J. 2011. Influence of antimicrobial feed additives on broiler commensal posthatch gut microbiota development and performance. *Appl Environ Microb, 77*: 3380-3390.

Wallace, R.; Oleszek, W.; Franz, C.; Hahn, I.; Baser, K.H., Mathe, A. and Teichmann, K. 2010. Dietary plant bioactives for poultry health and productivity. *Brit Poultry Sci,* 51: 461-487.

Yang, C.J.; Yang, I.Y.; Oh, D.H.; Bae, I.H.; Cho, S.G.; Kong, I.G.; Uuganbayar, D.; Nou, I.S. and Choi, K.S. 2003. Effect of green tea by-product on performance and body composition in broiler chicks. *Asian Australas J Anim Sci,* 16: 867-872.

Yang, X.; Guo, Y.; He, X.; Yuan, J.; Yang, Y. and Wang, Z. 2008. Growth performance and immune responses in chickens after challenge with lipopolysaccharide and modulation by dietary different oils. *Animal,* 2: 216-223.

Yang, X.; Zhang, B.; Guo, Y.; Jiao, P. and Long, F. 2010. Effects of dietary lipids and *Clostridium butyricum* on fat deposition and meat quality of broiler chickens. *Poult Sci,* 89: 254-260.