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

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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 intestinal.
Ciego.
Ileon.
Molleja.
Té verde.

INFORMACIÓN

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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, phytophagenic feed additives (see the review of Wallace et al., 2010), e.g. phytophobiotics such as green tea (Camellia sinensis L.; GT), also 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; Ereneter et al., 2011) or anticoxidial (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 eicosapentaeanoic 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) with 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% (Jean-Horgg 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 included 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 determine bacterial growth and colony counts, the agar plates streaked on the site were used.

Collecting tubes were wrapped, 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 create anaerobic condition. 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.

**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), 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.
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 Tork 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.

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)).

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

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)).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aerobic bacteria total</th>
<th>Lactic acid-producing bacteria</th>
<th>Coliform bacteria</th>
<th>Intestinal negative lactose bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish oil (%) in diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.370</td>
<td>6.205</td>
<td>6.082</td>
<td>5.550</td>
</tr>
<tr>
<td>1.5</td>
<td>8.311 (±0.928)</td>
<td>8.181 (±0.903)</td>
<td>7.885 (±0.901)</td>
<td>7.049 (±0.814)</td>
</tr>
<tr>
<td>2.0</td>
<td>7.424</td>
<td>7.332</td>
<td>7.163</td>
<td>6.290</td>
</tr>
<tr>
<td>Green tea powder (%) in diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.414</td>
<td>7.257</td>
<td>7.171</td>
<td>6.494</td>
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<tr>
<td>1.0</td>
<td>7.348 (±0.928)</td>
<td>7.214 (±0.903)</td>
<td>6.959 (±0.901)</td>
<td>6.212 (±0.814)</td>
</tr>
<tr>
<td>1.5</td>
<td>7.343</td>
<td>7.247</td>
<td>7.053</td>
<td>6.183</td>
</tr>
</tbody>
</table>

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)).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aerobic bacteria total</th>
<th>Lactic acid-producing bacteria</th>
<th>Coliform bacteria</th>
<th>Intestinal negative lactose bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish oil (%) in diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.507</td>
<td>6.364</td>
<td>6.236</td>
<td>4.803</td>
</tr>
<tr>
<td>1.5</td>
<td>8.326 (±0.931)</td>
<td>7.288 (±0.900)</td>
<td>7.908 (±0.892)</td>
<td>7.043 (±0.823)</td>
</tr>
<tr>
<td>2.0</td>
<td>7.396</td>
<td>8.165</td>
<td>7.033</td>
<td>6.559</td>
</tr>
<tr>
<td>Green tea powder (%) in diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.493</td>
<td>7.336</td>
<td>7.226</td>
<td>5.801</td>
</tr>
<tr>
<td>1.0</td>
<td>6.487 (±0.931)</td>
<td>7.263 (±0.900)</td>
<td>6.114 (±0.892)</td>
<td>5.661 (±0.823)</td>
</tr>
<tr>
<td>1.5</td>
<td>8.248</td>
<td>7.218</td>
<td>7.838</td>
<td>6.944</td>
</tr>
</tbody>
</table>
The ß-glucosidase enzyme 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 occur 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 intravascular 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.

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