Effect of an ethanolic extract from Enterolobium cyclocarpum pods on feed intake, egg production, and plasma lipid profile of laying hens

Efecto de un extracto etanólico de vainas de Enterolobium cyclocarpum sobre el consumo de alimento, producción de huevo y perfil de lípidos plasmáticos de gallinas de postura

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Abstract

Existe preocupación sobre el contenido de colesterol en el huevo. En este estudio, se investigó el efecto de un extracto etanólico de vainas de Enterolobium cyclocarpum (EEEC) en dietas de gallinas ponedoras sobre la producción de huevo y perfil de lípidos en sangre. Se agregó 0 mg/kg, 60 mg/kg, 120 mg/kg, 240 mg/kg y 480 mg/kg de EEEC en las dietas. El consumo de alimento fue menor en las gallinas alimentadas con 480 mg/kg EEEC ($p < 0.05$). La albúmina fue menos pesada y extendida en las alimentadas con EEEC, en comparación con las de la dieta control ($p < 0.05$). No se observó efecto de EEEC sobre el colesterol en plasma ($p < 0.08$). Se observó un aumento de lípidos de alta densidad (HDL) en proporción del colesterol total en gallinas suplementadas con 120 mg/kg de EEEC ($p < 0.05$). En conclusión, EEEC en la dieta redujo el peso y amplitud de la albúmina, y 120 mg/kg de EEEC en la dieta aumentó el HDL en proporción al colesterol total en sangre.

Keywords: Enterolobium cyclocarpum; gallinas; calidad de huevo; lípidos plasmáticos.

Resumen

Hay preocupación sobre el contenido de colesterol en el huevo. En este estudio, se investigó el efecto de un extracto etanólico de vainas de Enterolobium cyclocarpum (EEEC) en dietas de gallinas ponedoras sobre la producción de huevo y perfil de lípidos en sangre. Se agregó 0 mg/kg, 60 mg/kg, 120 mg/kg, 240 mg/kg y 480 mg/kg de EEEC en las dietas. El consumo de alimento fue menor en las gallinas alimentadas con 480 mg/kg EEEC ($p < 0.05$). La albúmina fue menos pesada y extendida en las alimentadas con EEEC, en comparación con las de la dieta control ($p < 0.05$). No se observó efecto de EEEC sobre el colesterol en plasma ($p < 0.08$); sin embargo, se observó un aumento de lípidos de alta densidad (HDL) en proporción del colesterol total en gallinas suplementadas con 120 mg/kg de EEEC ($p < 0.05$). En conclusión, EEEC en la dieta redujo el peso y amplitud de la albúmina, y 120 mg/kg de EEEC en la dieta aumentó el HDL en proporción al colesterol total en sangre.

Palabras clave: Enterolobium cyclocarpum; gallinas; calidad de huevo; lípidos plasmáticos.

Introduction

Chicken eggs are the most widely consumed animal product in the world. Mexico occupies one of the first places in consumption per capita of egg, with more than 22 kg/year (Unión Nacional de Avicultores [UNA], 2016). Chicken eggs are a cheap source of protein and vitamins; however, they have high cholesterol content. Eggs contain in average 200 mg of cholesterol (Virtanen, Mursu, Toumainen, Virtanen & Voutilainen, 2015), which constitute a risk for cardiovascular pathologies associated to cholesterol.

Among the strategies to reduce or modify the cholesterol content of eggs, the utilization of plant origin products is an option. Aydin, Karaman, Cicek & Yardibi (2008) reported a significant reduction of cholesterol in the egg yolk of laying hens fed diets with seeds of Nigella sativa. Similarly, the inclusion of Gynura procumbens or olive leaf (Olea europaea) in diets reduced egg yolk cholesterol in laying hens (Cayan & Erener, 2015; Lokhande et al., 2014) due to the secondary metabolites of these plants (v.gr. saponins). Some studies have reported that saponins from Karaya (Sterculina urens) (Afrose, Hossain, Maki & Tsujii, 2010a), Yucca schidigera (Wang & Kim, 2011), Ilex latifolia (Feng et al., 2015), and Alfalfa (Medicago sativa) (Fan, Du, Zhou, Shi & Wang, 2015; Liang et al., 2015) reduce egg and blood plasma cholesterol, and they regulate the expression of genes associated to cholesterol metabolism. According to many of these studies, there is a close relationship between plasma and egg cholesterol in laying hens, because egg cholesterol decreases as plasma cholesterol decreases (Afrose, Hossain, & Tsujii, 2010b; Fan et al., 2015; Lokhande et al., 2014).

Foliage as well as fruits of many tropical shrubs and trees are rich in saponins. One tree that produces fruits with high content of saponins is Enterolobium cyclocarpum. It contains between 29 mg/g to 43 mg/g of crude saponins depending on which part of the plant is sampled (Albores-Moreno et al., 2017; Rodríguez & Fondevila, 2012). Some authors (Albores-Moreno et al., 2017; Rodríguez & Fondevila, 2012) have reported that rich saponin extracts of E. cyclocarpum improve energy utilization in the rumen. Saponins reduce rumen protozoa population, increase propionate proportion, and decrease methane production (Albores-Moreno et al., 2017; Guo et al., 2008; Patra & Saxena, 2009; Rodríguez & Fondevila, 2012). However, there is no knowledge on the effect of E. cyclocarpum and its saponin extracts in the diet on productive performance or plasma cholesterol profile of laying hens. Therefore, the objective of this study was to evaluate the effect of an ethanolic extract from E. cyclocarpum cyclocarpum pods on feed intake, egg production, and plasma lipid profile in laying hens.

Materials and methods

The experiment was conducted at the Faculty of Medicine and Animal Science of the Universidad Autónoma de Yucatán, located Southeast Mexico.

Animals

One hundred Bovans White laying hens of 32 weeks of age were used. The birds were handled in couples in 50 wire cages (40 cm x 40 cm x 40 cm), kept in an open sided laying house. Cages had individual feeders and water troughs. Hens had free access to water and feed. The experiment lasted eight weeks, from January to February 2018. The hens had a regimen of 16 h of light, 12 h of natural light and 4 h of artificial light. During the experiment, the minimum and maximum ambient temperature were 23 °C and 36 °C, respectively. The average relative humidity was 85%.
Ethanolic extraction from Enterolobium cyclocarpum (EEEC) pods

The pods were manually fractionated into small pieces and then soaked in absolute ethanol (5 L of ethanol per kilogram of sample) at room temperature. Three extractions were made, leaving the material immersed in ethanol during 24 h. The supernatant was filtered and dried using an industrial rotary evaporator. The extract was dried in an oven during 24 h at 45 °C and, after that, stored at 4 °C in a refrigerator.

Determination of saponins and tannins in EEEC

Saponin content in the ethanolic extract from pods of *E. cyclocarpum* (EEEC) was determined based on methods described by Wall, Krider, Rothman & Eddy (1952) and Hess *et al.* (2003) with some modification. Briefly, 5 g of ethanolic extract were dissolved in 30 mL of distilled water. The solution was transferred to a filtration funnel, where it was added 30 mL of hexane, and then shaken. The process was repeated twice, obtaining an aqueous phase free of lipids. The aqueous phase was extracted twice with butanol (BuOH), and the BuOH phase was dried in a rotary evaporator. The dried BuOH extract was dissolved in methanol, followed by precipitation of the saponins by the addition of diethyl ether. The precipitate was again dissolved in methanol (small amount), and the final crude saponin was precipitated with acetone (yield = 38 mg/g). Hemolysis and foam tests were performed, both tested positive, which indicated the presence of saponins.

The total phenol content was determined using the Folin–Ciocalteau assay (Makkar, 2003), and the condensed tannins content was measured using the Vanillin assay (Price, Van Scoyoc & Butler, 1978).

Experimental diets

The laying hens were divided into five treatment (10 replicates per treatment, with two birds per replicate). A commercial diet for laying hens was used and supplemented with 0 mg/kg, 60 mg/kg, 120 mg/kg, 240 mg/kg, and 480 mg/kg of EEEC. The diet utilized had 18% crude protein, 2.8 Mcal of metabolizable energy, 4.2% calcium and 2.5% crude fiber. It included maize, soybean meal, oil, calcium, orthophosphate, vitamins, and minerals (table 1).

**Table 1.** Composition of the basal diet utilized.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
<th>Calculated analysis</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>54</td>
<td>Crude protein %</td>
<td>17.7</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>24</td>
<td>Calcium %</td>
<td>4.2</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>10</td>
<td>Phosphorous total %</td>
<td>0.7</td>
</tr>
<tr>
<td>Canola</td>
<td>5</td>
<td>Lysine %</td>
<td>1</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>3</td>
<td>Methionine + Cystine%</td>
<td>0.8</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>1</td>
<td>Metabolizable energy kcal/kg</td>
<td>2779</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common salt (NaCl)</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline chloride1</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minerals and vitamins Premix2</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1700 g/kg of choline chloride.
2Minerals and vitamins premix: Mn; 65 mg; I; 1 mg; Fe; 55 mg; Cu; 6 mg; Zn; 55 mg; Se; 0.3 mg; vitamin A; 8000 UI; vitamin D; 2500 UI; vitamin E; 8 UI; vitamin K; 2 mg; vitamin B12; 0.002 mg; riboflavin; 5.5 mg; calcium pantothenate; 13 mg; niacin; 3.6 mg; choline chloride; 500 mg; folic acid; 0.5 mg; thiamine; 1 mg; pyridoxine; 2.2 mg; biotin; 0.05 mg.

Source: Authors' own elaboration.
Variables evaluated

The birds were fed *ad libitum* and the eggs were collected daily. Every two weeks feed intake and feed conversion were calculated. In addition, every two weeks two eggs were collected from each of the 50 cages. The eggs were weighed and broken to take the weight, length, width, and height of the albumin; as well as the weight, width, and height of the yolk; using a scale and a micrometer. The measures were taken at 2, 4, 6, and 8 weeks of the experimental period. At week 4, 2 ml of blood from the brachial vein of five hens from each experimental group were taken to evaluate the plasma lipid profile. The hens were randomly selected in each group. After that, blood samples were left 1 h at room temperature and then were centrifuged at 1150 rpm. The blood plasma obtained was stored at -10 °C until its analysis. The blood plasma samples from week 4 were analyzed in a Cobas Integra 400 Plus analyzer, for cholesterol, high density lipids (HDL), low density lipids (LDL), very low-density lipids (VLDL), and triglycerides.

Statistical Analysis

Body weight, feed intake, feed conversion, and egg traits data were analyzed using completely randomized designs with repeated measures using the mixed procedure established by Statistical Analysis Software (SAS, 2010). The experimental unit was the cage with two birds. The statistical model describing those variables was:

\[ Y_{ijkl} = M + T_i + C(T)_{ij} + W_k + e_{ijkl} \]

where \( Y_{ijkl} \) = the observation for a given egg trait; \( M \) = the general mean; \( T_i \) = the fixed effect of treatment (\( i = 0 \) mg/kg, 60 mg/kg, 120 mg/kg, 240 mg/kg, 480 mg/kg of EEEC); \( C(T)_{ij} \) = the random effect of cage within treatment (experimental error), to test the effect of treatment; \( W_k \) = the effect of week of measure (2, 4, 6, 8 weeks); and \( e_{ijkl} \) = the residual error, test for week of measure effect.

Both the experimental and the residual error term were normal and independently distributed with means of 0 and variances \( \sigma^2_{C(T)} \) and \( \sigma^2_e \), respectively.

The selection of the best variance-covariance structure was based on the value of the Akaike information criterion (AIC; the lower the better). The variance components, compound symmetry, autoregressive (AR(1)), and unstructured variance-covariance structures were compared; AR(1) appeared to be the best. The AR(1) structure has homogenous variances and correlations which decline exponentially with distance. Treatment and week of measure means were carried using the Lsmeans test provided in the MIXED procedure of SAS (2010). Data for plasma samples were taken at week 4 of the study and statistically analyzed using a completely randomized design using the ANOVA procedure (SAS, 2010). Here, treatment means comparisons were carried out using the Tukey test.

Results

Ethanolic extract from pods of *E. cyclocarpum* had a content of 38 mg/g of saponins, 13 mg/g of condensed phenols, and 18 mg/g of tannins.

Significant reduction in feed intake was observed (\( p < 0.05 \)) in laying hens fed diets added 480 mg/kg of EEEC. Laying hens in this treatment consumed 6% less than hens in the control treatment (table 2).
Table 2. Performance of laying hens fed diets added ethanolic extract of *E. cyclocarpum* pods.

<table>
<thead>
<tr>
<th>Extract of <em>E. cyclocarpum</em> (mg/g)</th>
<th>0</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>480</th>
<th>SEM1</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (g/day)</td>
<td>99.0a</td>
<td>100.6a</td>
<td>99.4a</td>
<td>99.1a</td>
<td>93.1b</td>
<td>1.56</td>
<td>0.026</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>1.7</td>
<td>1.8</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>0.03</td>
<td>0.061</td>
</tr>
<tr>
<td>Liveweight (kg)</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.5</td>
<td>1.4</td>
<td>0.03</td>
<td>0.541</td>
</tr>
</tbody>
</table>

*a, b Means with different literal in the same row are statistically different. SEM: Standard error of the mean.
Source: Authors’ own elaboration.

Albumen of laying hens fed diets added EEEC had less weight and width (*p < 0.05*), in comparison to laying hens under the control diet (table 3). No statistical differences were observed in the other egg traits evaluated (*p > 0.05*).

Table 3. Quality of eggs from laying hens fed diets added ethanolic extract of *E. cyclocarpum* pods.

<table>
<thead>
<tr>
<th>Extract of <em>E. cyclocarpum</em> (mg/g)</th>
<th>0</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>480</th>
<th>SEM1</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg weight (g)</td>
<td>59.8</td>
<td>57.7</td>
<td>58.0</td>
<td>58.0</td>
<td>58.5</td>
<td>0.65</td>
<td>0.234</td>
</tr>
<tr>
<td>Albumen weight (g)</td>
<td>37.0a</td>
<td>34.6b</td>
<td>35.2b</td>
<td>35.6b</td>
<td>35.1b</td>
<td>0.52</td>
<td>0.043</td>
</tr>
<tr>
<td>Albumen length (cm)</td>
<td>7.7</td>
<td>7.6</td>
<td>7.6</td>
<td>7.7</td>
<td>7.6</td>
<td>0.08</td>
<td>0.886</td>
</tr>
<tr>
<td>Albumen width (cm)</td>
<td>6.3a</td>
<td>6.0b</td>
<td>6.0b</td>
<td>6.1b</td>
<td>5.9b</td>
<td>0.06</td>
<td>0.001</td>
</tr>
<tr>
<td>Albumen height (cm)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>0.02</td>
<td>0.423</td>
</tr>
<tr>
<td>Yolk weight (g)</td>
<td>14.8</td>
<td>15.2</td>
<td>14.6</td>
<td>14.8</td>
<td>15.1</td>
<td>0.23</td>
<td>0.422</td>
</tr>
<tr>
<td>Yolk diameter (cm)</td>
<td>4.1</td>
<td>4.1</td>
<td>4.1</td>
<td>4.1</td>
<td>4.1</td>
<td>0.04</td>
<td>0.324</td>
</tr>
</tbody>
</table>

*a, b, c Means with different literal in the same row are statistically different. SEM: Standard error of the mean.
Source: Authors’ own elaboration.

No significant trend to reduce cholesterol in plasma of laying hens fed diets supplemented with 60 mg/kg, 120 mg/kg, and 240 mg/kg of EEEC was observed (*p > 0.05*). However, a significant increase was observed in HDL when expressed as a proportion of total cholesterol (*p < 0.05*), in laying hens supplemented with 120 mg/kg of EEEC (table 4). No other significant effects due to the addition of EEEC on plasma lipid profile were observed (*p > 0.05*).
Table 4. Cholesterol profile in blood plasma of laying hens fed diets added ethanolic extract of *E. cyclocarpum* pods.

<table>
<thead>
<tr>
<th>Extract of <em>E. cyclocarpum</em> (mg/g)</th>
<th>0</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>480</th>
<th>SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>113.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.59</td>
<td>0.079</td>
</tr>
<tr>
<td>HDL&lt;sup&gt;2&lt;/sup&gt; (mg/dL)</td>
<td>10.6</td>
<td>10.2</td>
<td>15.1</td>
<td>11.8</td>
<td>13.2</td>
<td>0.71</td>
<td>0.218</td>
</tr>
<tr>
<td>LDL&lt;sup&gt;3&lt;/sup&gt; (mg/dL)</td>
<td>1.9</td>
<td>3.2</td>
<td>2.8</td>
<td>2.1</td>
<td>3.9</td>
<td>0.38</td>
<td>0.504</td>
</tr>
<tr>
<td>VLDL&lt;sup&gt;4&lt;/sup&gt; (mg/dL)</td>
<td>209.8</td>
<td>157.2</td>
<td>126.3</td>
<td>146.1</td>
<td>181.5</td>
<td>14.1</td>
<td>0.406</td>
</tr>
<tr>
<td>HDL/Cholesterol (%)</td>
<td>9.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58</td>
<td>0.008</td>
</tr>
<tr>
<td>LDL/Cholesterol (%)</td>
<td>2.0</td>
<td>3.5</td>
<td>3.3</td>
<td>2.2</td>
<td>3.7</td>
<td>0.46</td>
<td>0.688</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>1114.0</td>
<td>786.0</td>
<td>631.3</td>
<td>730.7</td>
<td>907.3</td>
<td>0.02</td>
<td>0.313</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means with different literal in the same row are statistically different. SEM: Standard error of the mean. HDL: High density lipids. LDL: Low density lipids. VLDL: Very low density lipids.

Source: Authors' own elaboration.

Discussion

Inclusion of 480 mg/kg of EEEC in the diets of laying hens affected negatively feed intake. However, there are no reports about the use and effects of this extract on poultry performance. This reduction in feed intake is attributable to the inclusion of the EEEC in the diet. There are reports about the bitter taste that saponins from legumes confer to food (Frikha, Valencia, de Coca-Sinova, Lázaro & Mateos, 2013). However, it is mentioned that extracts of raw pods of *Enterolobium cyclocarpum* may contain other compounds, such as phenols (Raya-González et al., 2013), that could also affect feed consumption. In this experiment, a concentration of 13 mg/g of phenols and 18 mg/g of condensed tannins were found in the EEEC, which could have affected feed intake.

The treatment with 480 mg/kg of EEEC had an evident reduction in feed intake that has been discussed before. This reduction in feed intake was related to the reduction in albumen weight and width. However, feed intake was similar between the other treatments with the EEEC; therefore, feed intake itself does not explain the reduction in albumen weight and width observed in treatments with 60 mg/kg, 120 mg/kg, and 240 mg/kg of EEEC. There is no evidence of saponins interference with digestive enzymes and digestion process (Frikha et al., 2013). However, as it has been mentioned before, those raw herbal extracts may contain other antinutritive compounds. In addition, there was a concentration of 13 mg/g of phenols and 18 mg/g of condensed tannins in the EEEC. The phenols and tannins could inhibit protease activities that reduce protein digestion in the gastrointestinal tract (McDougall et al., 2005; McDougall & Stewart, 2005).

In this study, inclusion of EEEC in the diet did not affect total cholesterol concentration in plasma. However, significant increase in HDL in proportion to total cholesterol in laying hens fed 120 mg/kg of EEEC in the diet was observed (p < 0.05). The capacity of herbal extracts rich in saponins to regulate lipid metabolism and to modify plasma lipid profile has been studied. It has been found that Karaya saponins extract reduces serum cholesterol in laying hens (Afrose et al., 2010b). Fan et al. (2015) and Zhou et al. (2014) reported an efflux reduction of cholesterol from liver and reduction of cholesterol in the yolk of laying hens fed diets added saponin extract from alfalfa. Similar results have been reported for plants extract rich in saponins added in laying hen diets (Deng, Dong, Tong, Xie & Zhang 2012; Wang & Kim, 2011). In mice,
reduction in plasma cholesterol using saponins from *Ilex latifoli* was reported (Feng et al., 2015). Studies carried out on alfalfa showed that saponin extract regulates expression of genes involved in cholesterol metabolism (Fan et al., 2018, Liang et al., 2015). Probably, the increase of HDL proportional to total cholesterol, in laying hens fed diets supplemented 120 mg/kg of EEEC, could have not a relevant effect on egg quality. However, it could be of interest to study its effect on human health. Ethanolic extract from *E. cyclocarpum* seems to show potential to modify plasma lipid profile in laying hens.

**Conclusions**

Inclusion of EEEC in the diet of laying hens reduced albumen weight and width, and 120 mg/kg of EEEC in the diet increased HDL in proportion to total cholesterol in the lipid plasma profile of laying hens.

**Conflicts of interest**

The authors declare that there is not conflict of interest.

**References**


