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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

There is concern about egg cholesterol content. In this study, the effect of an ethanolic extract from *Enterolobium cyclocarpum* (EEEC) pods in laying hen diets on egg production and plasma lipid profile was investigated. EEEC was added in 0 mg/kg, 60 mg/kg, 120 mg/kg, 240 mg/kg, and 480 mg/kg to the diets. Feed intake was lower in laying hens fed diets with 480 mg/kg of EEEC ($p < 0.05$). Albumen was less heavy and wide in laying hens fed diets with EEEC, in comparison to those in the control diet ($p < 0.05$). No significant effect of EEEC on cholesterol in plasma was observed ($p < 0.08$); however, a significant increase of high-density lipids (HDL) in proportion of total cholesterol was observed in hens supplemented with 120 mg/kg of EEEC ($p < 0.05$). In conclusion, EEEC reduced albumen weight and wide in eggs, and 120 mg/kg of EEEC in the diet increased HDL in proportion to total cholesterol in blood.

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

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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 europea*) 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%.

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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.

Ingredient	%	Calculated analysis	%
Corn	54	Crude protein %	17.7
Soybean meal	24	Calcium %	4.2
Calcium carbonate	10	Phosphorous total %	0.7
Canola	5	Lysine %	1
Wheat bran	3	Methionine + Cystine %	0.8
Soybean oil	1	Metabolizable energy kcal/kg	2779
Di-calcium phosphate	0.5		
Common salt (NaCl)	0.3		
Methionine	1.2		
Choline chloride[1]	0.5		
Minerals and vitamins Premix[2]	0.5		

[1]700 g/kg of choline chloride.
[2]Minerals 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 B_{12}, 0.002 mg; riboflavin, 5.5 mg; calcium pantothenate, 13 mg; niacine, 36 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.

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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 σ^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).

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Table 2. Performance of laying hens fed diets added ethanolic extract of *E. cyclocarpum* pods.

	Extract of *E. cyclocarpum* (mg/g)						
	0	60	120	240	480	SEM[1]	*p*
Feed intake (g/day)	99.0[a]	100.6[a]	99.4[a]	99.1[a]	93.1[b]	1.56	0.026
Feed conversion ratio	1.7	1.8	1.7	1.7	1.7	0.03	0.061
Liveweight (kg)	1.4	1.4	1.4	1.5	1.4	0.03	0.541

[a, b] Means with different literal in the same row are statistically different. [1]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.

	Extract of *E. cyclocarpum* (mg/g)						
	0	60	120	240	480	SEM[1]	*p*
Egg weight (g)	59.8	57.7	58.0	58.0	58.5	0.65	0.234
Albumen weight (g)	37.0[a]	34.6[b]	35.2[b]	35.6[b]	35.1[b]	0.52	0.043
Albumen length (cm)	7.7	7.6	7.6	7.7	7.6	0.08	0.886
Albumen width (cm)	6.3[a]	6.0[bc]	6.0[bc]	6.1[b]	5.9[c]	0.06	0.001
Albumen height (cm)	1.2	1.2	1.2	1.2	1.2	0.02	0.423
Yolk weight (g)	14.8	15.2	14.6	14.8	15.1	0.23	0.422
Yolk diameter (cm)	4.1	4.1	4.1	4.1	4.1	0.04	0.324

[a, b, c] Means with different literal in the same row are statistically different. [1]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 ($p > 0.05$).

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Table 4. Cholesterol profile in blood plasma of laying hens fed diets added ethanolic extract of *E. cyclocarpum* pods.

	Extract of *E. cyclocarpum* (mg/g)						
	0	60	120	240	480	SEM[1]	*p*
Cholesterol (mg/dL)	113.3[a]	89.7[b]	85.9[b]	89.1[b]	108.3[a]	3.59	0.079
HDL[2] (mg/dL)	10.6	10.2	15.1	11.8	13.2	0.71	0.218
LDL[3] (mg/dL)	1.9	3.2	2.8	2.1	3.9	0.38	0.504
VLDL[4] (mg/dL)	209.8	157.2	126.3	146.1	181.5	14.1	0.406
HDL/Cholesterol (%)	9.8[a]	11.2[a]	17.4[b]	13.3[a]	11.8[a]	0.58	0.008
LDL/Cholesterol (%)	2.0	3.5	3.3	2.2	3.7	0.46	0.688
Triglycerides (mg/dL)	1114.0	786.0	631.3	730.7	907.3	0.02	0.313

[a, b] Means with different literal in the same row are statistically different. [1]Standard error of the mean. [2]High density lipids. [3]Low density lipids. [4]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

Afrose, S., Hossain, M. S., Maki, T., & Tsujii H. (2010a). Effects of karaya saponin and *Rhodobacter capsulatus* on yolk cholesterol in laying hens. *British Poultry Science, 51*(3), 409-418. doi: https://doi.org/10.1080/00071668.2010.495469

Afrose, S., Hossain, M. S., & Tsujii, H. (2010b). Effect of dietary karaya saponin on serum and egg yolk cholesterol in laying hens. *British Poultry Science, 51*(6), 797-804. doi: https://doi.org/10.1080/00071668.2010.526924

Albores-Moreno, S., Alayón-Gamboa, J. A., Ayala-Burgos, A. J., Solorio-Sánchez, F. J., Aguilar-Pérez, C. F., Olivera-Castillo, L., & Ku-Vera, J. C. (2017). Effects of feeding ground pods of *Enterolobium cyclocarpum* Jacq. Griseb on dry matter intake, rumen fermentation, and enteric methane production by Pelibuey sheep fed tropical grass. *Tropical Animal Health and Production, 49*(4), 857-866. doi: https://doi.org/10.1007/s11250-017-1275-y

Aydin, R., Karaman, M., Cicek, T., & Yardibi, H. (2008). Black cumin (*Nigella sativa L.*) supplementation into the diet of the laying hen positively influences egg yield parameters, shell quality, and decreases egg cholesterol. *Poultry Science, 87*(12), 2590-2595. doi: https://doi.org/10.3382/ps.2008-00097

Cayan, H., & Erener, G. (2015). Effect of olive leaf (*Olea europaea*) powder on laying hens performance, egg quality and egg yolk cholesterol levels. *Asian-Australasian Journal of Animal Sciences, 28*(4), 538-543. doi: http://dx.doi.org/10.5713/ajas.14.0369

Deng, W., Dong, X. F., Tong, J. M., Xie T. H., & Zhang, Q. (2012). Effects of an aqueous alfalfa extract on production performance, egg quality and lipid metabolism of laying hens. *Journal of Animal Physiology and Animal Nutrition, 96*(1), 85–94. doi: https://doi.org/10.1111/j.1439-0396.2010.01125.x

Fan, W., Du, H., Zhou, L., Shi, P., & Wang, C. (2015). Digital gene-expression of alfalfa saponin extract on laying hens. *Genomics Data, 3*, 97-99. doi: https//doi.org/10.1016/j.gdata.2014.12.003

Fan, W., Zhang, X., Shi, P., Li J., Wang, C., Li, D., & Zhu, X. (2018). Effects of dietary alfalfa saponins on laying performance, egg cholesterol concentration, and ATP-binding cassete transporters G5 and G8 expression in laying hens. *Journal of Applied Animal Research, 46*(1), 1051-1058. doi: https://doi.org/10.1080/09712119.2018.1454323

Feng, R., Fan, C., Liu, Q., Liu, Z., Zhang, W., Li, Y., Tang, W., Wang, Y., Li, M., & Ye, W. (2015). Crude triterpenoid saponins from *Ilex latifolia* (*Da Ye Dong Qing*) ameliorate lipid accumulation by inhibiting SREBP expression via activation of AMPK in a non-alcoholic fatty liver disease model. *Chinese Medicine, 10*(23). doi: https://doi.org/10.1186/s13020-015-0054-9

Frikha, M., Valencia, D. G., de Coca-Sinova, A., Lázaro, R., & Mateos, G. G. (2013). Ileal digestibility of amino acids of unheated and autoclaved pea protein concentrate in broilers. *Poultry Science, 92*(7), 1848–1857. doi: http://dx.doi.org/10.3382/ps.2013-03007

Guo, Y. Q., Liu, J., Lu, Y., Zhu, W. Y., Denman, S. E., & McSweeney, C. S. (2008). Effect of tea saponin on methanogenesis, microbial community structure and expression of *mcrA* gene, in cultures of rumen micro-organisms. *Letters in Applied Microbiology, 47*(5), 421-426. doi: https://doi.org/10.1111/j.1472-765X.2008.02459.x

Hess, H. D., Kreuzer, M., Diaz, T. E., Lascano, C. E., Carulla, J. E., Soliva, C. R., & Machmüller, A. (2003). Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. *Animal Feed Science and Technology, 109*(1-4), 79-94. doi: https://doi.org/10.1016/S0377-8401(03)00212-8

Liang, X., Zhang, D., Chen, Y., Guo, R., Wang, J., Wang, C., & Shi Y. (2015). Effects of alfalfa saponin extract on mRNA expression of *Ldlr*, *LXRα*, and *FXR* in BRL cells. *Journal of Zhejiang University Science B, 16*, 479-486. doi: https://doi.org/10.1631/jzus.B1400343

Lokhande, A., Ingale, S. L., Lee, S. H., Sen, S., Khong, C., Chae, B. J., & Kwon, I. K. (2014). Effects of dietary supplementation with *Gynura procumbens* (Merr.) on egg yolk cholesterol, excreta microflora and laying hen performance. *British Poultry Science, 55*(4), 524-531. doi: https://doi.org/10.1080/00071668.2014.938020

Makkar, H. P. S. (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research, 49*(3), 241–256. doi: http://dx.doi.org/10.1016/S0921-4488(03)00142-1

McDougall, G. J., & Stewart, D. (2005). The inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors, 23*(4), 189-195. doi: https://doi.org/10.1002/biof.5520230403

McDougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. *Journal of Agricultural and Food Chemistry, 53*(7), 2760-2766. doi: https://doi.org/10.1021/jf0489926

Patra, A. K., & Saxena, J. (2009). The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. *Nutrition Research Review, 22*(2), 204-219. doi: https://doi.org/10.1017/S0954422409990163

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 26*(5), 1214-1218. doi: https://doi.org/10.1021/jf60219a031

Raya-González, D., Martínez-Muñoz, R. E., Ron-Echeverría, O. A., Flores-García, A., Macias-Rodríguez, L. I., & Martínez-Pacheco, M. M. (2013). Dissuasive effect of an aqueous extract from *Enterolobium cyclocarpum* (Jacq) Griseb on the drywood termite *Incisitermes marginipennis* (Isoptera:Kalotermitidae) (Latreille). *Emirates Journal of Food and Agriculture,* 25(7), 524-530. doi: https://doi.org/10.9755/ejfa.v25i7.15987

Rodríguez, R., & Fondevila, M. (2012). Effect of saponins from *Enterolobium cyclocarpum* on in vitro microbial fermentation of the tropical grass *Pennisetum purpureum*. *Journal of Animal Physiology and Animal Nutrition (Berl), 96*(5), 762-769. doi: https://doi.org/10.1111/j.1439-0396.2011.01161.x

Statistical Analysis Software (SAS). (2010). *SAS / STAT user's guide* (Version 9.3) [manual]. SAS Institute.

Unión Nacional De Avicultores (UNA). (2016). *Indicadores económicos. Compendio de indicadores económicos del sector avícola 2016*. *http://www.una.org.mx/index.php/component/content/article/2-uncategorised/19-indicadores-economicos*

Virtanen, J. K., Mursu, J., Tuomainen, T., Virtanen, H., & Voutilainen, S. (2015). Egg consumption and risk of incident type 2 diabetes in men: The Kuopio Ischaemic Heart Disease Risk Factor Study. *The American Journal of Clinic Nutrition, 101*(5), 1088–1096. doi: https://doi.org/10.3945/ajcn.114.104109

Wall, M. E., Krider, M. M., Rothman, E. S., & Eddy, C. R. (1952). Steroidal sapogenins. I. Extraction, isolation, and identification. *Journal of Biology and Chemistry, 198*, 533-543. https://www.jbc.org/content/198/2/533.full.pdf

Wang, J. P., & Kim, I. H. (2011). Effect of caprylic acid and *Yucca schidigera* extract on production performance, egg quality, blood characteristics, and excreta microflora in laying hens. *British Poultry Science, 52*(6), 711-717. doi: https://doi.org/10.1080/00071668.2011.635638

Zhou, L., Shi, Y., Guo, R., Liang, M., Zhu, X., & Wang, G. (2014). Digital gene expression profiling analysis of the cholesterol-lowering effects of alfalfa saponin extract on laying hens. *PLoS ONE, 9*(6), e98578. doi: https://doi.org/10.1371/journal.pone.0098578