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Effect of *Stevia rebaudiana* Bertonni Leaves powder on Lipid Profiles and Productive Parameters of Laying Hens

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Abstract

Eggs are sources of high nutritious value; however, their intake is associated with hypercholesterolemia in consumers. *Stevia rebaudiana,* among other properties, has a hypoglycemic and hypocholesterolemic effect in humans, rats and broilers chicken. The objectives of this research were to determine the effect of the inclusion of 0, 1, 2 and 3% of *Stevia rebaudiana* leaves powder in the diet on the lipid profile and productive parameters of Isa Brown hens. One hundred, 17 - 24 week old, Isa Brown laying hens were used and distributed in a completely randomized design with four treatments and five repetitions. The increase in the levels of powder from Stevia leaves decreased the cholesterol profile ($p < 0.05$), without changing other profiles and the productive parameters ($p > 0.05$). Additionally, the triglyceride profile and weight gain showed quadratic and negative linear tendencies, respectively associated with the increase of powder from Stevia leaves in the diet. It is concluded that the levels of cholesterol, triglycerides, final weight and total weight gain of Isa Brown laying hens has a tendency to decrease when the levels of powder from Stevia leaves is increased in the diet.

Keywords: biochemical profiles; stevia; total cholesterol; triglycerides.

1. Introduction

With today's tendencies oriented at bettering food security, diminishing environmental contamination and general health risks, the necessity to find ways to lower the fat content in products of animal origin is emphasized. The chemical composition of eggs, meanly from the yolk, is based in saturated and unsaturated lipids and it may be influenced basically, by the feeding sources, the space available for rearing and at the same time, their genotype (Steenfeldt and Hammershoj, 2015; Hammershoj and Steenfeldt, 2015; Hassan *et al.,* 2013; Nicole *et al.*, 2009; Matt *et al.*, 2009).

The consumption of eggs has been associated with hypercholesteremia disorder in consumers (Spence, 2016; Spence *et al.,* 2012), due to the high lipid content in the yolk which represents 84% of the dry matter, composed of triglycerides (65%) and cholesterol (4%) (Nys and Guyot, 2011), and they come from the blood serum (Vieira *et al.*, 1995). These components restrict the consumption of eggs by the general population (McNamara, 2015; Novello *et al.*, 2006).

Nutrition and phytomedicine have come together to search for products that can reduce the lipids, particularly cholesterol in the blood, for which many experiments have been done and feed stuffs based in alfalfa, oats and barley that substantially reduce cholesterol have been used in laying hens (Deng *et al.*, 2011; Jacob and Pescatore, 2012; Newman *et al.*, 1992); however, the use of these feed stuffs comes at a high cost.

Stevia rebaudiana is a perennial shrub native to the tropical region of South America and expanding its cultivation in the High Jungle from the Amazonian region of Peru as an alternative of income for very small producers to replace those coming from the coca cultivation. Leaves of this plant possesses diverse substances such as polyphenols, flavonoids and proteins (Gawel-Beben *et al.*, 2015; Hazirah *et al.*, 2017) which bestow an antioxidant, antimicrobial, anticarcinogenic and in particular a hypoglycemic effect and reduce cholesterol in the blood of rats, humans and broiler chickens (Ruiz-Ruiz *et al.*, 2015a; Assaei *et al.,* 2016; Ruiz-Ruiz *et al.*, 2017; Bender *et al.,* 2015; Atteh *et al.*, 2008).

Likewise, potential nutritional attributes have been reported for animal diets (Atteh *et al.*, 2011). As a result, it is important to carry

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out studies that allow determination of *S. rebaudiana* effect in reducing the levels of total lipids in the blood of laying hens.
The objective was to determine the effects of the inclusion of different levels of *Stevia rebaudiana* leaves powder on the lipid profile and productive parameters in laying hens from the Isa Brown line during the laying stage.

2. Materials and methods

One hundred Isa Brown laying hens at 17 to 24 weeks' old were distributed into four treatments, with five replicates and five hens each. All of the birds were located in twenty experimental cages 1.2 x 1.0 x 0.6 m. The diets were established based on the nutritional requirements for Brown egg hens and diets composition and nutritive values (Rostagno *et al.*, 2017). Stevia leaves powder was added to the diets without altering the nutritional content. Diets were offered in a controlled form, given 110 grams per bird per day; meanwhile, the drinking water was given at livitum.
Stevia leaves were, acquired from the Stevia Peru S.A.C., located in the Rupa Rupa district, Leoncio Prado province of Peru. Leaves were dried in a forced ventilation oven at 60 oC for twenty-four hours, the grinding procedure was made using a 1 mm diameter sieve in a model 4 Thomas Willey brand grinder, USA. Once powdered it was added to the diet according to the Table 1.

Determination of the hematological, Biochemical profile and productive parameters

Twenty blood samples with EDTA and twenty with no anticoagulant were collected, from the wing vein, at 17, 20 and 24 weeks old in the four treatments. Determinations of hemoglobin by the cyanmetahemoglobin technique and the hematocrit by the micro hematocrit technique (Samour *et al.*, 2016) at 11,000 rpm for 3 min in a Kert Lab Tom's centrifuge (USA Science Tech Group) were done. In the sixty samples, the blood serum was extracted by centrifugation at 1500 rpm, and triglycerides, total cholesterol, total serum protein and glucose were determined using Wiener Lab reagents (2012), in a spectrophotometer DIALAB DTN 405 and 515 and 450 nm filters. Final weight, total weight gain, number of eggs per day, laying rate, daily egg weight per hen, daily feed intake and Feed conversion rate were determined.

Statistical analysis

The animals were distributed using the completely randomized design (CRD) with four treatments and five replicates each. The results were analyzed using the Software Statistics Infostat (Universidad Nacional de Córdova, 2016), for the variance analysis and the 5% Tukey significance test. Additionally, the data was analyzed using the 5% orthogonal contrast test to verify the tendency thereof.

3. Results and discussion

Biochemical blood profiles

Glucose and triglyceride profiles of laying hens were not influenced ($p > 0.05$) by the different levels of Stevia leaves powder (Table 1), contrasting with those obtained in rats and mice (Assaei *et al.,* 2016; Elnaga *et al.*, 2016; Inga, 2009) and in broilers (Atteh *et al.*, 2008). The hypoglycemic mechanisms of Stevia have been documented in mammals, including humans, (Marcinek and Krejpcio, 2016; Ruiz-Ruiz *et al.* 2015b; Chatsudthipong and Muanprasat, 2009; Jeppezen, 2000); nonetheless, it has still not been reported in laying hens.
Hypoglycemic substances, decreasing the level of glucose in blood, they can decrease the level of fatty acids, which are the structure of different fractions of lipids in tissues, like triglycerides, lipoproteins, cholesterol and phospholipids (Bruss, 2008). Studies in this direction have shown that the administration of diets with elevated levels of carbohydrates have induced to an increase up to 150% in the synthesis of total fatty acids and up to a 270% of this synthesis comes from the liver glucose (Menezes *et al.*, 2013).
The lack of variation of glucose and triglycerides could be associated to the physiological mechanisms of these substances in hens, in which these profiles are two to three times higher and more stable than those of mammals, due to a greater capacity of the gluconeogenesis mechanisms (Lewis, 2004) and for the action of estrogens on the triglycerides in birds (Flórez and Osorio, 2013; Lien *et al.*, 2001); thus, being much less sensitive to external factors.
These physiologic mechanisms, at the same time, can be added up to the energetic effect of the powder from Stevia leaves in the diet (Atteh *et al.*, 2011), up to a certain level; and later at a greater level, activate a hypoglycemic effect and jointly, may have established the negative quadratic tendencies of the triglyceride profiles (Figure 1).

Table 1
Hematological and serum biochemical profiles of Isa Brown hens 17-24 weeks' old fed with diets containing 0, 1, 2 and 3% S. Rebaudiana leaves powder

Blood and biochemical profiles	Factors					Level of Stevia leaves powder				Age of the hens in weeks		
	Contrast	Stevia level	Age in weeks	Stevia x Age	CV (%)	0%	1%	2%	3%	17	20	24
Glucose (g/dL)	p>0.05	0.93	0.23	0.90	18.1	187	180	182	183	189	173	187
Triglyceride (g/dL)	C	0.17	0.001	0.27	37.7	750	988	863	768	478 [a]	1012 [b]	1037 [b]
T. Cholesterol (mg/dL)	C	0.031	0.001	0.52	28.1	186 [ab]	204 [ab]	224 [b]	165 [a]	78.6 [a]	245.6 [b]	260.1 [b]
T. Protein (g/dL)	p>0.05	0.95	0.002	0.86	13.3	5.11	5.08	4.97	5.1	4.9 [a]	4.8 [a]	5.5 [b]
Hematocrit (%)	p>0.05	0.71	0.13	0.71	10.3	26.0	25.5	24.9	25.4	24.8	25.2	26.4
Hemoglobin (g/dl)	p>0.05	0.90	0.001	0.64	19.6	11.5	11.6	11.9	11.3	17.7 [b]	8.3 [a]	8.7 [a]

NS: Not significant, C: Contrast with a quadratic tendency.

Total cholesterol profile diminished with the increase of the powder from *S. rebaudiana* leaves in the diet ($p < 0.05$) (Table 1). The level of cholesterol in hens generally depends on the genetic line (Flórez *et al.*, 2013); on the action of the estrogen, but at a lower level than the triglycerides (Flórez and Osorio, 2013; Lien *et al.*, 2001), so that it could be more sensitive to the external factors, as the hypoglycemic effect of the components of the powder from S. Rebaudiana, being able to diminish it, in contrast to the results obtained for the triglycerides.

These results are similar to those obtained by Atteh *et al.* (2008), in which the powder from *S. rebaudiana* diminished the levels of serum cholesterol in broiler chickens, and as those obtained by Elnaga *et al.* (2016) in overweight rats. However, they contrast with those reported by Martínez and Poveda (2010), in which the effect of the artichoke did not decrease the levels of serum cholesterol in Cornish hens, and those obtained by Sizmaz *et al.* (2014) in which the effect of the addition of different levels of boric acid and plant extracts, separate or mixed, did not modify the levels of cholesterol in the serum of laying hens. In addition, these profiles of triglycerides and cholesterol were higher than those obtained in creole and Hy-line Brown laying hens at 68 and 56 weeks old respectively (Paredes-López *et al.*, 2018).

The effect of the Stevia powder also influenced causing the serum cholesterol profiles to adopt a quadratic tendency (Figure 2). This could be associated to the considerably energetic value of the powder from Stevia leaves (Atteh *et al.*, 2011), but as the level of the powder increases, the same mechanisms for the triglycerides above described may be developed.

The total protein in the serum is generally associated with non-specific immunity (Ortuño, 2001) and its variance is associated with a stimulant effect of the immune system (Dominguez *et al.*, 2011). The total protein profiles of laying hens were not influenced ($p > 0.05$) by the different levels of inclusion of Stevia leaves powder in the diet (Table 1).

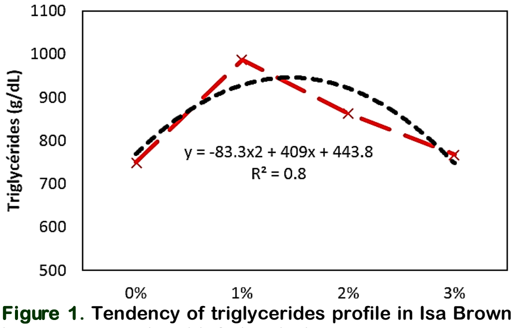

Figure 1. Tendency of triglycerides profile in Isa Brown hens 17-24 weeks old, fed with diets containing 0, 1, 2 and 3% *S. rebaudiana* leaves powder.

Similar results have been reported in male rats treated with low and high doses of stevioside, compared to the control group (Awney *et al.*, 2010). Other similar results to those obtained in the present study are those by Hurtado-Ramírez *et al.* (2014); Reategui *et al.* (2015); Paredes *et al.* (2015) who reported no variation in the plasmatic protein profiles of laying hens from the Hy Line Brown line and from broiler chickens of the Cobb 500 line.

On the contrary, results in which a lowering of the level of total protein and albumin in serum were evidenced have been reported in overweight rats treated with different levels of Stevia as a sweetener, in comparison to the negative control (Elnaga *et al.*, 2016).

The age influenced ($p < 0.05$) the triglycerides, total cholesterol and total protein blood profiles, but not glucose (Table 1). With respect to the triglycerides and cholesterol, their level in the blood of birds is associated with the levels of estrogen and these increases with age, and specifically in the production stage of layers (Lien *et al.*, 2001; Yang *et al.*, 2013). From there, in the present study, the levels of triglycerides and cholesterol double or triple between 20 and 24 weeks, production start period, in comparison to the levels at 17 weeks old, the growth period (Table 1).

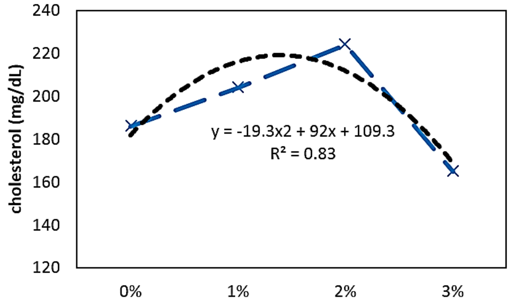

Figure 2. Tendency of the total cholesterol of Isa Brown hens 17-24 weeks old, fed with diets containing 0, 1, 2 and 3% *S. rebaudiana* leaves powder.

Levels of protein in laying birds plasma, in the same manner, directed by the levels of estrogen in the blood, which is to say, it increases with age and specifically in the production stage (Lewis, 2004; Lin and Chan, 1981). In the present study, the protein profile increased at 24 weeks ($p < 0.05$), the age of increasing in the production, in comparison to the levels at 17 to 20 weeks, ending the growth period (Table 1).

Hematological parameters

The values of hematocrit and hemoglobin did not vary as an effect of the inclusion of *S. rebaudiana* powder ($p > 0.05$) (Table 1). Important levels of phytochemicals with antioxidant properties such as total pigments, total phenolic compounds and polyphenols have been found in *S, reubadiana* (Ruiz Ruiz *et al.*, 2015a). These antioxidants act by directly sequestering the peroxyl radical, a potent cellular oxidant (Pilarski *et al.*, 2006) and other free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Hazirah *et al.*, 2017; Gawel-Beben *et al.*, 2015).

The polyphenols not only act as attackers of free radicals and inhibitors of the peroxidation of lipids, but also have the capacity to directly interact with the biological membranes; making them more resistant to alteration by oxidation (Dreifuss *et al.*, 2010). These same mechanisms permit human erythrocytes to increase in membrane thickness, and as a result, increase in size and morphologic variation (Bors *et al.*, 2012). Apparently, none of these mechanisms will develop due to the effect of the components of the *S. rebaudiana* powder, and as a result, no changes were shown in the hematocrit and hemoglobin profiles in the present study.

Variable results for hematocrit and hemoglobin levels have been reported in rats under the effect of different levels of *S. rebaudiana* as a sweetener. Elnaga *et al.*, (2016) reported that hemoglobin levels did not vary with the increase of *S. Rebaudiana*; meanwhile, hematocrit increased in relation to *S. rebaudiana* increasing level. Awney *et al.* (2010), on the contrary, reported an increase in hemoglobin and no variation in hematocrit in male rats treated with low and high doses of stevioside, compared to the control. Other studies using *Dracontium spruceanun* and *Uncaria tomentosa* extracts in broilers chicken have shown the hematocrit and hemoglobin levels increased as the level of such extract in the drinking water increased (Paredes-López *et al.*, 2018; Paredes-López *et al.*, 2017).

The age of the birds influenced, diminishing hemoglobin level ($p < 0.05$) at 20 and 24 weeks old in comparison to 17 weeks old, not withstanding, hematocrit had no variation ($p > 0.05$) (Table 1). These results contrast those obtained by Albokhadain (2012), in broilers, in which hemoglobin and hematocrit levels did not vary with age; likewise, those found by Islam *et al.* (2014) in laying hens during the production phase, where these parameters increased to the same extent to the increase in age production.

Similarly, Hurtado-Ramírez *et al.* (2014) found that in Hy Line Brown hens, from 13 to 16 weeks old, the level of hematocrit progressively increased with the birds' age. The elevated level of hemoglobin at 17 weeks of age in the present study is possibly associated to the state of hem concentration. The blood and biochemical profile obtained in the Isa Brown hens are within the normal values (Reece, 2015).

Productive parameters

No influence from the levels of Stevia leaves powder was evidenced in the daily feed intake, weight gain, total weight and feed conversion rate of hens in the laying phase ($p > 0.05$) (Table 2). However, the final weight and total weight gain showed a negative linear tendency as the level of Stevia leaves powder increased in the diet (Figure 3 and 4).

These results are similar to those obtained by Atteh *et al.* (2008) in broiler chickens during the initial phase, when fed without and with a 2% inclusion of *S. Rebaudiana* leaves powder, no effect was found on daily feed consumption, weight gain and final weight. Meanwhile, in the growth phase, the feed consumption, feed conversion rate and amount of abdominal fat increased and the weight gain and final weight diminished significantly in the birds fed with a 2% inclusion *S. rebaudiana* leaves powder in the diet.

Table 2
Productive parameters of laying hens 17-24 weeks' old fed with diets containing 0, 1, 2 and 3% *S. rebaudiana* leaves poder

Measurement	Treatments				Statistics	
	0%	1%	2%	3%	VC (%)	Contrast
IW (g)	1558	1527	1554	1577	2.1	p>0.05
FW (g)	1812	1786	1783	1737	2.3	L
TWG (g)	256	230	227	181	18.2	L
NEHD (n°)	0.8	0.8	0.8	0.8	6.2	p>0.05
LR (%)	80.6	77.9	84.4	80.2	6.1	p>0.05
DEWH (g)	49.4	48.20	50.6	48.9	6.2	p>0.05
DFI (g)	110	110	110	110	-	-
FCR (g)	2.2	2.3	2.1	2.2	6.3	p>0.05

IW: Initial weight, FW: Final weight, TWG: Total weight gain, NEHD: Number of eggs per hen/day, LR: Laying rate, DEWH: Daily Egg Weight per hen, DFI: Daily feed intake, FCR: Feed conversion rate, NS: Not significant, L: Contrast with linear tendency, VC: variation coefficient.

In another study on male rats fed with diets containing 1500 mg/kg live weight per day of stevioside, a significant decrease in the food consumption was obtained during the twelve weeks and the weight gain decreased in the last six weeks of the study (Awney *et al.*, 2010).
This also resembles other results obtained in female rats fed 25, 250, 500 and 1000 mg/kg of live weight, per day, with Stevia as sweetener; where the final weight, as well as the weight gain, decreased as the level of sweetener used in the diet increased (Elnaga *et al.*, 2016).

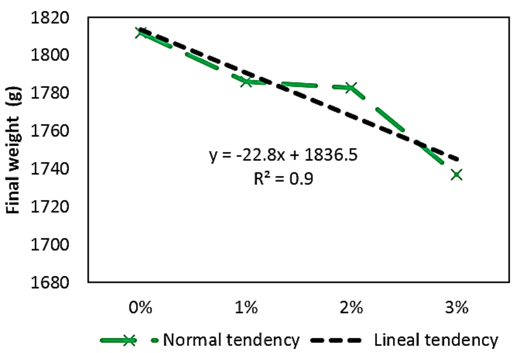

Figure 3. Tendency of final weight in the Isa Brown hens at 17-24 weeks old, fed with diets containing 0, 1, 2 and 3% *S. Rebaudiana* leaves powder.

While the results of the present study contrast those obtained in studies of other animal species, the negative linear tendency for weight gain and final weight (Figure 3 and 4) maintain a relationship with them. This relationship could be associated with the hypoglycemic effect of the components of Stevia (Marcinek and Krejpcio, 2016; Ruiz-Ruiz *et al.*, 2015a; Chatsudthipong and Muanprasat, 2009; Jeppezen, 2000), but in birds, due to having their own mechanisms for maintaining higher and more stable levels of glucose than mammals, (Lewis, 2004) it would make them much less sensitive to weight loss caused by the hypoglycemic effect of Stevia.
The indices of laying hens were not influenced by the different levels of Stevia leaves powder ($p > 0.05$). In general, the percentage of laying (Table 2) is lower than those reported by Hendrix Genetics (2002), who obtained 93.9% of lying in the Isa Brown line 23 to 51 weeks old; and to those by Sizmaz *et al.* (2014) who reported 91.4% of laying in Hy Line White 98, 23 to 31 weeks old.

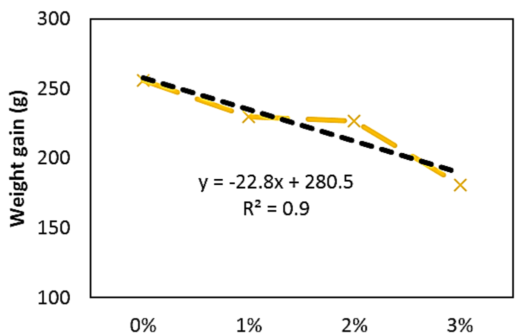

Figure 4. Tendency of weight gain in Isa Brown hens at 17-24 weeks old, fed with diets containing 0, 1, 2 and 3% *S. rebaudiana* leaves powder.

The number of eggs/day/bird and weight of egg/bird/day (Table 2) are similar to those obtained by Mendonça *et al.* (1999), who reported 50.81 in weight of egg/day/bird, fed with a commercial diet, and are different from those reported by Salvador and Guevara (2013) who obtained 57.7 g/day of egg weight in Isa Brown hens at 40 weeks old.
These results were also less than the 57.7 g/bird/day egg weight reported by Sizmaz *et al.* (2014), and also, less than the 57.8 g egg/day/bird reported by Hendrix Genetics (2002). The low production indices of the hens in the present study, compared to those reported in previous studies may be due to the climate conditions and handling used in the tropical zone.

4. Conclusions

This study has explored the way for improving the lipid profiles in the blood of laying hens, with the purpose of improving the quality of eggs with regards to the level of lipids. *Stevia rebaudiana* leaves powder added at a 3% level in the diet diminished the levels of cholesterol in the blood without changing glucose, serum protein and haematocrit and hemoglobin profiles in laying hens at 17 to 24 weeks old. The triglyceride profile, final weight and total weight gain did not vary; however, the triglycerides showed a negative quadratic tendency and the final weight and total weight gain showed a negative linear tendency associated with the increase in Stevia leaves powder in the diet. The effect of Stevia leaves powder on blood lipid profiles in relation to the lipid content in eggs should still be investigated.

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References

Albokhadain, I. 2012. Hematological and some biochemical values of indigenous chickens in Al-Ahsa, Saudi Arabia during summer season. Asian Journal of Poultry Science 6(4): 138-145.

Assaei, R.; Mokarram, P.; Dastghaib, S.; Darbandi, S.; Darbandi, M.; Zal, F.; Akmali, M.; Omrani, G.H.R. 2016. Hypoglycemic Effect of Aquatic Extract of Stevia in Pancreas of Diabetic Rats: PPARγ -dependent Regulation or Antioxidant Potential. Avicenna Journal of Medical Biotechnology 8(2): 65-74.

Atteh, J.; Onagbesan, O.; Tona, K.; Buys, E.J.; Decuypere, E.; Geuns, J. 2011. Potential use of *Stevia rebaudiana* in animal feeds, Archivos de zootecnia 60(229): 133-136.

Atteh, J.; Onagbesan, O.; Tona, K.; Decuypere, E.; Geuns, J.; Buyse, J. 2008. Evaluation of supplementary stevia (*Stevia rebaudiana*, bertoni) leaves and stevioside in broiler diets: effects on feed intake, nutrient metabolism, blood parameters and growth performance. Journal of Animal Physiology and Animal Nutrition. 92: 640-649.

Awney, H.A.; Massoud, M.I.; El-Maghrabi, S. 2011. Long-term feeding effects of stevioside sweetener on some toxicological parameters of growing male rats. Journal of Applied Toxicology 31: 431-438.

Bender, C.; Graziano, S.; Zimmermann, B.F. 2015. Study of *Stevia rebaudiana* Bertoni antioxidant activities and cellular properties. International Journal of Food Science and Nutrition 66(5): 553-558

Bors, M.; Cisinska, P.; Michalowicz, J.; Wieteska, P.; Gulewicz, K. and Bucouska, B. 2012. Evaluation of the effect of *Uncaria tomentosa* extract on the size and shape of human erythrocyte (*in vitro*); Environmental toxicology and pharmacology 33: 127-134.

Bruss, M.L. 2008. Lipids and ketones. In: Clinical Biochemistry of Domestic Animals by Kaneko J.; Harvey J.M.; Bruss M.L. Fifth ed. Academia Press, London. pp. 81-115

Chatsudthipong, V.; Muanprasat, C. 2009. Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacology y Therapeutics 121: 41-54.

Deng, W.; Dong., X.F.; Tong, J.M.; Xie, T.H.; Zhang, Q. 2011. Effects of an aqueous alfalfa extract on production performance, egg quality and lipid metabolism of laying hens. Journal of Animal Physiology and Nutrition 96(1): 85-94.

Dominguez, A.; Sartori, A.; Marino Valente, L.M.; Golim, A.M.; Siani, A.C.; Viero, R.M. 2011. *Uncaria tomentosa* Aqueous-ethanol extract triggers an immunomodulation towards Th2 cytokine profile. Phytotherapy Research 25: 1229-1235.

Dreifuss, AA.; Bastos-Pereira, A.L.; Avila, T.V.; Da Silva B.; Rivero, A.J.; Aguilar, J.L.; Acco, A. 2010 Antitumoral and antioxidant effects of a hidroalcoholic extract of cat's claw (*Uncaria tomentosa*) (Willd Ex Roem & Schult) in an in vivo carcinoma sarcoma model. Journal of Etnopharmacology 130(1): 127-133.

Elnaga, N.I.E.A.; Massoud, M.I.; Yousef, M.I.; Mohamed, H.H.A. 2016. Effect of stevia sweetener consumption as non-caloric sweetening on body weight gain and biochemical's parameters in overweight female rats. Annals of Agriculture Science 61(1): 155-163.

Flórez, J.; Osorio, J. 2013. Perfil metabólico de aves comerciales mediante métodos directos. Revista de Investigaciones Veterinarias del Perú 24(2): 162-167.

Flórez, J.; Osorio, J.; Pérez, J. 2013. Variabilidad y correlación en la concentración de lípidos sanguíneos en dos líneas genéticas de Gallinas ponedoras. Rev. U.D.CA Act. & Div. Cient. 16(1): 151 – 158.

Gaweł-Bęben, K.; Bujak, T.; Nizioł-Łukaszewska, Z.; Antosiewicz, B.; Jakubczyk, A.; Karaś, M.; Rybczyńska, K. 2015. *Stevia Rebaudiana* Bert. Leaf Extracts as a Multifunctional Source of Natural Antioxidants. Moléculas 20: 5468-5486

Hassan, R.; Choe, H.S.; Jeong, Y.D; Hwangbo, J. y Ryu, K.S. 2013. Effect of Dietary Energy and Protein on the Performance, Egg Quality, Bone Mineral Density, Blood Properties and Yolk Fatty Acid Composition of Organic Laying Hens. Italian Journal of Animal Science 12(10): 60-65.

Hazirah, A.R.; Siti, S.S.; Syakinah, A.; Siti, A.J.; Zainal, B.; Abdah, M.A. 2017. Total phenolic content and antioxidant activities of *Corchorus capsularis* and *Stevia rebaudiana* extracts. The International Journal of medicine and Sciences 2(1): 41-48.

Hendrix Genetics Company. 2002. Productividad de gallinas de postura Isa Brown. Jornadas Profesionales de avicultura. 10 pp. Disponible en: https://www.hendrix-genetics.com/en/

Hammershoj, M.; Steenfeldt, S. 2015. Organic egg production. II: The quality of organic eggs is influenced by hen genotype, diet and forage material analyzed by physical parameters, functional properties and sensory evaluation. Animal feed Science and Technology 208:182-197

Hurtado-Ramírez, L.; Paredes-López, D.; Robles-Huaynate, R. 2014. Efecto de la torta de sacha inchi (*plukenetia volúbilis* L.) en el perfil bioquímico sanguíneo e histopatología del hígado de aves de postura. Ciencia Amazónica (Iquitos) 4(1): 60-66.

Inga, C.H. 2009. Evaluación preliminar del nivel de glucosa en la sangre de ratones consumiendo dietas con estevia (*Stevia rebaudiana* bertoni). Tesis para optar el grado de Mag. Sc. Lima, Perú, Universidad Nacional Agraria la Molina. 80 pp.
Islam, S.; Howlader, M.M.R.; Saha, S.; Bhowmik, N: 2014. Hemato-Biochemical Changes of Layer Birds during Egg Production Reared in Sylhet Region of Bangladesh. International Journal of Agriculture Innovations and Research 2(4): 491-494.
Jacob, J.P.; Pescatore, P.J. 2012. Using barley in poultry diets: A review. Journal Appllied Poultry Research 21: 915-940
Jeppezen, R. 2000. Stevioside acts directly on pancreatic beta cells to secrete insulin. Metabolism 49(2): 208-214
Lewis, S. 2004. Avian biochemistry and molecular biology. Cambridge University Press. UK. Pp. 29-79.
Lien, T.F.; Lu, J.J.; Jan, D.F. 2001. Alterations in lipid metabolism between the growing and laying periods of white Leghorn Layers. Disponible en: https://www.ajas.info/upload/pdf/14-217.pdf
Lin, C.T.; Chan, L. 1981. Estrogen regulation of yolk and non-yolk protein synthesis in the avian liver. An immunocytochemical study. Differentiation; Research in Biological Diversity 18(2): 105-114
Marcinek, K.; Krejpcio, Z. 2016. *Stevia rebaudiana* Bertoni: health promoting properties and therapeutic applications. Journal of Consumer Protection and Food Safety 11: 3-8.
Martínez, I. and Poveda, C.A. 2010. Evaluación del valor nutricional de la alcachofa (*Cynara scolymus*) en la producción de codornices de postura. Revista Colombiana de Ciencia Animal 3(1): 15-21.
Matt, D.; Veromann, E. and Luik, A. 2009. Effect of housing systems on biochemical composition of chicken eggs. Agronomy Research 7: 662-667.
McNamara, D.J. 2015. The Fifty Year Rehabilitation of the Egg. Nutrients 7: 816-822
Mendonça, Jr.C.; Martins, A.; Mori, A.; Silva, E.; Mori, C. 2000. Efeito da adição de óleo de peixe a dieta sobre o desempenho e níveis de lipídeos plasmáticos e de colesterol no ovo de galinhas poedeiras. Brazilian Journal Veterinary Animal Science 37(1): 79-83.
Menezes, A.L.; Pereira, M.P.; Buzelle, S.L.; Dos Santos, M.P.; De França, S.A.; Baviera, A.M.; Andrade, CM.; Garófalo, M.A.; Kettelhutido, C.; Cháves, V.E.; Kawashita, N.H. 2013. A low-protein, high-carbohydrate diet increases de novo fatty acid synthesis from glycerol and glycerokinase content in the liver of growing rats. Nutrition research 33: 494-502
Newman, R.K.; Klopfenstein, C.F.; Newman, C.W.; Guritno, N.; Hofer, P.J. 1992. Comparison of the Cholesterol-Lowering Properties of Whole Barley Oat Bran, and Wheat Red Dog in Chicks and Rats. Cereal Chemistry 69(3): 240-244.
Nicole, C.J.; Caplen, G.; Edgar, J.; Browne, W.J. 2009. Associations between welfare indicators and environmental choice in laying hens. Animal Behavior 78: 413-424.
Novello, D.; Franceschini, P.; Quintiliano, D.P.; Ost, P.R. 2006. Egg: Concepts, analyses and controversies in the human health. Archivos latinoamericanos de nutrición 56(4): 315-320.
Nys, Y.; Guyot, N. 2011. Egg formation and chemistry. In: Nys, Y., Bain, M. and Van Inmmerseel, M. Improving the safety and quality of eggs and egg products. Woodhead Publishing Limited pp. 83-132
Ortuño, J.; Esteban, M.A.; Meseguer, J. 2001. Effect of shorth-term crowding stress on the gilthead seabream (*Sparus aurata* L.) innate immune response. Fish & shelfish immunology 11: 187-197
Paredes, D.; Valencia, T.; Saavedra, H. 2015. Efectos de la torta de sacha inchi (*Plukenetia volubilis*) precocida sobre la estructura histológica del higado, ileon y el nivel de proteína total en sangre de pollos broiler. Investigación y Amazonía 5 (1 y 2): 25-29.
Paredes-López, D.; Robles-Huaynate, R.; Valles-Tananta, R. 2018. A comparative evaluation of the hematological parameters, biochemical profile and chemical composition of eggs of creole and Hy-line Brown laying hens. Livestock Research for Rural Development 30 (1): #3.
Paredes-López, D.; Robles-Huaynate, R.; Mendoza-Isla, D.; Mendoza-Pérez, C.; Saavedra-Rodríguez, H. 2018. The effect of the ethanol extract from the Dracontium spruceanum rhizome on hematologic and biochemical profiles and performance parameters of broiler chickens. Scientia Agropecuaria 9(2):411-416
Paredes López, D.; Robles Huaynate, R.; Sandoval Cueva, C.; Sandoval Chacón, M. 2018. Effect of *Uncaria tomentosa* aqueous extract on biochemical and hematological profiles and live performance parameters in broiler chickens. Livestock research for Rural development 30(5): #79.
Pilarski, R.; Zielinski, H.; Ciesiolka, D.; and Gulewicz, K. 2006. Antioxidant activity of ethanolic and aquos extract of *Uncaria tomentosa* (Willd.) DC. Journal of Etnopharmacology 104: 18-23
Reece, W. 2015. The composition and functions of blood. In: Reece, W.O.; Erickson, H.H.; Goff, J.P.; Uemura, E.E. Duckes' Physiology of Domestic Animals 13th Edition, Wiley Blackwell, OX, UK. P. 114-136.
Reategui-Inga, R.; Paredes López, D.; Robles Huaynate, R: 2014: Determination of the effect of sacha inchi (*Plukenetia volúbilis* L.) cake feeding on blood biochemical profile of broilers. Folia Amazonica 24(2): 131-138.
Rostagno, H.; Albino, L.; Donzele, J.; Gomes, P.; De Oliveira, R.; Lopez, D.; Ferreira, A.; Barreto, S.; Euclides, R. 2017.Tablas Brasileñas para aves y cerdos. Composición de alimentos y requerimentos nutricionales. 4a Edición. Universidad Federal de Viçosa–Departamento de Zootecnia. 488 pp.
Ruiz-Ruiz, J.C.; Moguel-Ordoñez, Y.B.; Matus-Basto, A.J.; Segura-Campos, M.R. 2015a. Antidiabetic and antioxidant activity of *Stevia rebaudiana* extracts (Var. Morita) and their

incorporation into a potential functional bread. Journal of Food Science and Technol 52(12): 7894-7903.

Ruiz-Ruiz, J.C.; Moguel-Ordoñez, Y.B.; Matus-Basto, A.J.; Segura-Campos, M.R. 2015b. Antioxidant capacity of leaf extracts from two *Stevia rebaudiana* Bertoni varieties adapted to cultivation in Mexico. Nutrición Hospitalaria 31(3):1163-1170

Ruiz-Ruiz, J.C.; Moguel-Ordoñez, Y.B.; Segura-Campos, M.R. 2017. Biological activity of *Stevia rebaudiana* Bertoni and their relationship to health. Critical Reviews in Food Science and Nutrition 57(12): 2680-2690.

Salvador, E.; Guevara, V. 2013. Desarrollo y validación de un modelo de predicción del requerimiento óptimo de aminoácidos esenciales y del comportamiento productivos en ponedoras comerciales. Revista de Investigaciones Veterinarias del Perú 24(3): 264-276

Samour, J.; Silvanose, C.; Pendl, H. 2016. Clinical and diagnostic procedures. In: Samour, J. Avian Medicine 3ed Edition, Mosby, pp.73-175

Sizmaz, O.; Yildiz, G.; Koksal, B. 2014. Effects of single of combined dietary supplementation of boric acid and plant extract mixture on egg production, egg quality and blood cholesterolemia in laying hens. Kafkas University Vet Fak Derg. 20 (4): 599-604.

Spence, J.D. 2016. Dietary cholesterol and egg yolk should be avoided by patients at risk of vascular disease. Journal of Translational Internal Medicine 4(1): 20-24.

Spence, J.D.; Jenkings, D.J.A.; Davignon, J. 2012. Egg yolk comsumption and carotid plaque. Atherosclerosis 224: 469-473

Steenfeldt, S.; Hammershøj, M. 2015. Organic egg production. I: Effects of different dietary protein contents and forage material on organic egg production, nitrogen and mineral retention and total tract digestibility of nutrients of two hen genotypes. Animal feed Science and Technology 209:186-201

Universidad Nacional de Córdova. 2016. Corporation Analytical Service: InfoStat

Vieira, P.M.; Vieira, A.V.; Sanders, E.J.; Steyrer, E.; Nimpf, J.; Schneider, W.J. 1995. Chicken yolk contains bona fide high density lipoprotein particles. Journal of Lipids Research 36: 601-610.

Wiener Lab reagents. 2012. Vademecum vsp, Rosario – Argentina, 13 pp. Disponible en: http://files.wiener-lab.com/Vademecum_completo_espanol.pdf

Yang, P.K.; Tian, Y.D.; Sun, G.R.; Jiang, R.R.; Han, R.L.; Kang, X.T. 2013. Deposition rule of yolk cholesterol in two different breeds of laying hens. Genetic Molecular Research 12(4): 5786-5792.