EFFECT OF DIETARY INCLUSION OF PURSLANE (*Portulaca oleracea L.*) ON YOLK OMEGA-3 FATTY ACIDS CONTENT, EGG QUALITY AND PRODUCTIVE PERFORMANCE OF RHODE ISLAND RED HENS

[EFECTO DE LA INCLUSION DE VERDOLAGA (*Portulaca oleracea L.*) SOBRE EL CONTENIDO DE ACIDOS GRASOS OMEGA-3 EN LA YEMA DE HUEVO, LA CALIDAD DE HUEVO Y EL RENDIMIENTO PRODUCTIVO DE GALLINAS RHODE ISLAND RED]

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SUMMARY

This study aimed to determine the effects of dietary inclusion of 100 g/kg and 200 g/kg purslane meal on yolk ω-3 fatty acids content, egg quality and productive performance in Rhode Island Red laying hens. Sixty-four hens of 30 weeks of age, with an average body weight of 2100 g, were randomly allocated into four treatments: 0 g/kg (negative control), 100 g/kg and 200 g/kg purslane meal, and 14.7 g/kg flaxseed oil (positive control) in the diet. Hens were individually housed in cages equipped with feeders and drinking troughs. They received food and water *ad libitum* and 18 h of light during 56 days. The inclusion of 200 g/kg purslane and 14.7 g/kg flaxseed oil in laying hens diet showed higher ($P < 0.05$) content of ω-3 fatty acids (α-linolenic acid and docosahexaenoic acid) in egg-yolks compared with those of the groups that received 0 and 100 g/kg purslane. Treatments did not influence ($P>0.05$) parameters of egg quality such as egg longitudinal and transverse diameter, albumin and yolk diameter, yolk height, and yolk weight. Hens that received 200 g/kg purslane in their diet increased ($P < 0.05$) feed intake, egg production, egg mass, and feed conversion compared with those fed with the negative control. It was concluded that the dietary inclusion of both 100 g/kg and 200 g/kg purslane increases yolk ω-3 fatty acids content without any adverse effect on egg quality traits and productive performance of Rhode Island Red hens.

Keywords: ω-3 fatty acids; egg quality; purslane; laying hens; flaxseed oil.

RESUMEN

Se evaluó los efectos de la inclusión de 100 y 200 g/kg de harina de verdolaga sobre el contenido de ácidos grasos ω-3 en yema de huevos, la calidad del huevo y el rendimiento productivo en gallinas ponedoras Rhode Island Red. Sesenta y cuatro gallinas de 30 semanas de edad, con un peso promedio de 2100 g, se asignaron al azar a cuatro tratamientos: 0 g/kg (control negativo), 100 g/kg y 200 g/kg de harina de verdolaga, y 14.7 g/kg de aceite de linaza (control positivo) en la dieta. Las gallinas fueron alojadas en jaulas individuales recibiendo alimentos y agua ad libitum y 18 horas de luz durante 56 días. La inclusión de 200 g/kg verdolaga y 14.7 g/kg de aceite de linaza en la dieta aumentó ($P <0.05$) el contenido de ácidos grasos ω-3 (α-linolénico y ácido docosahexaenoico) en yemas de huevo en comparación con los producidos por las gallinas que recibieron 0 y 100 g/kg de verdolaga. Los tratamientos no influyeron ($P> 0.05$) sobre el diámetro longitudinal y transversal del huevo, diámetro de la albúmina y de la yema, altura y peso de la yema. Las gallinas que recibieron 200 g/kg de verdolaga en su dieta aumentaron ($P <0,05$) el consumo, la producción de huevo, la masa de huevo, y la conversión de alimento en comparación con las alimentadas con el control negativo. Se concluyó que la inclusión en la dieta de 100 g/kg como 200 g/kg de verdolaga aumentó el contenido de ácidos grasos ω-3 en la yema sin afectar negativamente a la calidad del huevo y el comportamiento productivo de gallinas Rhode Island Red.

Palabras clave: ácidos grasos ω-3; calidad del huevo; verdolaga; gallinas ponedoras; aceite de linaza.

INTRODUCTION

The ω-3 fatty acids ALA, EPA and DHA are essential nutrients from the standpoint of human health (Harris et al., 2009), they have been proven effective in preventing cancer (Pandalai et al., 1996) and cardiovascular problems and lowering blood triglycerides level. These three fatty acids are important in human diet; that is why Simopoulos et al. (1999) recommended a minimum intake of 2.22 g/d ALA; 0.65 g/d EPA and DHA for adults. These amounts should be higher in infants and elderly people. Nowadays, the consumption of egg yolk enriched with ω-3 fatty acids could be a good alternative to increase consumption of these essential fatty acids. Several studies showed that the inclusion of ingredients rich in ω-3 fatty acids in laying hens diet had lead to the incorporation of these nutrients in egg-yolks (Zotte et al., 2005; Betancourt and Díaz, 2009).

Purslane (*Portulaca oleracea* L.) is one of the most widespread herbaceous in the world. It yields up to 20 tons of fresh matter/ha (Cros et al., 2007). Besides of being a non-toxic plant (Gao et al., 2010), purslane is one of the richest plants in ω-3 fatty acids content (Mohamed and Hussein, 1994; Simopoulos 2004). In addition, purslane is a good source of vitamins and antioxidants. One hundred grams of fresh purslane leaves contain approximately 0.3-0.4 g ALA; 0.0122 g α-tocopherol (vitamin E); 0.0266 g ascorbic acid (vitamin C); 0.0019 g β-carotene (precursor of vitamin A) and 0.0148 g glutathione (Simopoulos et al., 1992). The abundance of nutrients in purslane plant makes it an important resource to be used in laying hens diet. The aim of this study was to determine the effects of dietary inclusion of either 100 or 200 g/kg purslane meal on egg yolk ω-3 fatty acids content, egg quality and productive performance of Rhode Island Red hens.

MATERIALS AND METHODS

Animals and diets

The study was conducted for 56 days at the Faculty of Veterinary Medicine and Animal Science (FMVZ) of the University of Yucatán (UADY), in Yucatán, Mexico. Sixty-four Rhode Island Red hens of 30 weeks of age and 2100 g body weight were randomly allocated into four dietary treatments: 0 g/kg (as negative control), 100 g/kg and 200 g/kg of purslane meal and 14.7 g/kg flaxseed oil (positive control). Each treatment consisted of 16 hens. Hens were individually housed in cages with individual feeding and drinking troughs; they received food and water *ad libitum* and 18 h of light. Fresh purslane leaves were collected at the FMVZ-UADY in a flat landscape (Rendzinas and Lithosols soils), dried at 60 ^{o}C for 72 h, ground (3 mm mesh) and added in proportions of 100 and 200 g/kg to the diets. Flaxseed oil was purchased from a Canadian provider. Diets were formulated to be isonitrogenous with 160 g/kg of crude protein and isocaloric with 11.7 MJ/kg of metabolizable energy. Ingredients, calculated composition, and fatty acids composition of experimental diets are shown in Table 1.

Samples preparation and oil extraction

During days 27-28 (sample one) and 55-56 (sample two) of the experiment, 24 fresh eggs from each treatment and sampling period (a total of 192 eggs) were randomly collected for fatty acids analyses; from eggs were clustered into 6 samples of 4 egg yolks each, and homogeneously mixed. From this mixture, 10 g were taken, and oil was extracted according to the method proposed by Bligh and Dyer (1959). The lipids extracted were kept at 4 ^{o}C until analysis.

Fatty acids composition

FAMEs were prepared according to the method proposed by Joseph and Ackman (1992) and quantified by gas chromatography using a Varian 450-GC gas chromatographer fitted with a flame ionization detector and a TR-FAME capillary column (120m, × 0.25 mm i.d., 0.25 µm film thickness thermo P/N:260M166L Thermo Scientific, Asheville, NC, USA). The conditions were as follow: detector temperature, 280 ^{o}C; injector temperature, 250 ^{o}C; column temperature, 150 ^{o}C for 0.5 minute at 10 ^{o}C/minute increased to 180 ^{o}C, at 1.5 ^{o}C/minute increased to 220 ^{o}C, and at 30 ^{o}C/minute increased to 260 ^{o}C and held at the final time of 46.50 minutes. Helium was used as the carrier and make-up gas at 1.3 µL/minute and 25 µL/minute, respectively. The injection volume was 1µl with a split ratio of 1:5. FAMEs were identified by comparison with retention times of standards Supelco® 37 Component FAME Mix (Sigma-Aldrich 47885; Saint Louis, MO, USA), Fluka α- Linolenic acid (Sigma-Aldrich 62160, Saint Louis, MO, USA), Fluka Eicosapentaenoic Acid Methyl Ester (Sigma-Aldrich 17266; Saint Louis, MO, USA), and Fluka Docosahexaenoic Acid Methyl Ester (Sigma-Aldrich 05832; Saint Louis, MO, USA). Galaxie data integration system was used to integrate peak areas. Yolk ω-3 fatty acids content were calculated using Heptadecanoic acid (C17:0) as an internal standard.

Table 1. Ingredients, calculated composition and fatty acids composition (g/kg of total fatty acids, as dry matter) of the experimental diets.

	Experimental diets (g/kg)			
	Purslane			Flaxseed oil
Ingredients	**0**	**100**	**200**	**14.7**
Sorghum	694.2	632.5	561.9	645.4
Flaxseed oil[a]	0	0	0	14.7
Purslane[bc]	0	100	200	0
Soya meal	200.7	175.3	144.8	196.2
Wheat bran	11	0	0	51.3
Corn oil	1.6	1.7	4.8	0
Salt	2.5	2.5	2.5	2.5
Premix minerals[d]	0.5	0.5	0.5	0.5
Premix vitamins[d]	0.5	0.5	0.5	0.5
Calcium carbonate	89	87	85	88.9
Calculated Composition				
Crude protein	160	160	160	160
Metabolizable energy (MJ/kg)	11.7	11.7	11.7	11.7
Crude fiber	29.2	28.3	28.1	28.2
Lysine	7.5	7.5	7.7	76
Methionine + Cystine	59	59	59	59
Methionine	0.32	0.33	0.33	0.32
Calcium	35	35	35	35
Total phosphorus	0.30	0.30	0.27	0.27
Linoleic acid	18.4	15	13.4	19.1
Linolenic acid	0	4.1	8.2	8.3
Fatty Acids Composition (g/kg fat)				
Palmitic	190.1	170.4	164	175.7
Stearic	32.9	34.5	34.3	45.8
Oleic	336.2	290.5	285.4	298.9
Linoleic	415.8	460.1	448.2	348.2
α-Linolenic	24.9	44.5	64.1	129.7

[a]Fatty acids profile (g/L FAMEs) of flaxseed oil: C16:0, 50.5; C17:0, 42.1; C18:0, 34.3; C18:1(ω-9c), 158.8; C18:2(ω-6c), 161.9; α-C18:3(ω-3), 549.7; C20:0, 1.7; C22:0, 0.7; C22:6(ω-3), 0.3.

[b]Fatty acids profile (g/L FAMEs) of dry purslane: C16:0, 240.6; C18:0, 72.4; C18:1(ω-9c), 110; C18:2(ω-6c), 220; C18:2(ω-6t), 9.5; α-C18:3(ω-3), 312.3; C20:0, 15.6; C22:0, 13.4; C22:6(ω-3), 6.1.

[c]Proximate analysis (g/kg) of purslane as dry matter : dry matter, 89.7; ash, 213; crude protein, 192; crude fiber 62.6; ether extract, 29.4; nitrogen free extract, 503.

[d]Minerals and Vitamins Premix: Content kg^{-1} of diet: Manganese, 65 mg; iodine, 1 mg; iron, 55 mg, copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg; [b]: Content kg^{-1}: 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; pantothenate of calcium, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; thiamine, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg.

Egg quality

From each treatment, 16 eggs were collected to determine the effect of the experimental diets on egg quality traits such as egg longitudinal and transverse diameter (cm), albumin diameter (cm), yolk diameter (cm), and yolk height (cm) measured with a Vernier and yolk weight (g) measured with an electrical scale.

Productive performance

Egg production was recorded daily and calculated at the end of each week. Daily, an electronic scale was used to weigh the eggs individually according to their replication and treatment. Egg mass was estimated as grams of eggs produced by hens per day. Feed intake was calculated by the difference between offered and refused feed, weighing the refused feed every 72 h.

Feed conversion was determined weekly as grams of feed consumed for a gram of egg produced. Hens' body weight was estimated as weight at the end of the experiment.

Statistical analysis

The statistical replicates for yolk's ω-3 fatty acids content, egg quality and productive performance were 6 samples, 16 eggs, and 16 hens by treatment, respectively. Data were analyzed using analysis of variance (ANOVA) followed by Tukey multiple comparison test. P-values <0.05 were considered as statistically significant. It should be noted that because of no statistically significant difference between samples one and two, analyzed data were the average of both samples. All statistical tests were performed with the SAS program (SAS, 2000).

RESULTS

Yolk ω-3 fatty acids composition

The effects of experimental diets on yolk's *ω*-3 fatty acids content are shown in Table 2. Yolk ALA's content increased as purslane in the diet increased. Eggs from hens that were fed with the flaxseed oil diet were richer in ALA than those produced by hens that received the other treatments. The difference between treatments was significant. On the other hand, DHA content increased (P=0.001) in egg-yolks produced by hens that fed the diet supplemented with 200 g/kg purslane meal compared with those from hens that received the diets with 0 or 100 g/kg purslane meal. Flaxseed oil increased significantly DHA in egg-yolks. As for EPA, the difference between treatments was not significant.

Egg quality

The experimental diets did not affect (P>0.05) the parameters used to evaluate egg quality such as egg longitudinal and transverse diameter, albumin diameter, yolk diameter, yolk height and yolk weight (Table 2).

Productive performance

Results for egg production, egg weight, egg mass, feed intake, feed conversion and body weight are shown in Table 3. The diet supplemented with 200 g/kg purslane increased (P<0.05) egg production, feed intake, and feed conversion compared with the negative control diet. Moreover, egg mass increased (P=0.001) for hens fed with 100 and 200 g/kg purslane compared with those that received the negative control diet. Experimental diets did not affect egg weight and hens body weight (P>0.05).

Table 2. Effects of experimental diets on yolk *ω*-3 fatty acids content (g/kg of total fatty acids)[a] and egg longitudinal diameter (ELD), egg transverse diameter (ETD), albumin diameter (AD), yolk diameter (YD), yolk height (YH) and yolk weight (YW) of Rhode Island Red laying hens[b]

	Dietary levels (g/kg) of					
	Purslane			Flaxseed oil		
Yolk *ω*-3 fatty acids	**0**	**100**	**200**	**14.7**	**SEM**	***P-Value***
ALA	3^{w}	4.5^{x}	7^{y}	28.5^{z}	2.16	0.001
DHA	10.6^{w}	11.2^{w}	16.04^{x}	30.7^{y}	1.73	0.001
EPA	0	0	0.04	0.05	0.01	0.001
Egg quality parameters						
ELD (cm)	5.65	5.64	5.76	5.74	0.03	0.460
ETD (cm)	4.29	4.39	4.35	4.37	0.04	0.169
AD (cm)	8.50	8.35	8.60	8.64	0.10	0.738
YD (cm)	4.05	4.09	4.15	4.15	0.02	0.427
YH (cm)	1.65	1.66	1.63	1.65	0.01	0.742
YW (g)	15.86	16.03	16.13	16.9	0.16	0.133

Means with different letters (w, x, y, z) within a row are significantly different at P<0.05.
[a]Results are means and standard errors of the means of 6 samples/treatment.
[b]Results are means and standard errors of the means of 16 eggs/treatment.

Table 3. Effects of experimental diets on productive performance of Rhode Island Red laying hens[a].

	Dietary levels (g/kg) of					
	Purslane			Flaxseed oil		
Parameters	**0**	**100**	**200**	**14.7**	**S.E.M**	***P- Value***
Egg production (%)	65.28^{w}	73.61^{wx}	77.77^{x}	70.80^{wx}	1.36	0.005
Egg weight (g)	56.11	60.16	60.82	57.90	1.18	0.497
Egg mass (g/hen/day)	36.55^{w}	43.82^{xy}	47.23^{y}	40.90^{wxy}	1.06	0.001
Feed intake (g/hen/day)	127.94^{w}	144.81^{wx}	147.23^{x}	135.30^{wx}	2.74	0.039
Feed conversion	3.27^{w}	3.06^{wx}	2.71^{x}	3.10^{wx}	0.07	0.049
Body weight (g)	2168.62	2171.25	2172.62	2166.15	25.89	0.988

Means with different letters (w, x, y) within a row are significantly different at P<0.05.
[a]Results are means and standard errors of the means of 16 laying hens/treatment fed for 56 days.

DISCUSSION

In this study, ALA and DHA on egg yolk increased significantly with 200 g/kg purslane in the diet. Similar results were reported by Zotte et al. (2005). However, in the current study, yolks ALA and DHA contents were higher than those reported by these researchers. The increase of ALA in the yolks could be due to the increase of purslane in diet, for other studies have shown that the level of yolk ALA increases with the amount of ALA rich materials in hens' diets (Caston and Leeson, 1990; Van Elswyk, 1997). The increase of DHA in the yolks could be due to the desaturation and elongation of ALA in hen's liver, since it has been shown that laying hens can convert ALA to DHA and deposit it in the egg yolk (Kralik et al., 2008). The few amount of EPA in the yolks can be explained by the fact that laying hens have the unusual ability of rapidly converting ALA into DHA in significant quantities, and into EPA in lesser amounts (Farrell, 2010).

The increase of egg production found in this study could arise because of the increase of feed intake of the hens fed with the diet supplemented with 200 g/kg purslane. The significant increase of egg mass can be justified by the fact that hens fed with 100 and 200 g/kg purslane had the highest egg production and egg weight. The results for feed intake in this study disagree with those reported by Aydin and Dogan (2010). The high feed intake reported in this study could be attributed to a lower concentration of dietary metabolizable energy since it has been reported that hens tend to increase feed intake when there is a low energy concentration in the diet (NRC, 1987; Ferket and Gernat, 2006). However, in this study, a trial was not performed in order to verify the metabolizable energy of the experimental diets.

CONCLUSION

The dietary inclusion of purslane meal up to 200 g/kg increased yolk's ω-3 fatty acids content without any adverse effect on egg quality parameters and productive performance of Rhode Island Red hens.

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Submitted December 30, 2013 – Accepted November 26, 2014