Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2020 / v.22 / n.1 / 001-008

http://dx.doi.org/10.1590/1806-9061-2019-1014

Original Article

Effect of Essential Fatty Acid Proportion in Feed on Productive and Reproductive Performance of Japanese Quail (*Coturnix coturnix japonica*)

■Author(s)

Castro-Tamayo CB[I] https://orcid.org/0000-0002-5639-3371
Rios-Rincón FG[I] https://orcid.org/0000-0001-6674-4318
Castillo-Lopez RI[II] https://orcid.org/0000-0003-1494-7863
Contreras-Pérez G[I] https://orcid.org/0000-0001-8388-0485
Molina-Barrios RM[III] https://orcid.org/0000-0002-3285-5738
Heredia JB[IV] https://orcid.org/0000-0003-1017-957X
Muy-Rangel MD[IV] https://orcid.org/0000-0002-6971-535X
Portillo-Loera JJ[I] https://orcid.org/0000-0002-5990-7841

[I] Universidad Autonoma de Sinaloa - FMVZ Blvd. San Angel 3886 Fracc. San Benito, Culiacan, Sinaloa 80246 Mexico.
[II] Facultad de Ciencias Químico Biologicas Blvd. de las Américas y Josefa Ortiz de Domínguez S/N. Ciudad Universitaria, Culiacan, Sinaloa 80013 Mexico.
[III] Ciencias Agronómicas y Veterinarias Antonio Caso 2266, Villa ITSON, Cd. Obregon, Sonora 85130 Mexico.
[IV] Centro de Investigación en Alimentación y Desarrollo, AC - Functional Foods and Nutraceuticals Carretera a El Dorado Km 5.5 Col. Campo el Diez , Culiacán, Sinaloa 80110 Mexico.

■Mail Address

Corresponding author e-mail address
Jesus Jose Portillo-Loera
Unidad Avicola, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Sinaloa Bivd. San Angel 3886 Fracc. San Benito, Culiacan, Sinaloa 80246 México.
Phone: +52 (667)7181650
Email: portillo6422@uas.edu.mx

■Keywords

Omega-3, n-6:n-3 ratio, linolenic acid, hatchability, embryonic mortality.

Submitted: 19/August/2019
Approved: 23/November/2019

ABSTRACT

This investigation was carried out to determine the effect of Essential Fatty Acids proportion (EFAs [n-6, n-3]) in feed through the mixture of soy, olive, canola or chia oil on EFA profile in eggs as well as productive and reproductive performance of Japanese quail. We used 120 quail from 7 to 22 weeks of age, in 15 cages in groups of 6 females and 2 males assigned according to the completely randomized design to 3 treatments with 5 replicates. The treatments were n-6:n-3 proportions 10:1 (control), 4:1 and 1:1. FA profile in yolk, feed intake, laying rate, egg weight, fertility, hatchability, and embryonic mortality were measured. In the egg yolk, n-6 content was similar in the proportions (p>0.05), while n-3 content increased (p<0.01) as n-6:n-3 ratio decreased in the feed. Feed consumption per quail was similar between treatments (p>0.05). In 4:1 and 1:1 proportion laying percentage was greater, but egg weight was lower (p<0.01). Fertility and hatchability were similar between proportions n-6, n-3 (p>0.68). Early and total embryonic mortality was lower in 10:1 and 4:1 proportion (p<0.01); while intermediate and late mortality was similar (p>0.30). The results of the experiment indicate that the mixture of soy, olive, canola or chia oil, to obtain n-6:n-3 proportion of 1:1, 4:1 and 10:1 does not modify feed consumption, laying rate, egg weight, fertility, and hatchability; but, 4:1 and 10:1 proportions favor a lower embryonic mortality.

INTRODUCTION

During the incubation process in birds, egg yolk lipids are the energy reserves and provide the embryo the essential fatty acids (Cherian, 2015), necessary for the formation of cell membranes (Cherian *et al*., 1997). The polyunsaturated fatty acids (PUFA) linoleic acid (AL 18: 2n-6) and α-linolenic acid (ALA; 18: 3n-3) are obtained by birds in feed. However, the ability to incorporate n-3 to the yolk can vary according to the source of PUFA and bird type: chicken, quail, turkey or geese (Nadia *et al*., 2012). Chickens have the liver enzymes delta-6-desaturase and delta-5-desaturase that allows them to synthesize from linolenic acid (n-3), eicosapentaenoic acid and docosahexaenoic acid (DHA) (Barceló-Coblijn & Murphy, 2009), and from linoleic acid (n-6) arachidonic acid (AA) (Spector, 2000); however, n-6 and n-3 compete for liver enzymes in the biochemical pathways of desaturation and elongation (Jing *et al*., 2013). AA and DHA are important during the post-hatching period due to rapid cell proliferation and intense tissue accumulation of these during this time (Cherian & Sim, 1992), as well as favoring the maturation of lymphoid organs (Cherian *et al*., 1997), therefore their function is likely important during incubation (Cherian & Sim, 1992).

The Japanese quail (*Coturnix coturnix japonica*) is native to Europe, North Africa and Asia, (Neumann, 2001); it is of rapid growth, precocity, resistance to diseases and high productivity (Lucotte, 1980). Quail meat production is concentrated in Spain, France, and the United States, and egg production in China, Japan and Brazil (Minvielle, 2004). Studies have been conducted in quails to enrich the egg with n-3 by including up to 4% fish oil or flaxseed in feed (Güçlü, 2008; Al-Daraji *et al*., 2010). However, the results of supplementation with sources containing n-3 showed no effect on fertility and hatchability of lightweight breeder hens (Nadia *et al*., 2012) or quails (Manohar, 2017); and even decreased in heavy reproductive hens (Herstad *et al*., 2000). There was increased fertility in turkey (Shamma *et al*., 2016) and quail hens (Manohar, 2017), as well as an increase in hatchability in quails (Al-Daraji *et al*., 2010; Manohar, 2017). Al-Daraji *et al*. (2010) observed that quail fertility and hatchability improved by including 3% fish or flaxseed oil compared to sunflower oil. It is possible to attribute this effect to the narrowest n-6:n-3 proportion in fish and flaxseed oil than in sunflower oil. However, in the studies conducted, the effect of the source of EFA was determined regardless of the n-6:n-3 proportion; therefore, the objective of this study was to determine the effect of n-6:n-3 proportion in the feed on EFA profile of the egg as well as productive and reproductive performance of Japanese quail.

MATERIALS AND METHODS

The experiment was carried out in the Poultry Unit of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autonoma de Sinaloa, in Culiacan Sinaloa, Mexico (24 46’13’’ LN and 107 21’14’’ LO). The climate of the zone is BS (h’) w (w) (e), semi-dry very hot, with rains in the summer, Köppen classification; with an annual average temperature of 25.9 °C; average relative humidity of 68%, maximum of 81% and minimum 51%; average annual precipitation of 688.5 mm.

The experiment was conducted according to the technical specifications for the production, care and use of laboratory animals of the Mexican official standard (NOM-062-ZOO-1999); and the specifications of the Institutional Committee for the Care and Use of Animals of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autonoma de Sinaloa (Protocol FMVZ-171/11-11-2016). The experimental period comprised four periods of 21 days for productive response and nine periods of 24 days for reproductive response. Before initiating data collection, the quails were adapted to cage management for seven days. 120 quails (90 females and 30 males) were utilized. Egg collection was performed twice a day (08:00 a.m. and 18:00 p.m.). The temperature and relative humidity (RH) in the coop was 25.2 ± 3.7°C and 40.7 ± 7.2%, respectively.

The experiment was established under a completely randomized design with three treatments corresponding to diets with n-6:n-3 proportions in feed: (control) 10:1, 4:1 and 1:1, with five replicates of 8 quails (6 females and 2 males) per treatment, and weeks as a cross-factor. The wire battery cages (60 x 50 x 20 cm) allowed 375 cm^2 per quail. The lighting period was 16 h per day and the feed and water were offered *ad libitum*.

To formulate the diets, fatty acid (FA) profile of soy, olive, canola or chia oil (Table 1); as well as proximal chemical composition (AOAC, 2000) of corn and soybean meal was determined. Metabolizable energy of corn and soybean meal was estimated with the equation: MS (kcal/kg) = 3.75 x crude protein + 8.09 x ether extract - 6.95 x crude fiber + 3.94 x nitrogen-free extract (Moir *et al*., 1980).

Table 1 – Fatty acids profile of oils included in the diet.

	Fatty acid (%)[1]		
Oil	Linolenic	Linoleic	Oleic
Soy	5.63±0.71	50.91±0.10	31.67±0.99
Olive	0.71±0.01	5.01±0.03	82.55±0.04
Canola	13.62±3.88	17.79±1.90	46.19±2.10
Chia	57.23±1.73	19.88±0.37	15.23±1.72

[1]Mean ± standard deviation (n=3).

The diets (Table 2) were formulated according to the nutritional requirements for Japanese quail breeders (NRC 1994), and the composition of EFA profile of every oil was considered. A flour-based feed was prepared every week and stored in plastic boxes at 20 to 22°C, subsamples were taken from each batch of feed to determine FA (Table 3) and Proximate chemical analysis. The peroxide index in the feed (NMX-F-154-1987) was measured in the samples after 7 days of storage (Table 3).

Three weeks into the laying cycle, based on shape, size and egg color, collection and selection were initiated. The eggs to be incubated were kept at 11.0 ± 0.41°C (Nieto Refrigerator, Critotec CFX-8 Model, Guadalajara, Jalisco, Mexico). An automatic incubator (Huacuja, Model 1200, Guadalajara, Jalisco, Mexico) was used, and the eggs were maintained at 37.44 ± 0.22°C and 74.04 ± 2.08% RH for 334 hours, then they were transferred to a hatchery, where the eggs spent 3 to 4 days at 37.5 ± 0.4°C and 90.2 ± 0.41%

Table 2 – Composition and nutritional contribution of experimental diets.

Ingredient (g/100 g)	Proportion n-6:n-3		
	10:1[2]	4:1	1:1
Corn	50.05	50.10	50.70
Soybean 46%	35.90	36.10	35.70
Soybean oil	3.45	2.65	0
Olive oil	1.32	0	0
Canola oil	0	1.20	0.90
Chia oil	0	0.65	3.50
Salt	0.25	0.25	0.25
L-lysine 78%	0.43	0.50	0.45
L-threonine 98%	0.35	0.35	0.35
DL-methionine 98%	0.50	0.50	0.50
Limestone	5.80	5.70	5.70
Dicalcium phosphate	1.15	1.20	1.10
Vitamins and mineral premix[1, 3]	0.25	0.25	0.25
Pigment[4]	0.10	0.10	0.10
Probioticyeast (*Saccharomyces cerevisiae*)	0.20	0.20	0.20
Adsorbent[5]	0.10	0.10	0.10
Phytase[6]	0.20	0.20	0.20
Calculated composition			
Crude protein (%)	20.28	20.03	20.00
Metabolizable energy (kcal/kg)	3202	3149	3088
Lysine (%)	0.66	0.73	0.68
Methionine (%)	0.75	0.75	0.75
Cysteine (%)	0.33	0.33	0.33
Threonine (%)	1.11	1.11	1.11
Tryptophan (%)	0.30	0.30	0.29
Calcium (%)	2.53	2.51	2.52
Non-phytate phosphorous (%)	0.35	0.40	0.38
Crude fiber (%)	3.61	3.63	3.61
Ether extract (%)	6.96	6.63	6.61
Linoleic acid (%)	3.24	3.06	2.11
Dry matter (%)	89.85	90.15	90.19
Analyzed composition			
Crude protein (%)	21.75	21.08	21.04
Ether extract (%)	6.31	5.87	4.40
Ash (%)	9.77	9.21	10.25
Moisture (%)	10.02	8.40	8.61
Crude fiber (%)	1.99	2.25	1.52

[1]Composition of vitamin premix per kg: 12,500 IU (retinol); 4,480 IU (cholecalciferol); 30 IU (tocopherol acetate); 3 mg Menadione sodium bisulfide; 1.5 mg thiamin; 6 mg riboflavin; 3 mg pyridoxine; 15 mg cyanocobalamin; 1.5 mg folic acid; 55 mg niacin; 15 mg Ca pantothenate; 180 µg biotin; 600 mg choline; 120 mg Banox (BHA + BHT).

[2] Control treatment.

[3]Composition of mineral premix per kg: 75 mg Mn; 75 mg Zn; 75 mg Fe; 900 mg Mo; 750 µg Co; 105 mg Se.

[4]Florafil HP, Industrias Vepinsa, S.A. de C.V.

[5]Aluminosilicate, Zeolex.

[6]Natuphos* 5000 GP Fitasa, Basf Mexicana, S.A. de C.V.

RH. Withdrawal of chicks began around 12 h after hatching began. The unhatched eggs were broken and observed with the naked eye to determine if they were fertile, as well as the stage of embryonic death; and classified as early, intermediate, late and total embryonic death according to the classification proposed by Dalton, (2000). At week 20 of the laying cycle, three eggs from every treatment were randomly collected for FA determination.

Fatty acid profile of feed yolk and oils was carried out at the Food Technology Laboratory in the Research Center for Food Development Food in Culiacan Sinaloa utilizing the methods developed by Folch *et al*. (1957) and AOAC (1998) standard 963.22 with modifications; subsequently they were dry evaporated in a rotary evaporator, after methylation the filtrate was recovered in a 2 mL vial, stored in a nitrogen atmosphere and placed in the freezer. Subsequently, 1 µL of the sample was injected into a gas chromatograph. The methyl esters dissolved in hexane were analyzed with a chromatograph (Varian CP-3800, USA), with flame ionization detector (FID) equipped with Omegawax 320 column of 30 m x 0.32 mm, 0.25 mm internal diameter (Supelco , USA). Helium was used as a carrier gas at a rate of 3 mL/min. The oven temperature was maintained at 140°C for 5 minutes, presetat a maximum temperature of 240°C with an increase of 4°C every 90 seconds. Both the temperature of the injector and the detector were set at 260°C. For the identification and quantification of fatty acids, the retention time of sample was compared with those of a standard mixture consisting of 37 methyl esters of fatty acids (Supelco, Bellefonte, USA).

FA results were expressed in percentage of fatty acid with respect to the percentage of fat contained in the sample. The peroxide value was expressed in meq O_2/kg. In productive response, after every feed consumption period, egg number and weight were recorded. For reproductive response after every egg collection, fertility rate, hatchability of fertile eggs and early, intermediate, late and total mortality were recorded.

The statistical analysis of FA results in egg yolk, feed intake, laying percentage, egg weight, fertility and hatchability were performed under a model for a completely randomized experimental design. The comparison of means was made with the Tukey test. The proportions of embryonic mortality were analyzed with the Chi-square test. The maximum alpha level to accept statistical difference was 0.05.

Table 3 – EFA composition and contribution of n-6:n-3 as well as in dexperoxide index of quail diet.

		Proportion n-6:n-3				
Fatty acids (%)[1]	Nomenclature	10:1[2]	4:1	1:1	SEM[3]	*p*-value
Palmitic	C16:0	12.97[a]	11.73[ab]	10.76[b]	0.33	0.0095
Oleic	C18:1, cis-n-9	35.65[a]	30.55[b]	23.52[c]	0.83	0.0001
Linoleic	C18:2, cis-9,12n-6	46.05[a]	46.18[a]	34.59[b]	0.42	0.0001
Linolenic	C18:3, cis-9,12,15n-3	4.78[c]	10.93[b]	30.57[a]	0.19	0.0001
Arachidic	C20:0	0.543	0.613	0.560	0.04	0.4905
Saturated fatty acids (SFA)		12.97[a]	11.73[ab]	10.75[b]	0.33	0.0095
Monounsaturated fatty acids (MFA)		35.65[a]	30.54[b]	23.52[c]	0.83	0.0001
Polyunsaturated fatty acids (PFA)		51.37[c]	57.72[b]	65.72[a]	0.56	0.0001
SFA/PFA		0.252[a]	0.203[b]	0.163[c]	0.004	0.0001
n-6		46.17[a]	46.07[a]	34.59[b]	0.40	0.0001
n-3		4.78[c]	10.93[b]	30.57[a]	0.19	0.0001
n-6:n-3		9.79[a]	4.28[b]	1.14[c]	0.26	0.0001
Peroxide index, mEqO$_2$/kg		2.01[b]	1.06[c]	4.79[a]	0.51	0.0001

[a,b,c]Different letters in row indicate statistical difference ($p<0.05$).

[1]As of total lipids (%).

[2]Control treatment.

[3]Standard error of the mean (n= 3).

RESULTS AND DISCUSSION

Fatty acids in the yolk

FA composition in egg yolk is shown in Table 4. According to the FA group, monounsaturated FA were found to be in the highest percentage, close to 50%, due to its content of oleic and palmitoleic acids, followed by saturated FA that were present in about 30% and finally polyunsaturated FA at 20%. Saturated FA were in greater percentage ($p<0.01$) in the 4:1 n-6:n-3 proportion than in 10:1 and 1:1 proportions; where as myristic acid and stearic acid were detected in a similar reduced proportion between treatments; while erucic acid appeared in a greater percentage ($p<0.03$) in the 1:1 proportion. Monounsaturated FA content was similar ($p>0.05$); However, oleic and palmitoleic acids were in a greater percentage, nonetheless palmitoleic acid percentage was greater ($p<0.02$) in the 4:1 proportion than in the 10:1 and 1:1 proportion. Polyunsaturated FA content was similar, although the 1:1 proportion had a higher content and was close to having a statistical difference ($p>0.08$). Linoleic acid content had the greatest percentage and was similar between proportions ($p>0.05$). Linolenic and docosadienoic acids were in greater percentage ($p<0.02$) in the1:1 proportion, which revealed a greater percentage of n-3 fatty acids ($p<0.01$), in accordance to feed proportion and n-6:n-3 proportion also differed. Chen & Hsu (2003) supplemented 2 to 6% refined cod liver oil to duck hens and observed that yolk concentration of saturated fatty acids decreased and while polyunsaturated fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) increased, compared to animal fat controls. In this study the n-6:n-3 proportion in feed remained in the egg yolk. Based on the amount of n-6 and n-3 fatty acids reported by Neijat *et al*. (2016) in egg yolk and chicken feed after the inclusion of hemp seed or oil as a source of n-3 it can be deduced that n-6:n-3 proportions in the diets is kept constant from 1.1 to 1.5 from feed to the egg yolk; this coincides with the results of Navas *et al*. (2001) in bass eggs (*Dicentrarchus labrax* L) where there was constant of 1.2 to 1.7 from feed to the egg yolk. In addition, arachidonic acid and eicosadienoic acid were detected in the yolk and were not detected in feed analysis; this is explained by the bird's ability to lengthen fatty acid chains (Spector, 2000; Barceló-Coblijn & Murphy, 2009). It has been observed that lineages or strains can modify EFA profiles, (Mao *et al*., 1998). Alessandri *et al*. (2012) reported that slow-growing egg-type lines of chickens or layers appear to have greater efficiency in the deposition of EPA and DHA with respect to meat-type chickens since elongation is affected in part by estrogen levels. Arantes da Silva *et al*. (2009) after the inclusion of 5% flax seed to quail diets reported that n-3 incorporation into the yolk was 20%. Mennicken *et al*. (2005) made a divergent selection in chickens for n-3: n-6 proportions and mentioned that n-3 increased 34.7% in the yolk with respect to feed content. These differences in the ability of these birds to incorporate n-3 to yolk fat can vary according to n-3 source and bird species (Nadia *et al*., 2012), due to the competition between the enzymes involved in lengthening and desaturation of linoleic and linolenic acid. A 4:1 proportion or lower has been shown to be optimal for elongating 11 g of

Table 4 – Fatty acid profile and n-6:n-3 proportions in quail egg olk.

Fatty acids (%)[1]	Nomenclature	Proportion n-6:n-3			SEM[3]	*p*-value
		10:1[2]	4:1	1:1		
Myristic	C14:0	0.34	0.46	0.33	0.046	0.160
Myristoleic	C14:1	0.03[b]	0.09[a]	0.05[b]	0.005	0.0008
Palmitoleic	C16:1, cis-9	27.32[b]	31.25[a]	27.93[b]	0.717	0.017
Stearic	C18:0	0.26	0.20	0.21	0.024	0.248
Oleic	C18:1, cis-n-9	49.02	49.55	47.56	0.965	0.382
Linoleic	C18:2 ,cis-9,12n-6	15.85	13.46	12.78	1.079	0.189
Gamma-Linoleic	C18:3n-6	ND	ND	0.08	-----	-----
Linolenic	C18:3, cis-9,12,15n-3	0.43[b]	0.97[b]	3.14[a]	0.381	0.005
Eicosadienoic	C20:2, cis-11, 4n-9	0.77	2.14	2.01	0.429	0.119
Arachidonic	C20:4n-9	0.12	ND	0.14	-----	0.498
Behenic	C22:0	3.01	ND	ND	-----	-----
Timnodonic or Eicosapentaenoic	C20:5, cis-5,8n-3	ND	ND	0.56	-----	-----
Erucic	C21:0	0.29[b]	0.29[b]	0.62[a]	0.070	0.026
Docosadienoic	C22:2, cis-13,16n-6	2.57[ab]	1.61[b]	4.61[a]	0.533	0.019
Saturated fatty acids (SFA)		27.92[b]	31.90[a]	28.47[b]	0.743	0.018
Monounsaturated fatty acids (MFA)		50.22	52.06	50.37	1.247	0.546
Polyunsaturated fatty acids (PFA)		18.84	16.04	21.16	1.275	0.077
SFA/PFA		1.52	2.02	1.35	0.161	0.058
n-6		18.42	15.07	17.39	1.240	0.228
n-3		0.43[c]	0.97[b]	3.70[a]	0.380	0.002
n-6:n-3		43.86[a]	16.96[b]	4.89[c]	2.451	0.0001

[a,b,c]Different letters in row indicate statistical difference (*p*<0.05).

[1] As % of total lipid.

[2]Control treatment.

[3]Values are expressed as means ± pooled standard error (n=3).

ND= Not determined.

linolenic acid to 1 g of eicosapentaenoic acid (Nadia *et al*., 2012), this relationship is important in foods that have a higher linoleic acid content and lower linolenic acid content, since it will reduce the conversion to EPA which is biologically more active than linoleic acid. Therefore, the optimal intake of linoleic in relation to linolenic is crucial for normal metabolism (Simopoulos, 2000), which may be related to FA source, linseed and chia contain more LAN and algae and fish oils are a source of EPA, DHA that are not present in land-based plant or animal sources.

Productive response

The results for productive response are presented in Table 5. Quail feed consumption was similar between treatments (*p*>0.05). These results coincide with the results observed by Morales-Barrera *et al*. (2013) who included 3% tuna oil (*Thunnus albacares*) as a source of n-3 in White Leghorn chicken diets, and with Baucells *et al*. (2000) who replaced fish oil with linseed oil or grape oil and tallow. Rodriguez-Michel *et al*. (2018) observed that after fish oil inclusion feed consumption decreased. A decrease in feed consumption when adding fish oil is related to a reduction in palatability (Hulan *et al*., 1989), although this may not happen as indicated by the results of Baucells *et al*. (2000). The inclusion of essential fatty acids sources of plant origin such as oils or seeds, may not affect feed palatability; Regarding this Al-Daraji *et al*. (2010) included 3% sunflower, flax or corn oils in quail feed where n-6:n-3 proportion ranged from 0.08:1 to 251:1 and recorded a similar feed intake.

Table 5 – Effect n-6:n-3 proportion on productive performance of quail.

Proportion	FI (g d^{-1})	LR (%)	HEW (g)
10:1[1]	32.94	87.90[b]	14.15[a]
4:1	30.78	90.96[a]	13.63[b]
1:1	30.59	91.28[a]	13.63[b]
EEM[2]	3.42	0.71	0.06
p-value	0.33	0.01	0.01

[a,b,c]Different letters in column indicate statistical difference (*p*<0.05).

FI= Feed intake, LR= Laying rate, HEW= Hatching ability egg weight.

[1]Control treatment.

[2]Standard error ofthe mean (n= 9).

Quails fed 4:1 and 1:1 proportion had a higher laying rate than the 10:1 proportion (*p*<0.01). The results obtained in other experiments are not consistent and do not give a definite response, since Baucells *et al*. (2000) reported that laying rate was similar after the inclusion of fish, flaxseed, and grape oils as well as tallow, where PUFA n-6:n-3 proportions ranged from 1 to 38 in chicken feed, on the other hand, Betancourt & Díaz (2009) reported that in broader proportions (7:1) laying rate was greater than in the narrowest proportion (2:1), 93.1% and 86%, respectively.

In 4:1 and 1:1 proportion egg weight was lower ($p<0.01$) with respect to the 10:1 proportion. These results are in agreement with those of Güclü *et al*. (2008) who added 4% sunflower, corn, fish, soy, sesame, olive, cotton or walnut oils to quail feed and obtained eggs with a greater weight (12 g) in the n6:n3 200:1 proportion, compared with the 53:1 and 7:1 proportions of sunflower, corn or soybean oil which weighed 11.5 and 11.3 g, respectively. The greater weight seen in the 10:1 ratio is explained by the lower laying rate (87%) since there is a genetic and phenotypic negative correlation between these two parameters; in this respect, Hagger (1994) estimated a negative genetic correlation in hens (-0.267).

Reproductive response

Results in reproductive response are shown in Table 6. In this study, fertility was similar between treatments ($p>0.680$). In studies where different sources containing EFA are supplemented, discrepancies on the effect on fertility are reported. Nadia *et al*. (2012) used 1.73% flaxseed oil in light reproductive hens, and Manohar (2017) included 4% fish oil in quails and did not find any difference. In turkeys Fertility increased by 5.39% when 2% fish oil was supplemented and by 3.43% when 2% flaxseed oil was added (Shamma *et al*., 2016), fertility also increased by 12.75% after the inclusion of 2% fish oil in quail diets (Manohar, 2017), however Herstad *et al*. (2000) with diets that had 3% recycled vegetable oil or no oil at all observed that in diets for heavy reproductive hens with n-6:n-3 proportions of 1.03:1 to 1.12:1 with 3% fish oil fertility rate decreased (76.3 to 83.7%) compared to 7.6:1 to 8.31:1 proportion (89.5 to 92.1%). The source, quantity and lipid type in the diets are important. Bleisbois *et al*. (1997) mentions changes in the proportions of n-6:n-3 or phospholipid ratios affect sperm membrane structure and fluidity; this can alter fertility by modifying viability and ability of the sperm to interact with the reproductive tract of the female and thereby the union of the sperm with the ovum (Bongalhardo *et al*., 2009).

Hatchability of fertile eggs was similar between the treatments ($p>0.95$). Discrepancies were also found on the effect of EFA supplementation on hatchability in the studies. Nadia *et al*. (2012) after the inclusion of 1.73% of flax seed oil and n-6:n-3 proportions that varied from 2:1 to 10:1 in lightweight reproductive hens; and Manohar (2017) in quails with 2% flaxseed, 4% fish and 2% and 4% linseed and fish oil combinations, did not observe differences in hatchability. Hatchability increased 3.2% and 6.17% when flaxseed or fish oil with n-6:n-3 proportions of 0.22:1 and 0.08:1 were supplemented in quails, compared to corn oil (42:1) (Al-Darji *et al*., 2010); Manohar (2017) supplemented quail diets with 2% fish oil and observed 5.4% greater hatchability compared to a zero oil control. On the other hand, Herstad *et al*. (2000) observed that in heavy reproductive hens, diets with n-6:n-3 proportions of 1.03:1 to 1.12:1 from 3% fish oil, hatchability decreased (73.2 to 77.5%) compared to 7.55:1 to 8.31:1 proportion (88.5 to 92.4%) obtained from diets with 3% of recycled vegetable oil or zero oil.

The n-6:n-3 proportion 1:1 had higher early and total embryo mortality ($p<0.01$), while 10:1 and 4:1 proportions were similar ($p>0.05$) (Figure 1). Al-Daraji *et al*. (2010) observed that in quails supplemented with 3% fish oil total embryonic mortality was 2.92% compared to 12.32% with 3% sunflower oil inclusion. After supplementation with fish oil n-6:n-3 proportion was narrow (0.08:1) with respect to that of sunflower oil (251:1). When EFA content is higher and more double bonds exist, greater oxidation is possible. In this study, the peroxide index in feed a week after being prepared was 4.79 mEqO_2/kg in the n-6:n-3 1:1

Table 6 – Effect of n-6:n-3 proportion in diets in fertility and hatchability fertile eggs.

Proportion	Transferred eggs	Fertile eggs	Chickens born	Fertility (%)	Hatchability fertile eggs (%)
10:1[1]	1191	1154	943	95.13	83.56
4:1	1265	1210	999	94.50	83.87
1:1	1293	1235	920	94.24	84.18
SEM[2]				0.74	1.44
p-value Fisher				0.680	0.950

[1]Control treatment.

[2]Values are expressed as means ± pooled standard error, (n= 9).

proportion while in the 10:1 proportion a 2.01 $mEqO_2$/kg (*p*<0.01) was recorded. Calder (2002) mentions that a greater proportion of n-3 consumption can diminish immune response due to a susceptibility to oxidation due to its unsaturation. In this study, the highest mortality in the 1:1 proportion can be attributed to greater peroxidation of linolenic acid, in addition to the possibility of peroxidation of its products. Zanini *et al*. (2003) observed that when the n-6:n-3 proportion in their diet was narrow because it contained 32.3% linolenic acid, fertility in cockerels decreased, however, after vitamin E was administered, fertility increased.

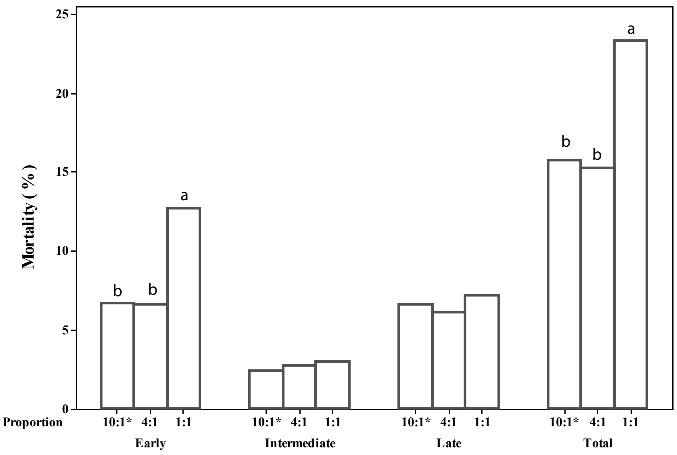

Figure 1 – Effect of n-6:n-3 proportion of in diets in embryo diagnosis an non eclosionated quail egg. [ab]Significant at $p \leq 0.01$ with chi square test. *Control treatment.

CONCLUSION

The results of the experiment indicate that the mixture of soy, olive, canola or chia oil, to obtain n-6:n-3 proportions of 1:1, 4:1 and 10:1 does not modify feed consumption, laying rate, egg weight, fertility or hatchability; but, 4:1 and 10:1 proportion favor a diminished embryonic mortality.

Favoring breeder bird feeds that have n-6 and n-3 proportions close to 1:1 is relative; as shown by the results of this experiment which concludes that reproduction did not improve, therefore it is recommended that n-6 and n-3 content be taken into account and estimate feed consumption in milligrams or daily ingested feed percentage, more than proportion contained in diet.

ACKNOWLEDGEMENTS

We gratefully acknowledge to Laboratory of Antioxidants and Functional Foods. Centro de Investigación en Alimentación y Desarrollo, AC, forallowing the use of the Chromatography equipment for the fatty acid profile. The authors would also like to thank CONACYT-México for the scholarship granted.

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