

# EFFECT OF DIETARY ASPIRIN ON LAYING PERFORMANCES AND EGG YOLK FATTY ACID COMPOSITION IN JAPANESE QUAIL

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## Summary

In avian species, addition of aspirin to the diet was shown to improve the egg production and to elevate the proportion of essential fatty acid contents in several body tissues. This study was conducted to investigate the effect of dietary aspirin on the accumulation of essential fatty acids in egg yolk. Laying Japanese quail at 170 days of age were fed practical diets supplemented with graded levels (0, 0.4 and 0.8%) of aspirin for 2 weeks. There were no significant differences in final body weight and liver weight. Food intake and egg weight on the 0.8% aspirin diet were significantly lower than those on the 0 or 0.4% aspirin diet. In the liver and egg yolk lipids, the 16:0 in birds fed the 0.8% aspirin diet was significantly higher than that in birds fed the aspirin-free diet. However, the proportion of n-6 poly-unsaturated fatty acids was not affected by feeding aspirin diets.

(Key Words: Aspirin, Laying Performances, Fatty Acid Composition, Japanese Quail)

## Introduction

Aspirin has been known as an inhibitor of cyclooxygenase which converts dihomogamma-linolenic acid (DGLA; 20:3n-6) and arachidonic acid (AA; 20:4n-6), synthesized from linoleic acid (LA; 18:2n-6), to the respective prostaglandin. In chickens, antipyretic effect of aspirin has been emphasized. Addition of 0.3% aspirin to the diet reduced body weight losses of growing chickens under heat stress (Glick, 1963). Other studies using aspirin levels ranging from 0.005% to 0.9% found no improvement or negative effects on growth (Reid et al., 1964; Adams and Rogler, 1968). Hens fed diet containing 0.05% aspirin had increased egg production, shell percentage and in some cases food efficiency (Reid et al., 1964; Thomas et al., 1966).

Bruckner et al. (1983) reported that dietary aspirin (0.1%) supplementation increased serum AA level in growing chickens, because the flux from AA to prostaglandin was inhibited by aspirin treatment. In the liver of growing Japanese quail, increase in n-6 poly-unsaturated fatty acid (PUFA) and decrease in saturated fatty acid

(SFA) were induced by 0.4% aspirin addition to the diet compared with the aspirin-free diet (Murai, Furuse and Okumura, unpublished data). The fatty acid composition of egg yolk is similar to that of liver, because the lipoprotein complexes synthesized in the liver are transported by the blood to the ovary and that the lipoprotein complexes are deposited intact in the follicles (Christie and Moore, 1972). Therefore, addition of aspirin to laying Japanese quail might induce the accumulation of n-6 PUFA called to be essential fatty acids in egg yolk lipids.

The present study was conducted to examine the effect of aspirin addition on egg weight, fatty acid compositions of liver lipids and egg yolk lipids in Japanese quail fed a practical diet.

## Materials and Methods

Laying Japanese quail (156 days old) were housed in a stainless steel cages placed in a room with constant temperature ( $24 \pm 2^\circ\text{C}$ ) and 14 hr light 10 hr dark light cycle. They were given a practical layer diet (Power Uzura®, Crude Fat 3%, Toyohashi Feed Mills Co. Ltd, Toyohashi) for 2 weeks as a preliminary period. Fatty acid composition of this diet is shown in table 1. On 170 days of age, the quail were distributed into 3 groups of 6 birds each, so that the mean egg weight and body weight were as uniform as

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possible. Each group was fed the diet supplemented with 0, 0.4 or 0.8% aspirin *ad libitum* for 2 weeks. Eggs were collected daily at 10:00. On the final experimental day, all birds were weighed and killed by decapitation. The livers were immediately removed and weighed, and stored at  $-20^{\circ}\text{C}$  until analysis. Eggs on the final day in each treatment were used for analysis of fatty acid composition. After separating the yolks from these eggs, the yolk membranes were cut, and the liquid yolks were stored individually at  $-20^{\circ}\text{C}$ .

TABLE 1. FATTY ACID COMPOSITION OF THE DIETARY FAT

Fatty acids	(%)
14:0	0.93
16:0	16.53
16:1n-7	1.56
18:0	4.18
18:1n-9	29.61
18:2n-6	44.07
18:3n-6	0.14
18:3n-3	2.23
20:3n-9	0.11
20:3n-6	0.02
20:4n-6	0.43
22:4n-6	0.07

Lipids in the diet, liver and egg yolk were extracted by chloroform:methanol (2:1, v/v) solvent. The total lipid extract was filtered and dried under a gentle stream of nitrogen, and the total lipid was redissolved in 2.8% sodium methylate. The solution was incubated at  $45^{\circ}\text{C}$  for 2 hr and mixed 3 times with 2 mL of hexane. A fraction

of hexane was used for the determination of fatty acid composition. The fatty acid composition of the diet, liver and egg yolk lipids was determined by using a gas chromatography (GC-14A, Shimadzu Co., Kyoto) fitted with a  $25\text{ m} \times 0.25\text{ mm}$  i.d. PEG-20M capillary column (Gasukuro Kogyo, Inc., Tokyo) and data analytical system (CR-4A, Shimadzu Co., Kyoto).

The analyses of data were performed by one way analysis of variance, and significance of differences between means was assessed by the Duncan's multiple range test. Statistical procedure was done using a commercially available statistical package (SAS, 1985).

## Results

Body weight, liver weight, food intake and egg weight in laying Japanese quail fed the diets containing various levels of aspirin are shown in table 2. There were no significant differences in final body weight and liver weight in any treatment. However, final body weight in birds fed the diet containing 0.8% aspirin decreased from 142 g (initial body weight) to 129 g. Food intake and egg weight in birds fed the diet containing 0.8% aspirin were significantly lower than those in birds fed the diets containing 0 or 0.4% aspirin.

Fatty acid composition of liver is shown in table 3. Significant difference was observed in the 16:0 and total amounts of SFA (14:0 + 16:0 + 18:0) between control and 0.8% aspirin treatment. The 18:3n-6 and 18:3n-3 in birds fed the diet containing 0.4% aspirin were significantly higher than those in birds fed the diet containing 0.8% aspirin.

TABLE 2. EFFECT OF DIETARY ASPIRIN ON BODY WEIGHT, LIVER WEIGHT, FOOD INTAKE AND EGG WEIGHT IN LAYING JAPANESE QUAIL

Parameters	Aspirin (%)			Pooled SEM
	0	0.4	0.8	
Number of birds	5	6	6	
Initial body weight (g)	138	141	142	6.18
Final body weight (g)	135	142	129	6.51
Liver weight (g/kg body weight)	27.6	23.3	25.7	1.41
Food intake (g/day)	26.4 <sup>a</sup>	25.9 <sup>a</sup>	21.3 <sup>b</sup>	1.26
Egg weight (g)	10.5 <sup>a</sup>	10.4 <sup>a</sup>	9.2 <sup>b</sup>	0.32

<sup>ab</sup> Means not having the same superscript within a row are significantly different at  $p < 0.05$ .

FATTY ACID COMPOSITION IN JAPANESE QUAIL

TABLE 3. EFFECT OF DIETARY ASPIRIN ON FATTY ACID COMPOSITION OF LIVER LIPIDS IN LAYING JAPANESE QUAIL

	Aspirin (%)			Pooled SEM
	0	0.4	0.8	
Number of birds	5	6	6	
Fatty acids	..... (%) .....			
14:0	0.31	0.30	0.32	0.060
16:0	24.36 <sup>a</sup>	24.61 <sup>ab</sup>	27.13 <sup>b</sup>	0.866
16:1n-7	3.17	2.87	2.60	0.497
18:0	16.10	15.08	17.50	1.503
18:1n-9	34.23	32.91	31.79	3.607
18:2n-6	15.19	17.91	13.67	1.937
18:3n-6	0.21 <sup>ab</sup>	0.28 <sup>a</sup>	0.17 <sup>b</sup>	0.030
18:3n-3	0.18 <sup>ab</sup>	0.21 <sup>a</sup>	0.16 <sup>b</sup>	0.025
20:3n-9	0.23	0.17	0.16	0.040
20:3n-6	0.24	0.16	0.19	0.056
20:4n-6	5.64	5.33	6.24	1.261
22:4n-6	0.15	0.17	0.14	0.037
SFA <sup>1</sup>	40.76 <sup>a</sup>	39.99 <sup>a</sup>	44.95 <sup>b</sup>	1.337
MUFA <sup>2</sup>	37.40	35.78	34.38	4.062
PUFA (n-6) <sup>3</sup>	21.42	23.84	20.40	3.150
SFA/MUFA	1.16	1.19	1.44	0.183

<sup>1</sup> Saturated fatty acids = 14:0 + 16:0 + 18:0.

<sup>2</sup> Mono-unsaturated fatty acids = 16:1n-7 + 18:1n-9.

<sup>3</sup> Poly-unsaturated fatty acids (n-6) = 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6.

<sup>a,b</sup> Means not having the same superscript within a row are significantly different at  $p < 0.05$ .

Fatty acid composition of egg yolk is shown in table 4. The 16:0 and SFA in birds fed the diets containing 0.4% or 0.8% aspirin were significantly higher than those in birds fed the aspirin-free diet. Significant differences were not found in other fatty acid composition between treatments.

**Discussion**

Reid et al. (1964) and Thomas et al. (1966) reported that total egg production increased when hens were fed diets containing 0.05% aspirin. Moreover, the production of shell-less egg was reduced in hens fed 0.05% aspirin, although egg weight was not influenced (Balog and Hester, 1991). In the present study, the 0.8% aspirin level, but not 0.4% level, reduced egg size accompanied by the reduction of food intake. It is well known that the ingestion of a high level of aspirin may result in epigastric distress, nausea, and vomiting (Insel, 1991). Therefore, a high dietary aspirin

level reduced food intake, which in turn resulted in a decrease in egg weight. However, the resistance for aspirin might be stronger in Japanese quail than in chickens, because Brenes and Jensen (1982) reported that a practical diet supplemented with 0.2% aspirin reduced food intake in growing chickens. So far, it has been well recognized that prostaglandin controls oviposition of laying hens (Hertelendy and Biellier, 1978) and Japanese quail (Hertelendy, 1972), though the relationship between inhibition of prostaglandin synthesis by aspirin and egg size has not been well understood. Further experiment remains to be done.

Fatty acid composition in the liver and egg yolk was reflected by dietary fat source (Skellon and Windsor, 1962; Menge et al., 1965; Calvert, 1969). In avian species, liver is the major site of fatty acid synthesis (Goodridge and Ball, 1967; Leveille, 1969), and lipids accumulated in egg yolk were mainly derived from liver (Ranney and Chaikoff, 1951; Flickinger and Rounds, 1956). In addition, Hargis et al. (1991) suggested

that a period longer than 2 weeks of feeding a test diet was needed to attain a steady state alteration in yolk fatty acids for laying hens. The experimental period of 2 weeks, in this experiment, was probably reasonable for alteration of egg yolk fatty acid composition. Therefore, the change in fatty acid composition of egg yolk in the present study could be influenced by the increase of 16:0 in the liver by aspirin supplementation. However, the reason for the increase in 16:0 by aspirin treatment was unclear.

Bruckner et al. (1983) investigated the serum fatty acid composition in growing chickens fed the diets containing 0, 0.1 or 0.2% aspirin for 2 weeks, and reported that there was a significant increase in serum LA and AA levels with an increase in dietary aspirin. In growing Japanese quail fed the diet containing 2% LA, the proportion of n-6 PUFA in the liver was increased with aspirin supplementation (Murai, Furuse and Okumura, unpublished data). These results might be due to the inhibitory effect of aspirin on prostaglandin synthesis from DGLA and AA.

Thus, dietary aspirin can be used as a regulator to increase in n-6 essential fatty acids in the body tissues of egg yolk. However, in this experiment, no significant difference was observed in the proportion of LA, AA or other n-6 PUFA in the liver from the birds fed the diets containing aspirin. The differences in aspirin response to hepatic fatty acid composition between two experiments might be due to the quality and quantity of dietary fat or age of birds used. In our previous experiment using growing Japanese quail, the diet containing 2% LA was made up with semi-purified ingredients and the LA level was adjusted with the safflower oil. On the other hand, the LA concentrations of practical diet used in the present study was approximately 1.3% in the diet. Moreover, it is reported that hepatic fatty acid metabolism are sometimes affected by aging. Biagi et al. (1991) reported that the enzyme activity of delta-6 desaturation in the conversion from LA to gamma-linolenic acid decreased with aging and AA content in the liver of young rats was higher than that of old rats.

TABLE 4. EFFECT OF DIETARY ASPIRIN ON FATTY ACID COMPOSITION OF EGG YOLK LIPIDS IN LAYING JAPANESE QUAIL

	Aspirin (%)			Pooled SEM
	0	0.4 <sup>1</sup>	0.8	
Number of birds	5	6	6	
Fatty acids	..... (%) .....			
14:0	0.40	0.47	0.47	0.028
16:0	26.41 <sup>a</sup>	28.72 <sup>b</sup>	29.43 <sup>b</sup>	0.708
16:1n-7	4.60	4.78	4.40	0.247
18:0	10.58	10.81	11.89	0.379
18:1n-9	45.44	43.43	41.57	1.299
18:2n-6	10.60	9.86	10.22	0.993
18:3n-6	0.16	0.16	0.13	0.014
18:3n-3	0.20	0.17	0.19	0.029
20:3n-9	0.10	0.13	0.10	0.019
20:3n-6	0.09	0.09	0.11	0.009
20:4n-6	1.25	1.25	1.40	0.080
22:4n-6	0.17	0.12	0.09	0.050
SFA <sup>1</sup>	37.40 <sup>a</sup>	40.00 <sup>ab</sup>	41.79 <sup>b</sup>	0.936
MUFA <sup>2</sup>	50.03	48.22	45.97	1.184
PUFA (n-6) <sup>3</sup>	12.27	11.48	11.95	1.013
SFA/MUFA	0.75	0.83	0.91	0.036

<sup>1</sup> Saturated fatty acids = 14:0 + 16:0 + 18:0.

<sup>2</sup> Mono-unsaturated fatty acids = 16:1n-7 + 18:1n-9.

<sup>3</sup> Poly-unsaturated fatty acids (n-6) = 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6.

<sup>ab</sup> Means not having the same superscript within a row are significantly different at  $p < 0.05$ .

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In conclusion, dietary aspirin was not useful to improve the egg yolk fatty acid composition, with special reference to essential fatty acids in Japanese quail.

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