キトサン添加飼料の給与がウズラの卵胞発育に及ぼす影響

誌名	香川大学農学部学術報告
ISSN	03685128
著者名	橋口,峰雄
発行元	香川大学農学部
巻/号	65巻
掲載ページ	p. 5-10
発行年月	2013年2月

Tech. Bull. Fac. Agr. Kagawa Univ., Vol. 65, 5 ~10, 2013

Effect of Dietary Chitosan on Ovarian Follicle Growth in Japanese Quail

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Abstract

The current study was performed to investigate the relationship between dietary chitosan and ovarian follicle growth in Japanese quail. Quail were divided into 3 treatment groups and supplied diets containing 0%, 2% and 4% chitosan from 3 weeks of age until the onset of lay. Age at the onset of lay was significantly delayed in birds fed a high chitosan diet. There was no difference in body weight due to the amounts of dietary chitosan. Ovary weight was lower for the quail fed a high percentage chitosan diet. The weights of the 3rd to 5th largest follicles in ovary were lighter in birds fed the high percentage chitosan diets. Egg yolk lipid and cholesterol concentrations were not effected by variations in dietary chitosan. Therefore, it may be stated that dietary chitosan depresses follicle growth in ovary without influencing the lipid and cholesterol concentrations of the yolk in Japanese quail.

Keyword : Chitosan, ovary, follicle growth, cholesterol, quail

Introduction

Chitin is a polysaccharide found naturally in natural world. It is the component of cell walls of some kinds of mold and the exoskeletons of arthropods, such as crabs and shrimps[1]. Chitin has an amino group in its chemical structure that is similar to cellulose in dietary fiber, and chitosan is an N-deacetylated derivative of chitin. It has been showed to have an array of impacts in various animals, with broiler chicken being widely studied: Higher chitosan concentrations in the diet reduced body growth and feed intakes[2] ; optical body growth was obtained by reduced chitosan concentrations[3]. Dietary chitosan reduced ileal fat digestibility without effecting plasma triacylglycerol concentrations[2] but did lower plasma cholesterol concentrations[2, 4]. Notable studies involving other animals have provided further information on the role played by chitosan. Plasma cholesterol is lowered through a higher intake of chitosan in human[5] and similarly in rat[6, 7]. Higher percentage dietary chitosan reduced total liver cholesterol in rat[6, 8]. It is considered that the hypocholesterolemic effect of chitosan is due to interruption of enterohepatic bile acid circulation and consequently decreased cholesterol pool in the body[5].

In domestic fowl ovary growth rapidly occurs immediately before the onset of lay. Ovary contains a hierarchy of follicles, proceeding large yellow ones to thousands smaller ones[9]. The ovarian follicle growth correlates directly with triglyceride-rich lipoprotein uptake into the follicle from the circulating blood. Principally this involves endocytosis of triglyceride-rich lipoprotein, which produced in liver, on the plasma membrane of the follicle [10, 11]. Since cholesterol is a vital component of triglyceride-rich lipoprotein, it is deemed an essential element in the growth of ovary follicles in domestic fowl. Consequently, feeding chitosan diet may cause follicle growth to lag in domestic fowl. The current study was performed to investigate the relationship between dietary chitosan and ovarian follicle growth in Japanese quail.

Materials and Methods

Animals and Diet

A total 27 one-day-old female Japanese quail chicks (*Coturnix coturnix japonica*) were purchased from a hatchery (Toukai Kigyou Ltd, Toyohashi, Japan) and were subsequently reared in battery cages equipped with an electric heater. At 21 days of age, the birds were divided into three treatment groups with almost the same average body weight, each group having 9 birds, and then they were maintained in individual cages in a controlled environment at 22 ± 3℃ and under 16 hour light and 8 hour dark. Corn-soybean basal diet was formulated to meet or exceed nutrient requirements of National Research Council for Japanese quail[13]. The composition of corn-soybean basal diet using in this experiment was shown in Table 1. Experimental diets were prepared by supplementing the corn-soybean basal diet with chitosan at 0%, 2% and 4% (Wako Pure Chemical Industry Ltd, Osaka, Japan). From 1 day to 20 days of age, the birds were fed the basal diet. There-

Table 1 Compositon of basal diets

Ingredients and analysis	%
Ground corn	58.75
Soybean meal	31.03
Fish meal	8.00
Corn oil	0.36
Dicalcium phosphate	0.13
Ground limestone	1.14
Salt (NaCl)	0.15
Choline chloride	0.07
DL-methionine	0.08
L-threonine	0.09
Vitamin+mineral premix[1)]	0.20
Calculated analyses	
ME (kcal/kg)	2902
Crude protein (%)	24.00
Methionine (%)	0.61
Methionine+cystine (%)	0.90
Lysine (%)	1.40
Linoleic acid (%)	1.62
Calcium (%)	0.88
Available phosphorus (%)	0.36

1) Vitamine + mineral premix supplied the following per kilogram of diet: vitamine A, 7,500 IU; vitamine D, 2,500 IU; vitamine E, 13 mg; menadione sodium bisulfate, 1.0 mg; vitamine B1, 1 mg; vitamine B2, 2 mg; vitamine B6, 1.5 mg; pantothenic acid, 7.5 mg; vitamine B12, 0.002 mg; niacin, 20 mg; folic acid, 0.5 mg; biotin, 0.5 mg; Fe, 0.3 mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg.

after until the onset of lay, the treatment groups were fed the experimental diets. There was free access to food and water. Feed intakes were measured on every 7 days during the experimental period. Animal care and usage was according to a protocol approved by the Animal Care and Use Committee of Kagawa University.

Sampling

Birds were monitored every day and age at lay was determined. At the onset of lay, quail were weighed and blood samples were obtained with heart puncture under ether anesthesia. Immediately the birds were put to death by decapitation and performed laparotomy. Ovary and oviduct samples were removed from their body and weighed respectively. Thereafter five larger follicles were taken from ovary and each follicle was weighed. Serum was separated from the blood sample and then stored at −29 ℃ until the cholesterol concentration was determined. Yolk samples were obtained from first-laid egg and stored at −29℃ to measure the lipid and cholesterol concentrations.

Cholesterol and Lipid Analysis

Serum cholesterol was determined with a commercial enzyme kit (Cholesterol E-Test Wako, Wako Pure Chemical Industry Ltd, Osaka, Japan). Yolk lipid was extracted with the method of Nielsen[12] according to the method of Folch *et al.*[14]. Half gram yolk sample was placed into a 50 ml screw-capped tube in duplicate. Eight ml methanol was added, and the samples were homogenized with a polytron at high speed. To this suspension 4 ml chloroform was added with further homogenizing. The polytron head was washed with 3 ml solution (methanol: chloroform, 2 : 1) that was then added to the homogenate and stored overnight. The following day 3 ml of aqueous salt solution (2.9 g sodium chloride and 0.2 g calcium chloride/l) was added to the homogenate and mixed. After centrifugation the precipitate was again extracted with 5 ml chloroform and centrifugation was performed again. In order to remove the nonlipid components, the two supernatants were combined and added a 5 ml salt solution, which was infused by shaking vigorously. After centrifugation the lipid solution was isolated and made up to 25 ml with methanol. Duplicate aliquots (6 ml) were then taken from the lipid solution and heated under a stream of nitrogen at 50℃ in a block heater to evaporate the solvent. The aliquots were then dried *in vacuo* at room temperature. Once dried, the aliquots were weighed to determine the amount of total lipid.

Duplicate aliquots of 1 ml were taken from the lipid solution for determination of yolk cholesterol. The lipid solution was hydrolyzed by a modification[15] of the method of Heuck *et al.*[16]. The lipid solution was taken into a 10 ml screw-capped tube and hydrolyzed with 50% potassium hydroxide solution (w/v) and absolute ethanol at 60℃ in a water bath for 15 min. After cooling to room temperature, the solution was mixed with hexane by shaking vigorously, followed by adding distilled water. Centrifugation was then performed. Next, an aliquot of the upper phase was taken and heated under a stream of nitrogen at 50℃ in a block heater, and then resolved in ethanol. Using the solution, yolk cholesterol was determined with a commercial enzyme kit (Cholesterol E-Test Wako, Wako Pure Chemical Industry Ltd, Osaka, Japan).

Statistical analysis

Statistical analysis for data was performed by one-way ANOVA[17]. Individual treatment differences were tested by the multiple range test[18]. A probability level of $P < 0.05$ was considered statistically significant.

Results

Body growth and feed intakes during the experimental pe-

Table 2 Body weight, daily gain and feed intake in quail fed diets containing chitosan

Item	Dietary chitosan (%)		
	0	2	4
Body weight (g)			
21 days of age	79.7 ± 1.4	79.1 ± 2.4	79.0 ± 1.9
Days at sexual maturity	134.1 ± 8.6	134.4 ± 7.4	138.4 ± 5.0
Feed intake (g/day)	16.37 ± 2.60	17.13 ± 1.42	16.78 ± 2.77

Mean ± SD of 9 birds

Table 3 Age and reproductive organ weights at the onset of lay in quail fed diets containing chitosan

Item	Dietary chitosan (%)		
	0	2	4
Age at onset of lay (days)	38.3 ± 2.2^{a}	40.8 ± 1.7^{ab}	42.6 ± 3.2^{b}
Ovary weight			
(g)	6.33 ± 1.26^{b}	5.34 ± 0.66^{ab}	4.43 ± 1.12^{a}
(% per body weight)	4.70 ± 0.78^{b}	3.97 ± 0.36^{a}	3.66 ± 0.95^{a}
Oviduct weight			
(g)	5.79 ± 0.59^{a}	5.71 ± 0.74^{a}	5.34 ± 0.26^{a}
(% per body weight)	4.33 ± 0.55^{a}	4.25 ± 0.45^{a}	3.87 ± 0.25^{a}

Mean ± SD of 9 birds
Means with no common superscripts are significantly different ($P < 0.05$).

riod in quail fed diets containing chitosan are shown in Table 2. Body weight at the onset of lay did not vary among birds due to amounts of dietary chitosan; however, the daily gain tended to be less in the 2% and 4% groups than in the 0% group ($P < 0.10$). There were no differences in daily feed intake among birds fed the different diets.

Age and reproductive organ weight at the onset of lay in quail are shown in Table 3. Age at the onset of lay was significantly later for quail fed the 4% chitosan diet than for those fed the 0% chitosan diet. Furthermore, age at lay was delayed with increased chitosan in diet. Ovary weight was significantly lower for birds fed the 4% chitosan diet than for those fed the 0% chitosan diet. Ovary weight also decreased in quail fed diets with increased concentrations of chitosan. There were significant differences in percentage of ovary weight per body weight between the 0% chitosan group and the 2% or 4 % group. In contrast, no significant differences were observed in oviduct weight and the percentage per body weight due to amounts of dietary chitosan. Ovarian follicle weight from the largest follicle to the 5th largest follicle is shown in Figure 1. There are no weight differences in two largest follicle weight between diet groups; however, the 3rd largest follicle tended to be lighter in the 2% and 4% chitosan groups ($P < 0.10$), when compared with the chitosan-free group. The 4th and 5th

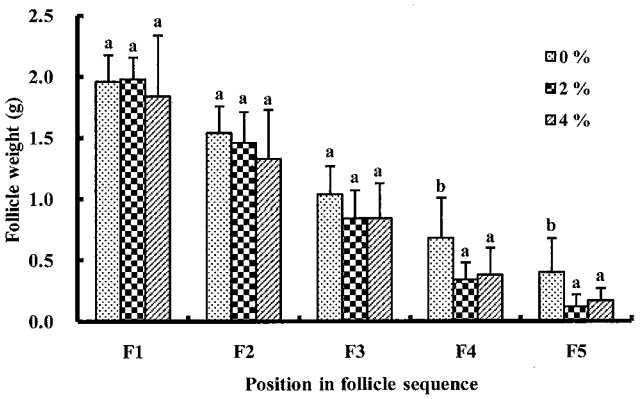

Figure 1 Follicle growth at the onset of lay in female quail fed diets containing chitosan. Bar shows Mean±SD and means with no common superscripts are significantly different ($P < 0.05$).

Table 4 Cholesterol and/or lipid concentrations in yolk and serum at the onset of lay in quail fed diets containing chitosan

Item	Dietarychitosan (%)		
	0	2	4
Yolk			
Cholesterol (mg/g)	12.74 ± 2.85	14.19 ± 1.65	12.81 ± 0.87
Lipid (mg/g)	319.8 ± 15.0	324.3 ± 8.8	326.2 ± 13.3
Serum			
Cholesterol (mg/dl)	248.9 ± 106.7	177.1 ± 49.1	179.8 ± 36.8

Mean±SD of 9 birds

largest follicle weights were significantly lighter in the 2% and 4% chitosan groups.

As shown in Table 4, there were no differences in yolk lipid concentration of egg between the diet groups, and the yolk cholesterol concentration did not also vary among birds due to the amounts of dietary chitosan. Serum cholesterol concentration tended to be lower in the 2% and 4% chitosan diet groups than in the 0% chitosan diet group ($P < 0.10$).

Discussion

It is not well known that dietary chitosan influences reproductive function in domestic fowls. In this study, age at the onset of lay of Japanese quail was later with increasing the amounts of chitosan in diets (Table 3). Body weight at the onset of lay, on the other hand, did not vary among birds due to the amounts of chitosan in diets (Table 2). It is suggested that the attainment of some minimum body weight is required for the onset of lay in female chicken[19, 20] and female Japanese quail[21]. Therefore, it seems that dietary chitosan may delay age at the attainment of this minimum body weight required for the

lay and consequently age at the onset of lay. Feeding chitosan diets to chicken is reported to reduce body weight[4]. In the present study, also, daily body gain tended to be decreased with dietary chitosan (Table 2).

In the present study, age at the onset of lay was later in birds fed diets containing chitosan (Table 3). The fact indicates that the growth of ovarian follicle is delayed by chitosan, since the onset of lay is generally dependent on the growth of follicle in domestic fowl. At the onset of lay, moreover, dietary chitosan lowered ovary weight (Table 3), and although dietary chitosan did not change the largest and 2nd largest follicle weights, it tended to lower or lowered the 3rd, 4th and 5th largest follicle weights, respectively (Figure 1). Thus, from these results feeding chiotsn diets is considered to depress the follicle growth of ovary during the process of sexual maturity in female quail.

Cholesterol is essential to ovarian follicle growth in domestic fowl, since it is an important component in the follicles. It is showed that dietary chitosan significantly reduces plasma or serum cholesterol concentration in broiler chicken[2, 4] and rats[6]. In this study, feeding chitosan diets to female quail tended to decrease serum cholesterol concentration at the onset of lay ($P < 0.10$; Table 4), being similar to results reported in laying chicken[22]. Maezaki *et al.*[5] suggested that chitosan excreted bile acids into the feces and decreased the re-absorption of the bile acids in humans. Consequently, they stated that the cholesterol pool in the body was decreased. Accordingly, the depressed ovarian follicle growth of quail fed the chitosan diets (Table 3, Figure 1) may be due to insufficient supply of cholesterol.

Feeing chitosan diet did not change yolk cholesterol concentration of egg in quail (Table 4). The result suggests that yolk cholesterol concentration is not regulated by dietary chitosan in female quail. On the other hand, yolk cholesterol concentration is showed to be decreased by feeding chitosan diet to laying hen[22]. Such conflicting results on dietary chitosan may be partially due to variation in the stage of egg production and difference in species. It remains to be solved. Also, dietary chitosan did not vary yolk lipid concentration in quail (Table 4), supporting the result reported in laying hen that chitosan diet did not influence yolk lipid concentration in egg[22]. The results indicate that dietary chitosan changes the concentrations of yolk lipid and cholesterol in quail.

In this study, I indicated dietary chitosan depressed the growth of ovarian follicles and consequently delayed age at sexual maturity in quail. In addition, dietary chitosan did not change the concentrations of yolk lipid and cholesterol. These findings suggested that dietary chitosan depressed ovarian follicle growth without changing yolk lipid and cholesterol concentrations in Japanese quail.

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キトサン添加飼料の給与がウズラの卵胞発育に及ぼす影響

橋口峰雄

要　　約

メスウズラに２％と４％のキトサンを添加した飼料を21日齢から性成熟（産卵開始）まで給与し，キトサンが性成熟の発現と卵胞の発育に及ぼす影響を調査した．性成熟到達日齢はキトサンの投与により有意に遅延した．しかし，性成熟時における体重はキトサンの投与量により差異はみられなかった．卵巣重量はキトサンの投与により低くなり，また第３から第５番目に大きい卵胞の重量はキトサンの投与により軽くなった．卵黄の脂肪とコレステロール濃度はキトサンの投与により変化しなかった．これらのことから，キトサンはウズラの卵胞の発育を抑制するが，卵黄の脂肪とコレステロール濃度には影響しないことが示唆された．