

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

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Effects of Chicory Fructans on Egg Cholesterol in Commercial Laying Hen

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Abstract: Supplementing layer diets with oligofructose (1.0% (w/w) Raftifeed@OPS) and 1.0% (w/w) inulin (in the form of 1.3% (w/w) Raftifeed@IPE) reduced ($P<0.05$) yolk cholesterol concentrations on average by 18.64% and 16.44%, respectively, when compared to the control. However, greater reductions in yolk cholesterol content were calculated. They were 20.68% and 22.39%, on average, for oligofructose and inulin, respectively. These prebiotics reduced ($P<0.05$) laying hen's blood serum cholesterol by 17.75% and 16.23%, respectively. At the same time, oligofructose and inulin increased ($P<0.05$) cholesterol concentrations of the small intestinal (jejunum) contents and total cholesterol excretion from the fecal material in layers compared with those of the controls. In studies of the linear relations of cholesterol concentrations in each pair of yolk, serum, jejunum contents, and fecal materials, the results indicated that lowered concentrations of yolk cholesterol might be attributed to lowered serum cholesterol that results from a greater content of unabsorbable cholesterol in jejunum contents and more cholesterol excretion. Hence, supplementation of a basal laying hen diet with either oligofructose or inulin reduced yolk cholesterol and may offer a way to produce eggs with lowered yolk cholesterol.

Key words: Cholesterol, yolk, serum, small intestinal (jejunum) content, fecal material, prebiotic

Introduction

It was assumed that a high concentration of cholesterol in the diet leads to a raised cholesterol level in blood serum and that, in turn, this exposes the consumer to the risk of arteriosclerosis and coronary heart disease (Grundy, 1990). As stated by Holden *et al.* (1989), the average cholesterol content of one large egg is 208 mg. Kritchevsky and Kritchevsky (2000) recommended that people should limit the consumption of eggs because of their high cholesterol content. Hence, the cholesterol scare may have created a severe negative influence on consumers' attitudes toward eggs.

A prebiotic has been defined as "a non-digestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995). Inulin and oligofructose are considered the archetypal prebiotics, and they occur naturally in many food plants. Inulin is extracted from chicory roots with hot water. It is composed of linear chains of $\beta(2-1)$ linked fructose molecules. The chain length of inulin varies between 2 and 65 fructose moieties, the average chain length is 10. Raftifeed@IPE is a spray-dried, unrefined chicory extract, and contains 74% (w/w) inulin. The remaining 26% is composed of mono- and disaccharides, mineral, and organic acids. Oligofructose is a partial enzymatic hydrolysate of inulin. The chain length varies between 3 and 8, with an average chain length of 4. Raftifeed@OPS is a refined oligofructose and contains >93.5% oligofructose, and the rest is mono-

and disaccharides (Van Loo *et al.*, 1995). Inulin and oligofructose are not digested by the enzymes of vertebrates in general, and humans (Ellegard *et al.*, 1997) and birds in particular. As such, they are completely available for fermentation by intestinal flora. The unique properties of inulin and oligofructose are that they selectively stimulate the growth of bifidobacteria, lactobacilli, and certain butyrate-producing bacteria (Hold *et al.*, 2003). At the same time, they suppress the growth of proteolytic bacteria such as the *Clostridium perfringens* group (Gibson *et al.*, 1995). Similar observations on gut flora were made in poultry (Oyarzabal and Conner, 1996). These properties (non-digestibility and selective interaction with intestinal bacteria) are considered to be at the basis of their use in animal feed. Recently, a serum cholesterol-lowering effect of inulin and oligofructose has been reported for some animal and human studies (Fiordaliso *et al.*, 1995; Diez *et al.*, 1997; Davidson *et al.*, 1998). A hypocholesterolaemic effect of certain soluble plant fiber material may be due to the increased neutral steroid and bile acid excretion or increased synthesis of fermentation by-products like propionic acid which, in turn, may decrease cholesterol synthesis in the liver (Chen *et al.*, 1984). Beyer and Jensen (1993) indicated a reduction of serum cholesterol and lipoprotein without concomitant reduction of egg cholesterol by adding dietary sorbose to the layers' diet. A reduction of serum cholesterol and abdominal fat of broilers was reported by supplementing inulin and oligofructose into broilers

diets (Yusrizal and Chen, 2003). Few, if any, reports evaluating the cholesterol metabolism in layers are available. Hence, the potential hypocholesterolaemic effects of oligofructose and inulin are of interest.

Egg consumption has decreased significantly since 1960 (United States Department of Agriculture, 1994). Bell (1999) indicated that in the USA, annual egg consumption per capita dropped by 125 eggs between 1955 and 1999. He also supposed that only 20% of this decrease was attributable to the cholesterol scare and more than \$10 billion loss in reduced sales was estimated for the egg industry because the cholesterol issue emerged. Thus, the egg industry is working hard to offer consumers low-cholesterol eggs. However, to the knowledge of the authors, no report of the effects of prebiotics on egg cholesterol content has appeared. The purpose of the current study was to assess the effects of commercial prebiotics on cholesterol metabolism in layers and the production of eggs with lower yolk cholesterol content.

Materials and Methods

Birds and diets: Two weeks prior to the feeding trial, White Leghorn layers with similar body weight and health conditions were selected. After selection, 60 White Leghorn hens at 57 wk of age were obtained and divided randomly into three groups. Twenty birds were assigned to each of the following diets, with two birds per cage.

- 1) Basal diet (antibiotic and wheat-free regular diet) (Table 1).
- 2) Basal diet with 1.0% (w/w) oligofructose containing at least $\geq 93.2\%$ β (2-1) fructans (Raftifeed@OPS, Orafti, Belgium) with degrees of polymerization (DP) varying between 2 and 8, the average is 4, and the remaining material is a mixture of glucose, fructose, and sucrose.
- 3) Basal diet with 1.0% (w/w) chicory inulin (DP ranging between 3 and 65; average 10) in the form of a semi-purified chicory root extract containing 74% inulin (Raftifeed@IPE, Orafti, Belgium). The remaining material contains minerals, organic acids, and lower-DP sugars (fructose, glucose, and sucrose).

The prebiotic supplementation was added manually and mixed into the basal diet and stored for less than 3 d. Both feed and water were provided *ad libitum*. A regimen of 16 h light:8 h dark/d was provided throughout the study, which lasted for 4 wk. Eggs were collected daily between 8:00 and 8:30 a.m.

Sample collections: Eggs and fecal material per cage in each treatment were collected on every seventh day for 28 d. Fecal material was collected for 24 h and used for fecal analyses. At the end of experiment, one layer per cage from each treatment was tested for serum cholesterol and small intestinal (jejunum) cholesterol

Table 1: Major ingredients of the basal layer diet

Ingredients	Composition (g/kg)
Corn	676
Soybean meal	190
Dicalcium phosphate	22
Limestone	90
dl-Methionine	0.5
Vitamin mixture	3.5
Salt	5
Fat	13
Calculated nutrient content	
Crude protein (g/kg)	150
Metabolizable energy (MJ/kg)	11.99

contents. About 5 ml of blood was collected in VACUTAINER® tubes (non-anticoagulant inclusion) (Becton Dickinson VACUTAINER Systems, Franklin Lakes, NJ) and left for 1 h. The tubes were centrifuged at 4000 rpm for 30 minutes to collect serum.

Cholesterol concentration analyses

Yolk: An enzyme method described by Pasin *et al.* (1998) was used to determine the cholesterol concentrations. Liquid egg yolk (3 g) was dispersed in 27 ml of 2% (w/v) NaCl and shaken at room temperature at 150 rpm (Junior Orbit Shaker cat. no. 3520, Lab-line Instruments Inc., Melrose Park, IL) for approximately 2 h in a tightly capped flask to prevent evaporation. A 1 ml sample of solubilized yolk was further diluted 10-fold with 2% (w/v) NaCl and used as a working sample. The test sample contained 0.5 ml of 2% (w/v) NaCl, 5 ml of commercial diagnostic cholesterol reagent (Sigma diagnostic cholesterol reagent procedure no. 401, Sigma Chemical Co., St Louis, MO) and 0.05 ml of working sample. Test samples were incubated for 5 min at 37°C and absorbance was read at 500 nm using a spectrophotometer. Final cholesterol concentrations were calculated by comparison with a standard (200mg/dl of Cholesterol Calibrator, Sigma Chemical Co., St Louis, MO).

Fecal material and intestinal (jejunum) contents: Fecal material or small intestinal (jejunum) contents were mixed homogeneously. The cholesterol concentrations were determined as described by Pasin *et al.* (1998), but yolk was replaced by fecal material or small intestinal (jejunum) contents.

Serum: One drop of serum was placed into a diagnostic cholesterol slide (CHOL DT Slides, Ortho-Clinical Diagnostics INC., Rochester, NY). The serum cholesterol content was analyzed by using an Ektachem DT60 calcium analyzer (Eastman Kodak Company, Rochester, NY).

Statistical analysis: The experiment was conducted

Table 2: Yolk weight, yolk cholesterol concentration, and yolk cholesterol contents as affected by dietary addition of oligofructose and inulin (mean values for 8 observations)

Parameter	Treatment		
	Control	Oligofructose	Inulin
Yolk weight (g/egg)			
Initial	16.66 ^a	17.68 ^a	17.55 ^a
1st week	16.83 ^a	17.48 ^a	16.01 ^a
2nd week	17.02 ^a	16.82 ^a	15.95 ^a
3rd week	17.95 ^a	16.78 ^a	15.87 ^a
4th week	17.59 ^a	16.51 ^a	16.75 ^a
Yolk cholesterol concentration (mg/g)			
Initial	14.03 ^a	14.00 ^a	14.03 ^a
1st week	13.09 ^a	10.19 ^b	10.27 ^b
2nd week	12.70 ^a	10.28 ^c	11.67 ^b
3rd week	13.22 ^a	11.41 ^b	10.97 ^b
4th week	11.32 ^a	9.16 ^b	9.16 ^b
Yolk cholesterol content (mg/egg)			
Initial	247.77 ^a	247.52 ^a	246.23 ^a
1st week	221.03 ^a	178.12 ^b	164.42 ^b
2nd week	216.15 ^a	173.04 ^b	186.10 ^b
3rd week	237.30 ^a	191.46 ^b	173.07 ^b
4th week	199.12 ^a	151.25 ^b	153.37 ^b

^{a,b} Mean values in a horizontal row with different letters were significantly different (least-squares difference test; P<0.05).

Table 3: Cholesterol levels of fecal material and total cholesterol excretion (mg) as affected by dietary oligofructose and inulin (mean values for 8 observations)

Week	Cholesterol levels in fecal material (mg/g)			Total fecal cholesterol excretion (mg/bird)		
	Control	Oligofructose	Inulin	Control	Oligofructose	Inulin
1	4.00 ^b	4.94 ^a	5.41 ^a	273.85 ^b	311.78 ^b	387.68 ^a
2	3.54 ^a	4.31 ^a	5.18 ^a	276.41 ^b	332.20 ^{ab}	360.35 ^a
3	3.98 ^c	7.43 ^a	5.18 ^b	210.39 ^b	431.74 ^a	336.49 ^a
4	2.51 ^b	3.98 ^a	4.58 ^a	177.67 ^b	313.55 ^a	279.39 ^a

^{a,c} Mean values in a horizontal row with the same parameter with different letters were significantly different (least-squares difference test; P<0.05).

using a completely random design (CRD) (Steel and Torrie, 1980). Data were analyzed using analysis of variance (ANOVA) (SAS Institute, Inc., 1993). A significant difference was used at the 0.05 probability level, and differences between treatments were tested using the least significant difference (LSD) test (Freud and Wilson, 1997). Linear relations of each pair of cholesterol concentrations in yolk, serum, small intestinal (jejunum) contents, and fecal materials were calculated using a linear regression. Regression parameters at 0.05 probability were considered significant. All statistical analyses of data were performed using SAS (SAS Institute, Inc., 1993).

Results and Discussion

Yolk cholesterol and weight: Supplementing a layers' diet with chicory oligofructose or inulin reduced (P<0.05) the cholesterol concentrations and cholesterol amount per yolk over a 4-wk feeding trial compared with feeding

the basal diet (Table 2). Oligofructose-type dietary supplementation reduced (P<0.05) yolk cholesterol concentrations by 22.16, 19.05, 13.72 and 19.08%, respectively, over the 4-wk trial when compared with controls. A similar lowering effect for the inulin dietary supplementation was observed (21.57% 1 wk; 8.16% 2 wk; 16.99% 3 wk; 19.08% 4 wk). The reduction of cholesterol content for both oligofructose and inulin were calculated (20.68% and 22.39%, respectively). There was no difference (P >0.05) among the control and two prebiotic groups with respect to the mean yolk weight (Table 2).

Serum cholesterol: At the end of a 4-wk feeding trial, supplementing layers with oligofructose and inulin reduced (P<0.05) mean serum cholesterol (Fig. 1). Concentrations of serum cholesterol in oligofructose-fed and inulin-fed groups were 17.75% and 16.23% lower (P<0.05) than controls, respectively. A similar lowering

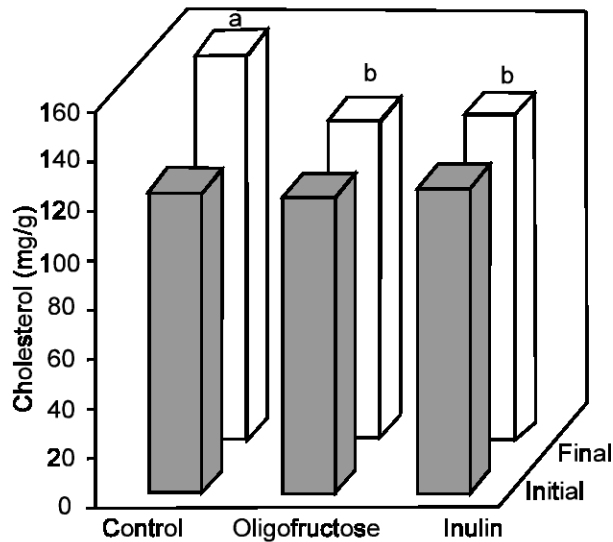


Fig. 1: Serum cholesterol concentration as affected by dietary oligofructose and inulin. Mean values in the initial or final of experiment with different letters were significantly different (least-squares difference test; $P < 0.05$).

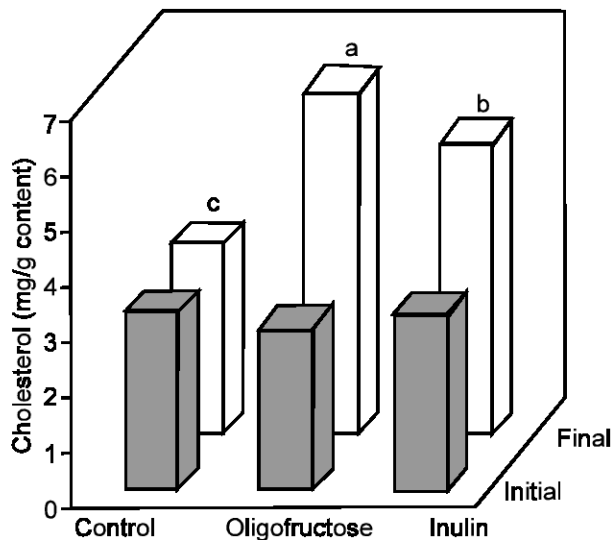


Fig. 2: Cholesterol concentration in jejunum content as affected by dietary oligofructose and inulin a-c Mean values at the start (initial) or end (final) of experiment with different letters were significantly different (least-squares difference test; $P < 0.05$).

effect of serum cholesterol by supplementing inulin and oligofructose into broiler diet has been reported (Yusrizal and Chen, 2003). Unfortunately, no relation between yolk and serum cholesterol concentrations was available. However, it might be hypothesized that lowered serum cholesterol concentration results in lower yolk cholesterol concentration (Table 2 and Fig. 1).

Cholesterol in fecal material and intestinal (jejunum) content:

Oligofructose and inulin resulted in higher ($P < 0.05$) cholesterol concentrations in excreted fecal material than those of the control group (Table 3). Meanwhile, higher ($P < 0.05$) total fecal cholesterol excretion per bird was obtained in the inulin group when compared with those of control. The oligofructose group showed the higher ($P < 0.05$) cholesterol excretion after 2 wk of feeding. At the end of the experiment, layers fed oligofructose showed the highest ($P < 0.05$) concentration of cholesterol in the jejunum contents, followed by inulin-fed and the control group (Fig. 2). A similar result has been reported by Vanhoof and De Schrijver (1995). They indicated that addition of unprocessed and baked inulin with 1% cholesterol and 0.1% cholic acid to rat's diet led to a higher fecal output of cholesterol. The higher concentration of cholesterol in jejunum contents might be interpreted as cholesterol uptake by lactobacillus or as coprecipitation of cholesterol with deconjugated bile salts (Gilliland *et al.*, 1985). Therefore, we speculated that an increase in cholesterol excretion might be due to the unabsorbable cholesterol in jejunum contents.

Linear relations on cholesterol concentrations in different test parameters:

Positive linear relationships between the concentrations of serum and yolk cholesterol ($P < 0.05$, $R^2 = 0.6453$), and the concentrations of cholesterol in fecal materials and in jejunum contents ($P < 0.05$, $R^2 = 0.6135$) are shown in Fig. 3a and e. These results indicated that higher concentrations of serum cholesterol are coupled with higher concentrations of yolk cholesterol, and add support to our previous hypothesis, that higher concentrations of cholesterol in the jejunum content might reflect higher concentrations of cholesterol in the fecal material. Either the relationship between the concentrations of cholesterol in jejunum contents and in the yolk ($P < 0.05$, $R^2 = 0.6479$) (Fig. 3b) or between the levels of cholesterol in fecal material and in the yolk ($P < 0.05$, $R^2 = 0.4390$) (Fig. 3c) indicated higher concentrations of cholesterol in jejunum contents or fecal material corresponded with lower concentrations of cholesterol in the yolk. Moreover, two negative linear relationships, (1) between the concentrations of cholesterol in jejunum content and the concentration of serum cholesterol ($P < 0.05$, $R^2 = 0.6319$) (Fig. 3d), and (2) between the concentration of cholesterol in fecal materials and the concentration of serum cholesterol ($P < 0.05$, $R^2 = 0.7659$) (Fig. 3f.) were analyzed statistically. However, fewer studies related to cholesterol metabolism in layers were available. Therefore, the linear relations reported here support our previous hypotheses, that lower concentrations of serum cholesterol are due to higher concentrations of unabsorbable cholesterol in jejunum contents and higher concentrations of cholesterol in fecal materials,

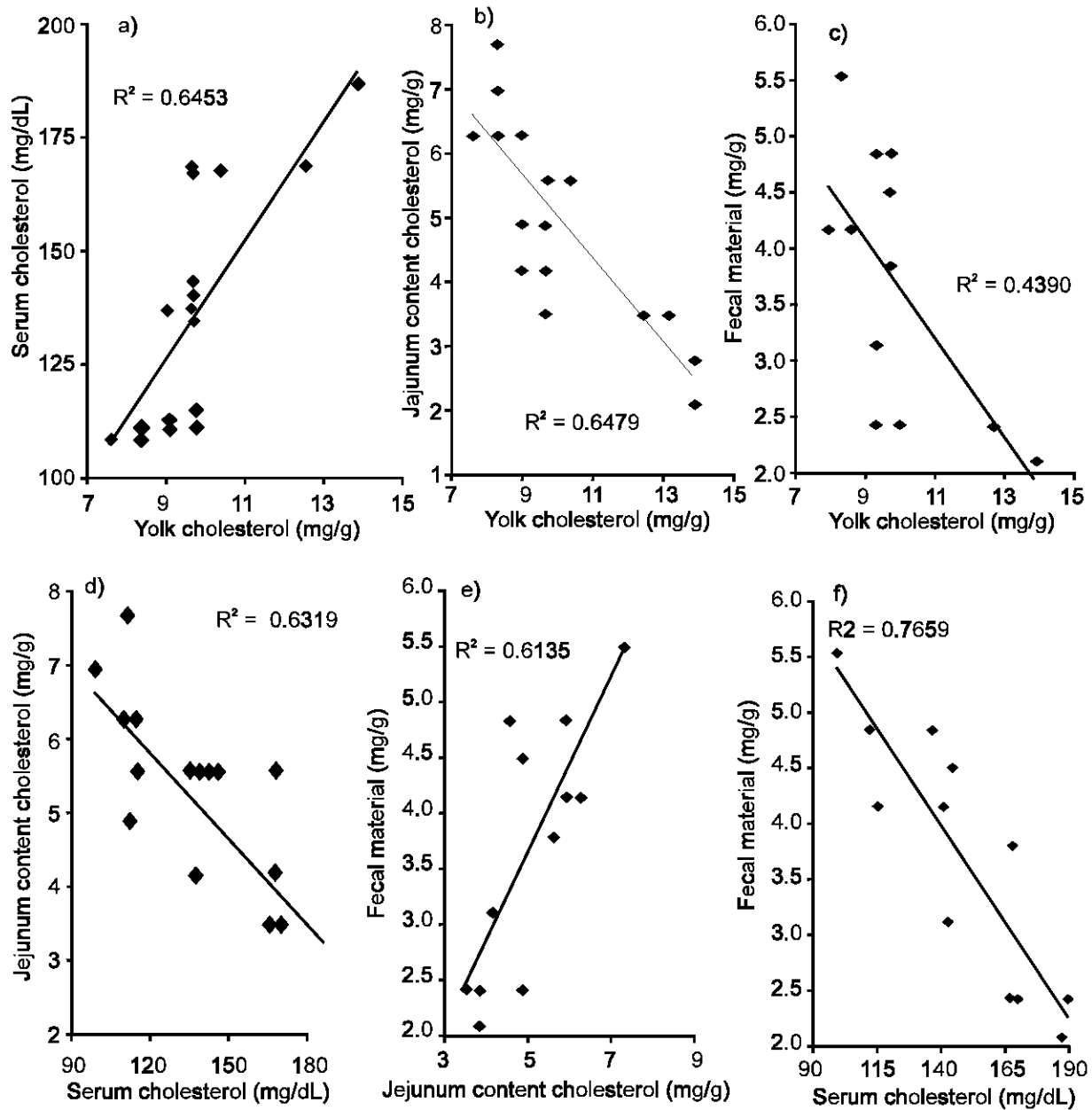


Fig. 3: Linear relations on cholesterol concentration of test parameters. (a) Serum versus yolk; (b) jejunum content versus yolk; (c) fecal material versus yolk; (d) jejunum content versus serum; (e) fecal material versus jejunum content; (f) fecal material versus serum

and suggested that production of eggs with a lower yolk-cholesterol might result from several factors, such as a higher concentration of unabsorbable cholesterol in jejunum contents and fecal materials, and a lower concentration of serum cholesterol when adding oligofructose-type and inulin-type prebiotic dietary supplementations in the layer diet.

Summary: Lowering cholesterol content should be

helpful in the marketing of eggs and egg products. Layers fed diets supplemented with either chicory oligofructose or inulin can produce low-cholesterol eggs, while serum cholesterol concentrations could be reduced by feedig either one of these prebiotic dietary supplementations. Moreover, oligofructose and inulin prebiotic dietary supplementations resulted in higher concentrations of unabsorbable cholesterol in the jejunum contents and more cholesterol excretion. The

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results of this study provide further evidence that lowered concentrations of cholesterol in the yolk and in serum seem to be related to the concentration of unabsorbable cholesterol in jejunum contents and cholesterol excretion. Future research should focus on elucidating the exact mechanism underlying this observation.

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