Chitosan as an Ingredient for Domestic Animal Feeds

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Chitosan was tested for use as an ingredient for domestic animal feeds. (1) No abnormal symptom was observed with hens and broilers by feeding <1.4 g of chitosan/kg of body weight per day for up to 239 days, and with rabbits by feeding <0.8 g of chitosan/kg of body weight per day for up to 239 days. (2) Both chitin and chitosan were digested 35–83% by rabbits and 88–98% by hens and broilers. (3) An increase in the serum cholesterol, triacylglycerol, and free fatty acid values of these domestic animals fed cholesterol-additive diets was suppressed by feeding 1.2–1.4 g of chitosan/kg of body weight per day for hens and broilers and 0.7–0.8 g of chitosan/kg of body weight per day for rabbits, but not by feeding chitin. (4) Hen's appetite and egg-laying rate decreased by feeding an excessive amount of chitosan for a long term (3.6–4.2 g of chitosan/kg of body weight per day for 189 days) because of incomplete digestion of chitosan.

Chitin is (1→4)-linked 2-acetamido-2-deoxy-β-D-glucan, and chitosan is an N-deacetylated product of chitin. Chitin and chitosan are nontoxic (Arai et al., 1968). Chitosan has hypocholesterolemic activity (Sugano et al., 1978, 1980, 1988; Nagyvary et al., 1979; Kobayashi et al., 1979; Vahouny et al., 1983), immunoadjuvant activity, and protecting activity against disease infection (Suzuki et al., 1982, 1984; Nishimura et al., 1984) as demonstrated in rats. An enhancement of lactose digestion by feeding chitosan is also demonstrated in broilers (Austin et al., 1981). These reports suggest that chitosan is a useable ingredient for domestic animal feeds, but little is known about the biological safety, digestibility, and functionality of chitosan by its oral administration for long term in other domestic animals.

We now report that chitosan is safe, digestible, and hypolipidemic at an appropriate dosage by oral administration in rabbits, hens, and broilers, but feeding of excessive chitosan brings about physiological disorders because of incomplete digestion of chitosan.

MATERIALS AND METHODS

Crab shell chitosan (Flonac-N) was offered from Kyowa Yushi, Chiba, and the degree of substitution was 0.25 for the *N*-acetyl group. *N*-Stearoylchitosan was prepared in our laboratory by N-acylation of chitosan with stearic anhydride (Hirano et al., 1976).

Animals and Feeding. Rabbits, hens, and broilers were raised with fresh drinking water freely. As basal diets (A), a commercial granular diet was used for feeding rabbits and commercial mealy diets were used for feeding hens and broilers. Cholesterol content in these basal diets was analyzed, and cholesterol content was adjusted by addition of cholesterol.

Male rabbits weighing 2.4–2.9 kg (Kitayama Rabesu, Kyoto) were individually raised in cages by feeding 100 g of a basal diet (A)/rabbit per day, and the diet (0.2% cholesterol) (Labo-R-Stock; Nihon Nosan Industry Corp., Kanagawa) consisted of 8% moisture, 17% crude protein, 4% crude fat, 17% crude fiber, 10% ash, and 44% nitrogen-free extract. Chitosan-supplemental diets (A + 1–5% S) were prepared: chitosan (1.0–5.0 g) was dissolved in aqueous lactic acid (0.9 g of lactic acid in 30 mL of distilled water), and the diet (94–98 g) was added to the solution. The mixture was stirred for few seconds, chitosan adsorbed into the granular diet, and the diet air-dried at room temperature. A cholesterol-additive diet (A + 0.7% C) was prepared: cholesterol (0.7 g) was partially dissolved in corn oil (1.0 g), and to the suspension was added a commercial granular diet (98.3 g). The mixture was stirred for a few minutes, and cholesterol and corn oil were adsorbed into the granular diets. Chitosan-supplemental and cholesterol-additive diets (A + 0.7% C + 1–2% S) were prepared by treating the chitosan-supplemental diet with cholesterol–corn oil as described above. A chitin-supplemental and cholesterol-additive diet was prepared by coating 94 g of granular diet (A + 0.7% C) with a chitin-suspended starch paste (2 g of chitin was suspended into 2 g of starch dissolved in 30 mL of boiling water).

Five-month-old egg-laying hens (white leghorn) weighing 1.2–1.4 kg were raised in groups of two to five on a flat floor by feeding 70–100 g of a basal diet (A)/hen per day, and the feed (0.2% cholesterol) (egg mash 17; Kinki Kumiai Corp., Osaka) consisted of 9% moisture, 17% crude protein, 4% crude fat, 5% crude fiber, 13% ash, and 52% nitrogen-free extract. A cholesterol-additive diet (A + 0.7% C) was prepared by mixing 0.7% cholesterol with A. Chitosan-supplemental diets were prepared by mixing A or A + 0.7% C with the corresponding amount of powder chitosan. A chitosan-supplemental and cholesterol-additive diet was prepared by mixing chitosan and cholesterol with A.

White broilers (Tottori livestock breeding station, Tottori) were raised in groups of five to six. Three basal diets A1 (0.8% cholesterol), A2 (1.1% cholesterol), and A3 (1.5% cholesterol) were obtained from Kinki Kumiai Corp. These diets consisted of 9% moisture, 18–22% protein, 4% crude fat, 5% crude fiber, 8% ash, and 52–56% nitrogen-free extract. After being raised in an incubator at 35 °C by feeding 1.4–55 g of A1/chicken per day from hatch to day 19, chickens were raised at room temperature on a flat floor from the day 20 to 60 by feeding 62–120 g of A2/chicken per day and from the day 61 to 85 by feeding 120 g of A3/broiler per day. Cholesterol-additive diets (1.1–1.8% cholesterol) were prepared by adding 0.3% cholesterol to each of the basal diets (A1, A2, A3). Chitosan-supplemental diets, and chitosan-supplemental and cholesterol-additive diets were prepared as described in hen's diets.

Acid Hydrolysis of Feces. Fresh feces were collected daily and air-dried at 100 °C for 5 h, followed by drying in a vacuum oven over P_2O_5 at 100 °C for 24 h. A portion (20 mg) of the

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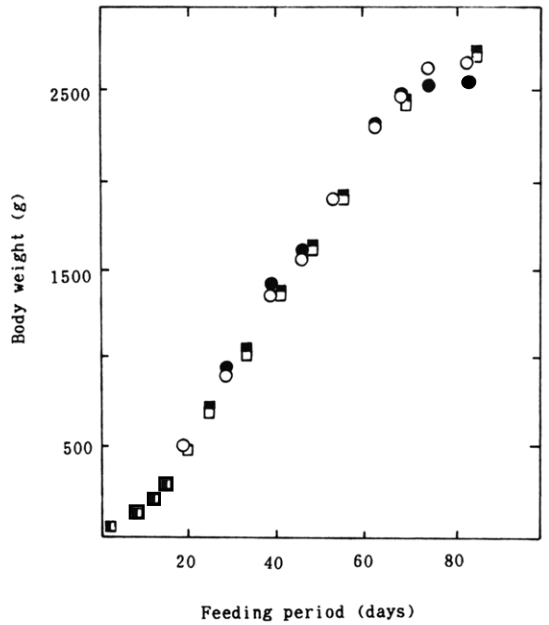

Figure 1. Comparative growth in body weight from chickens to broilers by feeding chitosan: ○, commercial diets (A1 + A2 + A3); ●, A1 + A2 + A3 + 2% S; □, A1 + A2 + A3 + 0.3% C; ■, A1 + A2 + A3 + 2% S + 0.3% C. Each of the values is the average of three broilers.

dry feces was suspended in 4 mL of 6 N HCl in a sealed glass tube under nitrogen gas, kept at 50–60 °C for 6 h, and then heated at 100 °C for 10 h. The hydrolysates were used for analysis for hexosamine by the Blix–Gardell method (Blix, 1948) with D-glucosamine hydrochloride as a standard. The digestibility was calculated by the equation [(D-glucosamine calculated from chitosan added to diet–hexosamine excreted in feces)/(D-glucosamine calculated from chitosan added to diet)] × 100.

Analyses of Cholesterol, Triacylglycerol, and Free Fatty Acids. A portion (3–4 mL) of blood was drawn from a caudal auricular vein of rabbits and from an ulnar cutaneous vein of hens and broilers. The portion of blood was put into a small plastic centrifuging tube, kept at 4 °C for 20–30 min, and centrifuged at 1000*g* at 4 °C for 20 min to give serum. A portion (2.0 g) from the fresh livers and muscles of rabbits and broilers and hen's thigh and pectoral muscles was homogenized in 20 mL of chloroform–methanol (2:1, v/v) by a homogenizer (Type PED2, Masuda Rika, Osaka), and the mixture was centrifuged at 1000*g* for 20 min at 4 °C to give a supernatant. The total cholesterol value of the supernatant was determined by the Cholesterol-B-Test, the triacylglycerol value by the Triacylglyceride-Test, and the free fatty acid value by the NEFA C-Test-Wako (Wako Pure Chemical Industries, Ltd., Osaka). The analytical value was expressed by the average of values from two to five animals.

RESULTS AND DISCUSSION

Biological Safety. We tested the growth, appetite, and appearance of these domestic animals; the color, size, weight, and fatty deposit of animal livers; and the ratio of animal live/body weight. No abnormal symptom was observed with hens by feeding 1.2–1.4 g of chitosan/kg of body weight per day and with rabbits by feeding 0.7–0.8 g of chitosan/kg of body weight per day for up to 239 days. The feeding of 2% chitosan did not significantly affect the growth rate of chickens (Figure 1). However, a decrease in their egg-laying rate and appetite was observed with these five hens by feeding an excessive amount of chitosan for a long term (3.6–4.2 g of chitosan/kg of body weight per day for 140 days or 14–18 g of chitosan/kg of body weight per day for 56 days) due to incomplete digestion of chitosan (Figure 2). One of the

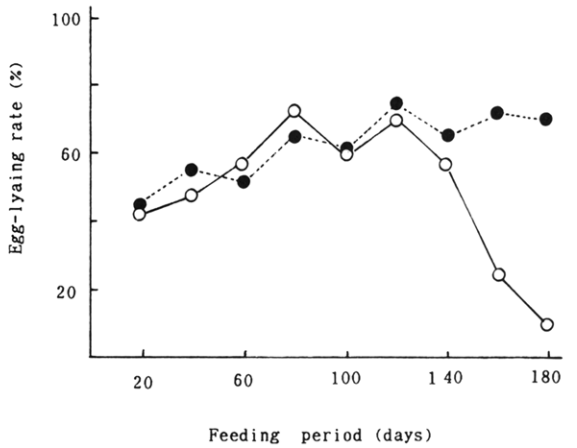

Figure 2. Effect of the feeding of chitosan on hen's egg-laying rate: commercial diet (A) as a control; A + 5% S. The egg-laying rate (%) was calculated by the equation (laying egg number/(number of hens × feeding days) × 100. Each of the groups consisted of five hens.

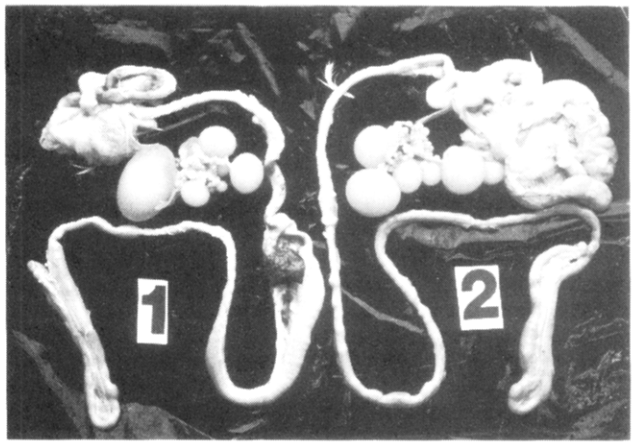

Figure 3. Anatomical view of the digestive organs of hens: 1, the physiologically disordered hen; 2, a normal hen (see text for details).

hens in the latter group was anatomized and compared with a normal hen. The whole body was thin, and the thigh muscles were about 50% smaller than that of control hens. A constriction was found in Meckel's diverticulum part of the jejunum, and the upper part of the intestinal canal was distended up to the duodenum filled with dark-green diet residues containing chitosan (Figure 3). No visible injury was found on the surface of mucous membranes in the constriction and distended parts. The liver weight and color were normal. The hen's mature ovarian follicles were about 50% low in number and were slightly opaque, and a mature egg having no hard egg shell was present in a tubovaginal part. However, the physiological trouble of hens disappeared within 1 week after the feeding of chitosan was stopped.

Digestibility. The weight of dry feces did not significantly increase by feeding 2% chitosan in rabbits for 15 days and by feeding 6–7% chitosan in broilers for 69 days (Table I). Chitosan could not be extracted with aqueous acetic acid or with formic acid from these animal's feces because of strong polyelectrolyte complexes as demonstrated in chitosan–heparin complexes (Nakajima and Sata, 1974). The cationic amino group of chitosan is known to inhibit the acid hydrolysis of glycosidic linkages (Horton, 1969). N-Acetylation of chitosan in the polyelectrolyte feces by stirring with acetic anhydride in aqueous acetic acid–methanol was also unsuccessful (Hirano et al., 1976). Therefore, the dry feces were directly hydro-

Table I. Digestibility of Chitin, Chitosan, and *N*-Stearoylchitosan by Rabbits, Hens, and Broilers

supplement (%)	basal diet[a]	feeding period, days	digestibility, % rabbits	hens	broilers
chitin (2)	A	25	35		
		12		92	
chitosan (2)	A	5	41 [28 ± 4][b]		
		15	82 [24 ± 5]		
		39	83		
chitosan (5)	A	12		98	
chitosan (10)	A	12		67	
chitosan (6)	A2	32			88 [32]
chitosan (7)	A2 and A3	69			94 [35]
chitosan (20)	A3	12			67
N-stearoyl-chitosan (5)	A	12		0	

[a] Key: A, basal diets for rabbits and hens; A2 and A3, basal diets for broilers. See text for details. [b] The average value in the weight of dry feces (grams/head per day) is shown in brackets as calculated from three individuals for rabbits and from a group of five for broilers.

lyzed with 6 N HCl at 100 °C in a sealed tube under nitrogen gas, and the degrees of acid hydrolysis were 62 ± 3% for chitosan and 79 ± 5% for chitin under the present conditions. Based on these degrees of hydrolysis, the digestibility of chitin and chitosan by these domestic animals was estimated, and the digestibility varied 25–38% for chitin and 41–83% for chitosan by rabbits and 67–98% for chitin and chitosan by both hens and broilers (Table I). However, *N*-stearoylchitosan was not digested in agreement with an in vitro experiment (Hirano and Yagi, 1980). Chitosan was more digestible than chitin by rabbits. The digestibility of chitosan was 41% on day 5 and 82% on day 15, indicating an increase in the digestibility of chitosan with adaptation to feeding. The digestion probably occurs by hydrolysis of chitosan by chitinase and chitosanase, which are secreted from intestinal bacteria. In addition, plant ingredients present in the commercial diets have chitinase activity (Hirano et al., 1988), from 910 to 1200 mU/100 g of dry diet, and the enzymic activity probably plays a minor role in the digestion of chitin and chitosan. Orally administrated chitosan dissolves or swells in the acidic digestive fluid of the glandular stomach and gizzard of hens, and chitosan coagulates in the alkaline fluid of the small intestines (the duodenum and the jejunum) and then is digested by chitinase and chitosanase (Figure 3).

Hypolipidemic Activity. As shown in Table II, cholesterol and triacylglycerol values (mg/dL) in rabbit's serum increased to 850 and 320, respectively, by feeding a 0.7% cholesterol-additive diet for 39 days, and they were suppressed to 300 and 210, respectively, by feeding 2% chitosan. These values in hen's serum increased to 670 and 34, respectively, by feeding a 0.7% cholesterol-additive diet for 189 days, and they were suppressed to 420 and 25, respectively, by feeding 2% chitosan. Similarly these values in broiler's serum increased to 330 and 89, respectively, by feeding 0.3% cholesterol-additive diets for 84 days, and they were suppressed to 210 and 35, respectively, by feeding 2% chitosan. As demonstrated in the present study, the serum cholesterol and triacylglycerol values of rabbits, hens, and broilers were kept low by feeding 2% chitosan, but they were not kept low by feeding 1% chitosan or 2% chitin (Figure 2). This indicates that an increase in serum cholesterol value is suppressed by orally administrated chitosan at an appropriate dosage in agreement with data in rats (Furda, 1983; Sugano et al., 1978, 1980, 1988; Nagyvary et al., 1979; Kobayashi et al., 1979; Vahouny et al., 1983). It is of

Table II. Effect of the Feeding of Chitosan on the Values of Total Cholesterol, Triacylglycerol, and Free Fatty Acids in the Serums of Rabbits, Hens, and Broilers

diet[a]	feeding period, days	serum,[b] mg/dL total cholesterol	HDL cholesterol	triacyl-glycerol
		Rabbits		
A		79 ± 4	37 ± 5	140 ± 8
A + 1% S	15	76 ± 0		110 ± 11
A + 2% S	15	76 ± 0		110 ± 12
A + 5% S	15	72 ± 3		100 ± 9
A + 0.7% C	39	850 ± 21	53 ± 5	320 ± 62
	69	1260 ± 28		810 ± 110
A + 0.7% C + 1% S	39	690 ± 13		
	69	960 ± 25		1200 ± 120
A + 0.7% C + 2% S	39	300 ± 13	58 ± 5	210 ± 40
A + 0.7% + 2% chitin	39			
		Hens		
A		210 ± 10		26 ± 2
A + 5% S	74	120 ± 10		27 ± 2
A + 0.7% C	81	370 ± 15		28 ± 3
	125	520 ± 18		32 ± 2
	189	670 ± 0		34 ± 2
A + 0.7% C + 2% S	74	153 ± 15		28 ± 3
	81	250 ± 15		21 ± 2
	125	370 ± 12		
	189	420 ± 0		25 ± 4
		Broilers		
A1 + A2	51	100 ± 9		74 ± 0
A1 + A2 + A3	66	160 ± 10		46 ± 15
	78	210 ± 3		73 ± 20
	83	250 ± 11		82 ± 8
A1 + A2 + 2% S	51	100 ± 3		nd[c]
A1 + A2 + A3 + 2% S	66	150 ± 10		55 ± 7
	78	190 ± 11		65 ± 10
	83	230 ± 12		87 ± 8
A1 + A2 + 0.3% C	42	260 ± 11		60 ± 8
	55	380 ± 45		nd
A1 + A2 + A3 + 0.3% C	66	330 ± 34		56 ± 14
	75	320 ± 34		85 ± 14
	84	330 ± 47		49 ± 0
A1 + A2 + 0.3% C + 2% S	42	220 ± 14		65 ± 7
	55	290 ± 48		nd
A1 + A2 + A3 + 0.3% C + 2% S	66	280 ± 48		51 ± 1
	75	250 ± 37		51 ± 0
	84	210 ± 9		35 ± 2

[a] Key: A, control; S, chitosan supplement; C, cholesterol addition. See Materials and Methods for details. [b] HDL cholesterol = high-density lipoprotein cholesterol. [c] Not determined.

Table III. Effect of the Feeding of Chitosan on the Values of Total Cholesterol and Triacylglycerol in the Livers of Rabbits, Hens, and Broilers

diet[a]	feeding period, (days)	liver,[b] mg/dL total cholesterol	triacyl-glycerol
	Rabbits		
A + 0.9% C	39	14 ± 2	12 ± 2
A + 0.9% C + 2% S	39	8 ± 2	8 ± 2
	Broilers		
A1 + A2 + A3	83	8 ± 1	8 ± 2
A1 + A2 + A3 + 2% S	83	6 ± 1	6 ± 1
A1 + A2 + A3 + 0.3% C	84	13 ± 1	10 ± 2
A1 + A2 + A3 + 0.3% C + 2% S	84	8 ± 2	6 ± 2

[a] Key: A, control; S, chitosan supplement; C, cholesterol addition. See Materials and Methods for details. [b] By fresh weight.

significance to note that high-density lipoprotein (HDL) cholesterol values in rabbit's serums were maintained even when the total cholesterol value decreased by feeding 2% chitosan. Free fatty acid in rabbit's serum decreased lit-

Table IV. Effect of the Feeding of Chitosan on the Values of Total Cholesterol, Triacylglycerol, and Free Fatty Acid in Hen's Thigh and Pectoral Muscles[a]

	thigh muscle			pectoral muscle		
diet[b]	cholesterol, mg/g	triacylglycerol, mg/g	free fatty acids, mequiv/kg	cholesterol, mg/g	triacylglycerol, mg/g	free fatty acids, mequiv/kg
A1 + A2 + A3	2.0 ± 0.5	9.7 ± 0.9	8.1 ± 0.5	1.5 ± 0.2	11 ± 3	5.0 ± 1.5
A1 + A2 + A3 + 2% S	2.1 ± 0.4	7.9 ± 1.2	5.0 ± 0.4	1.3 ± 0.1	6 ± 1	5.5 ± 2.0
A1 + A2 + A3 + 0.3% C	5.7 ± 0.9	9.2 ± 0.0	12 ± 2	3.5 ± 0.4	11 ± 2	6.6 ± 0.0
A1 + A2 + A3 + 0.3% C + 2% S	1.9 ± 0.0	7.6 ● 1.3	2.8 ± 0.4	3.7 ± 0.4	9 ± 1	6.7 ± 0.0

[a] The period of feeding was 83 days. These values are expressed by fresh weight, and each of the experimental groups consisted of three to five. [b] See footnote *a* in Table II.

tle by feeding 2% chitosan (the data are not shown). An increase in total cholesterol and triacylglycerol values in these animal livers was suppressed by feeding 2% chitosan (Table III). An increase in the values of cholesterol, triacylglycerol, and free fatty acid in hen's thigh muscles was also suppressed by feeding 2% chitosan, but those in hen's pectoral muscles were changed little (Table IV), indicating a possible production of low-cholesterol meats.

A synthetic cholestyramine (Dowex resin), which is now used as an orally hypocholesterolemic agent in clinical field, is nondigestible and relatively toxic (Vahouny et al., 1983; Goodman and Noble, 1968), but chitosan is safe and digestible in domestic animals as demonstrated in the present study. These data indicate that chitosan can be useable as an ingredient at an appropriate dosage for domestic animal feeds, and the safety dosage varies with animals and is <1.4 g/kg of body weight per day for hens and broilers and <0.8 g/kg of body weight per day for rabbits.

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