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Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens

BY A. RAZDAN AND D. PETTERSSON

Department of Food Science, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

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Broiler chickens were fed on a control diet based on maize and maize starch or diets containing chitin, or 94, 82 or 76% deacetylated chitin (chitosans) with different viscosities (360, 590 and 620 m Pa.s respectively) at an inclusion level of 30 g/kg. Animals had free access to feed and water for the whole experimental period. On days 10 and 18 of the experiment chickens given the control and chitin-containing diets weighed more, had consumed more feed and had lower feed conversion ratios (g feed/g weight gain) than chitosan-fed birds. Feeding of chitosan-containing diets generally reduced total plasma cholesterol and high-density-lipoprotein (HDL)-cholesterol concentrations and gave an increased HDL:total cholesterol ratio in comparison with chickens given the control and chitin-containing diets. However, no significant reductions in plasma triacylglycerol concentrations resulting from feeding of the chitosan-containing diets were observed. The reduction in total cholesterol concentration and increased HDL:total cholesterol ratio were probably caused by enhanced reverse cholesterol transport in response to intestinal losses of dietary fats. The suggestion that dietary fat absorption was impeded by the chitosans was strengthened by the observation that ileal fat digestibility was reduced by 26% in comparison with control and chitin-fed animals. In a plasma triacylglycerol response study on day 21, feeding of 94 and 76%-chitosan-containing diets generally reduced postprandial triacylglycerol concentrations compared with chickens given the chitin-containing diet. Duodenal digestibilities of nutrients amongst chickens given the chitin-containing diet were generally lower than those of control and chitosan-fed birds indicating decreased intestinal transit time. The reduced caecal short-chain fatty acid concentrations of chickens given chitosan diets compared with the control diet illustrates the antimicrobial nature of chitosan. The fact that the three chitosan-containing diets affected the registered variables similarly indicated that the level of inclusion of chitosans in the diet exceeded the level at which the effect of the different viscosities could be significant.

Chitosan: Chitin: Plasma lipids: Digestibility: Chickens

The considerable current interest in the hypocholesterolaemic properties of new sources of dietary fibre necessitates the analysis and assessment of these 'alternative' dietary fibres. One such dietary fibre is chitosan, a polygluocosamine derived from chitin, a cellulose-like polymer located in the exoskeletons of arthropods such as crabs, shrimps, lobsters and insects (Furda, 1983). It is generally accepted that chitin is extensively acetylated while chitosan is largely deacetylated (Furda, 1983).

The solubilities of dietary fibres in water and dilute acids are physiologically important since water-soluble fibres, such as pectins, exhibit hypocholesterolaemic properties in animal and human studies (Anderson *et al.* 1990; Pettersson & Razdan, 1993; Wood, 1993). Although the mechanisms by which soluble dietary fibres reduce blood lipid levels are not fully understood, it has been proposed that the increased viscosity associated with soluble fibres may increase the thickness of the intestinal lumen boundary layer thereby reducing lipid absorption (Johnson & Gee, 1981; Furda, 1990). Chitosan, although

relatively insoluble in water, is soluble in dilute acids in which highly viscous solutions are produced (Sugano *et al.* 1988), whereas chitin is insoluble in both water and acid. Moreover, chitosan, but not chitin, has a high anion-exchange capacity as a result of the quaternary ammonium ions associated with the polyglucosamine chains and consequently has a bile acid binding capacity (Sugano *et al.* 1980) which may interrupt enterohepatic bile acid circulation, decrease lipid absorption and increase faecal cholesterol excretion. This theory has been strengthened by the observed potent hypolipidaemic effects of chitosans on blood lipid levels in rats (Kobayashi *et al.* 1979; Sugano *et al.* 1980; Ikeda *et al.* 1993).

The present experiment was performed in order to establish the effects of *ad lib.* feeding of a control diet supplemented with chitin or three chitosan fractions with different levels of acetylation on production results and plasma lipid concentrations in broiler chickens. Duodenal and ileal digestibilities, plasma triacylglycerol concentrations over a 4-h postprandial period and caecal short-chain fatty acid (SCFA) concentrations in broiler chickens fed on control, chitosan- or chitin-containing diets were also measured.

MATERIALS AND METHODS

Chitin and chitosan

Chitin and 76, 82 and 94 % deacetylated chitin (C-76, C-82 and C-94 chitosans respectively) fractions were supplied by Protan Biopolymers, Drammen, Norway. The chitosan fractions were deacetylated from chitin according to Protan standard methods.

Diets

The chickens received a control diet based on maize (405 g/kg feed) and maize starch (244·8 g/kg feed) or diets in which part (30 g/kg) of the maize starch component was substituted with chitin or C-76, C-82 and C-94 chitosans respectively (Table 1). Lysine and methionine were included at a level of 1·6 g/kg in each of the mash diets, milled to pass a 3·5 mm screen.

Chickens

A total of 320 1-d-old broiler chickens (Ross) of mixed sex were divided into twenty groups of sixteen chickens with an average group weight of 704 g and a maximum difference in weight of 4 g between groups. The groups were randomly allotted to six four-tier battery cages with raised wire floors in a windowless, light- and temperature-controlled room (24 h light period, 30°). The five experimental diets were randomly assigned to four replicates (cages) each. Chickens were wing banded and their sex determined on day 1 of the experiment. All diets were given *ad lib.* and the birds had free access to feed except for 8 h before blood collection for analysis of plasma lipids on days 11 and 19, and 3 h before collection of blood on day 21. Chickens had free access to water for the duration of the experiment.

Production study

Individual chicken weights and group cumulative feed intakes were recorded at 10 and 18 d of age and group feed conversion ratios were calculated on a weight-gain basis.

Plasma lipid study

At both days 11 and 19, chickens were starved for 8 h after which two birds from each cage (one of each sex), with weights as close as possible to the average group weight, were slaughtered by cervical dislocation. Blood samples were collected from the jugular veins of each chicken for triacylglycerol and cholesterol analysis.

Table 1. *Composition of the control and chitosan- or chitin-containing broiler chicken diets (g/kg air dry basis)*

Diet...	Control	Chitosan- or chitin-containing
Maize	405·0	405·0
Soya-bean meal	200·0	200·0
Fish meal	40·5	40·5
Meat-and-bone meal	40·5	40·5
Animal fat	20·0	20·0
Maize starch	244·8	214·8
Chitosan or chitin	–	30·0
Limestone	17·0	17·0
Monocalcium phosphate	13·0	13·0
Vitamin and trace-element premix*	10·0	10·0
NaCl	3·0	3·0
Cholesterol	3·0	3·0
Lysine-hydrochloride	1·6	1·6
DL-Methionine	1·6	1·6

* For details, see Pettersson & Åman (1992).

Postprandial plasma triacylglycerol study

On day 20, chickens given the C-94 and C-76 chitosan- and chitin-containing diets were starved for 3 h after which one chicken from the four cages from each diet was chosen randomly and slaughtered by cervical dislocation at 08.00 hours. Blood samples were collected from the jugular veins of the birds for analysis of triacylglycerols. Feed was then given *ad lib.* to the remaining chickens for a 1 h period after which the feed was removed once more. Further blood sampling occurred in this manner (that is, one chicken from each of the four cages allotted to each diet per culling occasion) at 09.00, 10.00, 11.00, 12.00 and 13.00 hours. From 14.00 hours on day 21, diets were given *ad lib.* with a marker (Cr_2O_3) incorporated at a level of 4 g/kg to chickens given the control, C-94 and C-76 chitosan-containing and chitin-containing diets.

Digestibility study

On day 23 one randomly selected bird, from each of the four cages per diet containing chickens given control, C-94 and C-76 chitosan- and chitin-containing diets, was slaughtered by cervical dislocation. Thereafter, culling occurred every fourth hour for a further 20 h. At each slaughter the gastrointestinal tracts of the chickens were quickly removed and the contents of the duodenum and last third of the small intestine (denoted ileum) were collected separately, pooled for each group (cage), frozen (−25°) and freeze-dried. Digestibilities in the digesta samples were calculated relative to the Cr_2O_3 marker.

Chemical analysis

All analyses were carried out in duplicate and results are reported on a dry matter basis. Before analysis, representative feed samples were ground in a Tecator Cyclone Mill (Tecator AB, Höganäs, Sweden) to pass a 0·5 mm screen. Viscosities of the chitosan fractions were analysed by Protan Biopolymers according to standard methods used internally by the company (Brookfield RUTD viscometer at 20 s^{-1}/shear and 25°;

Brookfield Viscometers Ltd., Loughton, Essex). Samples of freeze-dried digesta were ground in a Retsch mill (0·5 mm screen size). Dry matter was determined by oven-drying at 105° for 16 h. Ash and crude protein (N × 6·25) were analysed according to standard methods of the Association of Official Analytical Chemists (1984). Crude fat was extracted with diethyl ether in a Tecator Soxtec System HT after 3 M-HCl acid hydrolysis (Anon, 1971). Starch was determined enzymically (Åman & Hesselman, 1984). Total dietary fibre, defined as the sum of non-starch polysaccharides and Klason lignin, was analysed according to Theander & Westerlund (1986). Cr_2O_3 was determined according to Fenton & Fenton (1979). Caecal samples were extracted in 50 mM-Tris-HCl buffer (pH 6·5) to a sample concentration of 1 mg/ml and analysis of caecal SCFA was performed by HPLC. A filtrate (20 μl) of the sample medium was injected onto a Biorad Amnex HPX-87H column (Biorad Laboratories AB, Solna, Sweden) at 60° with a flow rate of 0·4 ml eluent (0·05 M-H_2SO_4)/min, and the refractive index was measured using a Tecator RI 5902 refractometer (Kjell Larsson, personal communication). Plasma was isolated from blood samples by centrifugation (200 *g*) and triacylglycerol, total cholesterol and high-density lipoprotein (HDL)-cholesterol concentrations were analysed using enzymic colorimetric kits (Boehringer Mannheim Diagnostica).

Calculations and statistical analysis

Statistical analysis of the registered variables (production results, plasma triacylglycerol and cholesterol concentrations, ileal and duodenal digestibility values) were performed by an analysis of variance procedure, the general linear model (GLM) supported by the statistical analysis system (Statistical Analysis Systems Institute Inc., 1985). In the statistical model the main effects of individual diet, class of diet (control, chitosan- or chitin-containing) and time (bird age or time after feeding) were considered. The effect of sex was not considered when analysing producing variables since these results are presented as cage means and live weights were not influenced by the effect of sex.

RESULTS

Diets

The viscosities of the C-94, C-82 and C-76 chitosan fractions were 360, 590 and 620 m Pa.s respectively.

The starch content accounted for approximately 560, 520 and 510 g/kg dry matter content of the control, chitosan- and chitin-containing diets respectively (Table 2). Crude protein content was approximately 200 g/kg dry matter content of the control diet and accounted for 210 g/kg of the glucosamine-containing diets. Crude fat and ash contents were similar between control and glucosamine-containing diets. The sum of non-starch polysaccharide residues was 80 and 70 g/kg for the control and glucosamine-containing diets respectively, with arabinose, xylose, glucose, uronic acids and Klason lignin as the dominating non-starch polysaccharide and dietary fibre residues for all diets.

Production study

The mortality for the entire experiment was 5% and was not significantly influenced by individual diet (control, C-76, C-82, C-94 chitosan-containing or chitin-containing) or bird age.

Over the whole experimental period, broiler chicken live weights, feed intakes and feed conversion ratios were influenced by both individual diet and age ($P = 0{\cdot}001$). In general, feeding of chitosan-containing diets significantly reduced ($P < 0{\cdot}05$) broiler chicken live weights and feed intakes, in comparison with chickens given control and chitin-containing diets, on days 10 and 18 (Table 3). Feed conversion ratios for these chickens were also

Table 2. *Chemical composition (g/kg dry matter) of the control, chitosan- or chitin-containing broiler chicken diets*

	Diets				
		Chitosan-containing diets			
	Control	C-94	C-82	C-76	Chitin
Starch	558·1	519·8	519·9	524·8	511·7
Crude protein	194·4	209·9	208·7	210·9	208·0
Crude fat (HCl)	59·6	58·5	58·9	59·0	58·5
Ash	78·2	78·7	78·9	78·5	79·2
Non-starch polysaccharide residues					
Arabinose	9·7	9·3	8·8	8·6	9·3
Xylose	12·1	10·0	10·0	10·5	10·5
Glucose	18·5	17·4	17·3	17·3	17·6
Uronic acids	16·1	13·2	12·1	11·6	13·4
Klason lignin	10·0	10·8	9·4	12·8	9·4
Total dietary fibre	79·2	73·7	70·5	73·6	74·4

C-94, C-82, C-76, diets containing chitin that was 94, 82 or 76% deacetylated respectively.

Table 3. *Live weight, cumulative feed intake and feed conversion ratio of broiler chickens given control, chitosan- or chitin-containing broiler chicken diets**

	Diets					
		Chitosan-containing diets				
	Control	C-94	C-82	C-76	Chitin	Pooled SEM
Body wt (g)						
Day 10	220[a]	186[b]	181[b]	193[b]	219[a]	5·3
Day 18	536[a]	451[b]	456[b]	482[bc]	523[ac]	15·9
Cumulative feed intake (g)						
Day 10	233[a]	209[b]	207[b]	216[b]	236[a]	5·1
Day 18	677[a]	606[b]	612[b]	629[b]	668[a]	15·6
Feed conversion ratio (g feed/g wt gain)						
Day 10	1·33[a]	1·49[b]	1·52[b]	1·45[b]	1·35[a]	0·028
Day 18	1·38[a]	1·50[b]	1·48[bc]	1·44[ab]	1·39[ac]	0·032

C-94, C-82, C-76, diets containing chitin that was 94, 82, or 76% deacetylated respectively.
[a,b,c] Means within a row not sharing a common superscript were significantly different ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2, and p. 278.

generally higher than for chickens given control and chitin-containing diets, the highest feed conversion ratios being observed amongst chickens given the C-94 and C-82 diets.

Plasma lipid study

Plasma triacylglycerol and total plasma cholesterol concentrations were influenced ($P = 0·001$) by individual diet and bird age while plasma HDL-cholesterol concentrations were affected ($P = 0·04$) by bird age only.

Plasma triacylglycerol concentrations were, on days 11 and 19, generally lower in chickens given chitosan- and chitin-containing diets compared with birds fed on the control

Table 4. *Plasma triacylglycerol, total plasma cholesterol, plasma high-density-lipoprotein (HDL)-cholesterol and HDL:total plasma cholesterol ratio (HDL:total) of broiler chickens given control, chitosan- or chitin-containing broiler chicken diets**

	Diets					
		Chitosan-containing diets				Pooled
	Control	C-94	C-82	C-76	Chitin	SEM
Day 11						
Triacylglycerols (mmol/l)	0·49[a]	0·40[ab]	0·30[b]	0·27[b]	0·27[b]	0·062
Plasma cholesterol (mmol/l)						
Total	5·13[a]	3·92[b]	3·32[b]	3·29[b]	4·85[a]	0·429
HDL	2·31[a]	2·18[a]	1·79[a]	1·94[a]	2·42[a]	0·240
HDL:total	0·49[a]	0·56[a]	0·54[a]	0·61[a]	0·53[a]	0·059
Day 19						
Triacylglycerols (mmol/l)	0·35[a]	0·30[a]	0·26[a]	0·26[a]	0·31[a]	0·045
Plasma cholesterol (mmol/l)						
Total	6·40[a]	4·40[b]	4·65[b]	4·64[b]	6·77[a]	0·549
HDL	2·29[a]	2·38[a]	2·42[a]	2·57[a]	2·51[a]	0·216
HDL:total	0·38[a]	0·56[b]	0·56[b]	0·58[b]	0·41[ab]	0·067

C-94, C-82, C-76, diets containing chitin that was 94, 82 or 76% deacetylated respectively.
[a,b] Means within a row not sharing a common superscript were significantly different ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2, and pp. 278–280.

diet (Table 4). Lower plasma total and HDL-cholesterol concentrations were observed for chickens given chitosan-containing diets in comparison with birds given the chitin-containing diet. Plasma HDL:total cholesterol ratio was generally increased by feeding chitosan-containing diets in comparison with feeding control and chitin-containing diets.

At day 11, feeding C-82 and C-76 chitosan- and the chitin-containing diets significantly reduced plasma triacylglycerol concentrations when compared with chickens given the control diet. The plasma triacylglycerol concentration of birds given the C-94-containing diet was, however, similar to that of birds fed on the control diet and not significantly different from that of chickens receiving C-82; C-76- and chitin-containing diets. Total plasma cholesterol concentrations were significantly lower amongst chickens given chitosan-containing diets in comparison with birds receiving control and chitin-containing diets. It is noteworthy that similar total cholesterol concentrations were observed for both control and chitin-containing diets. On day 19, similar plasma triacylglycerol concentrations were observed for chickens fed on each diet while plasma total cholesterol concentrations were significantly reduced by feeding chitosan-containing diets when compared with control and chitin-containing diets. Birds given chitosan-containing diets had significantly greater HDL:total cholesterol ratios compared with chickens given the control diet.

Postprandial plasma triacylglycerol study

Feed intakes for the 1 h feeding period were on average 5·45 g (range 5·17–5·70 g) feed/chicken for chickens given the C-94-, C-76- and chitin-containing diets and there were no significant differences in feed intake between diets.

Plasma triacylglycerol concentration during the triacylglycerol response study was influenced ($P = 0·001$) by individual diet and time after feeding.

Generally, chickens fed on the chitin-containing diet before the plasma triacylglycerol study were observed to have elevated plasma triacylglycerol concentrations for the duration

Table 5. *Plasma triacylglycerol concentrations (mmol/l) of broiler chickens given chitosan- or chitin-containing broiler chicken diets over a 5-h sampling period after being deprived of feed for 3 h**

	Diets			
	Chitosan-containing diets			
Time after feeding (h)	C-94	C-76	Chitin	Pooled SEM
0 (08·00)	0·19[a]	0·30[a]	1·02[b]	0·152
1 (09·00)	0·63[a]	0·69[a]	1·72[b]	0·169
2 (10.00)	0·91[a]	1·01[a]	1·15[a]	0·281
3 (11.00)	0·90[a]	0·68[a]	1·55[b]	0·139
4 (12.00)	0·30[a]	0·42[a]	0·75[a]	0·145
5 (13.00)	0·47[a]	0·32[a]	0·75[b]	0·080

C-94, C-76, diets containing chitin that was 94 or 76% deacetylated respectively.
[a,b] Means within row not sharing a common superscript were significantly different ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2, and pp. 278–280.

of this study in comparison with chickens fed on chitosan-containing diets (Table 5). similar postprandial triacylglycerol responses of chickens given the C-94- and C-76-containing diets were observed throughout this part of the experiment. Plasma triacylglycerol concentrations after feed deprivation of chickens given the chitin diet were significantly ($P < 0·05$) greater than those of birds given chitosan-containing diets. While the peak of triacylglycerol response was observed 1 h after feeding for chickens given the chitin-containing diet, peaks of triacylglycerol response amongst chickens given the chitosan-containing diets were observed 2 h after feeding. Additionally, feeding chitosan-containing diets significantly lowered the triacylglycerol response at 3 and 5 h after feeding compared with responses observed for the chitin-fed chickens.

Digestibility study

Individual diet significantly influenced ($P = 0·04$) duodenal digesta dry matter content as well as duodenal digestibility of starch and of organic matter ($P = 0·04$). Ileal digesta dry matter content was also significantly influenced ($P = 0·003$) by individual diet. Individual diet significantly influenced ileal digestibility of crude protein ($P = 0·003$), crude fat ($P = 0·001$), and organic matter ($P = 0·001$) as well as arabinose, xylose, uronic acids ($P = 0·02$) and glucose ($P = 0·04$).

Feeding a chitin-containing diet to broiler chickens significantly ($P < 0·05$) reduced duodenal dry matter content and digestibilities of crude protein and organic matter in relation to birds given the control diet, while the chitin-containing diet reduced duodenal digestibility of starch compared with chickens given the C-94- and C-76-containing diets (Table 6).

Ileal digesta dry matter content was found to be significantly higher in the control-fed broiler chickens than in chickens given chitosan-containing diets (Table 7). The C-76-containing diet significantly increased ileal digestibility of crude protein compared with the C-94-containing diet. The ileal digestibility of crude fat amongst chickens given chitosan-containing diets was approximately 26% lower than that of birds given control and chitin-containing diets. When the ileal digestibility of non-starch polysaccharide residues of chickens given C-94- and C-76-containing diets were compared it was found that ileal

Table 6. *Digesta dry matter content (g/kg) and apparent ileal digestibilities of nutrients in duodenum of broiler chickens given control, chitosan- or chitin-containing diets**

	Diets				
		Chitosan-containing			Pooled
	Control	C-94	C-76	Chitin	SEM
Digesta dry matter content	228[a]	223[a]	224[a]	202[b]	5·88
Duodenal digestibility					
Crude protein	0·36[a]	0·29[ab]	0·22[ab]	0·14[b]	0·063
Starch	0·40[ab]	0·48[a]	0·42[a]	0·30[b]	0·038
Organic matter	0·30[a]	0·30[a]	0·26[ab]	0·16[b]	0·033

C-94, C-76, diets containing chitin that was 94 or 76% deacetylated respectively.
[a,b] Means within a row not sharing a common superscript were significantly different ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2, and pp. 278–280.

Table 7. *Digesta dry matter content (g/kg) and apparent ileal digestibility of nutrients and non-starch polysaccharide residues in the last third of the small intestine of broiler chickens given control, chitosan- or chitin-containing broiler chicken diets**

	Diets				
		Chitosan-containing diets			Pooled
	Control	C-94	C-76	Chitin	SEM
Digesta dry matter content	250[a]	220[bc]	212[c]	233[ab]	5·7
Ileal digestibility					
Crude protein	0·76[ac]	0·72[ab]	0·76[c]	0·71[b]	0·141
Crude fat	0·79[a]	0·59[b]	0·59[b]	0·80[a]	0·330
Starch	0·97[a]	0·97[a]	0·97[a]	0·97[a]	0·027
Organic matter	0·77[a]	0·72[b]	0·72[b]	0·73[b]	0·049
Non-starch polysaccharide residues					
Arabinose	−0·17[a]	−0·44[b]	−0·38[b]	−0·42[b]	0·058
Xylose	−0·17[a]	−0·44[b]	−0·42[b]	−0·45[b]	0·063
Glucose	−0·08[a]	−0·30[b]	−0·28[b]	−0·30[b]	0·055
Uronic acids	0·10[a]	−0·01[b]	−0·12[c]	−0·07[bc]	0·032
Total dietary fibre	−0·01[a]	−0·18[b]	−0·15[b]	−0·17[b]	0·041

C-94, C-76, diets containing chitin that was 94 or 76% deacetylated respectively.
[a,b,c] Means within a row not sharing a common superscript were significantly different ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2, and pp. 278–280.

uronic acid digestibility was significantly lower in birds receiving the C-76-containing diet. It is also noteworthy that feeding glucosamine-containing diets significantly reduced digestibilities of organic matter and non-starch polysaccharide residues in comparison with feeding the control diet.

Chickens given the control diet were observed to have numerically although not significantly higher caecal acetic and butyric acid levels compared with birds receiving glucosamine-containing diets. Furthermore, acetic acid concentrations were significantly reduced amongst chickens given the C-76-containing diet relative to control-fed animals (Table 8).

Table 8. *Caecal short-chain fatty acid content (mg/g dry matter) from the caeca of broiler chickens given control, chitosan- or chitin-containing diets**

	Diets				
		Chitosan-containing			Pooled
	Control	C-94	C-76	Chitin	SEM
Acetic acid	36·2[a]	26·0[ab]	18·9[b]	26·6[ab]	3·22
Butyric acid	6·76[a]	4·90[a]	5·24[a]	5·10[a]	1·53

C-94, C-76, diets containing chitin that was 94 or 76% deacetylated respectively.
[a,b] Means within a row not sharing a common superscript were significantly different ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2, and pp. 278–280.

DISCUSSION

Compared with the control diet, feeding chitosan-containing diets generally lowered body weights and feed intakes, resulted in poorer feed conversion, reduced plasma lipid concentrations, reduced duodenal digestibilities of nutrients, ileal digestibilities of crude fat, organic matter and non-starch polysaccharide residues and reduced caecal SCFA concentrations. In comparison with the chitin-containing diet, chitosan-containing diets tended to reduce body weights and feed intakes, result in poorer feed conversion, reduce plasma lipid concentrations, reduce postprandial triacylglycerol response, increase duodenal nutrient digestibilities, increase duodenal digesta dry matter content and reduce ileal digestibility of crude fat.

Chitosans, by virtue of their solubility in dilute acids, form highly viscous solutions (Sugano *et al.* 1988) which may cause distension of the duodenum in animals (Sellers, 1977) and thereby increase satiety. This effect could account for the reduced feed intakes and lower body weights observed for chickens fed on chitosan-containing diets in the present experiment. The lower digesta dry matter contents observed for these chickens in comparison with control-fed chickens illustrate the high water-holding capacity of chitosan and suggest increased viscosity in the duodenum and small intestine. Feeding the chitin-containing diet, however, did not reduce body weights or feed intakes relative to control-fed birds since these highly acetylated glucosamines are insoluble and hence have very low viscosity (Furda, 1983).

The physiological importance of soluble dietary fibres lies in their ability to reduce the diffusion and absorption of nutrients (most notably dietary fat) in the small intestine (Furda, 1990). The mechanism by which soluble dietary fibres reduce nutrient absorption is not fully understood but the increased viscosity associated with soluble dietary fibres has been shown to increase the thickness of the intestinal boundary layer and consequently impede nutrient uptake (Johnson & Gee, 1981; Flourie *et al.* 1984). Soluble chitosan would therefore be expected to reduce nutrient uptake due to the highly viscous environment produced on reaction with stomach acids. Furthermore, the observation that feeding chitosan-containing diets to rats decreased weight gain and increased faecal neutral sterol excretion (Sugano *et al.* 1978) has contributed to the theory that the polycationic nature of chitosans facilitates binding of the polyglucosamine to mixed lipid micelles, thereby preventing fat uptake while increasing hepatic synthesis of bile acids (Furda, 1983). However, since chitosans are insoluble at the pH of the site of lipid uptake in the ileum and no increases in bile acid excretion were observed in studies on chitosan-fed rats (Sugano *et al.* 1980), the proposal that precipitated chitosans disintegrate mixed micelles in the ileum

rather than bind them *in toto* is currently favoured (Ebihara & Schneeman, 1989). Restriction of the availability of lipid to lipase hydrolysis as a result of binding and disintegration would result in reductions in the size of the enterohepatic lipid pool (Furda, 1990).

It is noteworthy that plasma triacylglycerol concentrations of chickens given chitosan-containing diets were lower than those of their control-fed counterparts on day 11 but not on day 19 when chickens given the various diets were observed to have similar plasma triacylglycerol concentrations. The reduction in plasma triacylglycerol concentrations observed amongst chickens given chitosan-containing diets in comparison with control-fed birds on day 11 but not on day 19 may be an indication of the age-related changes in fat metabolism which are known to occur in broiler chickens (Krogdahl, 1985; Inarrea *et al.* 1989).

The reduced cholesterol concentrations of chickens fed on chitosan-containing diets in comparison with birds given the control diet on days 11 and 19 may indicate intestinal adaptation to fat absorption as well as changes in plasma lipoprotein metabolism, and these changes may be attributed to increased duodenal viscosity and binding of micelles. In the present experiment a 26% reduction in ileal crude fat digestibility was observed amongst chickens given chitosan-containing diets compared with birds given control and chitin-containing diets, probably as a result of chitosan binding to, and disintegration of, micelle components in the ileum or a delay in gastric emptying caused by increased viscosity in the glandular stomach (Edwards, 1990). Moreover, the elevated HDL:total cholesterol ratios for chickens given chitosan-containing diets in comparison with control- and chitin-fed birds on day 19 suggests enhanced reverse cholesterol transport whereby HDL returns cholesterol from peripheral tissues to the liver for catabolism and excretion (Gotto, 1992).

The generally reduced triacylglycerol response of chickens given chitosan-containing diets compared with chitin-fed birds on day 21 illustrates the effects of increased viscosity and micelle binding. It is interesting to note that triacylglycerol concentrations directly after feed deprivation of chickens given chitosan-containing diets were lower than those of chickens fed on the chitin-containing diet. The fasting period for the triacylglycerol response study was only 3 h in comparison with 8 h fasting periods before the plasma lipid studies on days 11 and 19. It is therefore apparent that the hypotriacylglycerolaemic response of chickens given chitosan-containing diets is an adaptive response to feeding soluble dietary fibre and is presumably related to increased duodenal viscosity, delayed gastric emptying, reduced intestinal motility, reduced fat absorption and increased micelle binding.

Insoluble dietary fibres such as cellulose tend to decrease intestinal transit time and increase faecal bulking in humans (Anderson *et al.* 1990). This may explain the reduced duodenal digesta dry matter content and nutrient digestibilities of chickens fed on the chitin-containing diet in comparison with the control- and chitosan-fed birds since both water and nutrients would be flushed more rapidly through the duodenum.

The general reduction observed in SCFA concentrations of chickens given polyglucosamine-containing diets may have been due to antimicrobial effects since chitosan may be capable of integrating with and changing the form of microbial DNA thereby hindering RNA and consequently protein synthesis (Hadwinger & Loschke, 1981). Other evidence suggests that binding of chitosan to microbial cell-wall polysaccharides or membrane proteins and phospholipids may be possible due to the cationic nature of the polyglucosamine chain (Young *et al.* 1982).

The nutritional effects of inclusion of chitosans in diets for broiler chickens were indicated by the reduced plasma cholesterol concentrations, improved HDL:total cholesterol ratio, generally reduced postprandial triacylglycerol response and reduced ileal

fat digestibility, probably achieved with the assistance of increased gastric and duodenal viscosities, binding of duodenal micelle components and delayed gastric emptying. Although the chitosans used in this experiment were of different viscosities, it is noteworthy that there were no significant differences between them regarding their effect upon live weight, feed intakes, plasma lipid concentrations, postprandial triacylglycerol response, and duodenal digestibility or caecal SCFA concentration. It should also be borne in mind that the antimicrobial effects of chitosans may have adverse consequences in the long term for animals dependent on gut microflora for fermentative metabolism. Further research is therefore required to obtain better understanding of the lipid-reducing mechanisms and any potentially damaging consequences of chitosan to gut microflora.

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