Cholesterol-Lowering Effects of Dietary Lupin (*Lupinus albus* var Multolupa) in Chicken Diets

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ABSTRACT The present study was undertaken to investigate the effect of different concentrations of lupin seeds (0, 200, and 400 g/kg), with and without cholesterol added (10 g/kg), in chicken diets on performance, relative liver weight, liver fat, intestinal pH and viscosity, and different blood serum parameters (glucose, cholesterol, triglycerides, total biliary salts, amylase, total protein and albumin, and globulin fractions). Increasing the lupin content in the diet reduced weight gain and feed consumption and increased feed-to-gain ratio. A decrease in liver fat, cecal pH, serum glucose, cholesterol, total biliary salts, and total protein and an increase in jejunum viscosity were observed with increasing concentration of lupins. Serum albumin, β-globulin, γ-globulin, and albumin:globulin ratio were reduced by the addition of lupin in the diet. Cholesterol supplementation of diets had no effect on the performance, cecal pH, and serum triglycerides. Relative liver weight, liver fat, jejunum viscosity, serum cholesterol, total biliary salts, and total protein were increased, and serum glucose was reduced by addition of cholesterol. Cholesterol increased serum albumin, α-1 globulin, α-2 globulin, and β-globulin and reduced albumin:globulin ratio and amylase. These results indicate that inclusion of lupin seed in chicken diets causes a growth depression and a reduction of serum cholesterol and glucose and modifies other physiological parameters.

Key words: lupin, cholesterol, chicken

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INTRODUCTION

Evidence for a hypocholesterolemic effect of legumes on raised cholesterol level in animal models and man has accumulated in recent years (Martins et al., 2004). Legume seeds are typically rich in protein and nonstarch polysaccharides (**NSP**) and contain significant levels of oligosaccharides (Van Barneveld, 1999). Consumption of soy protein was effective in lowering the levels of serum triglycerides and cholesterol in humans and animals (Anderson et al., 1995; Sirtori et al., 1995). Such hypocholesterolemic effect is thought to be at least partially attributable to the amino acid profile of the plant protein itself (Carroll, 1991) or more recently to a complex system inducing enhanced expression of low-density lipoprotein (**LDL**) receptors (Sirtori et al., 2004). On the other hand, in soybean and in some other legume seeds, the dietary fiber fraction has also been reported to be an important component that could reduce serum cholesterol levels (Uberoi et al., 1992). Although soluble NSP of legume seeds are known to be an effective cholesterol-reducing agent, the insoluble NSP has also been reported to be effective in lowering serum cholesterol (Hughes, 1991; Lairon, 1996). Lupin has been recognized as a potentially valuable ingredient for livestock diets for many years and presents promising characteristics for becoming an element of the diet due to its high content in protein and a lack of antinutritional substances. The amount of antinutritional compounds found in other legumes, such as alkaloids, saponins, tannins. and trypsin inhibitor, is minimal in lupin (Van Barneveld, 1999). Particularly, the alkaloids that confer a bitter taste to lupin kernels have been reduced to very low levels in modern varieties through conventional breeding techniques (Duranti and Gius, 1997). Its protein content is comparable to that of soybean (32 to 45%). The carbohydrate chemistry of lupins is different to most legumes with negligible levels of starch and high levels (up to 500 g/kg) of soluble and insoluble NSP and oligosaccharides (Van Barneveld, 1999). The NSP of legumes is present in both the hull and the cotyledon, but the hulls tend to contain more cellulose and other insoluble NSP fractions than the cotyledon, which contains more soluble NSP (Champ et al., 1986). In general, there is more hemicellulose in the crude fiber content of lupins compared with legumes such as peas

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and fava beans, which have cellulose as the major component of fiber.

So far, there is limited information on the physiological effects of lupin seeds, particularly on the lipid metabolism. Rubio et al. (1998) reported a lower concentration of plasma cholesterol and triglycerides when lupins were included in chicken diets. It has also been demonstrated that the chicken liver is the major site of lipid biosynthesis and that avian adipose tissue is relatively unimportant as a site of fatty acid biosynthesis (Qureshi et al., 1980). Cockerels respond to a prolonged feeding of a high-cholesterol diet by developing an arterial process in the intimal layer that closely resembles atherosclerosis in other species, including man (Pick and Katz, 1965). This provides a useful model for studying the regulation of lipid metabolism in humans, because it has been shown that human liver is also the major site of fatty acid biosynthesis. Due to the interest in ingredients in human diets related mainly to possible health beneficial effect on serum cholesterol and diabetes, the purpose of this study was to determine the effect of lupin seeds in normal and hypercholesterolemic birds on different related biochemical parameters.

MATERIALS AND METHODS

Test Product, Birds, Feeding, and Management

The lupin cultivar used in the experiment was grown in Badajoz (Spain). Proximate composition of lupin seed is shown in Table 1. The experimental diets were formulated to meet or exceed the minimum NRC (1994) requirements for broiler chickens. A total of 108 one-day-old male broiler chicks were housed in electrically heated starter battery brooders in an environmentally controlled room. The chicks were allocated to 18 pens, each pen containing 6 chicks, to receive 6 dietary treatments with 3 replicates of each treatment. Diets in mash form and water were provided ad libitum. Celite (Celite Corp., Lompoc, CA), a source of acid insoluble ash, was added at 10 g/kg to all diets as an indigestible marker. The starter broiler diets (Table 2) with different inclusion levels of lupin (0, 200, and 400 g/kg) with and without cholesterol (Sigma-Aldrich Química SA, Tres Cantos, Spain) added (10 g/kg) were fed for 3 wk. At the end of the experimental period, birds were weighed, and feed consumption was recorded for feed efficiency computation.

At 21 d of age, 9 birds (3 per pen) were randomly selected from each treatment, and blood samples were obtained to determine different biochemical parameters. After the birds were killed by cervical dislocation, the liver, jejunum, and the ceca were removed for liver lipid, viscosity, and pH determination, respectively, using 9 randomly selected chicks per treatment. Experimental procedures were approved by the University Complutense of Madrid Animal Care and Ethics Committee in compliance with the Ministry of Agriculture, Fishery and Food for the Care and Use of Animals for Scientific Purposes.

Table 1. Determined chemical composition (g/kg as fed) of lupin seed

Item	(g/kg as fed)
Nutrient	
DM	938.5
AME[1] (kcal/kg)	2,368.0
CP	388.0
Ether extract	99.8
ADF[2]	147.0
NDF[3]	219.1
Insoluble dietary fiber	300.3
Soluble dietary fiber	16.1
Ash	37.4
Total alkaloids	0.2
Amino acids	
Asp	40.8
Thr	13.6
Ser	21.3
Glu	78.0
Gly	16.8
Ala	13.3
Cys	4.2
Val	14.4
Met	2.9
Ile	13.9
Leu	28.3
Tyr	15.9
Phe	15.5
His	9.1
Lys	14.1
Arg	37.1
Pro	16.3

[1]Brenes et al. (1993).
[2]Acid detergent fiber.
[3]Neutral detergent fiber.

Collection of Samples and Measurements

Blood samples were obtained by cardiac puncture for subsequent determination of glucose, cholesterol, triglyceride, biliary salt, total protein, amylase, and albumin and globulin fractions and albumin:globulin ratio in serum. For serum, the blood samples were allowed to clot in polypropylene tubes for 2 h at room temperature. The tubes were centrifuged at 1,500 × *g* for 10 min, and the supernatant was removed and stored at −20°C until assayed.

After the chicks were killed, the liver was removed, cleaned from adhering tissue, and weighed using 9 randomly selected chicks per treatment. Digesta contents were also collected from 9 birds per treatment for the viscosity determination (jejunum) and pH determination (Crison Micro pH 2000, Crison Instruments SA, Alella, Spain) was directly measured on cecal digesta (9 chicks per treatment).

Chemical Analysis

Dry matter (930.15), CP (976.05), crude fiber (978.10), and ash (942.05) were analyzed according to the methods of the AOAC (1995). Crude fat was determined by extraction in petroleum ether following acidification with 4 N

Table 2. Ingredients and nutrient composition of experimental diets (g/kg as fed) containing lupin seed

	Lupin seed (g/kg as fed)		
Item	0	200	400
Ingredients			
Corn	476.3	439.0	403.4
Soybean meal (48% CP)	421.0	256.0	87.9
Lupin meal[1]	—	200.0	400.0
Sunflower oil	62.7	63.3	64.0
Dicalcium phosphate	20.8	23.0	25.1
Calcium carbonate	9.8	8.7	7.6
NaCl	3.0	3.0	3.0
DL-Met	1.4	2.0	2.8
L-Lys	—	—	1.2
Vitamin and mineral premix[2]	5.0	5.0	5.0
Cholesterol	+/−	+/−	+/−
Analyzed composition			
Protein	228	229	232
Calculated analysis[3]			
AME (kcal/kg)	3,050	3,050	3,050
Lys	13.7	12.4	12.4
Met + Cys	9.0	9.0	9.0
Ca	10.0	10.0	10.0
Available P	4.5	4.5	4.5

[1]Protein content of lupin meal was 38.8%.

[2]Vitamin and mineral premix supplied the following per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B_{12}, 11.5 μg; riboflavin, 5.5 mg; Ca pantothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; delquin, 125 mg; DL-Met, 500 mg; amprol, 1 g; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; and NaCl, 2,500 mg.

[3]Estimated using FEDNA (2003) tables.

HCl solution (Wiseman et al., 1992). Soluble and insoluble NSP was analyzed by gas-liquid chromatography following the method of Englyst and Cummings (1984). Amino acids in the diets and ileal contents were analyzed following AOAC (1995) procedures and separated using a Beckman model 6300 AA autoanalyzer (Beckman Coulter, Monheim, Germany). Three replicates of all analyses were performed. Determination of the amino acid Trp was not possible under the conditions of analysis used. A linear regression equation was used for the determination of liver fat content based on the liver moisture determination (Mendonca and Jensen, 1983). Viscosity of jejunal digesta was measured at 25°C in a Brookfield DV-III viscometer (Brookfield Engineering Labs, Stoughton, MA). Two Eppendorf tubes (Eppendorf AG, Hamburg, Germany) were filled from each digesta sample and centrifuged at 12,000 × *g* for 3 min. The supernatant fluids were withdrawn, and the viscosity of a 0.5-mL aliquot was measured. Readings were taken at a speed of 0.004 × *g* and expressed in centipoises (cP). Blood serum was analyzed for glucose (glucose oxidase-peroxidase-enzymatic-colorimetric), cholesterol (cholesterol oxidase-peroxidase-enzymatic-colorimetric), triglycerides (glycerol-3-oxidase-peroxidase-enzymatic-colorimetric), total biliary salts (dimethyl sulfoxide colorimetric), amylase (CNPG3-cinética), and total protein (colorimetric Biuret method), following the methods described by Spinreact (St. Esteve de Bas, Girona, Spain). The gas and thin-layer chromatography methods (Muzquiz et al., 1989) were used to determine the total alkaloid content. Serum protein electrophoresis was performed using a cellulose acetate methodology (Lumeij, 1987; Cray and Tatum, 1998).

Statistical Analysis

Data were analyzed as a completely randomized design and subjected to ANOVA using the GLM procedure of SAS software (SAS Institute, 2001). The experiments were analyzed by ANOVA in 3 (lupin concentration) × 2 (cholesterol concentration) factorial arrangements of treatments. Significant differences among treatment means were determined at $P < 0.05$ by Duncan's multiple range test.

RESULTS

The chemical composition of lupin seed is shown in Table 1. The effects of dietary lupin concentration and cholesterol supplementation on growth performance, relative liver weight, liver fat, cecal pH, and jejunum viscosity are summarized in Table 3. The main effects data indicated that increasing amounts of lupin seed in the diet depressed BW (up to 12%; $P < 0.001$), feed-to-gain ratio (up to 7%; $P < 0.001$), liver fat (17%; $P < 0.001$; in the higher-lupin concentration), and cecal pH (up to 6%; $P < 0.01$) and increased intestinal viscosity (up to 14%; $P < 0.001$). Cholesterol concentration in the diet increased relative liver weight (10%; $P < 0.001$), liver fat (46%; $P < 0.001$), and intestinal viscosity (10%; 0.001). A significant interaction was only observed in liver fat ($P < 0.05$) and intestinal viscosity ($P < 0.001$).

Serum glucose, cholesterol, triglyceride, total biliary salts, and total protein contents were reported in Table 4. Increasing dietary lupin concentration reduced serum glucose (up to 20%; $P < 0.001$), cholesterol (up to 27%; P

Table 3. Effect of dietary lupin meal concentration with and without cholesterol on BW, feed consumption (FC), feed-to-gain ratio (FGR), relative liver weight (RLW), liver fat (LF), cecal pH (pH), and intestinal viscosity (IV) of broiler chicks at 3 wk of age

Item	Lupin (g/kg)	Cholesterol (g/kg)	BW[1] (g)	FC[1] (g)	FGR[1]	RLW[2] (g/100 g of BW)	LF[2] (%)	pH[2]	IV[2] (cP)
Treatments									
1	0	0	624[a]	826	1.30[c]	2.90[bc]	5.34[c]	6.25[a]	2.19[b]
2	200	0	580[bc]	748	1.37[ab]	3.08[abc]	6.56[b]	6.25[a]	2.33[b]
3	400	0	543[d]	761	1.40[a]	2.83[c]	4.57[c]	6.07[a]	2.32[b]
4	0	10	621[a]	813	1.33[ab]	3.13[abc]	8.74[a]	6.15[a]	2.20[b]
5	200	10	607[ab]	810	1.33[bc]	3.17[ab]	8.09[a]	6.28[a]	2.31[b]
6	400	10	555[cd]	782	1.41[a]	3.36[a]	7.09[b]	5.61[b]	2.68[a]
Pooled SEM			48.0	37.1	0.02	0.16	0.54	0.21	0.08
Main effects									
Lupin (L)									
0			622[a]	819	1.31[c]	3.12	7.04[a]	6.20[a]	2.19[c]
200			594[b]	779	1.35[b]	3.11	7.32[a]	6.26[a]	2.32[b]
400			549[c]	771	1.40[a]	3.01	5.83[b]	5.84[b]	2.50[a]
Cholesterol (CH)									
0			582	802	1.35	2.94[b]	5.49[b]	6.19	2.28[b]
10			595	778	1.35	3.22[a]	8.03[a]	6.01	2.50[a]
Source of variation			Probabilities						
L effect			0.001	NS	0.001	NS	0.001	0.01	0.001
CH effect			NS	NS	NS	0.001	0.001	NS	0.001
L × CH effect			NS	NS	NS	NS	0.05	NS	0.001

[a–d]Means in columns with no common superscript differ significantly ($P < 0.05$).

[1]Data are means of 3 pens of 6 chicks.

[2]Data are means of 3 pens of 3 chicks.

< 0.01), total biliary salts (up to 47%; $P < 0.01$), and total protein (up to 14%; $P < 0.001$) compared with those of chicks fed the control diet. The addition of 10 g/kg of cholesterol reduced serum glucose (14%; 0.001) and increased plasma cholesterol (60%; $P < 0.001$), total biliary salts (60%; $P < 0.001$), and total protein (39%; $P < 0.001$). A significant interaction was observed for glucose ($P < 0.01$), cholesterol ($P < 0.05$), and triglycerides ($P < 0.001$).

Serum albumin, α-1 globulin, α-2 globulin, β-globulin, albumin:globulin ratio, and amylase concentrations are shown in Table 5. The main effects data indicated that increasing concentration of lupin seed in the diets reduced serum albumin (up to 13%; $P < 0.05$), β-globulin (up to 24%; $P < 0.01$), γ-globulin (up to 32%; $P < 0.001$), and albumin:globulin ratio (up to 13%; $P < 0.01$). The addition of 10 g/kg of cholesterol increased serum albumin (36%;

Table 4. Effect of dietary lupin meal concentration with and without cholesterol on serum glucose (G), cholesterol (CH), triglyceride (T), total biliary salts (BS), and total protein (P) of broiler chicks at 3 wk of age[1]

Item	Lupin (g/kg)	Cholesterol (g/kg)	G (mg/dL)	CH (mg/dL)	T (mg/dL)	BS (nmol/dL)	P (mg/dL)
Treatments							
1	0	0	254[a]	103[c]	33[b]	12[bc]	2.66[cd]
2	200	0	249[a]	109[bc]	26[bc]	9[bc]	2.87[c]
3	400	0	199[c]	74[d]	15[d]	7[c]	2.44[d]
4	0	10	220[b]	182[a]	23[c]	22[a]	3.90[a]
5	200	10	207[c]	134[b]	20[cd]	15[ab]	3.87[a]
6	400	10	183[d]	137[b]	42[a]	11[bc]	3.28[b]
Pooled SEM			6.17	15.30	3.68	3.68	0.18
Main effects							
Lupin (L)							
0			239[a]	142[a]	28	17[a]	3.28[a]
200			228[b]	122[b]	23	12[b]	3.34[a]
400			191[c]	104[b]	29	9[b]	2.83[b]
Cholesterol (CH)							
0			236[a]	95[b]	25	10[b]	2.66[b]
10			203[b]	152[a]	28	16[a]	3.69[a]
Source of variation			Probabilities				
L effect			0.001	0.01	NS	0.01	0.001
CH effect			0.001	0.001	NS	0.001	0.001
L × CH effect			0.01	0.05	0.001	NS	NS

[a–d]Means in columns with no common superscript differ significantly ($P < 0.05$).

[1]Data are means of 3 pens of 3 chicks.

Table 5. Effect of dietary lupin meal concentration with and without cholesterol on serum albumin (ALB), α-1 globulin (α-1 GLB), α-2 globulin (α-2 GLB), β-globulin (β-GLB), albumin:globulin ratio (ALB:GLB), and amylase of broiler chicks at 3 wk of age[1]

Item	Lupin (g/kg)	Cholesterol (g/kg)	ALB (%)	α-1 GLB (%)	α-2 GLB (%)	β-GLB (%)	γ-GLB (%)	ALB:GLB ratio	Amylase (U/L)
Treatments									
1	0	0	1.43^{b}	0.16^{d}	0.29^{b}	0.32^{cd}	0.20^{bc}	1.33^{a}	40.75^{a}
2	200	0	1.38^{b}	0.20^{cd}	0.37^{ab}	0.39^{bcd}	0.25^{ab}	1.06^{b}	36.00^{b}
3	400	0	1.25^{b}	0.26^{c}	0.27^{b}	0.29^{d}	0.17^{c}	1.07^{b}	40.62^{a}
4	0	10	1.94^{a}	0.51^{a}	0.47^{a}	0.60^{a}	0.30^{a}	1.07^{b}	10.25^{c}
5	200	10	1.85^{a}	0.46^{ab}	0.42^{a}	0.44^{b}	0.27^{a}	1.05^{b}	10.71^{c}
6	400	10	1.70^{a}	0.38^{b}	0.40^{ab}	0.41^{bc}	0.17^{c}	1.14^{b}	9.87^{c}
Pooled SEM			0.14	0.04	0.07	0.05	0.04	0.06	1.65
Main effects									
Lupin (L)									
0			1.69^{a}	0.33	0.38	0.46^{a}	0.25^{a}	1.20^{a}	25.5
200			1.61^{ab}	0.33	0.40	0.42^{a}	0.26^{a}	1.05^{b}	24.2
400			1.47^{b}	0.32	0.34	0.35^{b}	0.17^{b}	1.11^{b}	25.2
Cholesterol (CH)									
0			1.35^{b}	0.20^{b}	0.31^{b}	0.33^{b}	0.21^{b}	1.16^{a}	39.12^{a}
10			1.83^{a}	0.45^{a}	0.43^{a}	0.48^{a}	0.25^{a}	1.09^{b}	10.26^{b}
Source of variation			Probabilities						
L effect			0.05	NS	NS	0.01	0.001	0.01	NS
CH effect			0.001	0.001	0.001	0.001	0.01	0.05	0.001
L × CH effect			NS	0.001	NS	0.01	NS	0.01	0.05

[a–d]Means in columns with no common superscript differ significantly ($P < 0.05$).

[1]Data are means of 3 pens of 3 chicks.

$P < 0.001$), α-1 globulin (125%; $P < 0.001$), α-2 globulin (39%; $P < 0.001$), γ-globulin ($P < 0.01$), and β-globulin (45%; $P < 0.001$) and reduced albumin:globulin ratio (6%; $P < 0.05$) and amylase (74%; $P < 0.001$). A significant interaction was observed between lupin seed concentration and cholesterol for serum α-1 globulin ($P < 0.05$), β-globulin ($P < 0.05$), albumin:globulin ratio ($P < 0.01$), and amylase ($P < 0.05$), indicating that the response of cholesterol was more effective, in the case of β-globulin, albumin:globulin ratio, and amylase, with 400 g/kg of lupin seed concentration, and, in the case of serum α-1 globulin, with the lowest lupin seed concentration.

DISCUSSION

Chemical analysis demonstrated that the lupin seed was a low-alkaloid cultivar and had a high content of protein (388 g/kg), fat (99.8 g/kg), and fiber (219.1 g/kg of neutral detergent fiber). The amino acid composition was similar to that reported by Aguilera et al. (1985) and Brenes et al. (1993). The current study also demonstrated that the inclusion of raw low-alkaloid lupin seeds up to 20% in diets for broilers depressed growth rate. Feed refusal, likely associated with poor palatability, is probably 1 of the most prominent adverse effects observed in animals fed lupins. Similar results have been obtained by Centeno et al. (1990) and Brenes et al. (2002). Part of this effect may have also been related to the higher fiber content found in the hulls, or the concentration of NSP within the cotyledon. The detrimental effect of fiber was further demonstrated by adding hulls to a dehulled lupin diet (Brenes et al., 1993, 2002; Gdala, 1998). Degradation of lupin fiber in the small intestine is very limited in birds (Brenes et al., 2003). However, a significant fraction of the total of NSP consists of pectin-like substances composed of branched β-(1 to ≥4) galactans, which are labile and probably highly susceptible to fermentative breakdown (Evans et al., 1993). The results obtained in the present experiment with an increase in the intestinal viscosity and a reduction in the cecal pH suggest that considerable amounts of NSP become soluble when ingested by the chicken. An increase in ileal viscosity related to an increase in ileal-soluble NSP levels was reported by Kocher et al. (2000). The adverse effects of soluble NSP on nutrient digestion and absorption in monogastric animals, especially in poultry, are due to their ability to increase the viscosity of the digesta (Annison et al., 1996), to modify the physiology of the gastrointestinal tract, and to change the ecosystem of the gut (Rubio et al., 1998). The net effects may include altered intestinal transit time, increased endogenous losses of nutrients, and a change of nutrient digestion and absorption pattern.

The results of the current study also demonstrated that dietary lupin seeds caused blood parameter changes in chicks, because a decrease in serum cholesterol, glucose, total biliary salts, and total protein concentrations as well as a reduction in liver fat were found after legume diet intake. These results are in agreement with the observations of Eder et al. (1996) and Rubio et al. (2003) in chickens; Rahman et al. (1996), Chango et al. (1998), and Sirtori et al. (2004) in rats; and Martins et al. (2005) in intact and ileorectal anastomosed pigs by using lupin seeds. The lupin seed contains high amounts of protein and viscous NSP that could modulate the intestinal absorption of sterols and thus cholesterolemia. Many dietary factors have been reported to contribute to the hypocholesterolemic effect of dietary legumes. The hypothesis that dietary protein can alter serum cholesterol concentrations contin-

ues to generate much research and discussion. The diets used in the present experiment were supplemented with Met and Lys to meet the minimum NRC (1994) requirements for broiler chickens. Lysine concentration in the control diet was different (13.7 g/kg) in relation to the lupin diets that contained similar concentrations (12.4 g/kg). Previous finding have demonstrated that Met content, Met:Gly ratio, and Lys:Arg ratio in dietary proteins were considered to be responsible factors in lowering the serum cholesterols in rats (Morita et al., 1997; Chango et al., 1998). In the present experiment, the Met content (0.54%) and the Met:Gly ratio (0.42) are approximately similar in all the diets. However, the Lys:Arg ratio is different among the different diets (0.77, 0.65, and 0.60, respectively), which could explain part of the hypocholesterolemic effect obtained with the lupin diets and to corroborate the results reported in rats. Sirtori et al. (2004) also showed that lupin protein isolates were able to reduce plasma total and very low density lipoprotein + LDL cholesterol concentrations in rats. This effect was associated with the stimulation of LDL receptors by a well-defined protein component of the lupin seeds, as demonstrated by in vitro studies. Yoshie-Stark and Wäsche (2004) also showed in in vitro studies that the application of lupin-isolated protein had the capacity to bind bile acids to nearly the same extent as cholestyramine. Similarly, Martins et al. (2004, 2005) found that feeding raw peas and whole blue lupin seeds to pigs exerted a marked hypocholesterolemic effect. This effect was mainly the consequence of a marked decrease in the intestinal absorption of cholesterol probably modulated by bile acid reabsorption and a higher content of dietary phytosterols.

The presence of NSP fraction in the diets is another factor that could affect the cholesterol metabolism. Elevated levels of NSP, in particular the soluble fraction, lead to decreased nutrient digestion and absorption in poultry (Choct and Annison, 1990). The current study showed an increase in the intestinal viscosity and a reduction in the cecal pH, suggesting that the carbohydrates in the lupin seeds could be responsible for these effects. Increased intestinal content supernatant viscosity is highly correlated with reduced serum and liver cholesterol (Gallaher et al., 1993a,b) and reductions in cholesterol absorption (Carr et al., 1996) in hamsters.

Increased bile acid excretion represents another mechanism by which a reduction in cholesterol can be produced. Ikegami et al. (1990) and Costa et al. (1994) demonstrated that viscous NSP can enhance bile secretion and subsequently result in significant loss of these acids in the feces of rats. The continued drain of bile acids and lipids by sequestration and increased elimination as fecal acidic and neutral esterols may ultimately influence the absorption of lipids and cholesterol in the intestine.

Although soluble dietary fiber was known to be an effective hypocholesterolemic agent, the insoluble dietary fiber of legume seeds has also been reported to be effective in lowering serum cholesterol in hypercholesterolemic men (Hughes, 1991). Addition of lupin kernel fiber to the diet provided favorable changes to some serum lipid (total cholesterol, high-density lipoprotein cholesterol; Hall et al., 2005). Similarly, findings on the insoluble fibers from soybean, chickpea, and bean correlated the magnitude of the hypocholesterolemic effects with some physicochemical properties such as water-holding capacity, viscosity, and cation exchange capacity of the fibers (Uberoi et al., 1992; Chau and Cheung, 1999).

The reduction of serum glucose level in the current experiment, by the increasing concentration of lupin in the diet, is in agreement with the in vitro and in vivo between a lupin seed protein (namely, conglutin γ) and insulin, reported recently by Magni et al. (2004). The effect of the oral administration of conglutin γ on the glycemic levels of rats subjected to glucose overloading resulted in a significant reduction in rat glycemia.

The reduction in serum cholesterol level observed with lupin diets in our study was also accompanied by a significant lower serum albumin, β-globulin, γ-globulin, and albumin:globulin ratio. Most of the circulating cholesterol is carried in birds by high-density lipoprotein cholesterol (present in α-2 globulin fraction) and LDL (present in β-globulin fraction; Zantop, 1997). These lipoproteins became the principal cholesterol transport and carried about 40 to 44% of the total serum protein.

The serum β-globulin fraction-lowering effect observed by the inclusion of lupin in the diet could be associated with a reduction in serum LDL cholesterol. Kingman et al. (1993) and Chau et al. (1998), in pigs and rats fed diets containing *Phaseolus vulgaris, Phaseolus lunatus, Pisum sativum,* and *Lens culinaris* (Kingman et al., 1993; Chau et al., 1998), also observed a reduction in serum LDL cholesterol. Likewise, Martins et al. (2005), in pigs fed dietary blue lupin, and Sirtori et al. (2004), in rats with isolated lupin protein fractions, corroborated that the cholesterol reduction appears to be associated with stimulation of LDL receptors, a widely accepted mechanism of cholesterol reduction related to the intake of vegetable proteins (Jones, 2002). The decrease observed in serum protein as well as the concentration of serum albumin and γ-globulin in response to increasing amount of lupin can be caused by the reduction of feed intake and growth rate observed in our experiment mainly in birds fed the higher concentration of lupin. These results corroborate the study of Rubio et al. (1995) in rats that suggested that the low nutritional value of lupin is caused by a decrease in protein synthesis and increased endogenous losses of protein.

Cholesterol is a major lipid that is a precursor of all the steroid hormones and bile acids as well as a component of the plasma membrane (Zantop, 1997). Considering the liver to be a major site of cholesterol metabolism and that this organ exhibited the greatest response to a cholesterol diet, the addition of cholesterol to the chicken diet could cause the increase in the relative liver weight and liver fat observed in this study. These results are similar to those obtained by Kruski and Narayan (1972) in chickens and Beynen et al. (1986) and Dabai et al. (1996) in rats. In the current experiment, the higher accumulation of

liver fat could induce a liver disfunction. If liver function is impaired, bile acids are not properly reabsorbed from the blood, and consequently, the proportion of excreted bile acids reaching the peripheral circulation increases. Elevations of bile acids have been shown to correlate with liver disease in chickens (Zantop, 1997). The increase in total serum protein and α- and β-globulin and the decrease in albumin:globulin ratio observed in response to cholesterol added could indicate inflammatory disease in the liver. Inflammatory disease state frequently results in increasing protein globulin fraction. Similarly, the increase in the acute-phase proteins (α-1 and α-2 globulins) is produced in the liver in response to inflammatory cytokines (Kendal, 2006). On the other hand, in the current study, significant reductions of serum α-amylase (more than 3 times) in response to cholesterol have also been observed. Serum α-amylase is a Ca-dependent metaloenzyme that catalyzes hydrolysis of complex carbohydrates at internal binding sites. The predominant sites of production are the pancreas, liver, and duodenum (Kendal, 2006). Serum α-amylase increase is sometimes seen with severe enteritis (Zantop, 1997).

In conclusion, the addition of lupin seed in chicken diets caused a negative effect on performance. The observed low concentrations of serum glucose, cholesterol, total biliary salts, and total protein and the modification in the electrophoretic bands of serum protein caused by lupin feeding may have some physiological importance and may be extrapolated to humans. The overall results suggest that lupin seed intake may be effective in lowering the cholesterol absorption as well as the serum glucose level in chickens and that this seed may have a potential application as a cholesterol-reducing agent.

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